TABLE OF CONTENTS VOLUME 12 ISSUE 2 2023 ANATOMY JOURNAL OF AFRICA

1. A REVIEW OF MODERN ANATOMY TEACHING PRACTICES: 2337-2339
   https://dx.doi.org/10.4314/aja.v12i2.1
2. HISTORY OF ANATOMY AND ITS INVOLVEMENT WITH MEDICAL SCIENCE AND PRACTICE: HISTORICAL REVIEW: 2340-2351
   https://dx.doi.org/10.4314/aja.v12i2.2
3. MORPHOLOGICAL VARIATIONS OF THE THYROID GLAND AND ITS RELATIONSHIP TO THE RECURRENT LARYNGEAL NERVE: A CADAVERIC STUDY:2352-2359
   https://dx.doi.org/10.4314/aja.v12i2.3
4. EFFECTS OF AQUEOUS LEAF EXTRACT OF MIMOSA PUDICA ON INSECT REPELLENT-INDUCED INJURY IN THE LUNGS OF ADULT WISTAR RATS: 2360-2374
   https://dx.doi.org/10.4314/aja.v12i2.4
5. FINGER PRINT PATTERNS DISTRIBUTION AMONG DIABETICS AND NON-DIABETICS IN WESTERN UGANDA POPULATION: 2375-2383
   https://dx.doi.org/10.4314/aja.v12i2.5
6. EVALUATION OF Abrus precatorius ON REPRODUCTIVE FUNCTION OF MALE WISTAR RAT: 2384-2392
   https://dx.doi.org/10.4314/aja.v12i2.6
7. HISTOMORPHOLOGICAL CHARACTERISTICS OF PROSTATE SPECIMENS ANALYZED AT A REFERRAL HOSPITAL IN KENYA: 2393-2401
   https://dx.doi.org/10.4314/aja.v12i2.7
8. ANTI-CANCER AND TISSUE-NEUROPROTECTIVE POTENTIALS OF MORINGA OLEIFERA AND MUSA SAPIENTUM AGAINST IN-VIVO CADMIUM CHLORIDEINDUCED SKIN TOXICITY: 2402-2413
   https://dx.doi.org/10.4314/aja.v12i2.8
9. GROSS MOPHOMETRY, HISTOMORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS ON LIVER OF ALBINO RATS FOLLOWING ADMINISTRATION OF RIFAMPICIN AND ISONIAZID: 2414-2421
   https://dx.doi.org/10.4314/aja.v12i2.9
10. RENAL GROSSMORPHOLOGICAL RESTORATIVE EFFECTS OF CURCUMA LONGA ON SILDENAFIL INDUCED NEPHROTOXICITY AMONG MALE ALBINO RATS: 2422-2431
    https://dx.doi.org/10.4314/aja.v12i2.10
11. VARIATIONS IN THE ORIGIN OF THE CORONARY ARTERY AMONG BLACK AFRICAN POPULATION: A CADAVERIC STUDY IN WESTERN KENYA: 2432-2437
    https://dx.doi.org/10.4314/aja.v12i2.11
12. ANATOMICAL VARIATIONS IN THE MORPHOLOGY OF THE BRACHIAL PLEXUS AMONG BLACK AFRICAN POPULATION; A CADAVERIC STUDY IN WESTERN KENYA: 2438-2447

https://dx.doi.org/10.4314/aja.v12i2.12
13. RESTORATIVE EFFECTS OF AZADICARACHTA INDICA ON THE KIDNEY HISTOMORPHOMETRY IN CISPLATIN-TREATED WISTER ALBINO RATS: 2448-2454
https://dx.doi.org/10.4314/aja.v12i2.13
14. STEREOLOGICAL EVALUATION OF VOLUME DENSITIES OF OVARIAN FOLLICULAR COMPONENTS IN HYPERPROLACTIN RATS TREATED WITH IMMATURE COCONUT WATER: 2455-2462
https://dx.doi.org/10.4314/aja.v12i2.14

EDITORIAL

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A REVIEW OF MODERN ANATOMY TEACHING PRACTICES

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INTRODUCTION

Human anatomy is considered to be the cornerstone of medicine. It is an essential part of the learning experience of undergraduate students and serves to equip the aspiring medical practitioner with adequate understanding of the human body and its intricacies; its various developmental stages and microscopic organization (Brenner et al., 2003). This provides the appreciation of the structures which form the functional unit that is composite of a healthy human being. Multiple models of teaching have been employed to dispense anatomical knowledge with each method utilizing different resources to achieve a specific objective. These methods in addition differ in the time it takes to administer knowledge. Different learning institutions thus select their preferred teaching method based on multiple considerations (Estai and Bunt, 2016).

Brenner et al grouped these teaching methods into 6 categories namely: dissection by students, inspection of prosected specimen, didacted teaching, use of models, computer-based learning and the teaching of living and radiological anatomy (Brenner et al., 2003). Multiple studies have reviewed the effectiveness and relevance of these models given the current educational climate including the just recent COVID-19 pandemic learning environment, ethical considerations, cost of teaching, time saved, student interest and preference, and overall memorability (Estai and Bunt, 2016), (Losco et al., 2017). It has also been reported that the quality of anatomical education has decreased over time as learning institutions dedicate less time and effort into anatomy teaching which supposedly has led to an increase in incidences of malpractice due to surgical incompetence related to anatomical errors (Cahill et al., 2000). It is therefore of interest to review these teaching methods to provide further insight into the current state of anatomy education, the impact of modern models and their reported effectiveness.

The first method under review, cadaveric dissection, is the oldest and most commonly practiced method of teaching. This method has been utilized for the past 400 years and has been the primary method of dispensing anatomical knowledge. Cadaveric dissection facilitates active learning, prepares students for the concepts of death, visualizes the classification of various bodily systems, and equips students with useful manual dexterity skills (Estai and Bunt, 2016). However, it is time consuming, expensive and poses a health risk to due to prolonged exposure to formalin. In addition, its validity has been put to question as studies claim it has no added benefit in preparation of students for the clinical experience and that modern teaching practices such as radiology based and computer associated learning provide a better understanding of patient illnesses in relation to anatomical structure (Azer and Eizenberg, 2007). It is for this reason that many developed countries have abandoned this teaching method in favour of other models. However, a review on anatomical teaching in Africa saw that most medical schools retained cadaveric dissection as the primary method of teaching due to easy

access and institutional preference (Kramer et al., 2008).

Prosection as a teaching method utilizes already dissected gross specimen. This method has the advantage over traditional dissection as it cuts down the hours required to visualize anatomical structures and thus is much more time efficient. In addition, it is not dependent on students' dissection skills to find deep set structures that would have otherwise been missed or severed by undergraduate students. This has thus made it highly favourable among anatomy students (Karaer and Barut, 2017). It is an increasingly common method of teaching and in some schools has wholly replaced dissection. However, a more common picture seen is an integrated form of learning where schools adopt both dissection and prosection and in addition, use other methods like computer assisted learning and anatomical models to supplement teaching.

Computer based learning is a model that utilizes computer software to visualize anatomical structures through simulated dissection presented in 3D, virtual or augmented reality. It also simultaneously offers additional information which includes radiological comparisons, histological organization and pathologic illustrations. This interactive method of teaching enables the exploration of the human body from a completely different perspective, displaying cross sections, neurovascular organizations, isolated systems to render an illustration that could not be displayed using traditional anatomical teachings. Computer assisted models include the Visible Human Project by the National Library of Medicine (Estai and Bunt, 2016), the Anatomage table, a virtual reality dissection table, BioDigital Human and various medical learning programs offered by the virtual reality headset developed by Meta, among others. This form of learning is becoming increasingly popular with many institutions due to its flexibility, easier access, student preference and reported effectiveness (Estai and Bunt, 2016). In addition, this teaching method does not require preservation, storage and does not raise any ethical issues. The effectiveness of this model over other methods has been repeatedly assessed with questions of how well it enables short term and long term retention (Losco et al., 2017). Computer assisted learning is also dependent on regular internet access, constant power supply, training to students by professionals and thus in resource limited settings like many medical institutions in Africa, this model is the least accessible and most expensive. It is due to these constraints that an integrated mode of learning has been promoted, focusing on problem based learning and clinical concepts over a specific method of learning.

The other methods of learning, including: didacted learning, the use of models and the teaching of radiological anatomy are methods that although not sufficient by themselves, serve to augment the understanding imparted by the other more practical methods discussed previously (Estai and Bunt, 2016). It is for this reason that they are often neglected and their importance undermined. Didacted learning solidifies anatomical illustrations and highlights the areas of emphasis in the vast field of gross anatomy. It enables discussions between learners and teachers that keep a student active and motivated. Anatomical models are the practical illustrations of various systems and have the advantage of longevity without the need for preservation, easy visualization of human structures without the complexity of organic material that hinders visualization and are readily available. Radiological images provide an abstract visualization of anatomical structure using 2D images and 3D reconstructions. With the massive relevance of imaging technology in the medical practice, this method gives students an introduction to radiographic anatomy in correlation with the gross specimen and equips them with

relevant skills for the interpretation of radiological images.

There is much speculation on the future of anatomy given the current educational climate. Reduced hours dedicated to anatomy, abandonment of manual dissection and the adoption of distance online learning due to the COVID pandemic are some of the current trends in anatomy teaching. However, given the increased emphasis by anatomists and specialists on the importance of maintaining high standards of anatomy education, institutions are adopting changes to their curricula that are more student-centered, problem-based, integrated, community-based, and systematic. This includes the enhancing of continuous anatomy learning throughout the medical course, with the encouragement of a peer system of teaching between advanced and younger learners. In addition, the vertical integration of anatomy into other clinical topics will be employed more and more, with increased emphasis on radiological anatomy and the training of instructors on this updated model of learning. A big part of the future of teaching involves the increased incorporation of technology-based learning models to reflect the increasing integration of technology into therapeutic practice. However, this should not come at the expense of previously utilized traditional methods such as cadaveric dissection which equips students with useful dissection skills. The understanding of anatomy is crucial to the development of a competent clinician and aspiring surgeon and should not be overlooked in order to promote better patient care. With the multiple methods available, institutions should dedicate enough time and utilize their available resources to provide an integrated method of learning that includes multiple models and has a focus on clinical aspects and problem-based learning so as to create a well-rounded student and clinician in order to enhance safe medical practice.

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REVIEW ARTICLE

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HISTORY OF ANATOMY AND ITS INVOLVEMENT WITH MEDICAL SCIENCE AND PRACTICE: HISTORICAL REVIEW.

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ABSTRACT

The medical practice starts from Patient's discomfort in his body structures due to distortion in morphology. This is located and examined by clinician so, medical practice and Anatomical knowledge are hand and gloves with each other. It is evident from history of Anatomy that Anatomy has been discovered by practicing physicians and surgeons. That clearly indicate that they could make the medical practice only after exploring the Anatomy. The source of knowledge of Anatomy were animal or human being as is evident from this review.

The medical practice might have been started from evolution of human-being. Earlier some people were exploring the herbal medicine on the results of experiments on themselves for the effect of these medicines to treat their own discomforts. When it shewed the impact, they chose it to treat others for the same discomfort. That time they might not be knowing Anatomy but it was an empirical result. As the location of discomforts was associated with remedy so, this might have involved Anatomy. History of clinical practice and thereby Anatomy is as old as 1600BC. Earliar the people experimented on animals and then on cadavers and executed criminals. Then came different Anatomical Laws governing the use of cadavers. Now, current scenario is that there is lack of cadevers all over the world. To compensate for this, new methods including anatomage table, models and charts have been used to teach anatomy. But these novel methods can only supplement and cannot replace cadaveric dissections. It is very essential to know how Anatomy evolved and took present shape. This encouraged the author to review history of Anatomy and correlate with the simultaneously running clinical practice. The development of Anatomy has been presented along with its advancement with development of science and technology involved with medical education and clinical practice. Thus, Anatomy is not merely an academic subject in medical education rather Anatomy is needed to grasp all the subjects of medical education used to develop clinical skill for clinical practice. This has also been supported by history of Anatomy as all other subjects of medical education evolved from Anatomy. The interwoven interrelations between these subjects with clinical practice are essential as revealed by history of Anatomy to analyse the diagnosis and treatment.

Keywords: History of Anatomy, morphology, Medical Education

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INTRODUCTION

The clinical practice, which is the end product of medical education, takes care of growth and maintenance of human body through diagnosis and treatment of diseases. The human body needs a balanced food intake to grow and maintain it healthy so that it can carry out all its day-today activities comfortably and successfully. But the body is exposed to the environment and subjected day to day activities having many menaces adversely affecting the health of body by creating diseases. Though nature provided well-equipped inbuilt safety network to counter any threat and to streamline its day to day functioning yet, when self-maintenance system fails to protect it from adverse effects of diseases, external aid has been developed in form of clinical practice to provide relief from discomforts of diseases. The methodology of clinical practice has been developed through medical education.

The discomforts of diseases are directly related with human body so, the clinician to understand diseases and their cures must have in depth knowledge of macro/microstructures, associated functions and process of converting food intake into providing energy to recoup the lost energy in day-today activities and work including growth and maintenance of the body (Luis-Alfonso et al., 2010). This knowledge can basically be achieved from basic sciences in general and Human Anatomy in particular. As the causatives of disease firstly distort the structures of the body, then, create discomforts in functions effecting activities creating signs and symptoms of discomforts of diseases. Thus, investigation of diseases and body structures and their functions are too interwoven to be separated from clinical practice. Not to speak of this, the elimination of pathogens by medicines/drugs or repair /removal of infected structures by surgery for restoration of structures to recoup its function demand the thorough knowledge of Human Anatomy (Beahrs, 1991).

The remedial measures need morphology and location of infected structure and configuration of surrounding structures including pathway to access infected/distorted macro/microstructures, or to understand reactions/ adverse effects of medicines/drugs and iatrogenic disorder in surgical treatment. Thus, the safe and successful clinical practice depends on Human Anatomy which are complementary to each other as described in the research literature. Therefore, the objective of study is to review the history of Anatomy and to examine development of Anatomy and successful clinical practice.

HISTORY OF ANATOMY

The medical practice might have been started from evolution of human-being. Earlier some people were exploring the herbal medicine on the results of experiments on themselves for the effect of these medicines to treat their own discomforts. When it shewed the impact, they chose it to treat others for the same discomfort. That time they might not be knowing Anatomy but it was an empirical result. As the location of discomforts was associated with remedy so, this might have involved Anatomy. The history of Anatomy has been divided in following segments chronologically-I.1600 BC-1550; II. 5th BCE to 4th BCE; III. 4th to 2nd BCE; IV 158 CE; VA. 980-1161-1288 AD; VI. Contemporary Europe 1200-15th century; 16th century-17th century; 18th century; 19th century anatomy; VII. Current Research:

I.1600 BC-1550:

In olden times, in Ancient Egypt, the complete bodies were preserved in the form of mummification due to religious beliefs that the dead were thought to live inside the tombs (Queiroz, 2005; Buchaim and Issa 2018). Written description of human organs and parts started thousands of years back in ancient Egyptian papyri where attention to the body was necessitated by their highly elaborated burial practices. But, the study of Anatomy started during *1600 BC at the date of Edwin Smith Surgical Papyrus*. This treatise shows that the *heart*, its *vessels*, *liver*, *spleen*, *kidneys*, *hypothalamus*, *uterus* and *bladder* were recognized, and that the blood vessels were known to emanate from the heart. The Ebers Papyrus (1550 BC) featured a treatise on the heart. It describes that the heart is the center of blood supply, and attached to it are vessels for every member of the body.

The Egyptians seem to have known little about the function of the kidneys and the brain and they made the heart the meeting point of a number of vessels which carried all the fluids of the body, blood, tears, urine and semen. However, they did not have a theory as to where saliva and sweat came from (Porter, 1999). This Anatomy may be of academic interest as it was not used to treat

people by clinical methods. However, the information was used by Edwin Smith during surgery. This would have been due to non-development of medicine at that time and it was not seen in relation to Anatomy. However, this started to build up knowledge of Anatomy. The application of Anatomy for surgery is an obvious thing. Whatsoever it be, these are the traces of history of human Anatomy in Egypt. Though Anatomy was developed to understand Human Body and organization of its organs yet it was found most essential for surgical treatment. Whatever be the reason on growing anatomical knowledge, but it was being used by clinicians including physicians.

II. 5th BCE to 4th BCE:

The nomenclature, methods, and applications for the study of Anatomy were started by the ancient Greeks (Singer, 1957). The Italian philosopher, Alcmaeon initiated dissection of animals for its use to strengthen anatomical knowledge in 5th BCE. He identified optic nerves and Eustachian tubes (Singer, 1957). Alcmaeon published a treatise entitled "On Nature" and it was the first document to describe and locate the optic nerve and eustachian tube. Furthermore, he proposed that the brain is the seat of consciousness, intelligence, and emotions (Malomo et al., 2006). Ancient *Greek physicians,* Acron, Pausanias and Philistion of Locri had probably, explored Anatomy and philosopher Empedocles viewed blood as the innate heat. Empedocles also stated that the heart was the chief organ of the body and was source from which blood was distributed by the blood vessels (Singer, 1957). *The Greek physicians, Acron, Pausanias and Philistion of Locri discovered Anatomy to be used in their clinical practice* and Empedocles accepted the blood as innate heat. He considered heart being main structure supplying blood through blood vessels to the whole body. *Hippocratic Corpus is the collection of information used by various authors but not by* Hippocrates (460-377 BCE) himself. This literature describes the musculoskeletal structure and initially understood functions of certain organs, such as the kidneys. The tricuspid valve of the heart and its function is documented in the *Treatise on the Heart.* Hippocrates of Cos (460 years BC) propounded the moral and ethical codes of medical practice, through his teachings and oaths in the Greek world (Queiroz, 2005; Malomo et val., 2006; Hipócrates). Furthermore, he is considered the "father" of medicine for stating that "the nature of the body is the beginning of medical science" and for the "humor theory of illness" (Queiroz 2005; Malomo et al., 2006). The work of philosopher Aristotle (4th century BCE) was pertaining to animal dissection and biology was regarding comparative anatomy through his empirical research. It is said that Praxagoras may have been the first to identify the difference between arteries and veins, with more accurate descriptions of organs than in previous works. During this period, philosophers and physicians explored Anatomy and besides contributing to Academic Anatomy, used this knowledge in their clinical practice. Human Anatomy was extracted by compared study of animal Anatomy.

III. 4th to 2nd BCE

There is a first record of school of Anatomy in Alexandria from the late *4th to 2nd BCE* (Siddiquey and Shamsuddin, 2009). Ptolemy I Soter of Hellenistic regime allowed medical officials for cadaver dissection for learning Anatomy. Herophilos and Erasistratus were *first to use human bodies for anatomical research*. They obtained permission to perform live dissections, or vivisection, on condemned criminals in Alexandria under the Ptolemaic dynasty specifically, Herophilos who developed anatomical knowledge directly extracted from human dissection rather than previous works. Herophilus described the liver, brain, sexual organs, the origin of some nerves (Queiroz,

2005; Buchaim and Issa, 2018). He also changed the concept of Aristotle regarding the 'seat of Intelligence', the brain instead of heart. Besides, he accurately explained the difference between veins and arteries including many other observations about the structure of human body, especially nervous system (Castro and Landeira-Fernandez, 2011). Erasistratus, established the School of Alexandria, which enhanced the progress of medical sciences (Queiroz 2005; Malomo et al., 2006; Castro and Landeira-Fernandez, 2011). Herophilos and Erasistratus were first to use dissection for studying human Anatomy. First school of Anatomy was started and ran in Alexandria. Earlier human Anatomy was derived from animal Anatomy but Herophilos started to know human Anatomy from cadaver dissection removing certain wrong concepts derived from animal Anatomy and started to develop factual Anatomy from human cadaver dissection. Around 150 BC, human dissection was prohibited for ethical and religious reasons (Queiroz, 2005). In Ancient Rome, Cornelius Celsus (25 BC) wrote chronologically the evolution of medicine from Hippocrates to the foundation of the Alexandria school. The four signs of inflammation were also elucidated by him (Queiroz, 2005.

IV. 158 CE:

In 158 CE, Galen served as chief physician to the gladiators in his native Pergamon. Anatomy was a prominent part of Galen's medical education. Galen elucidated the anatomy of the trachea and was the first to demonstrate that the larynx generates the voice. *Galen's anatomy and medicine were influenced by the 'humorism' theory practiced by many Greek physicians, including Hippocrates.* He wrote two great anatomical works, *on anatomical procedure* and *the uses of the parts of the body of man* (Bay and Bay, 2010; Singer, 1957).

The study on pigs and apes by Galen explored the organs and provided the basis for his *medical works*. Around 100 of these works guided many ancient Greek authors and 22 volumes of this work contributed to modern text. Galen discovered that the living arteries contain blood revoking the concept that these arteries contain air. However, Galen's concept of blood flow was back and forth from the heart in an ebb and flow motion (Pasipoularides, 2014). Circulatory system being open ended (Aird, 2011) believing that all the blood was absorbed by the body and had to be regenerated via liver using food and water (Neder, 2020) Galen considered cardiovascular system as a machine in which blood acts as fuel constantly recirculating rather than acting as a system. (Fleming, 1955) Although certain organs in the vascular system were identified by Galen, many of the functions of these organs were not rightly proved.

V. 980-1161-1288 AD:

At the fall of Roman Empire, the advancement in the knowledge of Anatomy was slowed down in Christian Europe but it was growing faster in the western Muslim countries. The physician Persian Avicenna (980-1037) followed the anatomical concepts of Galen by incorporating them in his "Principle of Medicine" (1020), a prominent research record of Anatomy in the Islamic world until the work of Ibn al-Nafis in the 13th century. *His book remained a guideline for medical education in Europe until the 16th century.* The *physician, Ibn Zuhr* (1091-1161) was the *first Arab to perform dissections in man, as well as necropsies* to study the cause of death. He recognized that scabies was caused by a parasite, a finding that was contrary to the 'mood theory' that came from the Greeks. Removal of the parasite from the patient's body produced healing and did not involve any purging of humor, bleeding, or any other traditional treatment. Physician, Abd-el-Latif from Arab examined human cadavers to identify bones such as jaws and sacra. Physician, *Ibn al-Nafis* discovered pulmonary and coronary circulation for the first time

during 1242 besides explaining metabolism and systems.
The physician, Ibn Jumay, also carried out *dissections of the human body* during 12th century and encouraged fellow-beings to directly dissect *to grasp medicine in a nice way*.

The studies of physician, Abd-el-Latif from Arab and during 1200 in Egypt, scanned human bodies concluding into the formation of bones like jaws and sacra separate from Galen's concept. Later, the physician, *Ibn al-Nafis* from Arab was also well known in *dissections of human bodies and necropsy* during (1213-1288). He investigated pulmonary and coronary circulation during 1242, for the first time so addressed as *the 'father of the circulation theory.'* Besides, he launched the explanation of metabolism and revolutionized the systems of anatomy countering the doctrine of the four humors of Avicenna and Galen. He explained the *pulse, bones, muscles, intestines, sensory organs, bile ducts, esophagus, stomach, and anatomy of almost every part of the human* body (Wikipedia*)*.

VI. Contemporary Europe 1200 AD-15th century

Emperor Frederick II made *human anatomy and surgery* (Crombie, 1967) mandatory for *students of medicine* at the establishment of universities in Italy during 1200 AD. The faculty used to guide the person doing dissection of the body in the universities. Whereas *Mondino de Luzzi did the dissection himself making him one of the first and few to use hands on approach to teaching human anatomy* (Persaud et al., 2014) specifically in 1315. *Mondino de' Liuzzi is known as first Western European to do human dissection* (Lindemann, 2010). He delivered lectures on human anatomy at Bologna University (Gordon, 1959) during 1314 to 1324. Mondino de'Luzzi *wrote a book, "Anathomia" in 1316* consisting of his detailed work on human anatomy. This book remained a lighthouse in universities for 250 years (Persaud et al., 2014). *"Mundinus" carried out the first systematic human dissections* since Herophilus of Chalcedon and Erasistratus of Ceos (Zimmerman and Veith, 1993; Crombie, 1959).
The anatomists started cadaver dissection at Bologna added correct description of organs and their function after decline of Roman empire. After the studies of de Liuzzi, other anatomists who contributed to Anatomy were Alessandro Achillini and Antonio Benivieni during 15th century (Zimmerman and Veith, 1993; Benivieni et al., 1529). During this period factual Anatomy has been extracted from human cadaver dissection and *Mondino de' Liuzzi* wrote a book on human Anatomy in 1316 in Europe. This book remained a guiding literature for about 250 years.

Leonardo da Vinci (1452–1519) received anatomical knowledge from Andrea del Verrocchio. Leonardo drew human forms for two decades in 1489 through his studies. He used his artwork for presenting his anatomical knowledge drawing sketches of skeletal structures, muscles, and heart and vascular system, sexual organ and other internal organs of humans and other vertebrates that he dissected (Boas, 1970; Mason, 1962). He sketched effects of aging and emotion on the human face, as well as the 'three-dimensional anatomy' of body segments. Da Vinci, firstly, followed Aristotle and then Galen. Later he used anatomical knowledge by directly dissecting around 30 human specimens until he was stopped. During this time, Anatomy was expressed through artwork/body painting and it remained a transition time from concepts of Aristotle and Galen and evolution of Anatomy by dissection (Wikipedia).

16th century-17th century

The knowledge from Galen was challenged by Vesalius and Harvey in the 16th century (Bay and Bay, 2010; Boas, 1970a). A large contribution to human Anatomy was made by Andreas Vesalius. Vesalius's success owed

to his concerted efforts to comprehend Anatomy from his own dissection. Anatomical knowledge was accumulated by Vesalius from his own dissections rather than to use works of other scholars which was most prevalent among the simultaneous medical community. Vesalius published a treatise, 'De Humani Corporis Fabrica' for the first time challenging Galen's anatomical teachings on the basis of dissection of other mammals and of human bodies in 1540 (Vesalius, 1540). Vesalius travelled all the way from Leuven to Padua for permission to dissect victims from the gallows without fear of persecution. His superbly executed drawings are triumphant descriptions of the differences between dogs and humans, but it took a century for Galen's influence to fade. Vesalius' work marked *a new era in the study of Anatomy and its relation to medicine*. Vesalius could develop Anatomy as a discipline. His research as well as teaching could establish dissection as most important through his publications and demonstrations (Klestinec, 2004). Thereafter, the anatomic knowledge was extended by the researchers in line and associated their names with their findings of Anatomic structures. The anatomists, to fully comprehend Anatomy, extended its study to natural philosophy but the students were interested in practical usage of Anatomy so they were interested to study technique of dissection rather than philosophy during late sixteenth century. Circulatory system was tried to be understood during 16th and 17th centuries. During this period, the purpose of valves in veins, flow of blood in the ventricles through the circulatory system was studied and hepatic veins described separate from circulatory system. The lymphatic system was also identified as a separate system at this time.

Harvey's anatomical researches from De Motu Cordis in 1628:

English physician William Harvey (1578-1657) studied blood circulation through *dissections of his father's and sister's bodies*. He published *De moto cordis et sanguinis*, a treatise in which he explained his theory (Ghosh, 2017). He gave exact description of the venous valves and analysed the function of the heart by pumping blood. He calculated the volume of the blood in the body and explained that blood cannot flow in reverse direction. Harvey could prove and described that blood flow was through the arteries and veins.

In the start of 17th century, the dissection of human cadaver created more interest in factual knowledge of Anatomy. However, this was an era of drawings of anatomical structures so the upcoming printing technology could compare/correlate the drawings and observed dissection. Contrary to popular belief, the Church neither objected to nor obstructed anatomical research.(Howse, 2009).

In Tuscany and Florence, Marcello Malpighi founded microscopic anatomy, and Nils Steensen studied the anatomy of lymph nodes and salivary glands. By the end of the 17th century, Gaetano Zumbo developed anatomical wax modelling techniques. Antonio Valsalva, a student of Malpighi and a professor of anatomy at University of Bologna, was one of the greatest anatomists of the time. He is called by many as the founder of anatomy and physiology of the ear (Wikipedia).

In the 17th century, many of the anatomical specimens were dried and stored in cabinets. In the Netherland, there were attempts to replicate Egyptian mummies by preserving soft tissue. This became known as Balsaming. In the 1660s the Dutch were also attempting to preserve organs by injecting wax to keep the organ's shape. Dyes and mercury were added to the wax to better differentiate and see various anatomical structures for academic and research anatomy (Wikipedia).

18th century Anatomy

During beginning of 18th century, William Cheselden challenged the Company of Barber Surgeon's exclusive rights on dissections. He was the first to hold regular anatomy lectures and demonstrations. He also wrote *The Anatomy of the Humane Body,* a student handbook of anatomy (Sanders, 1999). Thereafter in 1752, the rapid growth of medical schools in England created the pressing demand for cadavers, the laws to facilitate availability of cadavers were passed by then Government. The supply was still insufficient to meet the demand of cadavers for anatomical and medical training (Ghosh, 2017). So many criminal offences like *body-snatching, sneaking into a graveyard, digging up a corpse and using it including anatomy murder started for study of medicine (Rosner, 2006; Moore, 2006)*. In 17th and 18th centuries, the perception of dissections had evolved into a form of capital punishment. Dissections were considered a dishonour. Thus, shortage of human bodies, due to consideration of dissection as capital punishment and a dishonour, hampered the progress of anatomy in the late 17th and early 18th centuries. So, the concentration shifted from development of anatomical knowledge (Bynum, 1994) to discover the interrelation of anatomy with physiology and surgery.

Paris Medicine was notorious for its influence on medical thought and its contributions to medical knowledge. The new hospital medicine in France during the late 18th century was brought about in part by the Law of 1794 which made physicians and surgeons equals in the world of medical care. The law came as a response to the increase demand for medical professionals capable of caring for the increase in injuries and diseases brought about by French Revolution. The law also supplemented schools with bodies for anatomical lessons. Ultimately this created the opportunity for the field of medicine to grow in the direction of "localism of pathological anatomy, the development of appropriate diagnostic techniques, and the numerical approach to disease and therapeutics" (Bynum, 1994).

The British Parliament passed the Anatomy Act 1832, which finally provided for an adequate and legitimate supply of corpses by allowing legal dissection of executed murderers. The view of anatomist at the time, however, became similar to that of an executioner. Having one's body dissected was seen as a punishment worse than death, "if you stole a pig, you were hung. If you killed a man, you were hung and then dissected." Demand grew so great that some anatomists resorted to dissecting their own family members as well as robbing bodies from their graves (Roach, 2003).

Many Europeans interested in the study of anatomy travelled to Italy, then the centre of anatomy. Only in Italy could certain important research methods be used, such as dissections on women. Realdo Colombo (also known as Realdus Columbus) and Gabriele Falloppio were pupils of Vesalius. Columbus, as Vesalius's immediate successor in Padua, and afterwards professor at Rome, distinguished himself by describing the shape and cavities of the heart, the structure of the pulmonary artery and aorta and their valves, and tracing the course of the blood from the right to the left side of the heart (Boas, 1970b).

Giovanni Batista Morgagni related pre-mortem symptoms with post-mortem pathological findings using pathological anatomy in his book *De Sedibus* (Van den Tweel and Taylor, 2017). This led to the rise of morbid anatomy in France and Europe. The rise of morbid anatomy was one of the contributing factors to the shift in power between doctors and physicians, giving power to the physicians over patients (Harley, 1994). With the invention of the Stethoscope in 1816, *R.T.H. Laennec was able to help bridge the gap between a*

symptomatic approach to medicine and disease, to one based on anatomy and physiology. His treatments were based on "pathological anatomy" and because this approach to disease was rooted in anatomy instead of symptoms, the process of evaluation and treatment were also forced to evolve (Bynum, 1994). *From the late 18th century to the early 19th century, the work of professionals such as Morgagni, Scott Matthew Baillie, and Xavier Bichat served to demonstrate exactly how the detailed anatomical inspection of organs could lead to a more empirical means of understanding disease and health that would combine medical theory with medical practice.* This "pathological anatomy" paved the way for "clinical pathology that applied the knowledge of opening up corpses and quantifying illnesses to treatments"(Lindermann, 2010). Along with the popularity of anatomy and dissection an increasing interest came in the preservation of dissected specimens

By the late 18th century, Thomas Pole published *The Anatomic Instructor*, which detailed how to dry and preserve specimens and soft tissue (Mitchell et al., 2017).

19th century Anatomy

The anatomical research has covered histology and developmental biology of both humans and animals during 19th century. The women were permitted to attend medical school through anatomical theatres. The Royal College of Surgeons closed unregulated medical schools (Mclachlan and Patten, 2006) and museum were used for teaching comparative Anatomy (Reinarz, 2005).

In the nineteenth century, realism and sophistication predominated in portraits of the human body. Furthermore, with the discovery of the microscope, the studies explored the microanatomy of human body (Queiroz, 2005, Calkins et al.,1999). Sophisticated medical devices allowed to study anatomy in living people. Due to growing knowledge and the industrial revolution the advancement in science and technology generated many discoveries in the 20th century and ended with many erroneous thoughts and beliefs that still existed in daily medical life. The invention of the anatomage, synthetic parts, and the use of the internet are some of the new ways to understand over all Anatomy. However, there may be distorted information in the videos, as they are not made by reputable universities or institutions and, therefore, they often end up harming the student (Azer, 2021) thus, factual Anatomy can be grasped only by cadaveric dissection. These technologies have been increasingly used in studies of the human body, as the use of cadavers divides opinions in bioethics together with shortage of dead bodies. In general, the use of anatomical models, three-dimensional tables, YouTube, among other novelties in the study of anatomy, does not come to replace or extinguish the use of cadavers, as synthetic or electronic reproduction as these does not provide or fully represent the human figure (Calazans, 2013; Simão et al. 2016; Melo and Pinheiro, 2010; Buchaim et al., 2014). These methodologies may be used to supplement and expand access to the knowledge of human body, anywhere in the world, for anyone, whether a student or not.

As the anatomist, Gunther Von Hagens was questioned about the use of bodies without provenance in accordance with legal means. He discovered a new way of preserving dead bodies through plastination. This is a technique that replaces organic substances in the body with plastic materials. With that, the corpse became malleable, dry, odorless and with the same tone as the living individual (Queiroz, 2005; Calazans, 2013; Moore and Brown, 2004; Kim, 2012).

Now a days, progress in anatomy is mainly in the study of ontogenetic and phylogenetic development and the study of the function of specific structures, using techniques such as

immunohistochemistry/confocal microscopy by laser scanning, neuronal tracers or others.

Enhanced knowledge of cardiac anatomy and congenital heart disease led to the first surgery to treat congenital heart disease in November 1944 at Johns Hopkins Hospital. In 1938, the ductus arteriosus had been corrected, but now for the first time, there was a specific procedure to correct a congenital heart anomaly called the Blalock-Taussig shunt. This opened the door for new methods to be attempted in this area (Wikipedia).

Current Research:

During this century, there was very rapid progress in comprehension of medical science /clinical practice including evolutionary and molecular biology. Technological development facilitated to analyse comparative Anatomy between normal macro/microstructures of organs and systems and morbid Anatomy of these items together with transformation of metabolic and other processes through MRI machines and CAT scanners to relate with impairment of functions and activities of these structure thereby, signs and symptoms of discomforts to accurately, diagnose the diseases and safe and successful treatment. The endocrinology specialists could explain the function and utility of glands. Progress today in anatomy is centred in the development, evolution, and function of microanatomical features as the macroscopic aspects of human anatomy have largely been catalogued. *However, the variations in macro/microstructures, systems, organs and limbs still remained a challenge.*

With rapidly increasing medical institutions and poor contribution of body donation due to lack of awareness and ethical constraints, there had been an acute shortage of cadavers in medical education. Therefore, to alleviate the "crisis" of the use of unclaimed bodies and voluntary donation of dead bodies coupled with controversial biethics, the introduction of anatomical models, charts, plastinated bodies, prosections, anatomical table ("Anatomage") and use of the internet are used to complement the didactics. Therefore, some institute started teaching through preserved prosections so, the students need not to dissect cadavers. This had its own advantages and disadvantages. The perception of anatomical structures through colour images and 3D photography had good impact on 3D *visualization* of anatomical structures. Artificial anatomical models, surface landmarks were identified on patients and also on the student's self-body (McLachlan and Patten, 2006).

In modern time of advancement of new technology and updating the anatomical education with electronic information (Calazans, 2013; Melo and Pinheiro, 2010) is used for sharing the knowledge among students.

YouTube is an important information network that shares with students video lessons on various subjects such as dissection, body structures, autopsy and surgery (Calazans, 2013; Azer, 2021). Therefore, sites like YouTube and other web-based methods are alternative sources to consolidate anatomical content (Calazans, 2013). Medical Institutes have started to use body painting to give information regarding organization of organs, structures and systems inside the body on the surface. There are many sites like you tube, web-based resources for interaction and video lessons on dissection, body structures autopsy and surgery (Calazans, 2013; Azer, 2021). *However, there may be distorted information in the videos, as they are not made by reputable universities or institutions and, therefore, they often end up harming the student (*Azer, 2021).

There are multitude of variations in human body, therefore, there are hidden mysteries even today which is a challenge to surgeons to be supported by anatomists.

The current challenge of cardiovascular morphology is to characterize the exact role of stem cells. Stem cell research has been a field of increasing activity since the works of Ernest McCulloch and James till in the 1960s at the University of Toronto. There are two different types of stem cells in mammals: embryonic stem cells, isolated from the inner cell mass of the blastocyst and stem cells found in adult tissues. Possible mechanisms of stem cell activation in heart cell therapy include the generation of cardiomyocytes, stimulation and growth of new blood vessels (angiogenesis), secretion of growth factors, and possibly some other mechanism still unknown.

CONCLUSION

Medicine is as old as man and had come into being with the first awakening of human awareness regarding health care. So, Anatomy is the oldest and the most important of all medical subjects. The history of Anatomy reveals that in ancient times, the physicians or medical practitioners discovered the preliminary Anatomy either from surface of human body or through extraction of anatomical relation from animal dissection. This clearly establishes that no clinical care can be realized without relating to Anatomy of human body.

Thus, Anatomy is not merely a subject in medical education rather grasping of all the subjects of medical education can be used to develop clinical skill for clinical practice completely as it depends on Anatomy because these subjects evolved from Anatomy be it Physiology, Biochemistry, Pathology, Radiology and Pharmacology whereas Microbiology has very intimate relation with Anatomy. The study of these subjects along with Anatomy can be used in prescribing medicines/drugs without side-effects and minimal iatrogenic injuries. Presently, Anatomy is being ignored for multitude of reasons is a biggest challenge for safe and successful clinical practice as has been observed in history of Anatomy that the medical practice runs along with Anatomy

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ORIGINAL ARTICLE

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MORPHOLOGICAL VARIATIONS OF THE THYROID GLAND AND ITS RELATIONSHIP TO THE RECURRENT LARYNGEAL NERVE: A CADAVERIC STUDY.

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ABSTRACT

The thyroid gland is prone to variations due to its embryological origin and descent. These variations can cause distortion of its morphology and have been associated with thyroid disorders. This study aimed to provide evidence-based data on the morphological variations of the thyroid gland. In this study, the morphological variations of the thyroid gland pertained to the presence or absence of *pyramidal lobe (PL), levator glandulae thyroidae (LGT)* and *isthmus.* The main objective of this study was to assess the morphological variations of the thyroid gland and its relationship to the *recurrent laryngeal nerve* in cadavers. This was a cross-sectional descriptive study, conducted at the Pathology Department of the University Teaching Hospitals (UTH) in Lusaka, Zambia. In the study there were 46 cadavers; 36 males and 10 females aged between 20-64. The study involved dissection and removal of the hyoid bone, larynx, trachea, oesophagus, and thyroid gland en bloc. The thyroid gland was observed for the presence of the *PL* and *LGT,* and absence of *isthmus*. If present, the length, width and height were measured using a Vernier caliper. Location of the *isthmus* as well as the relationship of the thyroid gland to the *recurrent laryngeal nerves (RLNs)* were also observed. The most common variation was presence of *PL*, 32.61% (36% of males and 20% of females). The most common origin of the *PL* was both the *isthmus* (40%) and left lobe (40%). *Levator glandulae thyroideae* were commonly attached to the hyoid bone (72.7%). Statistically significant differences (<0.0001) were found in the mean length of *LGT* between males and females. Thickness of the *PL* was more in females than males and this difference was significant (< 0.015). The majority of the isthmi were found located on tracheal rings 1 and 2 (32.6%). The *RLNs* were mostly medial to the thyroid gland; *LRLN* (93.5%) and *RRLN* (91.3%). There was a high incidence of *RLNs* traversing the larynx posterior to the cricothyroid joint, 89.1% of *LRLN* and 93.5% of the *RRLN.* Knowledge of these variations, their measurements and the relationship of the thyroid gland to the *RLNs* may be of help to surgeons to perform safe and effective thyroid surgeries with reduced complications.

Keywords: Variations, thyroid gland, pyramidal lobe, levator glandulae thyroideae, isthmus, recurrent laryngeal nerve

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INTRODUCTION

The thyroid gland is an endocrine gland located anteriorly in the lower part of the neck (Drake *et al.*, 2015; Standring, 2016). It is the first endocrine gland to start developing in the embryo (Moore *et al.*, 2016). The thyroid gland has two (2) lobes connected by an isthmus. It is postulated that 50% of thyroid glands have a third lobe called the *pyramidal lobe* (Ranade *et al.,* 2008). The embryological origin and descent

of the thyroid gland predisposes it to multiple anatomical variations and congenital anomalies (Dessie, 2018). Some of these variations include, *pyramidal lobe* (*PL*), *levator glandulae thyroideae (LGT),* aberrant thyroid tissue and absence of isthmus. However, aberrant thyroid tissue is rare and occurs in 1 per 100 000-300 000 people (Noussios *et al*., 2011). Thyroid disorders are quite common and some have been linked to high incidence of variations such as the *pyramidal lobe* (Gurleyik, *et al*., 2015). Thyroid surgeries are one of the most common surgeries performed in the head and neck region (Raut *et al.,* 2018). Thus, knowledge of these variations is cardinal to surgeons to minimise iatrogenic injuries. The most common and serious complication that occurs after thyroid surgery is palsy of the recurrent laryngeal nerve due to its relation to the thyroid gland (Chaing, *et al*., 2010). Therefore, studying the relationship between *RLNs* and the thyroid gland and its variations, can help lower the risk of injuring these nerves. Several studies (Prakash *et al.,* 2012, Gaikwad and Joshi 2016, Diana *et al.,* 2019*,* Mitesh *et al.,* 2019, Al-Azzawi and Takahashi, 2021) have been done on the morphological variations of the thyroid gland. However, very little data was found in sub-Saharan Africa particularly, Zambia. In as much as studies agree that variations such as *PL*, *LGT* and absence of *isthmus* are common, their statistics vary. The studies also show differences in the morphometric measurements of the *PL, LGT* and *isthmi.* These differences are also seen when comparisons are made between gender. Therefore, it was important that evidence based data be obtained in a Zambian setting that would show if these variations were present, how common they occur and their measurements. Information on the point of entry of the *RLN* into the larynx and its relationship to the thyroid gland is scanty. Therefore, there also need to study this relationship as it has surgical implications.

MATERIALS AND METHODS

This was a cross sectional descriptive study conducted at the University Teaching Hospitals in Lusaka. The sample size was calculated using STATA. Assumptions using data from previous studies (Gurleyik *et al.,* 2015; Diana *et al.,* 2019; Mitesh *et al.,* 2019) were made. This data was imputed in STATA and an estimated sample size was calculated. Using systematic sampling, 46 non-embalmed cadavers, of which 36 were male and 10 were female, were selected. These were aged between 18-64 scheduled for post mortem examination. Due to age related changes that occur with age such as fibrosis and atrophy which leads to a reduction in volume (Ajish and Jayakumar, 2012, Lee *et al.,* 2016)) cadavers above the age of 70 were excluded from the study. Functional changes which result in increased risk of disorders of the thyroid gland which could affect morphology have been reported in individuals above the age of 60 (Gesing, 2015). The study also included cadavers that were collected within 48 hours of death to avoid the natural decay process which sets post-mortem. All cadavers were black and of Zambian origin. Excluded from the study were cadavers that had a history or evidence of neck surgery, trauma of neck region or had disease that distorted the morphology of the thyroid gland. This information was obtained from the files of the deceased, police reports and physical evidence obtained by forensic pathologists. The variations that were studied included presence of *PL, LGT* and absence of *isthmus.* A midline incision was made from the chin to the suprasternal notch. The infrahyoid and suprahyoid muscles were detached from there caudal attachments. The hyoid bone, larynx, trachea, oesophagus and thyroid gland were removed en bloc. Removal ensured that the inferior thyroid artery as well as the recurrent laryngeal nerve were

undisturbed. The thyroid was examined for absence or presence of the *levator glandulae thyroideae, pyramidal lobe* and *isthmus*. Location of *Isthmus* in relation to tracheal rings was also noted. The relation of the thyroid gland to the *RLN* at its point of entry into the larynx was also observed.

If *pyramidal lobe* was present, its origin was observed (right or left lobes or isthmus). Measurements in terms of length, width and thickness were done. The length of the *PL* was measured from the base to the apex, the width was taken as the transverse diameter of the base and thickness in the anteroposterior diameter of the base (Dessie, 2018). This was done using a Whitworth Vernier calliper. The vernier calliper was calibrated by Zambia Metrology Agency (ZMA), which is accredited by the Southern African Development Community Accreditation Service (SADCAS). To minimise intra-observer error, measurements were taken twice and an average was reported. The length of the *PL* was characterized as follows; short (≤15 mm), medium (16–30 mm), or long (≥31 mm) (Gurleyik, *et al*., 2015). If *levator glandulae thyroideae* was found, its origin as well as superior attachment and length were measured. The length, height and width of *isthmus* was also measured. Its location in relation to the tracheal rings was observed. The relationship of the *RLN*s in relation to the thyroid gland was also observed. Results were recorded in a data collection form. Data was analysed using STATA version 13 and all statistical analyses were significant if the P value was <0.05. To determine normality of the continuous variables, Shapiro-Wilk test was done which showed that data was normally distributed. Independent sample t-test was done to check for statistical significant differences in the morphometry of thyroid gland between males and females. This study was ethically approved by the Biomedical research ethics committee of the University of Zambia.

RESULTS

The number of cadavers with variations were 15 (32.6%) and those without variations were 31 (64.4%). Of the 15 cadavers with variations, 13 (86.7%) were male and 2 (13.3%) were females.

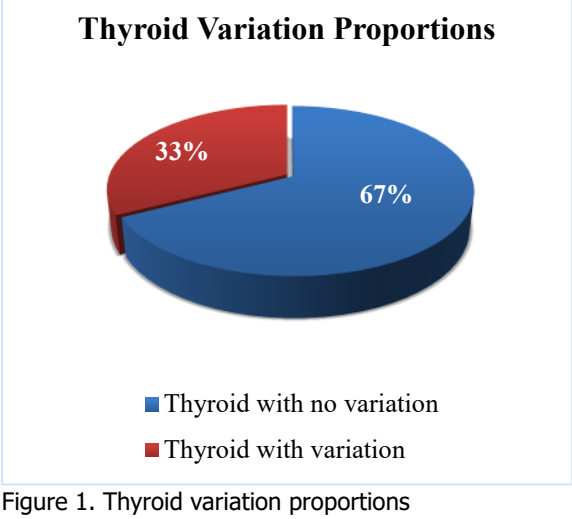


Figure 1. Thyroid variation proportions

Among the male cadavers (n=36) of the study, 23 (63.9%) had thyroid glands with no variations while 4 (11.1%) had a *PL* and 9 (25%) had both the *PL* and *LGT*. Thyroids without any variation were found in 8(80%) of female cadavers (n=10) of the study while 2(20%) had both *PL* and *LGT*. In this study, the presence of *PL* was associated with *LGT.* This is illustrated in the table below. Chi square test showed that there was no statistical significance (*p-value=0.474*) in the incidence of *PL* and *LGT* among males and females.

The common origin of the PL was both the left lobe (n=6, 40%) and the isthmus (n=6, 40%). The least common origin for PL was the right lobe. This is illustrated in figure 2. All the levator glandulae thyroideae were attached to the apex of the pyramidal lobe. The majority of the LGT were attached to the hyoid (n=8, 72.7%) superiorly and the rest

were attached to the thyroid cartilage (n=3, 27.3%) as shown in figure 3. The isthmus was commonly located in relation to tracheal rings 1 and 2 (n=15, 32.6%).

Table 1: Presence or absence of thyroid variations according to gender

Gender Cross tabulation						
				Gender		Total
	Male			Female		
	N	%	N	%	N	%
TG with no variation	23	63.9 %	8	80.0 %	31	67.4 %
Only *PL*	4	11.1 %	0	0.0%	4	8.7%
Both *PL* & LGT	9	25.0 %	2	20.0 %	11	23.9 %
Total	36	100.0 %	10	100.0 %	46	100.0 %

Key: TG=Thyroid gland; PL=pyramidal lobe; LGT=levator glandulae thyroideae

The pyramidal lobe (n=15, 32.61%) had a mean length of 15.29 mm, a width 8.75 mm and a thickness 2.34 mm. The thickness of the PL was more in females (3.85 mm) than males (1.97 mm) and this difference was significant with a p-value of 0.015. The mean length of the LGT was 22.26 mm, width 3.78 mm and thickness was 0.60 mm. The mean length of the LGT was more in males (23.86 mm) as compared to females (14.34 mm). This difference was statistically significant (p-value <0.0001). There was also a statistical difference in the mean of the width (p-value=0.033) and thickness (p-value=0.001) of the LGT. The isthmus was present in all the cadavers (n=46, 100%). It had a mean length of 9.85 mm, height was 10.99 mm and a thickness 2.32 mm. The mean thickness of the isthmus was more in females and this finding was statistically significant (p-value <0.001).

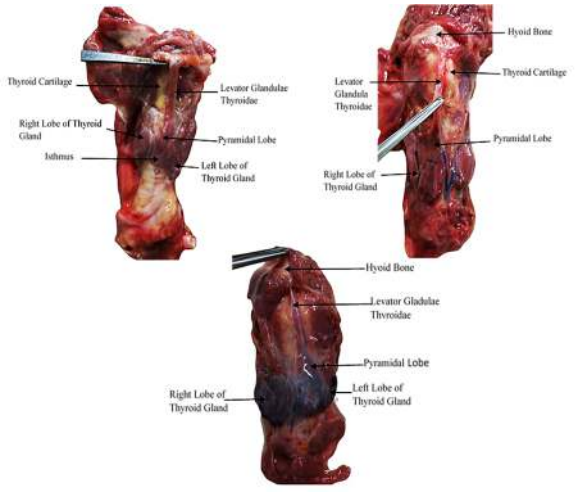


Figure 2: Anterior view showing origin of Pyramidal lobe; Isthmus (a), Right Lobe (B), Left Lobe (C)

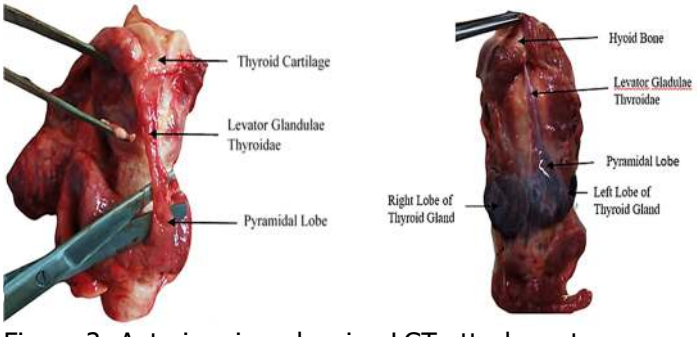


Figure 3: Anterior view showing LGT attachment to Thyroid cartilage (A) and Hyoid Bone (B)

Table 2: Descriptive Statistics of Morphometric Measurements in millimetres (mm)

	N	Minimum	Maximum	Mean	SD
PL Length	15	7.37	29.49	15.29	5.87
PL Width	15	5.11	15.77	8.75	3.19
PL Thickness	15	0.60	6.69	2.34	1.83
LGT Length	11	7.84	34.60	22.26	7.40
LGT Width	11	2.30	4.65	3.78	0.77
LGT Thickness	11	0.16	0.84	0.60	0.22
Isthmus Length	46	2.95	16.44	9.85	2.94
Isthmus Height	46	3.77	17.42	10.99	3.40
Isthmus Thickness	46	0.71	8.90	2.32	1.45

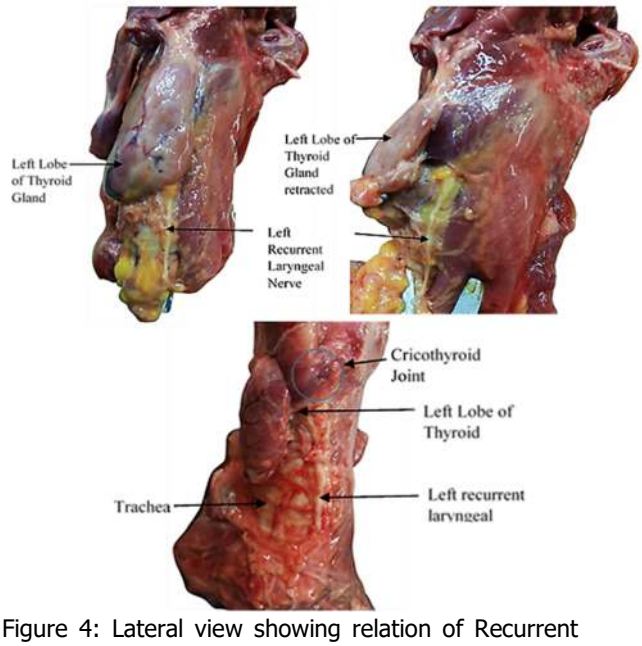


Figure 4: Lateral view showing relation of Recurrent laryngeal nerves to Thyroid Gland. Key: *LGT=Levator glandulae thyroideae*; *PL=Pyramidal lobe; N=Number*

The *recurrent laryngeal nerves* were commonly found medial to the thyroid gland, 43 (93.5%) for the *LRLN* and 42 (91.3%) for the *RRLN*. This is illustrated in *figure 4*. However, some of the nerves appeared as though they were posterior to the thyroid gland, 3 (6.5%) for the *LRLN* and 4 (8.7%) for the *RRLN*. However, this impression could have been spurious because the lobe appeared to be small (*figure 4C*).

The most common entry point into the larynx was posterior to the cricothyroid joint 41(89.1%) of *LRLN* and 43 (93.5%) of the *RRLN* (*figure* 4).

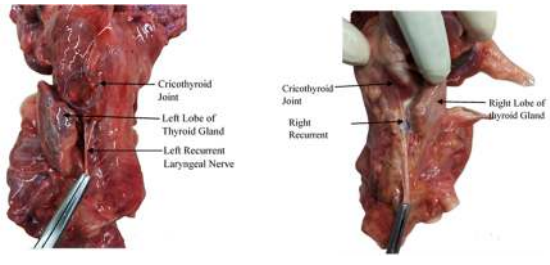


Figure 5: Lateral view showing recurrent laryngeal nerve; Branched (A) Not Branched (B); Point of entry

DISCUSSION

The thyroid gland is the first gland to develop approximately 24 days after fertilization (Maneenin *et al.,* 2019). It develops as a median endodermal thickening in the floor of the pharynx between tuberculum impar and copula. It descends in front of the pharyngeal gut as a bi-lobed diverticulum but remains attached to the tongue via the thyroglossal duct. As it descends the neck, it passes anterior to the developing hyoid and cartilages of the larynx. By week 7, the thyroid assumes its definitive shape and is located anteriorly on the lower part of the neck (Sadler, 2015; Moore *et al.,* 2016). Due to its origin and descent, the thyroid gland is prone to anatomical variations.

In the present study, there was a high incidence of the *pyramidal lobe* found in 15 (32.6%) of cadavers as compared to *LGT* found in 11(23.9%). A high incidence of *PL* than *LGT* was also found in studies done in 2018 by Dessie, (*PL* 52.5% and *LGT* 40 %) and Hemalatha *et al*., (*PL* 43.33% and *LGT* 36.66%). Another study conducted by Veerahanumaiah *et al., (2014),* found a higher incidence of *PL* (46%) than *LGT* (41%). However, a study done by Raut *et al.,* (2018) found a higher incidence of *LGT* (38.33%) than *PL* (25%). Geographical location and ethnicity could have contributed to differences in statistics (Gaikwad and Joshi, 2018).

In our study, there was a high incidence of *PL* in males (36.1%) than females (20%). That difference was not statistically significant. A similar study done by Gaikwad and Joshi *(2016),* found a high incidence of *PL* in male (37.77%) than female (16.66%) cadavers and this was also not statistically significant. In contrast, studies by Hemalatha and Subba-Rao (2018) and Mitesh *et al.,* (2019) found higher incidences of *PL* in females than males. The present study found a high incidence of *LGT* among males (81.8%) than females (18.2%) which was similar to a study by Prakash *et al.,* (2012).

However, a study by Mitesh *et al.,* (2019) found *LGT* more frequently in females than males. The *isthmus* was present in all cadavers and was commonly related to tracheal rings 1 and 2 in the present study.

Pyramidal lobe thickness was more in females than males and this was statistically significant (p-value=0.015). Mean length of *PL* was more in males but this was not significant. The mean length of the LGT was more in males than females and this was statistically significant (p-value <0.0001). Similar findings by Raut *et al.,* 2018. The findings were in contrast to others studies where the mean length of PL was 22.7mm and 23.1mm (Gurleyik *et al.,* 2015; Dessie, 2018 respectively). Differences in methodology when measuring the *PL* and *LGT* could contribute to the varying statistics.

The origin of the *pyramidal lobe* in this study were both the *isthmus* (40%) and left lobe (40%) with the least being the right lobe. Some other studies have found the most common origin was the *isthmus* (Raut *et al.,* 2018), others the left lobe (Prakash *et al.,* 2012; Rajkonwar and Kusre, 2016), and some the right lobe (Gurleyik *et al.,* 2015; Manneenin *et al.,* 2019). The results of the current study could have resulted from a small study sample. In males, the majority of the *LGT* were attached to the hyoid (n=7, 87.5%) while the rest were attached to the thyroid cartilage (n=2, 66.7%). In females, they were equally distributed. This is consistent with a study done by Raut *et al.,* (2018). In other studies, all cases of the *LGT* were found to be attached to the apex of the *PL* inferiorly and the hyoid superiorly (Begum *et al.,* 2009; Rajkonwar and Kusre, 2016).

In the current study, the majority of the *RLNs* were medial to the thyroid gland. Some of the observed *RLNs* branched before entering the larynx. However, despite the branching, the most common point of entry for the *RLNs* was posterior to the cricothyroid joint, 89.1% and 93.75% for *LRLNs* and *RRLNs* respectively. As a result of the *RLNs* branching, some of the nerves had branches that entered the larynx anterior and posterior to the cricothyroid joint, 8.7% for *LRLN* and 4.3% for *RRLN.*

Conclusion

The most common variation of the thyroid gland was the pyramidal lobe. Levator glandulae thyroideae were commonly found attached to the hyoid bone. All the cadavers had an isthmus and the majority were related to tracheal rings 1 and 2. The RLNs were mostly related to the thyroid gland medially.

Limitations of study

There were fewer post-mortem examinations done on females which explains the lower numbers.

With regard to the determination of relationship of thyroid gland to the *RLN* at its point of entry into the larynx, the anterior and posterior branching pattern was unexpected and requires further research

Recommendations

Surgeons, and radiologists to be conversant with the variations such as the *pyramidal lobe* for purposes of accurate diagnosis. We also recommend that during surgery, attention should be paid to the pre-laryngeal area for the presence of the *levator glandulae thyroideae.* Further research is required to determine if the risk of thyroid disorders has any relationship to morphological variations of the thyroid gland. Future studies to include the branching pattern of the *recurrent laryngeal nerve* at site of entry into the larynx.

Acknowledgements

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ORIGINAL ARTICLE

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EFFECTS OF AQUEOUS LEAF EXTRACT OF MIMOSA PUDICA ON INSECT REPELLENT-INDUCED INJURY IN THE LUNGS OF ADULT WISTAR RATS

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ABSTRACT

The aim of this study was to investigate the effects of aqueous leaf extract of *Mimosa pudica* in the lungs of adult Wistar rats exposed to insect-repellent mist. 30 adult Wistar rats were randomly assigned into 5 groups; group A-E comprising of 6 rats per group. Group A rats were placed on rat food and water only. Group B rats were exposed to insect repellant only via inhalation. Group C rats were given *Mimosa pudica* extract orally at 500mg/Kg body weight per day (BWT/D) and Group D rats were exposed to Mosquito repellent and received low dose of the plant extract at (250mg/kg BWT/D) and Group E rats were exposed to mosquito repellent and received (500mg/kg BWT/D) of extract. The dosages were given for 30 consecutive days via orogastric method. There was significant increase in body weight in all treated groups. The haematological outcome showed that insect repellent caused some derangements in haematological parameters especially haematocrit, plateletcrit and mean corpuscular hemoglobin concentration. Histologically, Group D showed an expanded lumen of the bronchioles and shrunken activated immune system. Group E showed a more activated immune system and contracted bronchial artery. Mosquito repellant induced vascular ulceration, bronchiolar mucosal ulceration and activated lymphoid tissue in Group B. These effects were reversed by *Mimosa pudica* leaf extract. In conclusion, *Mimosa pudica* had ameliorative effect against mosquito repellant induced injury in the lungs.

Keywords: *Mimosa pudica,* Mosquito repellant, Lungs, Insecticides

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INTRODUCTION

Mimosa pudica also called sensitive plant, touch-me-not or shame plant is a creeping annual or perennial flowering plant of the pea/legume family *Fabaceae*. *Mimosa pudica* was formally described by Carl Linnaeus in species plantarum in 1753 (Centre for Plants Biodiversity Research). The stem is erect in young plants, but becoming creeping or trailer with age.

The leaves are bi pinnately compound with one or two pinnae pairs, and 10-26 leaflets per pinna. The flowers are insect and wind pollinated (US Forest Service, 2008). *Mimosa pudica* is well known for its rapid plant movement. It undergoes changes in leaf orientation termed "sleep" or nyctinastic movement, the foliage closes during darkness and reopens in light (Raven *et al.,* 2005).

Mimosa pudica (*M. pudica)* contains the toxic alkaloid mimosine, which has been found to also have anti-poliferative and apoptotic effects (Restivo *et al., 2005*). *Mimosa pudica* contains various compounds, including "alkaloids, flavonoids C-glycosides, sterols, terenoids, tannis, saponin and fatty acids" (Genest, 2008). The roots of the plant have been shown to contain up to 10% tannin. A substance similar to adrenaline has been found within the plant's leaves. *Mimosa pudica's* seeds produce mucilage made up of D-glucuronic acid and D-xylene.

Additionally, extracts of M.pudica have been shown to contain crocetin dimethylester, tubulin and green-yellow fatty oils. A new class of phytohormone turgorines, which are derivatives of galli acid, have been discovered within the plant (Azmi, 2011).

The leaves of *M. pudica* also contain a wide range of carbon to mineral content as well as a large variation in (Carbon-13) values. The roots contain sac-like structures that release organic and organo-sulphur compounds including sulphur dioxide (SO_2), methylsulfinic acid, pyruvid acid, lactic acid, ethanosulphic acid, pyruvic, lactic acid, ethanesulfinic acid, propanesulphinic acid and thioformaldehyde (Volkov *et al*., 2010).

Insecticides are substances used to kill insects (IUPAC, 2006). Insecticides contain two types of chemicals; first is the synthetic insecticides assigned to groups based on the mode of toxic action, such as groups of organochlorines, organophosphate, carbamates, and pyrethroids insecticides; the second is natural insecticides such as Azadirachtin, rotenone, spinosad and abamectin. It causes adverse health effects to humans and ecosystems. Previous studies show that synthetic insecticides such as melathion, methomyl, chlorpyrifos, pirimiphos-methyl, dimethoate, and beta-cyfluthrin caused oxidative stress and liver and kidney damage in experimental animals (Witt *et al.,* 2014).

Sniper insecticide, a synthetic organophosphorus, which belongs to the DDVP chemical family (2, 2-dichlorovinyl dimethyl phosphate compound), is indiscriminately being used by many Nigerians as indoor insecticide, which is not the original purpose. Natural insecticides contains chemical, mineral, and biological materials and some products are available commercially e.g. pyrethrum, neem, spinosad, retenone, abamectin, *Bacillus thuringiensis* (Bt), garlic, cinnamon, pepper and essential oil products (Robinson *et al.,* 1990).

Respiratory diseases are pathological conditions affecting the organs and tissues that makes gas exchange difficult in air-breathing animals. Respiratory disease range from mild and self-limiting, such as the common cold, influenza and pharyngitis to life threatening disease such as bacterial pneumonia, pulmonary embolism, tuberculosis, acute asthma, lung cancer (Iyawe *et al.,* 2007).

MATERIALS AND METHODS

The leaves of *Mimosa pudica* that were used for this research work were collected at the back of nursing building, Medical Complex, University of Benin and identified as *Mimosa pudica* at the department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria.

The leaves were air-dried for two weeks. The air-dried leaves were grounded using the British milling Machine into fine powder and weighed. 500g of the powdered leaves were soaked with 1.5litres of distilled water for 24hours with constant shaking and stirring with a stirring rod. After 24 hours, it was filtered using filter paper. The residues were discarded, then the filterate was concentrated to paste level using crucible and water bath at 45°C to actualize the final crude extract.

Phytochemical constituents of *Mimosa pudica* include alkaloids e.g the toxic alkaloid 'mimosine'. It also contains a wide variety of compounds like flavonoids, C-glycosides, sterols, terpenoids, tannis, saponin and fatty acids"(Genest, 2008). Acute oral toxicity of the extract was evaluated and appropriate doses of the extract were made by diluting with distilled water into 250mg/kg body

weight and 500mg/kg body weight which were administered to the rats.

Experimental animals: 30 adult Wistar rats weighing between 180g and 280g were purchased from the animal house, Department of Anatomy, University of Benin, Benin City, Edo State Nigeria and were utilized for this experimental research. The rats were given a period of two weeks to adapt to their new environment before commencement of the experiment. During this period, the animals were allowed free access to standard animal feed (Vital grower's feed, manufactured by Bendel Flour Mill, Ewu) and clean water *ad libitum*.

Each animal procedure was carried out in accordance with approved protocols and in compliance with the recommendations for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010). Lung injury was induced by exposing the rats to sniper insect repellent daily for 30 days.

Experimental design: Thirty (30) experimental adult male Wistar rats were randomly assigned into Five (5) groups; group A-E comprising of six (6) rats per group. Animals in control group were designated as **Group A** and they received grower's mash and water *ad libitum* throughout the experimental period. Animals assigned to **Group B** were exposed to 15ml of insect repellent daily via inhalation. Animals assigned to **Group C** were given *Mimosa pudica* extract orally at 500mg/Kg body weight per day (BWT/D). Animals assigned to **Group D** were exposed to 15ml of insect repellent via inhalation and received low dose of *Mimosa pudica* plant extract 250mg/kg body weight per day (BWT/D) and **Group E** rats were also exposed to 15ml of insect repellent via inhalation and received high dose of *Mimosa pudica* plant extract 500mg/kg body weight per day (BWT/D). The experimental period lasted for 30days. Administration of plant extract was done orally via the use of an orogastric tube for 30 consecutive days.

Method of sacrifice and sample collection: After the 30th day, the animals were sacrificed under chloroform anesthesia. Blood samples were collected with plain specimen bottles for biochemical analysis and the lungs of each rat were harvested and immediately fixed in 10% formal saline for 24 hours before histological analysis.

The harvested tissues were histologically assessed using the following methods: fixation, embedding and tissue staining for microscopy. The tissue sections were examined under Leica DM750 research microscope with digital camera (Leica ICC50) attached. Digital photomicrographs of the tissue sections were taken at H & E x40 and x100 magnifications.

White blood cells (WBCs), lymphocytes, monocytes, granulocytes, red blood cells (RBCs), haemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, mean platelet volume were analyzed using an auto-analyzer (2006 model, manufactured by Hoddlier and Stoughton Group of company, London with a recognized biochemical kit (2010 model, Diagnostical Merek, London). The weights of the experimental animals were taken after 30 days and the difference between them and previous weights were noted. The data were subjected to statistical analysis and P values calculated using the students' t-test.

RESULTS

The results were presented as mean and standard error of mean (tables 1 and 2). Results based on statistical analysis showed that there was significant increase in body

weight across all groups when the initial weight was compared to the final weight. This could be as a result of growth of the rats after 30 days, whereas there was no significant change in the organ weight.

There was a statistically significant increase in the haematocrit value of rats administered *Mimosa pudica* only (**Group B**) indicating a condition known as polycythemia. *Mimosa pudica* that was administered at low dose (**Group D**) prevented this significant increase.

There was a statistically significant decrease in mean corpuscular haemoglobin concentration of rats in the group (**Group E**) exposed to insect repellant and 500mg/kg body weight of *Mimosa pudica* plant extract. There was a statistically significant increase in plateletcrit of rats in the group exposed to insect repellant and 500mg/kg body weight of *Mimosa pudica* plant extract (Group E). This is a condition where the bone marrows are caused to produce too many platelets. When it occurs due to an infection, it is referred to as secondary thrombocytosis.

Table 1: Comparison of Haematological Parameters in All Experimental Groups

	Control	Mosquito repellent only	M. pudica only	Mosquito repellent + M. pudica (low dose)	Mosquito repellent + M. pudica (high dose)	P-value
White blood cells (10^3/µL)	16.62±2.07	13.66±1.19	13.65±1.74	17.65±1.75	16.67±1.78	0.357
Lymphocytes (10^3/µL)	15.06±2.01	12.18±1.04	12.23±1.79	15.98±1.73	14.28±1.29	0.377
Monocytes (10^3/µL)	1.14±0.14	1.06±0.17	1.12±0.07	1.15±0.13	1.75±0.64	0.550
Granulocytes (10^3/µL)	0.38±0.07	0.44±0.11	0.33±0.07	0.53±0.20	0.45±0.12	0.766
Red blood cells (10^6/µL)	6.22±0.20	6.51±0.14	6.24±0.24	6.68±0.20	6.18±0.30	0.457
Haemoglobin (g/dL)	14.06±0.29	14.66±0.22	13.90±0.38	14.35±0.34	13.42±0.52	0.228
Haematocrit (%)	37.86±0.95	40.50±0.49*	38.03±0.79	39.45±0.65	38.38±0.95	0.157
Mean corpuscular volume (µm^3)	61.06±1.96	62.32±1.43	61.18±1.35	59.25±1.68	62.68±2.61	0.717
Mean corpuscular haemoglobin (pg)	22.68±0.62	22.54±0.34	22.33±0.40	21.53±0.51	21.80±0.43	0.381
Mean corpuscular haemoglobin concentration (g/dL)	37.14±0.34	36.18±0.53	36.53±0.39	36.35±0.44	34.93±0.77*	0.083
Platelets (10^3/µL)	653.40±55.61	634.40±37.14	712.00±12.00	662.17±35.35	804.00±85.40	0.174
Meanplatelet volume (µm^3)	7.64±0.19	8.06±0.27	7.70±0.25	8.17±0.26	8.03±0.21	0.446
Plateletcrit (%)	0.50±0.04	0.51±0.03	0.55±0.02	0.54±0.02	0.64±0.06	0.122

***Significantly different from the control group**

Table 2: Changes In Weights of Rats In All The Experimental Groups

GROUPS	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	P-VALU
Control	164.40±9.76	177.80±11.41*	0.017
Mosquito repellent only	145.83±6.49	162.00±8.47*	0.02
M. pudica only	149.50±3.24	168.00±4.97*	0.006
Mosquito repellent+M. pudica(low dose)	151.67±11.47	170.17±8.46*	0.009
Mosquito repellent+M. pudica(high dose)	148.50±4.55	161.50±3.33*	0.004

n=6, Mean value±SEM, **P<0.01

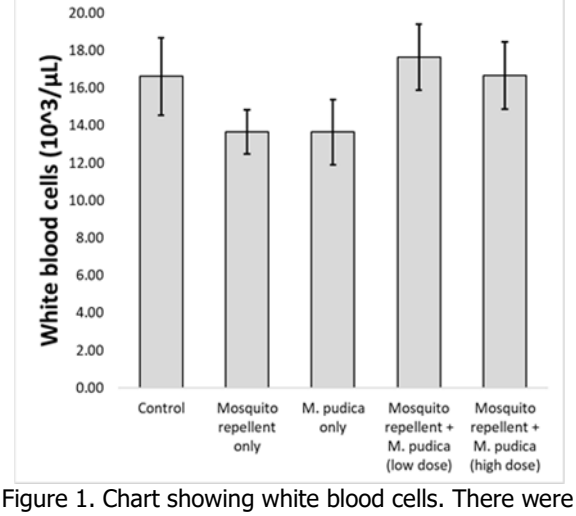


Figure 1. Chart showing white blood cells. There were no statistically significant differences (P>0.05) in the number of white blood cells across the groups

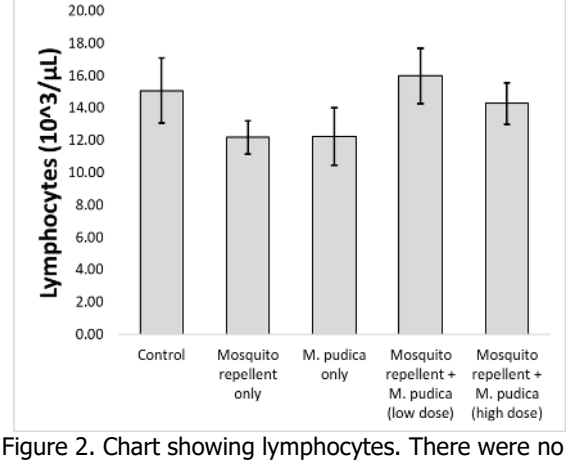


Figure 2. Chart showing lymphocytes. There were no statistically significant differences (P>0.05) in the number of lymphocytes across the groups

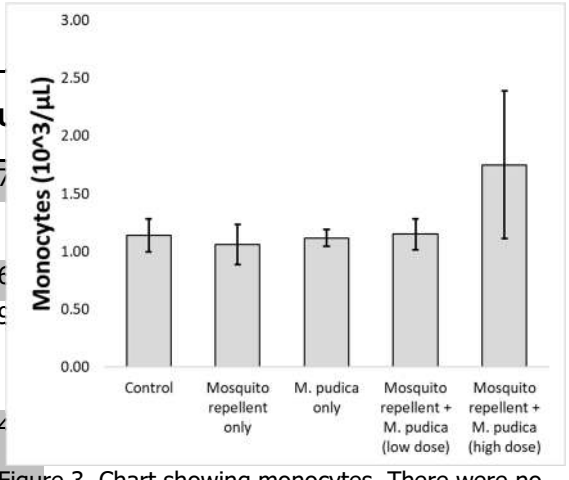


Figure 3. Chart showing monocytes. There were no statistically significant differences (P>0.05) in the number of monocytes across the groups

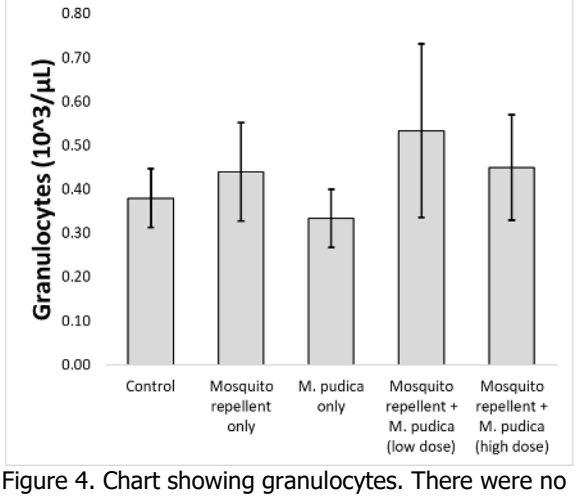


Figure 4. Chart showing granulocytes. There were no statistically significant differences (P>0.05) in the number of granulocytes across the groups

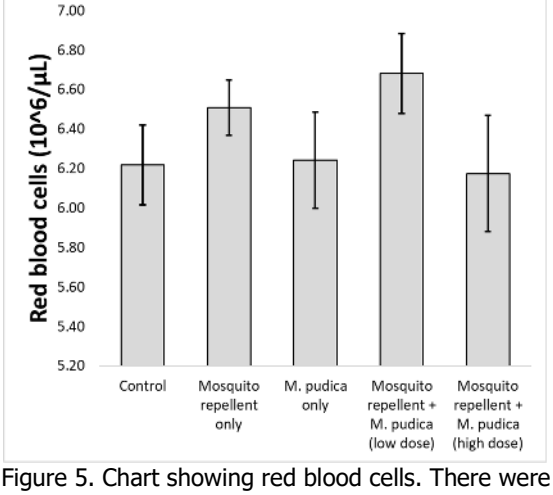


Figure 5. Chart showing red blood cells. There were no statistically significant differences (P>0.05) in the number of red blood cells across the groups.

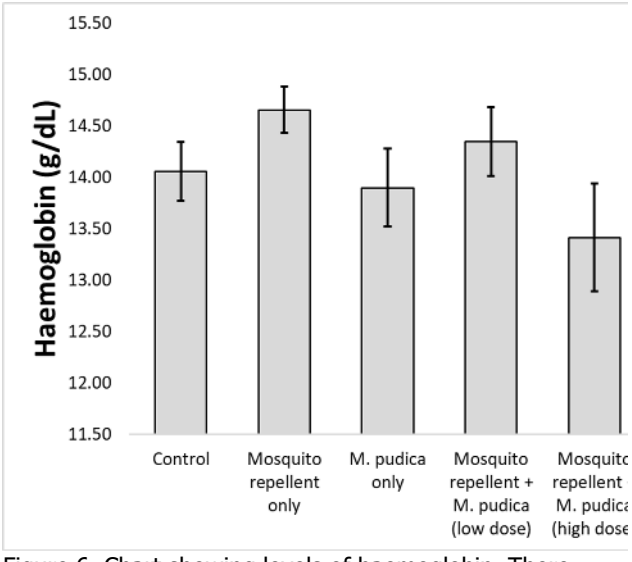


Figure 6. Chart showing levels of haemoglobin. There were no statistically significant differences (P>0.05) in the levels of haemoglobin across the groups

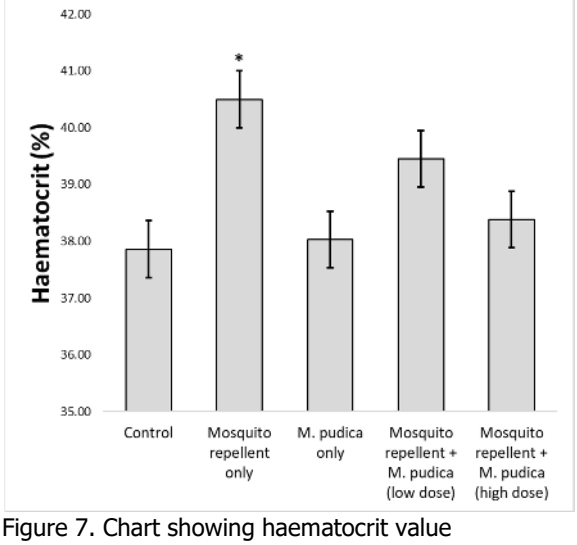


Figure 7. Chart showing haematocrit value
*Significantly different from the control group. There was a statistically significant increase (P<0.05) in the haematocrit value in Group B, when compared to the control group.

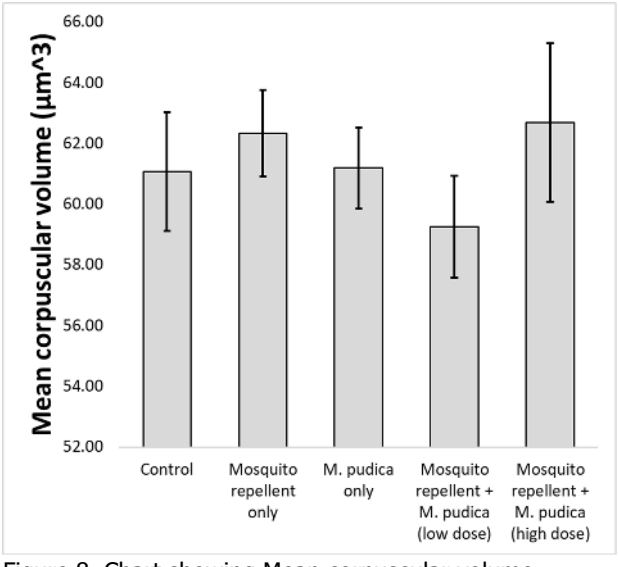


Figure 8. Chart showing Mean corpuscular volume. *There were no statistically significant differences (P>0.05) in Mean corpuscular volume across the groups*

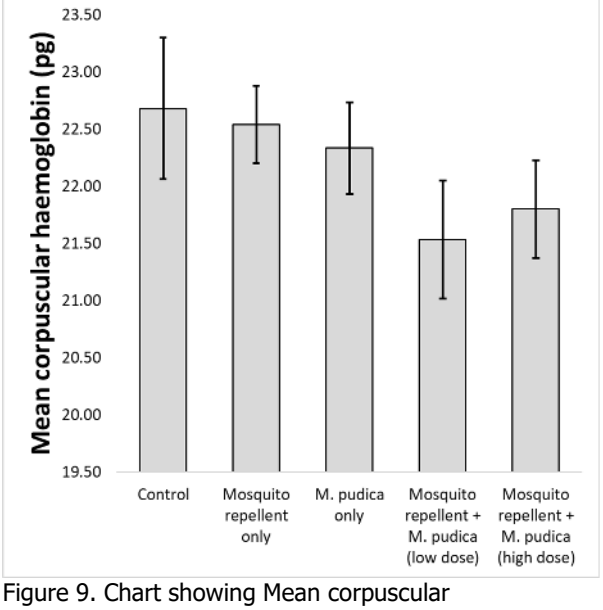


Figure 9. Chart showing Mean corpuscular haemoglobin. There were no statistically significant differences (P>0.05) in Mean corpuscular haemoglobin across the groups

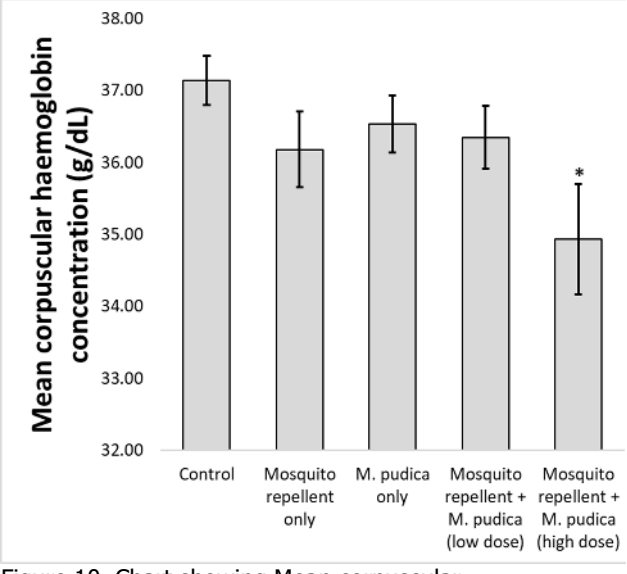


Figure 10. Chart showing Mean corpuscular haemoglobin concentration. *Significantly different from the control group. There was a statistically significant decrease (P<0.05) of Mean corpuscular haemoglobin concentration in in Group E, when compared to the control group.

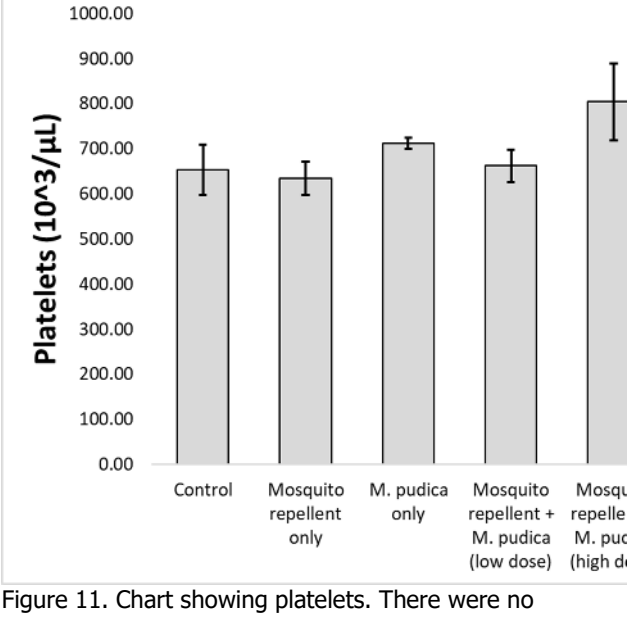


Figure 11. Chart showing platelets. There were no statistically significant differences (P>0.05) in the number of platelets across the groups

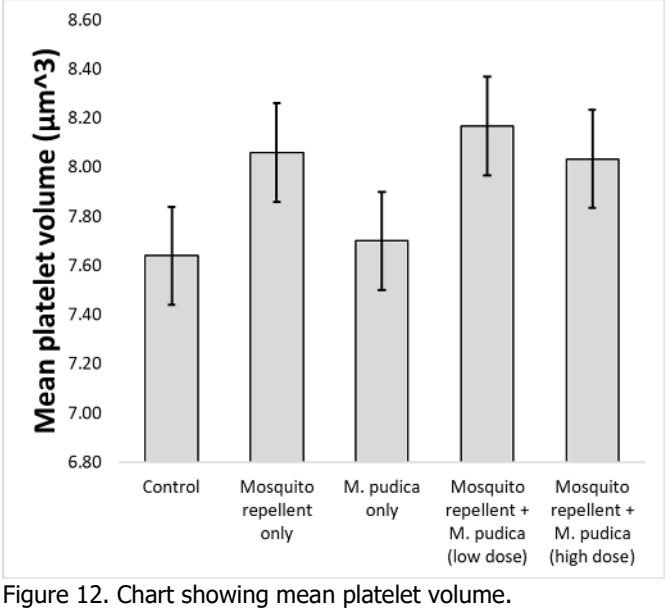


Figure 12. Chart showing mean platelet volume. There were no statistically significant differences (P>0.05) of mean platelet volume across the groups

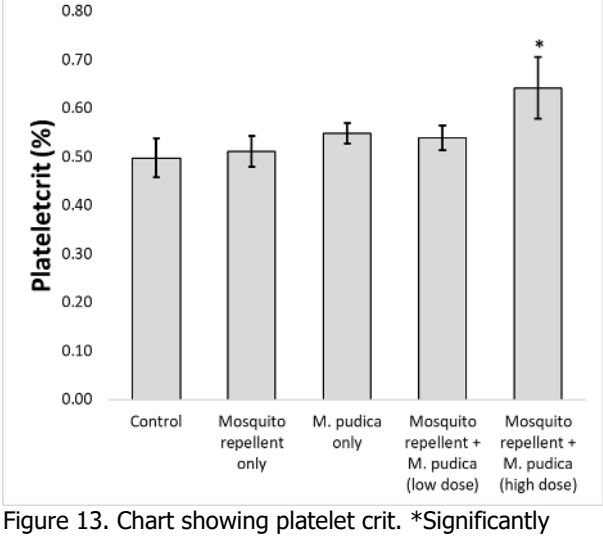


Figure 13. Chart showing platelet crit. *Significantly different from the control group. There was a statistically significant increase (P<0.05) of plateletcrit in Group E, when compared to the control group.

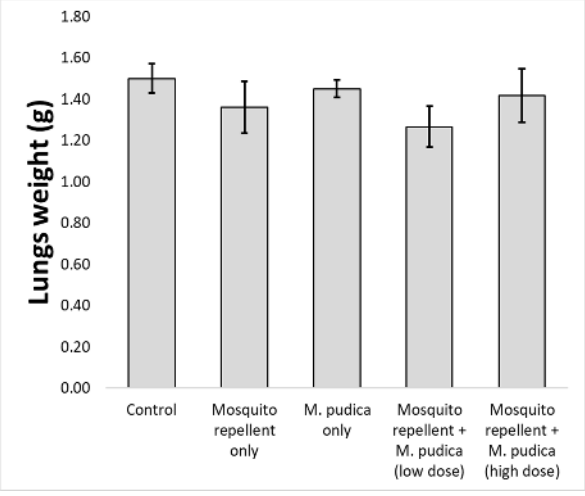


Figure 14. Chart showing lung weights. There were no statistically significant differences (P>0.05) of mean platelet volume across the groups

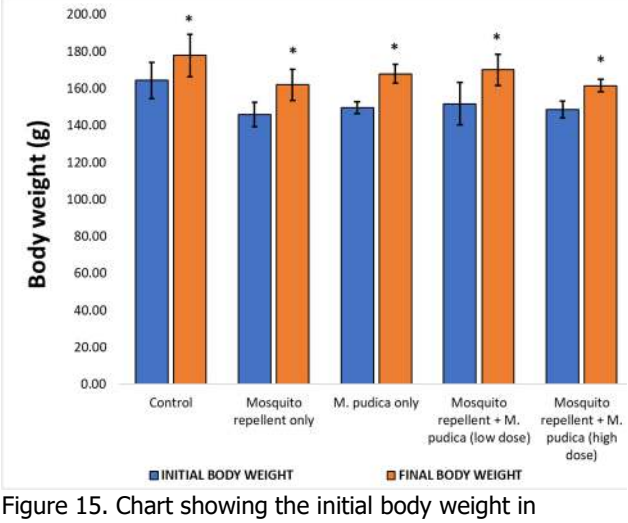


Figure 15. Chart showing the initial body weight in comparison with the final body weight. *Significantly different from the initial body weight. There were statistically significant increases (P<0.05) of body weights in all the groups when the initial body weights were compared to the final body weights.

Legends for photomicrograph

Figures 1 and 2 are photomicrographs of a section of rat lungs in the control group (Group A) at H&E ×40 and ×100 magnifications respectively showing **A.** Alveolar spaces **B.** interstitial space, **C.** terminal bronchiole and **D.** bronchial artery

Figures 3 & 4 are photomicrographs of a section of rat lungs given mosquito repellant only (Group B) at H&E ×40 and ×100 magnification respectively showing **A.** vascular ulceration **B.** bronchiolar mucosal ulceration **C.** activation of lymphoid tissue and **D.** bronchiolar dilation

Figures 5 & 6 are photomicrographs of a section of rat lungs given 500mg/kg body weight of plant extract only (Group C) at H&E ×40 and ×100 magnifications respectively showing **A.**normal alveoli, **B.** active interstitial congestion and **C.** normal bronchiole

Figures 7 & 8 are photomicrographs of a section of rat lungs given insect repellant + low dose of *Mimosa pudica* leaf extract (250mg/kg) (Group D) at H&E ×40 and ×100 magnifications respectively showing normal architecture of **A.** alveoli, **B.** bronchiole and **C.** vascular microstructure

Figures 9 & 10 are photomicrographs of a section of rat lungs given mosquito repellant + High dose of *Mimosa pudica* leaf extract (500 mg/kg) (Group D) at H&E ×40 and ×100 magnification respectively showing **A.** normal alveolar, **B.** bronchiolar microstructure and **C.** florid activation of bronchiolo-alveolarlymphoid aggregate and **D.** active vascular congestion and dilatation.

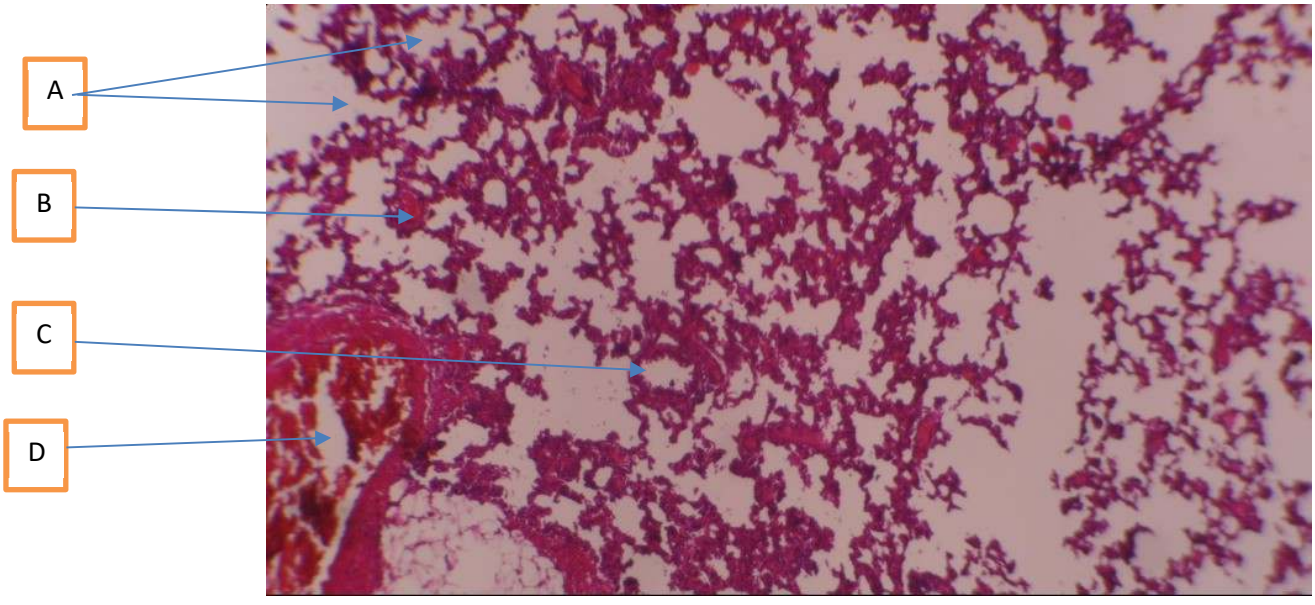


Figure 1: Lungs (Control). Composed of **A**: Alveolar spaces **B.** interstitial space **C**. terminal bronchiole and **D.** bronchial artery (H&E x 40)

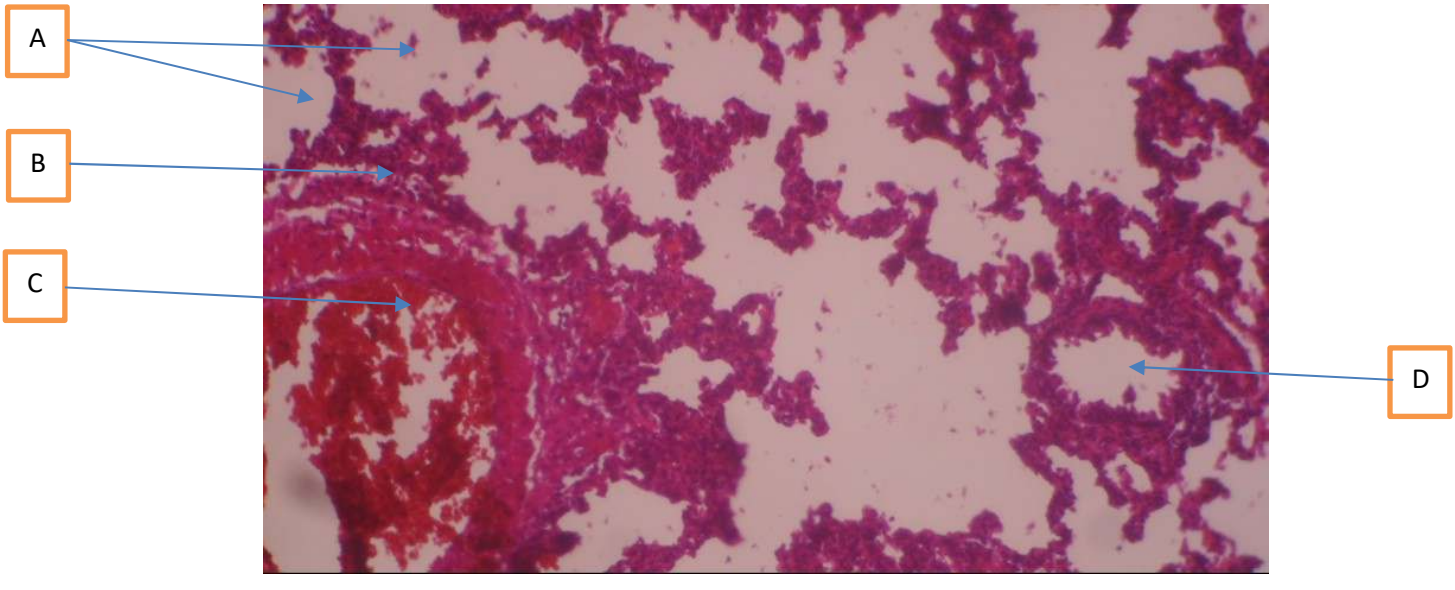


Figure 2: Higher magnification of the above: **A. B. C. D** (H&E x 100)

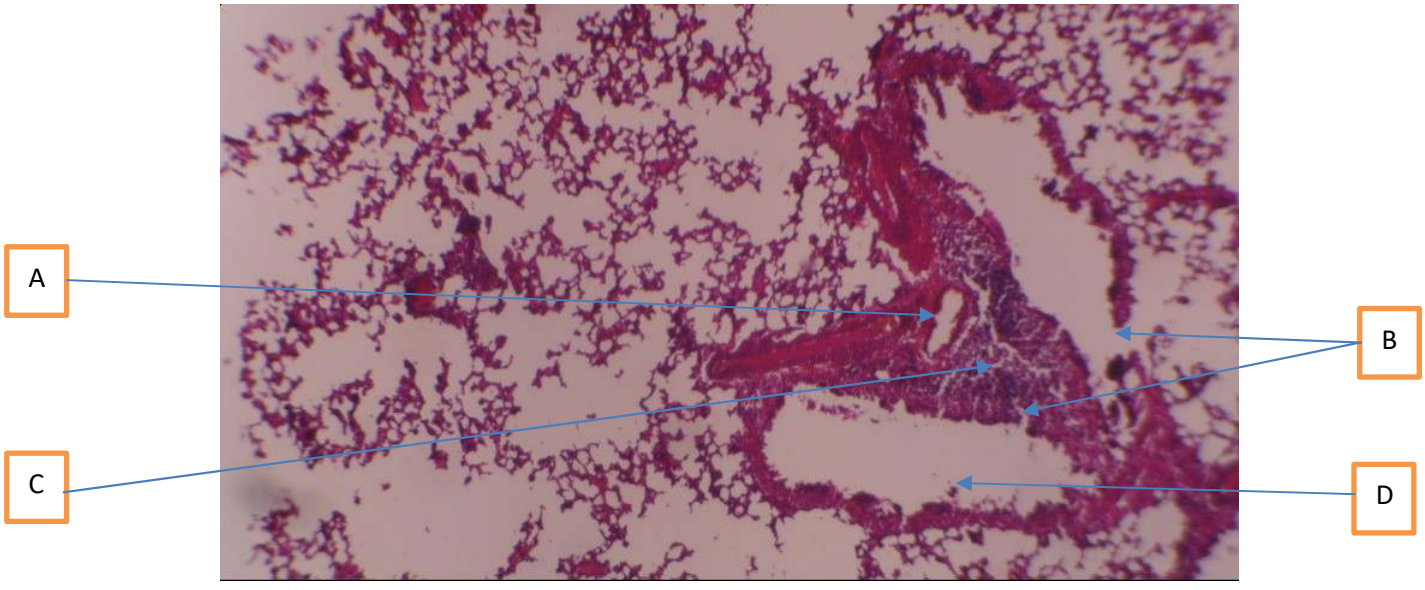


Figure 3: Rat given mosquito repellant: **A.** vascular ulceration **B.** bronchiolar mucosal ulceration **C.** activation of lymphoid tissue and **D.** bronchiolar dilation (H&E x 40)

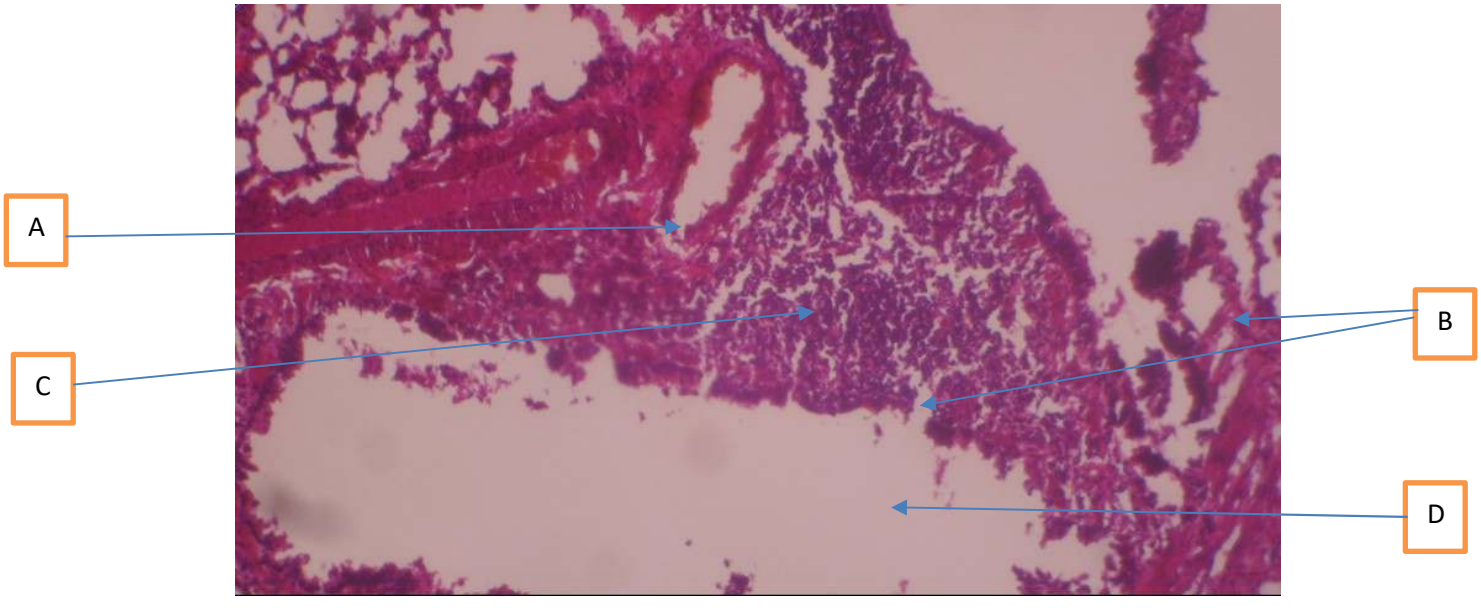


Figure 4: Higher magnification of the above: **A. B. C. D** (H&E x 100)

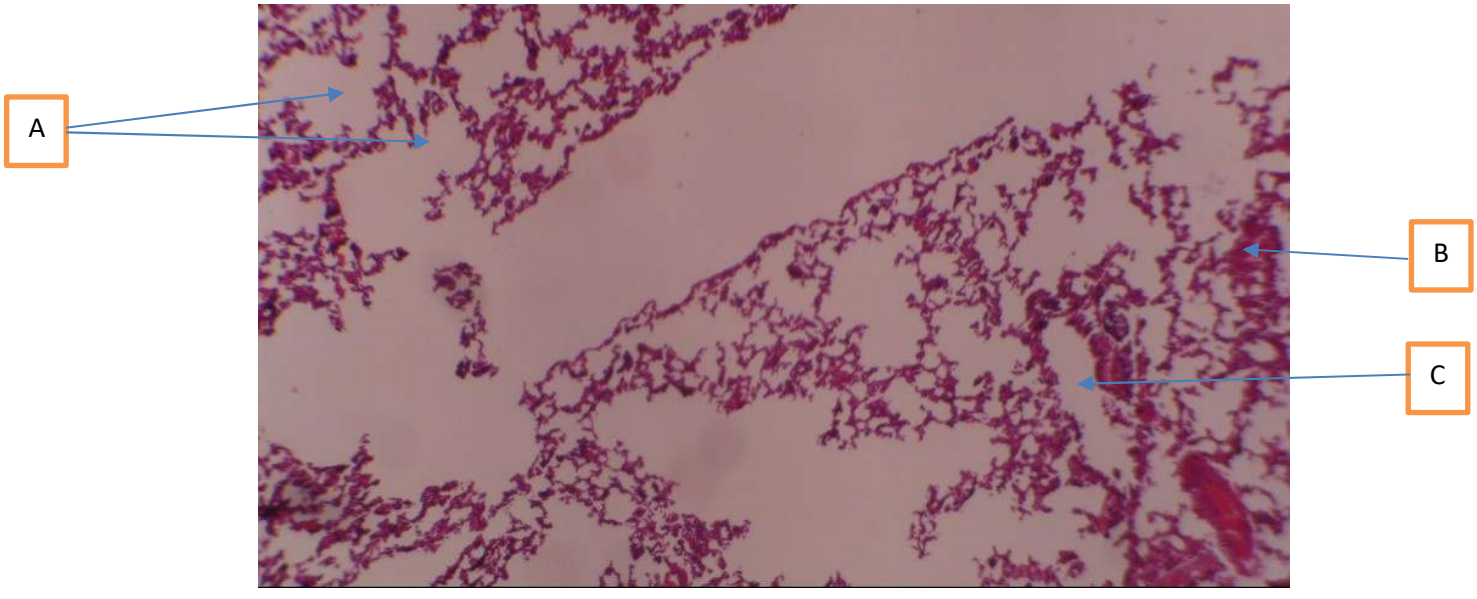


Figure 5: Rat given Extract only: **A**. normal alveoli **B.** active interstitial congestion and **C.** normal bronchiole (H&E x 40

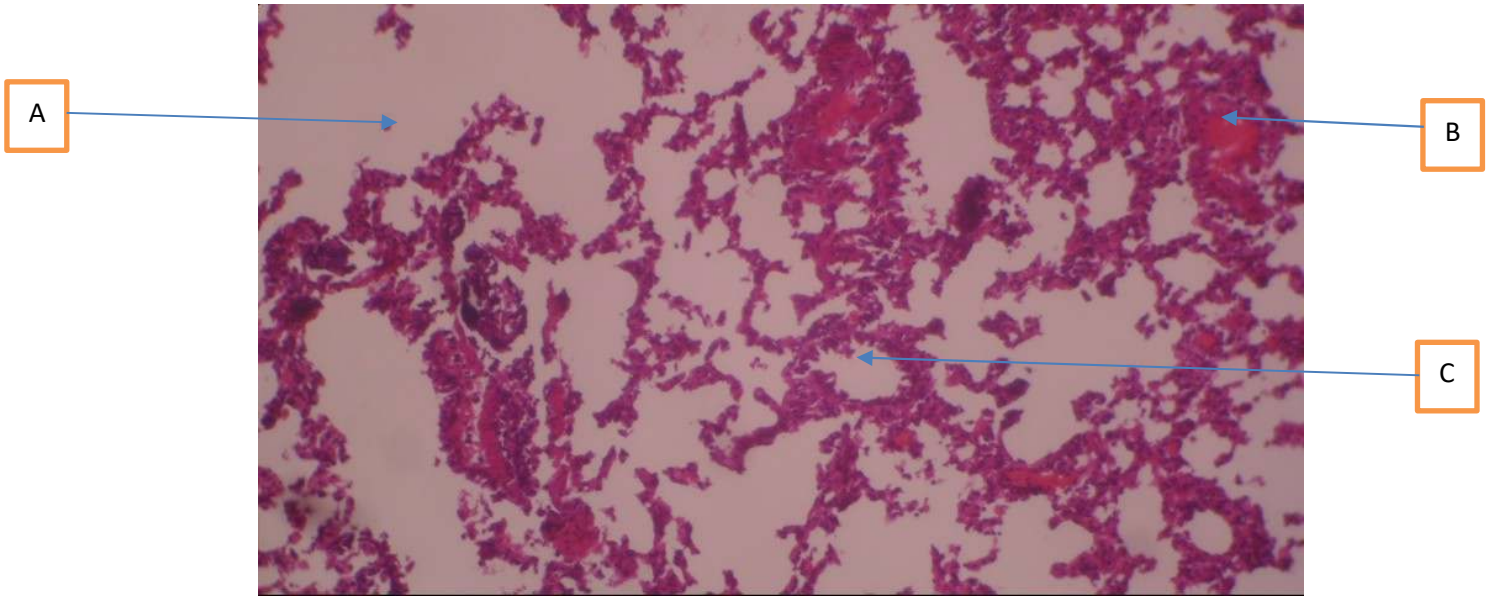


Figure 6: Higher magnification of the above: **A. B** and **C** (H&E x 100)

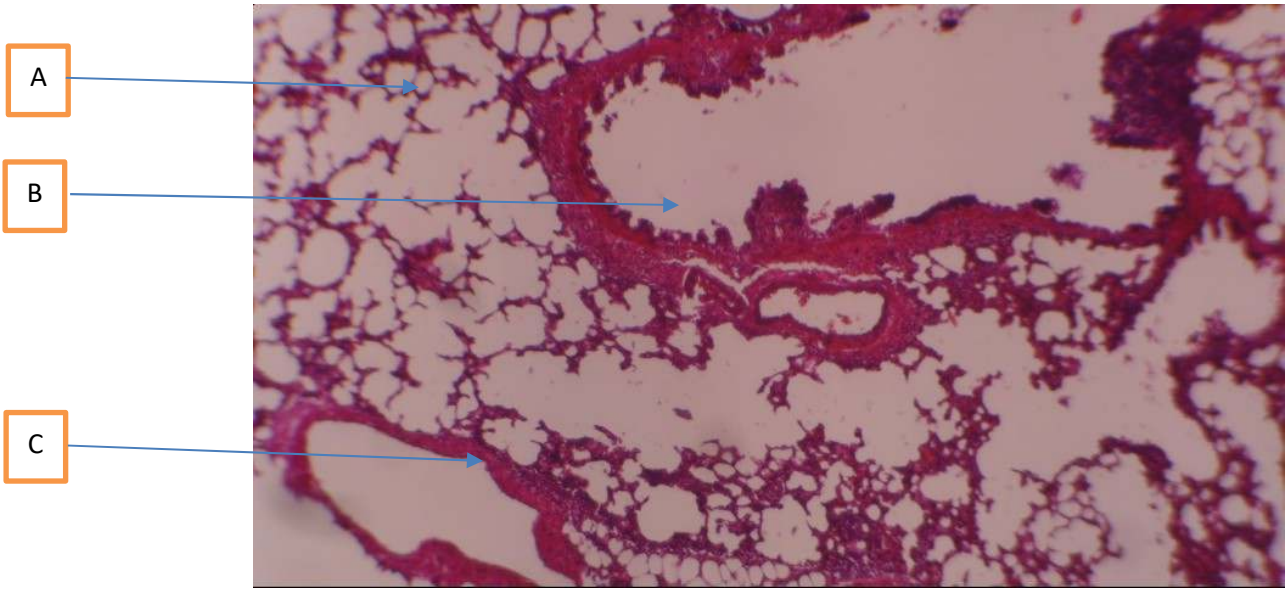


Figure 7: Rat given Toxicant + low dose extract: **A**. normal alveoli **B.** normal bronchiole and **C.** normal vascular microstructure (H&E x 40)

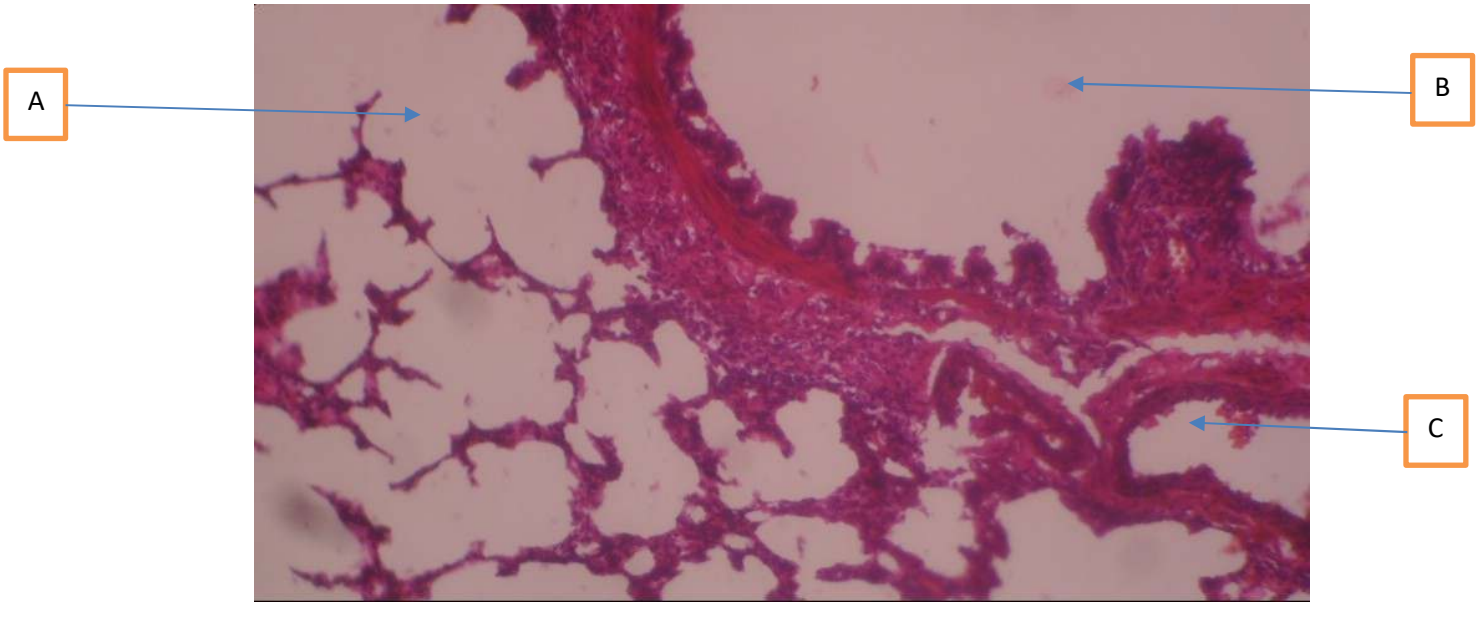


Figure 8: Higher magnification of the above: **A. B.** & **C** (H&E x 100)

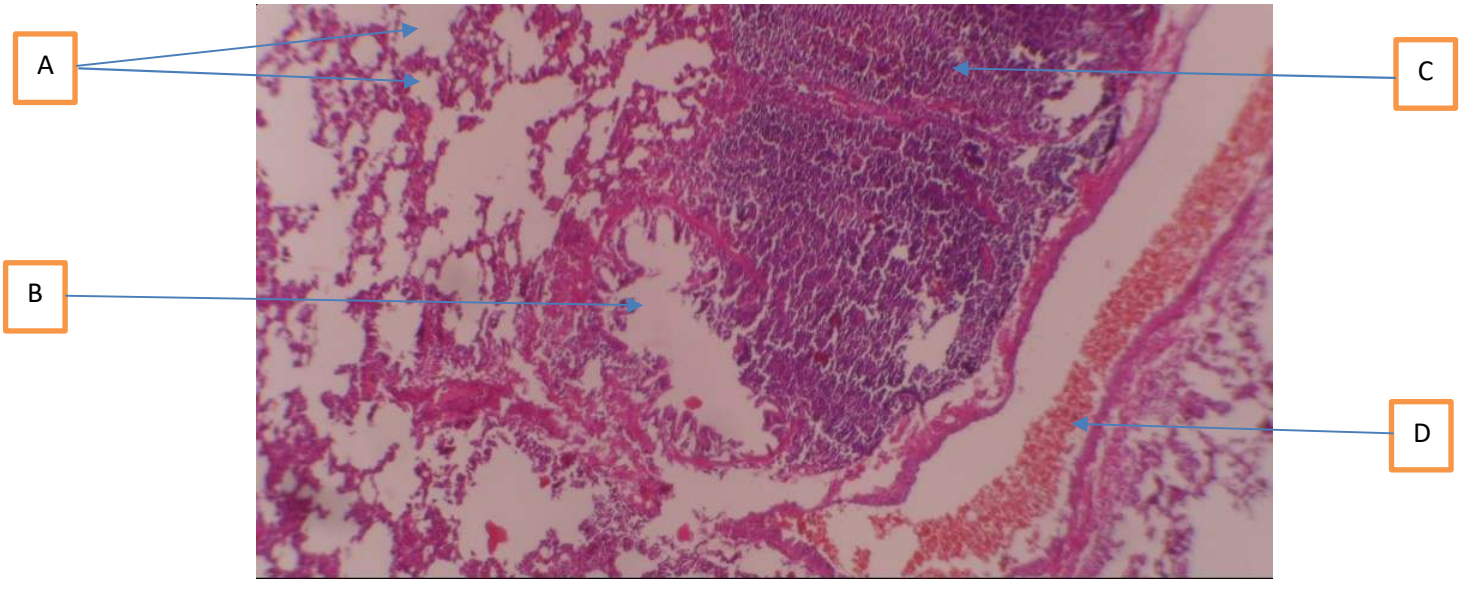


Figure 9: Rat given Toxicant + high dose extract: **A.** normal alveolar **B.** bronchiolar microstructure and **C.** florid activation of bronchiolo-alveolar lymphoid aggregate and **D.** active vascular congestion and dilatation (H&E x 40)

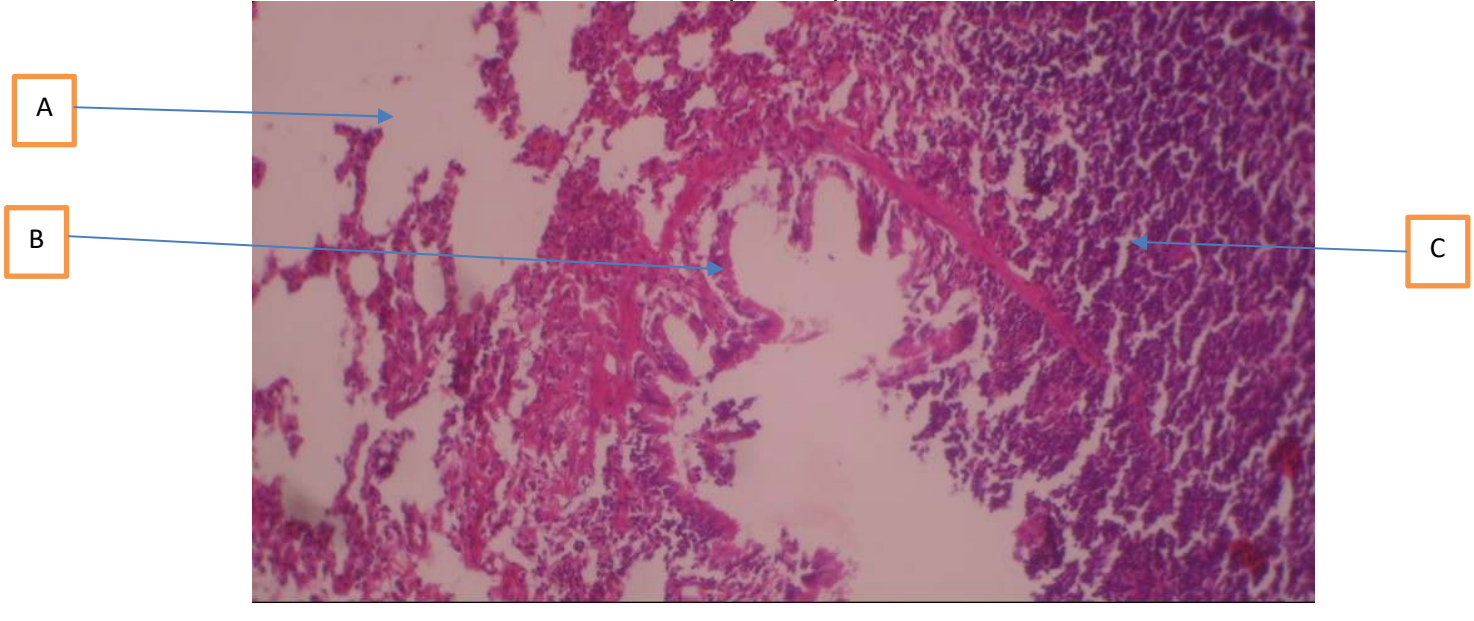


Figure 10: Higher magnification of the above: **A. B.** & **C** (H&E x 100)

DISCUSSION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practice since prehistoric times. This study was designed to evaluate the effects of aqueous leaf extract of *Mimosa pudica* in the lungs of adult Wistar rats exposed to insect repellant via inhalation.

The findings of this research for the body weight showed a significant increase in the body weight of the rats in all the groups. This can be attributed to normal growth during the period of the study.

The findings from this research showed that there was a statistically significant increase

in the hematocrit value of the rats treated with mosquito repellant only (Group B). This condition is known as polycythemia or erythrocytosis. It is associated with excessive production of red blood cells in the body. There was also a statistically significant decrease in mean corpuscular haemoglobin in the blood of rats exposed to insect repellant and treated with 500mg/kg body weight of *Mimosa pudica*plant extract (Group E), a condition that follows a reduced haemoglobin concentration in the blood. It may result from iron deficiency leading to anemia.

There was a significant increase in plateletcrit in the blood of rats exposed to mosquito repellant and treated with 500mg/kg body weight of *Mimosa pudica*plant extract (Group E). This usually arise from excessive production of platelets by the bone marrow in response to mucosal ulceration.

Histomorphological studies of the lungs of rats exposed to mosquito repellant only (Group B) shows that there was vascular ulceration, bronchiolar mucosal ulceration, activation of lymphoid tissue and bronchiolar dilatation (Figures 3 and 4). *Mimosa pudica* caused active interstitial congestion (Figures 5 and 6) as seen in the lungs of rats that received plant extract of *Mimosa pudica* only at 500mg/kg body weight (Group C). Conclusively, *Mimosa pudica* plant extract administered at 250mg/kg body weight after exposure to mosquito repellant was able to reverse the damage caused as seen in the photomicrographs of lung sections of rats in Group D (Figures 7 and 8). Photomicrographs taken from lung sections of rats that received 500mg/kg body weight of *Mimosa pudica* after exposure to mosquito repellant (Group E) shows that there was a less ameliorative effect at this dose. There was also a florid activation of bronchiolo-alveolar lymphoid aggregates and active vascular congestion and dilatation (Figures 9 and 10).

CONCLUSION

In conclusion, *Mimosa pudica* had ameliorative effects against mosquito repellant-induced vascular ulceration, bronchiolar mucosal ulceration and activation of bronchiolo-alveolar lymphoid tissue. These effects were seen to be inversely proportional to the dosage as the results were more desirable at lower doses and could be compared to the control group. It is therefore valuable in combating lung injuries.

RECOMMENDATION

Advanced studies should be carried out to develop this plant (*Mimosa pudica)* into a proper drug to combat lung diseases.

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ORIGINAL ARTICLE

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FINGER PRINT PATTERNS DISTRIBUTION AMONG DIABETICS AND NON-DIABETICS IN WESTERN UGANDA POPULATION

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ABSTRACT

Background: Dactyloscopy is a branch of dermatoglyphics (the study of epidermal ridges) which involves assessment and classification of fingerprint patterns for identification. All fingerprint patterns are laid down permanently from the third month of the intra-uterine life and they remain unchanged throughout the life. This is also the time when all organs in the body are finalizing their development. Therefore, a positive association of the dermatoglyphic features with different diseases like diabetes, mongolism, schizophrenia and leprosy have been well documented in the literature. The aim of the present study was to evaluate the dermatoglyphic patterns and the specific variations which may be used as a valuable diagnostic tool for early detection of diabetes mellitus. **Methods:** This was a prospective cross-sectional study, a total of 150 diabetic subjects and 150 non- diabetics (control) were selected from the KIUTH Western Uganda and their fingerprints were taken by the Indian ink method. The print patterns including whorls, arches, ulnar and radial loops were analysed and the variations were cross-tabulated between sex, the side of the hand and pattern distribution indexes. Data was analysed using SPSS version 26 and Dankmeijer's index and Furuhata's index were used to assess variability of the fingerprint patterns. Chi square tests was used to test for significance in variations at a confidence interval of 95%. **Results and conclusion:** Ulnar loops were the most predominant patterns among diabetic subjects at 35.2% followed closely by whorls at 35.1%. The most common pattern were whorls found in both hands of females at 21% (315/1500), whilst the most common pattern for male group were whorls at 17.5% (262). However, some fingers such as the ring finger completely lacked an arch pattern in both groups. The increase in arches was strongly supported by a significant increase in Dankmeijer's index. The presence of high number of ulnar loops can therefore maybe used as an early indicator of type two diabetes.

Keywords: Dermatoglyphics, Fingerprint, Diabetes

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INTRODUCTION

The importance of dermatoglyphics goes back to ancient China in 1839 where it was common practice in the sale of the land (Thoma, 1974). The deed of the land carried the impression of the finger prints as an acknowledgement of the deed. In addition, there was the prevailing custom of the sale of children in China and the prints of the palm and soles were recorded as a safeguard against the impersonation (Thoma, 1974).

Dermatoglyphics deals with the study of the epidermal ridges and their configurations on the fingers, palms and soles. The word "dermatoglyphics" is derived from the Greek word derma "meaning skin and "glyphic" meaning carvings (Kiran et al., 2010). Dermal ridge differentiation takes place early in foetal development. The resulting ridge configurations are genetically determined and influenced or modified by environmental forces (Schaumann & Alter, 1976).

Dermatoglyphics are of interest in anthropology, criminology, and medicine, including dysmorphology (the study of

congenital malformations) and the study of chromosome abnormalities such as trisomy 21 (Down syndrome) (Anitha et al., 2014). The study of fingerprints as a method of identification is also known as Dactylography or Dactyloscopy (Gutiérrez-Redomero et al., 2014), and currently it is also known as the Henry-Galton system of identification. Dactylography is the process of taking the impressions of papillary ridges of the fingertips for the purpose of identification of a person.

Epidermal ridges and their arrangement (dermatoglyphic patterns) exhibit a number of properties that reflect the biology of an individual (Crawford & Duggirala, 2014). Dermatoglyphic features statistically differ between the sexes, ethnic groups and age categories. Because dermatoglyphics and their components are genetically determined, and the arrangement of ridges remains constant throughout life, they have become of value as a supportive aid in the diagnosis of hereditary disorders (Crawford & Duggirala, 2014).

Considerable progress has been made in the understanding of the associations between dermatoglyphics and various medical disorders, and nearly all chromosomal disorders have been known to show dermatoglyphic patterns which can be used as part of preliminary diagnostic tools for such disorders (Yunis, 2012). Therefore, dermatoglyphic analysis has been established as a useful diagnostic and research tool in clinical disorders such as type two diabetic melitus (Anitha et al., 2014).

Currently, dermatoglyphics is involved in the forensic and medical field of research, such as Down's syndrome, psychosis, bipolar disorders and Diabetes mellitus (Anitha et al., 2014). However, some practitioners misuse dermatoglyphics and conduct fortune-telling instead of proper scientific analysis. This practice is considered a *faux pas* as dermatoglyphics is based on genetic research instead of baseless predictions (Valdez & Pathak, 2014).

The American Diabetes Association (2010), defines Diabetes Mellitus as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Funnell et al., 2011). The prevalence of diabetes mellitus is increasing, particularly in developing countries. It is one of the leading causes of morbidity and mortality and 90% of its etiology is genetically influenced (Whiting et al., 2011). The disease has a strong hereditary background. Offspring of two diabetic parents have an 80% lifetime risk of diabetes (Kenny et al., 1995)

Treatment of diabetes is expensive especially in third world countries, therefore early identification and management may reduce complications associated with it. Several significant population specific studies have been carried out to determine the association between dermatoglyphics and incidence of diabetes mellitus (Table 5). However, none so far have been undertaken in Western Uganda. Therefore, the present study intends to evaluate the relationship between the fingerprint patterns and the incidence of diabetes mellitus in the Western Uganda population. Widespread variation between the ethnic groups occurs, thus there is need to determine the parameter values for use in making a diagnosis in each ethnic group. There are 4 main finger print patterns although current techniques have divided some patterns into smaller sub groups (Figure 1).

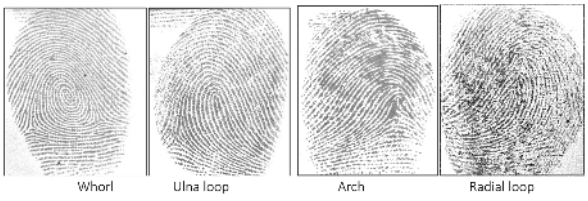


Figure 1: Digital finger print patterns

Frequency distribution indices of finger print patterns

The frequency distribution indices are unique fingerprint calculations that can be used to determine an individual's or a specific population distribution of patterns. These indices cannot be the same within individuals; thus, federal bureaus of investigation have frequently used it to identify persons of interest. However, these patterns can be used to determine the fingerprint distribution of one population to another (Issrani & Sinha, 2013). These indices include: Pattern intensity index (PII): $(2 \times \%\ \text{whorl} + \%\ \text{loop}) \div 10)$; arch/whorl index of Dankmeijer's; (% arches ÷% whorl) $\times 100$; and whorl/loop index of Furuhata's; (% whorl ÷% of loop) $\times 100$ (Mavalwala & Hunt, 1964).

The highest 'pattern intensity index' in the world is found in populations located in Australia and the Eskimos in Northern America - in these populations are whorls are predominant, resulting in an average 'pattern index' above 15.5 (while in most other populations loops are most common). And the lowest 'pattern intensity index' in the world is found in populations located in central Africa, such as the Bushmen and Pygmies - in these population groups arches and whorls are relatively common, resulting in an average 'pattern index' below 10 (while in other populations whorls are usually much more common than arches), (Mensvoort, 2013).

Value of dermatoglyphics in medicine

Considerable progress has been made in the understanding of the associations between dermatoglyphics and various medical disorders (Table 5). As a result of these advancements dermatoglyphic analysis has been established as a useful diagnostic and research tool in medicine, providing important insights into the inheritance and embryologic development of many studied clinical disorders (Anitha et al., 2014). Congenital anomalies like trisomy 21 and 46 XY female (Bosco et al., 2001) can have multiple effects on the phenotype, including the pattern of dermatoglyphics (Anitha et al., 2014).

Studies have also shown that dermatoglyphics may be an important feature in psychiatric illness. Schizophrenic cases showed reductions in palmar a-bridge counts (Stosljevic et al., 2013), whereas radial loops are increased in bipolar mood disorder (Chakraborty, 2001). Recent studies observed dermatoglyphics as diagnostic clues to various clinical conditions like acute lymphoblastic leukemia, occupational allergic bronchitis, locomotor disorder, coeliac disease, beta-thalassemia and many others (Mollik & Habib, 2012).

Currently, there are several ongoing studies on the relative frequencies of various dermatoglyphic features for chromosomal disorders. When combined with other clinical features of a particular disease, dermatoglyphics can serve to strengthen a diagnostic impression and may be useful in screening select individuals for additional diagnostic studies (Anitha et al., 2014).

MATERIALS AND METHODS

This was a cross sectional prospective study that included 300 residents (150 patients with diabetic mellitus and 150 control group) in Bushenyi District, Western Uganda. Diabetic patients were selected from outpatient department of Kampala International University Teaching Hospital (KIUTH) while the control groups with no family history of diabetes were randomly sampled from the surrounding community.

Only subjects who consented were included in the study. Any subjects with congenital conditions were excluded in the study. After obtaining consent from the respondents, all the subjects were asked to wash and dry their hands to remove dirt and grease.
For the collection of fingerprints, a plain glass plate of 12x12 inches was uniformly smeared with a thin layer of black printers' ink by using the inking roller, then the rolled impressions of each finger were stamped in the allotted space for that given finger on the data sheet. In this way for each and every individual the entire prints of ten fingers were prepared. The prints were then studied with the aid of a magnifying glass

In the present study, the arches, whorls, ulnar and radial loops were analyzed. All the types of whorls such as concentric, single spiral, double spiral, accidental, and also all the types of composite whorls such as twin loops, central pocket loops, lateral pocket loops, crested and knot-crested loops were grouped under the broad category of 'whorls'. On the other hand, radial and ulnar loops (RL and UL) were classified separately. The dermatoglyphic features for both hand and sexes were then evaluated and presented for the control group and diabetic patients separately.

Three indices were also calculated on the basis of the frequency distribution of different finger patterns. These included: (a) The pattern intensity index (PII) $(2 \times \%\ \text{whorl} + \%\ \text{loop}) \div 10)$; (b) Dankmeijer's index $(\%\ \text{arches} \div \%\ \text{whorl}) \times 100$; and Furuhata's index $(\%\ \text{whorl} \div \%\ \text{of loop}) \times 100$. Variations were subjected to chi-square tests and a 'p' value of <0.05 was recorded as significant (with the corresponding degree of freedom).

Ethical clearance was obtained from KIUTH ethics committee and gate keepers authorization obtained from the Hospital management. Patients were informed of the process, assured of confidentiality and anonymity throughout the process. All collected finger prints were disposed of after study as was agreed in the consent forms.

RESULTS

Demographics characteristics

Table 1: Comparison of frequency (%) distribution of fingerprint patterns between sexes of control and diabetic groups

	Pattern type (%)				
	Sex	**Arches (%)**	**U Loops (%)**	**R Loops (%)**	**Whorls (%)**
Control (N=1500 fingerprints)	**Male**	0.93 (14)	14.7 (221)	8.2 (123)	17.5(262)
	Female	2.27 (34)	22.67 (340)	13.33(200)	20.4 (306)
	Total	3.2 (48)	37.37 (561)	21.53 (323)	37.9 (568)
Diabetic (N=1500 fingerprints)	**Males**	2.73 (41)	14.6 (219)	11.87 (178)	14.13 (212)
	Female	3.27 (49)	20.6 (309)	11.8 (177)	21 (315)
	Total	**6 (90)**	**35.2 (528)**	**23.67 (355)**	**35.13 (527)**

A total of 3000 fingerprints (1500 diabetic and 1500 control groups) were analyzed from 300 subjects (150 Diabetic and 150 control groups) for different digital patterns. The mean age of the two groups was 32.7±4.2 and 29.7±5.1 for diabetic and control group respectively. The total number of males and females were 42.3% ($^{127}/_{300}$) and 57.7% ($^{173}/_{300}$) respectively (Table 1).

General fingerprint patterns

The general frequency (%) distribution of the 300 (3000 fingerprints) participants were as follows; 58.9% ($^{1767}/_{3000}$) loops (Ulnar loops 36.3% + Radial loops 22.6%): 4.6% ($^{138}/_{3000}$) arches, while 36.5% ($^{1095}/_{3000}$) were whorls. Of the total 138 arches, the left hand had 46.6% ($^{63}/_{138}$) and right was 54.4% (75/138). Total loops (U loops + R loops) on the left hand was 48.6% ($^{858}/_{1767}$) and right had 51.5% ($^{909}/_{1767}$), while the total whorls

were 52.9% ($^{579}/_{1095}$) for the left and 47.1% ($^{516}/_{1095}$) for the right hand (Figure 3).

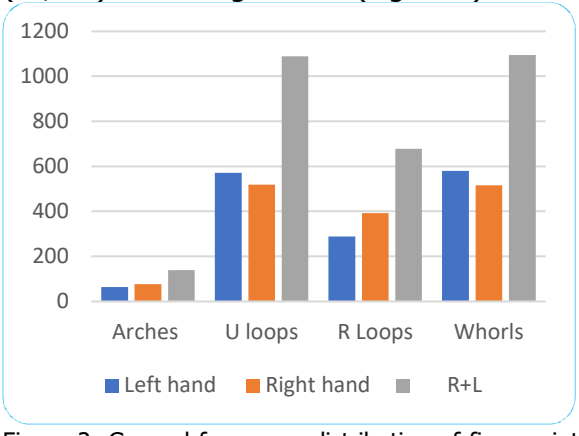


Figure 2: General frequency distribution of fingerprint patterns on the right and left hands

Frequency distribution of fingerprints between control and diabetic group

Ulnar loops were the most predominant patterns in both hands of diabetic subjects with a frequency of 35.2% ($^{528}/_{1500}$) followed closely by whorls at 35.1% ($^{527}/_{1500}$), however, whorls were most predominant in the control group at 37.9% ($^{568}/_{1500}$) (Table 2). The least pattern observed were arches in both groups; the Control group had the lowest at 3.2% (48) and diabetic with 6% (90) of the total 1500 fingerprints (Table 2).

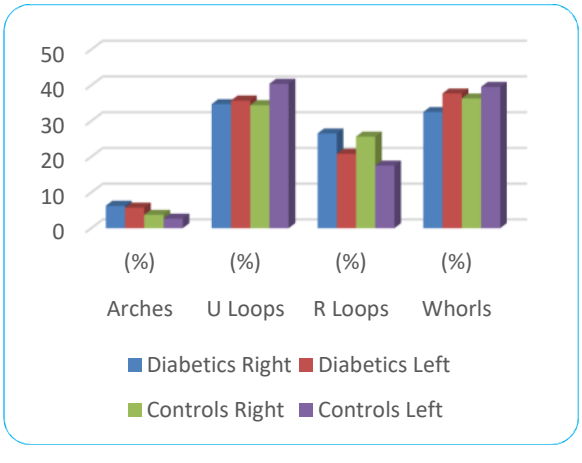


Figure 3: Frequency distribution of the right and left fingerprint patterns, between diabetic and control groups.

Table 2: Frequency distribution of fingerprint patterns between the control and diabetic groups

Pattern distribution	Subjects		
	Diabetic N=1500	**Control N=1500**	**Total (N=3000)**
Arches	6 (90)	3.2 (48)	4.6 (138)
Ulnar Loops	35.2 (528)	37.4 (561)	36.3 (1089)
Radial Loop	23.67 (355)	21.53 (323)	22.6 (678)
Whorls	35.1 (527)	37.9 (568)	36.5 (1095)
Total	100 (1500)	100 (1500)	100 (3000)

Comparison of distribution of fingerprint patterns between control and diabetic groups in relation to gender.

On the control group, (Table 1) ulnar loops were more common on the female side for both hands at 22.67% ($^{340}/_{1500}$), followed by whorls at 21% ($^{315}/_{1500}$). The most common pattern for male control group were whorls at 17.5% (262) followed by ulnar loops at 14.6% (219). Arches were the least common pattern in control group at 0.93% (14/1500) followed by arches in females at 2.27% ($^{34}/_{1500}$).

On the diabetic group (Table 1) the most common pattern were whorls found in both hands of females at 21% ($^{315}/_{1500}$), followed by ulnar loops at 20.6% ($^{309}/_{1500}$). The predominant pattern in male diabetic group were radial loops at 11.87% ($^{178}/_{1500}$). Arches were the least common pattern in diabetic subjects at 2.73% ($^{41}/_{1500}$) followed by arches in females at 3.27% ($^{49}/_{1500}$).

Frequency distribution indices of fingerprint patterns

The diabetic group had a total of 17.4% Dankmeijer's whilst the control group had less than half (8.22%) of the same for both male and female subjects (Table 3). Both hands (right and left for control and diabetic) were calculated together to ensure consistency. Descriptive statistics for fingerprint patterns including mean, Standard deviation and Standard of error

among diabetic and control subjects are presented in Table 4 respectively.

Table 3: Comparison of frequency (%) distribution indices between diabetic and control.

	Gender	Furuhata's index $\%_{Whorls}/\%_{Loops} \times 100$	Dankmeijer's index $\%_{Arches}/\%_{Whorls} \times 100$	PII $(2 \times \%\ whorls + \%\ loops) \div 10$
Diabetic	Male	53.38	19.32	8.12
	Female	64.81	15.57	10.68
	Total	**59.0**	**17.4**	**9.4**
Control	Male	76.42	5.31	8.08
	Female	56.67	11.13	11.28
	Total	**66.5**	**8.22**	**9.68**

Table 4: Descriptive statistics and a test of significance for control and diabetic subject

Parameters	**Diabetic (n=150)**		**Control (n= 150)**		**S. E**	**'*p*' value**	**Significance**
	Mean	SD	Mean	SD			
Arch	5.25	0.96	5.28	1.374	0.029	< 0.05	Significant
Ulnar Loop	5.0	1.44	5.0	1.49	0.485	> 0.05	Not Significant
Radial Loop	5.53	1.25	5.64	1.41	0.313	>0.05	Not Significant
Whorl	5.99	1.33	5.375	1.32	0.434	> 0.05	Not Significant

DISCUSSION

All the 300 (150 Diabetic and 150 control group) participants were adults of consenting age and with a mean 32.7±4.2 years and 29.7±5.1 years of age for diabetic and control groups respectively. However, most diabetic patients were much older than the control groups, this might be because most symptoms of diabetes begins to show in late adulthood except in the case of juvenile diabetes (Funnell et al., 2011). Although the onset and symptoms of diabetes commonly begins at late adulthood, age has no correlation with the fingerprint patterns as they form during intrauterine life and never change. There was a high number female participants for both control and diabetic groups although this was insignificant in the final results as the deference was very small.

In the current study, the most predominant fingerprint pattern were loops (Ulnar + Radial loops) for both diabetic and control groups at 58%. This conforms to findings of other authors who noted that loops were the most frequent patterns (Dhumal et al., 2021; Okeke et al., 2018; Sudharson et al., 2020; Tadesse et al., 2022). The general international distribution of fingerprint patterns as recorded in the literature (Mensvoort, 2013) also notes that loops are the most predominant. Arch patterns were rare in the general population and were completely absent on the thumbs of all diabetic patients and on the ring fingers of all the 300 participants, However, there was a general significant increase ($p<0.05$) of arch patterns in diabetic subjects as compared to the control group. After meta-analysis on

similar studies, several studies (Table 5) have reported a significant increase of arch pattern among diabetics than the control groups This findings however, were not in line with the results results of Tadesse et al. (2022); Tarigoppula et al. (2018) who noted that loop patterns were significantly higher in diabetic patients.

Most arch and Loop (U loops + R loops) patterns were predominant on the right hand of all subjects at 54.4% and 51.5% respectively, while the whorl pattern was more predominant on the left hand at 52.9% for both subjects and sexes (Figure 3). {Umana, 2014 #5073@@author-year} on the other hand reported consistent presence of high number of ulnar loop patterns in both right and left hands of diabetic patients, the authors did not correlate their findings to a control group thus no significant association was established.

The rarest pattern observed were arches (only 4.6% for the 300 respondents) in both groups although the control group had almost half the number of arches at only 3.2% as compared to the diabetic cases at 6% of the total 1500 fingerprints. This was statistically significant at a p value of 0.0932 (<0.05). Several Authors (Table 5) have also noted significant increase in Arch patterns among diabetic groups from different populations and therefore, diabetes mellitus seems to affect the distribution of fingerprint arch patterns.

On a more specific note, ulnar loops were more predominant finger print pattern and were found in the female control groups at 22.67%, followed by whorls at 21%, while the most predominant pattern in the diabetic group were whorls in females at 21%, followed by ulnar loops at 20.6%, The predominant pattern for the male control group were whorls at 17.5% (262) while for male diabetic group were radial loops at 11.87%. This indicates that the females on both diabetic and control groups had the most predominant ulnar and loop patters as compared to their male counterparts.

Table 5: Predominant fingerprint patterns as noted by various authors from different regions

Author	Sample size (n)	Region	Prevalent fingerprint pattern
Ravindranath and Thomas (1995)	150	India	Loops
Verbov (1973)	80	UK	Arches
Padmini et al. (2011)	200	India	Arches
Umana (2013)	101	Nigeria	Arches
Nezhad and Shah (2010)	30		Ulnar loop
Igbigbi et al. (2001)	99	Malawi	Arches
Marera et al. (2015)	150	Uganda	Arches

Arch patterns were the least common under the control and were found in the male side at only 0.93%. The same pattern (Arches) was still the least predominant pattern under diabetic group and found in males too at only 2.73%. The female control group had three times the number of arches as compared to their male counterparts at 2.27% of the total 1500 fingerprint studied.

On analysis of unique finger print frequency distribution indexes, Furuhata's index was lower in diabetic than the control groups; however, the Dankmeijer's index was twice as high (17.4%) in diabetic group than control group (8.22%), this was probably because of the significantly high number of arches in diabetic subjects. The pattern intensity index and Furuhata's index were high in the control group but with an insignificant percentile difference (Table 3), this was likely because the number of whorls and arches were almost near equal in both diabetic and control groups. The lowest pattern intensity indices are below 10% as stated in the above literature (Mensvoort, 2013), therefore the PPI in this study were well below the limit as commonly noted in African countries (Mensvoort, 2013).

CONCLUSION

Several studies done in different regions have significantly identified correlation between different fingerprint patterns and diabetes, however the type of pattern

identified varies from one region to another. This may be because of racial dermatoglyphic differences from one region to another. There are also different types of diabetes mellitus and each may present genetically with a different pattern.

Therefore, a more population and disease specific study may be required to confirm the value of dermatoglyphics in early diagnosis of diabetes mellitus. It would also be appropriate to subject results through calculations of frequency distribution indices such as PPI to confirm the significance of the predominant pattern. In the case of this study, statistically significant differences are evident in Arch pattern and Dankmeijer's finger print distribution index.

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ORIGINAL COMMUNICATION

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EVALUATION OF *Abrus precatorius* ON REPRODUCTIVE FUNCTION OF MALE WISTAR RAT.

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ABSTRACT

Background: In recent times, attention has been shifted from synthetic drugs to the use of medicinal plants and this has greatly improved reproductive functions. *Abrus precatorious* plant has different parts which are used as diverse sources of naturally occurring chemicals that have a variety of therapeutic effects on the body. However, in this present study, we consolidated the reproductive property of *Abrus precatorius* in paroxetine-induced male reproductive dysfunction. **Methods:** Twenty four (24) male rats were divided into four (4) groups each containing six animals was used for this experiment following paroxetine-induced erectile dysfunction. Group one, received 1ml of distilled water, Group two received 20mg/kg of Paroxetine and 50mg/kg of sildenafil, Group three received 20mg/kg of Paroxetine and 300mg/kg of A. *precatorius* extract, Group four received 20mg/kg of Paroxetine and 900mg/kg of A. *precatorius* extract for 21 days. **Results:** Results from the studies reveal that there was significant decrease in the sperm motility of the rats administered with *Abrus precatorius* when compared with control. Interestingly, the high dose extract increased the serum testosterone levels significantly while the low dose extract significantly reduce the level of testosterone when compared with the control. Histological examination of the testes of treated rats displayed noticeable atrophy, which was characterized by disruption of the seminiferous epithelium and reduction in cell population of the Leydig cells. **Conclusion:** It appears that very high dose of *Abrus precatorius* may induce infertility by increasing serum testosterone levels.

Keywords: Abrus precatorius, Fertility, Sildenafil, paroxetine, Testosterone, reproduction
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INTRODUCTION

The ancient literature of indigenous systems of medicine has noted that plant preparations have a significant role in fertility management. Over the new era, researchers became interested in the function of these native plant compounds in the induction of male and female fertility in experimental animals (Bhakta and Das 2020).

The use of medicinal plants is on the increase as a result of the move away of focus from synthetic drugs to traditional medicine (Zhang *et al*., 2015). The majority of conventional drugs are based on herbs that are utilized by about 80% of the global population (Bhakta and Das 2020). One of the uses of medicinal plants is to improve reproductive functions. In general, while other herbal remedies are used to treat different reproductive issues, natural aphrodisiacs are consumed to improve sexual performance, such as erectile dysfunction, oligospermia, azoospermia, hormonal imbalance, etc. (Ogbuehi et al., 2015).

Abrus precatorius has been widely used in various traditional systems for its potential effects on reproductive health (Zhang *et al*., 2015). It contains several bioactive compounds, including abrin, precatorine, abraline, abrectine, abrusogenin, and flavonoids such as kaempferol, quercetin, and isorhamnetin. These compounds exhibit various pharmacological properties and may contribute to the effects on fertility (Anant and Maitreyi, 2012; Sharma *et al*.2020). Several studies have investigated the effects of Abrus precatorius on sperm parameters. Singh *et al.* (2018) demonstrated that Abrus precatorius extract significantly improved sperm count, motility, and morphology in male rats. Because of the antioxidant property of *A. precatorius*, it has the potential to mitigate oxidative stress-induced damage to sperm, since oxidative stress is known to be a contributing factor to male infertility (Mishra *et al.* 2017).

As part of its role in reproductive health, it has been found to modulate testosterone levels by increasing serum testosterone levels in male rats suggesting its potential role in improving reproductive functions (Kumar *et al.* 2019). This underpins the potential effects of *A.precatorius* in regulating hormonal imbalances associated with female infertility (Pardhi *et al.* 2019).

According to Nemeroff (1993), paroxetine is a representative SSRI chemical that has been widely utilized in clinical practice. It has also been documented to cause sexual dysfunction, like other compounds in its class (Montejo *et al*.,). It has the ability to prevent the production of nitric oxide (NO), a crucial mediator of smooth muscle relaxation and penile erection, both in vitro and in vivo (Finkel *et al*., 1996). When given either chronically or suddenly to rats, paroxetine reduces their ability to erect after being stimulated by the carvernosal nerve (Kim *et al*., 1991; Finkel *et al*., 1996).

A powerful and specific type 5 phosphodiesterase (PDE5) inhibitor, sidenafil (Viagra), improves the relaxation of the human corpus carvernosum brought on by NO as well as the penile erection in rats when NO is administered (Saenz, 2001; Angulo, 2001).

This study intends to investigate and consolidate reproductive property of *Abrus precatorius* in paroxetine-induced male reproductive dysfunction in male Wistar rat.

MATERIALS AND METHODS

ANIMALS

The study was carried out on twenty-four male wistar rats weighing between 180g-200g after it has been acclimatized for two (2) weeks under normal temperature and unrestricted access to water, food and ventilation. The rats were bred and housed in the Faculty of Basic Medical Sciences Animal house, University of Ilorin, Ilorin, Nigeria. The animal house was well ventilated and the normal day light cycle was maintained.

PLANTS MATERIALS AND EXTRACT PREPARATION

Abrus precatorius was obtained from Dongari Etile in the Asa local government area of Kwara state, Nigeria, and was authenticated in the Botany department (herbarium), University of Ilorin. The leave of A*. precatorius* was air-dried for three weeks and ground into powdered form (66g) for extract preparation. Powdered A. *precatorius* was soaked in distilled water for 48 hours and thereafter filtered. The filtrate was evaporated to dryness using a hot plate set at 50-degree margin. The weight of the extract after evaporation to dryness was 31.9g.

ANIMAL GROUPING AND INDUCTION OF SEXUAL DYSFUNCTION

The twenty four (24) experimental animals (Male Wistar rats) were randomly divided

into four groups (6 per cage). Group one (1) served as the control, while group two to four (2-4) were induced with sexual dysfunction using a daily oral administration of 20mg/kg body weight of paroxetine (Saraswathi, 2020) for three weeks (21days). Assessment of sexual behaviour was carried out on the 14th and 19th days respectively. The male rats were subjected to healthy female rats in order to achieve this, and mating behaviour was assessed.

ASSESSMENT OF MATING BEHAVIOUR PARAMETERS IN RATS

Mating test was assessed in all the groups on the 14th day of induction between the hours of 11pm to 1am and observed for 30minutes. Female albino rats were bought and introduced into each of the group (four female rats per group) and the following mating behaviour were observed: Mount frequency (MF), Intromission frequency (IF), Ejaculation frequency (EF) and Post-ejaculatory interval (PEI). Some of the animals from groups 2-4 displayed all these sexual behaviour on day 14 while some do not at all. By day 19th the test was repeated again using the same procedure and all the animals from groups 2-4 that displayed none of the sexual behaviour listed above were considered to have erectile dysfunction and thereby included in the study. Also there were physical manifestations as the testes of animals in groups 2-4 become smaller when compared to the period of acclimatization. Male rats were often classified as sexually impaired if they had a minimum 25% drop in MF, IF, and EF as well as a minimum 25% increase in mount latency (ML), intromission latency (IL), erectile latency (EL), and PEI (Malviya et al., 2011).

EXTRACT ADMINISTRATION

Twenty four (24) male albino rats were divided into four groups of 6 rats each and treated for 3 weeks (21 days) as follows: Group 1 (Control) were fed with normal animal diet and water throughout the experimental period, Group 2 rats received 20mg/kg of Paroxetine (for three weeks) plus 50mg/kg of sildernafil (Viagra), Group 3 rats received 20mg/kg of Paroxetine (for three weeks) plus 300mg/kg of A. *precatorius* extract (low dose) for three weeks (21days) via oral route, Group 4 rats received 20mg/kg of Paroxetine (for three weeks) plus 900mg/kg of A. *precatorius* extract (high dose) for three weeks (21days) via the oral route.

DETERMINATION OF SERUM TESTOSTERONE

At the end of the experiment, rats were dissected and sacrificed following ketamine anesthesia. Blood samples were collected in a well labeled heparinized bottle and immediately centrifuged. Serum was stored for further assay. The quantitative determination of Total Testosterone concentration in Serum by a Microplate Enzyme Immunoassay was performed using Monobind assay kit (Lake Forest, USA).

SPERM ANALYSIS

The testes and epididymis of the rats were isolated by incising the abdomen of the rat, and thereafter the distal end of the epididymis was dissected out. An incision of 1mm was made in the distal end of the epididymis to collect the semen used to estimate sperm motility, sperm morphology and sperm count. Motility was expressed as the percentage of motile spermatozoa and their mean speed, or motile quality (scale 1 to 6, where 1= motile and 6= very fast progressive motile, that is >100 μm/s).

HISTOLOGICAL STUDIES

The testes were removed, dissected, and preserved in neutral buffered formalin at a 10% concentration. These formalin-fixed tissues were embedded in paraffin, cut into 5 micrometers sections, stained with hematoxylin and eosin (H&E), and analyzed histologically under a light microscope.

STATISTICS ANALYSIS

Data collected were expressed as mean ± SEM and analyzed using a statistical Software; Graph Pad Prism (version 5.0). Statistical significance between the means was attained using unpaired student's t-test. p-value < 0.05 was considered statistically significant.

RESULTS

EFFECT OF A. PRECATORIUS AND SILDENAFIL ON SEMEN PARAMETERS IN MALE WISTAR RATS TREATED FOR TWENTY-ONE DAYS

Treatments with plant extract and sildenafil for 21 days cause a significant increase in sperm count (fig.1a), decrease in sperm motility (fig.1b) and changes in sperm morphology like abnormal head shape (such as bent tail, double head, small head); abnormal tail structure (such as bent tail, multiple bend tails); cytoplasmic droplets, head vacuoles (fig.1c) when compared with the control.

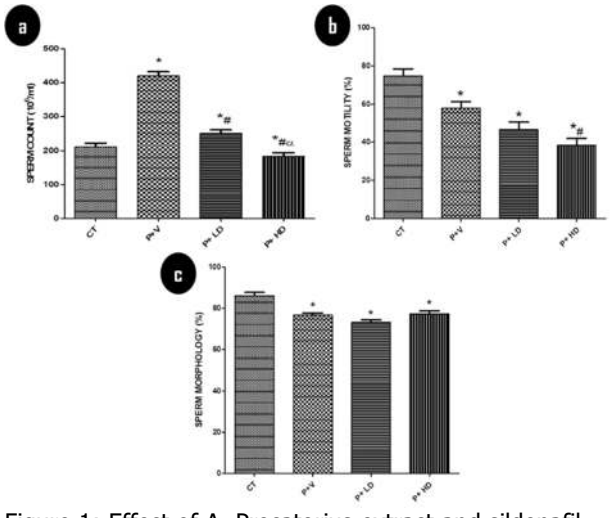


Figure 1: Effect of A. Precatorius extract and sildenafil on sperm parameter of male wistar rats. n=6; mean ± SEM, p<0.05 when compared with control group.
CT= Control, P +V = Paroxetine + Viagra, P + LD= Paroxetine + Low Dose extract, P + HD= Paroxetine + High Dose

EFFECT OF *ABRUS PRECARTORIOUS* ON SERUM TESTOSTERONE LEVEL OF MALE WISTAR RATS

Twenty one days treatment with A.Precatorius extract causes a significant increase in the high dose (HD) extract only when compared with the control. This might be the possible mechanism through which it improves sperm count.

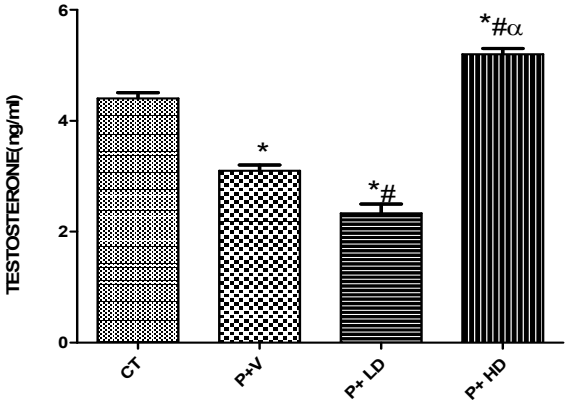


Figure 2: Effect of *Abrus precartorious* on serum testosterone level of male wistar rats. n=6; mean ± SEM, p< 0.05 when compared with control group. CT= Control, P +V = Paroxetine + Viagra, P + LD= Paroxetine + Low Dose extract, P + HD= Paroxetine + High Dose. NOTE: Values represent the Mean ± SEM (n=6); * p<0.05 significantly different from control, #p<0.05 significantly different from P + V, αp<0.05 P+HD significantly different from P+LD

HISTOLOGICAL OBSERVATIONS

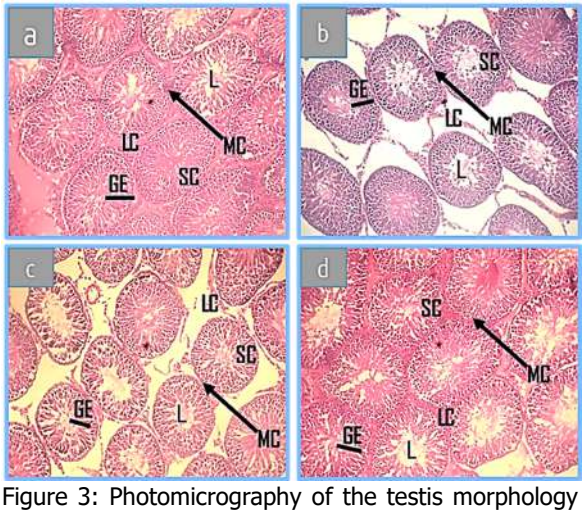


Figure 3: Photomicrography of the testis morphology stained with H&E at low magnification. The results show the Lumen (L), Leydig Cells (LC), Germinal Epithelium (GE), Myoid Cells (MC), and the Sertoli Cells (SC) across experimental groups.

The cellular assortment and the cytoachitecture of the group 1 (Control) reveals a well centralized lumen, sertoli cells with oval nucleus and dark nucleolus filled with abundant cytoplasm that extend from the basement membrane to the lumen. Furthermore, Leydig cells are properly arranged surrounded by connective tissues with the interstitial space. The germinal epithelium in these groups was properly arranged and myoid cells shows nucleus with dark pigmentation. The cellular assortments in the group 2 and 3 shows few vacuolated and decentralized lumen, with necrotic leydig and myoid cells and few pyknotic sertoli cells. Furthermore, there were few hypertrophy testes with germinal epithelium that are fragmented and disarrayed. The testicular histomorphology of the group 4 shows a slight similarity with that of Group 1 and only presented few hypertrophy testes and mild fragmentations in the germinal epithelium.

The cellular assortment and the cytoachitecture of the group 1 (Control) reveals a well centralized lumen, sertoli cells with oval nucleus and dark nucleolus filled with abundant cytoplasm that extend from the basement membrane to the lumen. The number of primary and secondary spermatocytes as well as spermatozoa decreased in the lumen of convoluted seminiferous tubules in group 2 and 3 respectively.

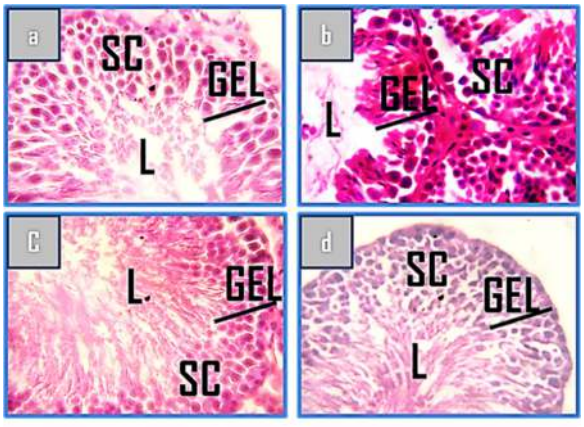


Figure 4: Photomicrography of the testis morphology stained with H&E at high magnification. The results show the Lumen (L), Germinal Epithelium (GE), and the Sertoli Cells (SC) across experimental groups.

The amount of seminiferous epithelium was reduced, with the majority of tubules being devoid of spermatids and just a small number of tubules harboring dead or degenerating tail pieces of spermatozoa. Around the tiny capillaries in the intertubular area, Leydig cells or interstitial cells were present, but their size and quantity were diminished. The interstitial cells displayed cytoplasmic atrophy and darkened nuclei. The same treated rats' epididymis displayed several empty tubules filled with degenerating spermatozoa or devoid of spermatozoa. The testes were normal, although there was cytoplasmic atrophy in the lining epithelial cells. The testicular histomorphology of the group 4 shows a slight similarity with that of Group 1 and only presented few hypertrophy testes and mild fragmentations in the germinal epithelium.

DISCUSSION

A.Precatorius extract has been reported to exhibit aphrodisiac property when administered chronically to experimental animals (Bhakta and Das 2020). Physical observation on mating performances such as mount latency (ML), mount frequency (MF), intromission latency and frequency (IL and IF), ejaculatory latency (EL) during the course of this present study shows that intake of this extract increases the libido of the experimental animals. They were more sexually active when compared with the control group; this could be attributed to the possible aphrodisiac property of *A.Precatorius.*

This result from this study shows that treatment with plant and Viagra increase significantly sperm count when compared to the control. However, there was a significant decrease of sperm motility

following treatment with plant extract across experimental group. The decrease in sperm motility seen in the high-dose extract group when compared with that of the control suggests that there is an alteration of sperm maturation in the epididymis and sperm production in the testis. This finding is corroborated by the report of Sinha et al., (1990) and Bhakta and Das (2015) in their studies that the *Abrus precatorius*' extract causes testicular anti-fertility effect and reduction of sperm motility in the cauda epididymis. Furthermore, methanol extract of *Abrus precatorius* seeds has been shown to deteriorate the motility of washed human spermatozoa with an EC 50 of 2.29 mg/ml irrevocably (Bhakta and Das, 2020). Sildenafil showed significant increase in the sperm motility when compared with LD and HD extract. This agrees with the study done by Glenn et al., (2007) which reported that Sildenafil citrate significantly increased both the number and velocity of progressively motile sperm.

The semen morphology when compared across experimental groups shows there is a considerable change in morphology like abnormal head shape, abnormal tail structure, cytoplasmic droplets and head vacuoles was noticed. These abnormalities can affect the sperm's ability to penetrate the egg, impair the sperm's motility making it difficult for them to reach and fertilize the and ultimately reduce fertility potential. However, there was an increase sperm count, decreases sperm motility in the treated group. This result is similar to Jahan et al., 2009 study. They reported that rats given *Abrus precatorious* showed decapitation, acrosome destruction, and swelling on the sperm at the midpiece region when observed under electron microscopy. The probable explanation of the observed morphological changes could be an alteration in energy metabolism caused by a decrease in ATPase and succinate dehydrogenase activity.

The result from the evaluation of serum testosterone shows an increase in serum testosterone in the high-dose (HD) extract when compared with the control. This might be the possible mechanism through which it improves sperm counts. Though, this increase looks contrary to other reports (Bhatt *et al*., 2007). While we used aqueous leave extract of *A. Precatorius* in the present study observe this increase in testosterone, Bhatt and colleagues used alcohol seed extract of *A. Precatorius* treated rats to describe its anti-fertility effect where it registered a significant reduction in testosterone levels that leads to functional sterility. There's however a study by Jahan et al (2009) that has also reported significant increase in serum testosterone levels after 60 days of *Abrus precatorious* administration. Additionally, Jahan and his team discovered an irreversible loss of sperm DNA integrity, which raised the possibility of teratogenicity. The increase observed in the high dose could be due to the negative feedback mechanism that stimulates the anterior pituitary gland to produce LH that act on the leydig cells to produce testosterone (Shama *et al*., 2022). It appears that very high dose of *Abrus precatorius* may induce infertility by increasing serum testosterone levels. Researchers have noted a decrease in testosterone levels, which may indicate that *A. precatorius* acts on the hypothalamus and pituitary (Bhakta *et al*., 2019). These findings may be related to gonadotrophin (LH, FSH) secretion that is below normal levels, which may result in fewer appropriate sperm in the lumen of convoluted seminiferous tubules. Also, the androgen starved effect could suggests that decreased testosterone is a possible cause of testicular and epididymal dysfunction (Khan *et al*., 2008). As a result, the mixed feeding had an impact on the development of sperm in each organ. This study demonstrated that a crude *A. precatorius*

mixture might produce functional infertility without causing harmful effects.

Tissue observation show normal testes in the control group when compared with the treated groups. In the treated groups there are mildly atrophied testes and degenerated cells. This could be due to the induction of paroxetine causing tissue dysfunctions. Tissue observation of control was compared with group two treated with sildenafil, it was observed that the control rat shows normal testis while the sildenafil showed some alterations in the seminiferous tubules of the testis and impaired spermatogenesis. This is due to the fact that sildenafil citrate induced abnormalities in sperm and an increase in sperm malformations, such as hypertrophic cells, necrosis of seminiferous tubules, testicular damage, and the presence of inflammatory cells. This observation is similar to the study of El-Kerdasy and Mohamed, 2019 who reported a comparable outcome. Furthermore, these consequences could result from modifications in the expression of different cGMP receptors or in the responsiveness of these receptors in the brain, damaging testicular tissue and impairing spermatogenesis (El-Kerdasy and Mohamed, 2019; Tocharus, 2005). Nitric oxide (NO) production may also vary as a result of the suppression of cGMP breakdown via negative feedback mechanisms (Kretschmann, 1999). NO may have varied effects on neurotransmitter activity in various brain systems.

Histology of the testis in group treated with HD and LD extract also show impairment in the testis specifically in the LD extract when compare with the control group as shown in fig 3. The number of primary and secondary spermatocytes as well as spermatozoa decreased in the lumen of convoluted seminiferous tubules. The amount of seminiferous epithelium was reduced, with the majority of tubules being devoid of spermatids and just a small number of tubules harboring dead or degenerating tail pieces of spermatozoa. Around the tiny capillaries in the intertubular area, Leydig cells or interstitial cells were present, but their size and quantity were diminished. The interstitial cells displayed cytoplasmic atrophy and darkened nuclei. The same treated rats' epididymis displayed several empty tubules filled with degenerating spermatozoa or devoid of spermatozoa. The testes were normal, although there was cytoplasmic atrophy in the lining epithelial cells. The implication of these could be that the treatment caused alterations in kinetics of spermatogenesis. Similar histological changes have also been reported in the work of Jahan *et al*., (2009) where they reported a significant reduction in the number of spermatozoa of animals treated with *A. Precatorius* extract which appear to be due to the suppressive effect of the treatment on spermatogenesis as it appears to possess a strong compound that decreases fertility mainly by decreasing the circulating androgen level.

CONCLUSION

Following the results of this research work, it can be suggested that A*. precatorius* extract at a very high dose may induce infertility by increasing serum testosterone levels, while at low dose a reduction in serum testosterone which could be linked to oxidative damage can possibly explicate the antifertility observed, although the mechanism of each dose appears dissimilar. Due to the fact that managing infertility and regulating fertility are two key aspects of reproductive health, crude extract of *Abrus precatorius* could be taken through clinical trials for possible therapeutic potentials.

RECOMMENDATION

It is recommended that further studies should be done to isolate the active ingredients of antifertility and aphrodisiac potentials of this plant.

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HISTOMORPHOLOGICAL CHARACTERISTICS OF PROSTATE SPECIMENS ANALYZED AT A REFERRAL HOSPITAL IN KENYA.

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ABSTRACT

Background: Global shift to high energy foods is important risk factor for new variants of prostate cancer. Some prostate tumors have an indolent course while others have an aggressive course; therefore, knowledge of tumor subtypes can help in clinical decision-making based on the patient profile. **Methods:** The main aim of the study was to determine the histomorphological characteristics of prostate specimens analyzed at regional referral hospital. The study was a cross-sectional retrospective study. The target specimens in this study consisted of prostate specimens that had prostate specific antigen level and were analyzed and reported between 2017 and 2022 at Jaramogi Odinga Oginga teaching and referral hospital, Kisumu Kenya. **Results:** Prostate color was not reported in eight reports. Of the 72 specimens observed, 50 (69.4%) were reported to be white, 12 (16.7%) were reported to be tan/white, 9 (12.5%) were tan brown, and 1 (1.4%) was tan grey. There was significant variation (p=0.001) in prostate specimen color. Of the 80 specimens, 47 (58.75%) had coarse surface, 16(20.00%) nodulated surfaces and 17 (21.25%) shrunken surfaces. There was variation in prostate surfaces (p<0.00001, X^2=23.275). Majority of specimens 55 (68.8%) measured between 0-29 mm, 12 (15%) measured 30-59 mm, 7 (8.8%) measured 60-89, 3 (3.8%) measured 90-119, 2 (2.5%) measured >150 mm and 1 (1.3%) measured 120-149 mm. There was no significant difference in the prostate biopsy sizes in comparison to the mean (p=0.984, t=0.020, 95% CI). The majority 20 (25%), of specimens had prostate cancer 15 (18.75%) had atypical findings, and 2 (2.5%) had high-grade proliferative intraepithelial neoplasia. The microscopic features of the groups did not differ significantly (F2, 34 = 1.469, p = 0.244). There is positive correlation between Gleason scores and Prostate specific antigen levels (p = 0.004, r = 0.474). **Conclusion:** There is variation of specimen color in prostate specimen with prostate cancer implying that advanced prostate disease causes changes in prostate color. Atypical prostate findings are common in age 50-59 which may suggest that targeted prevention and intervention should focus on this age group. Higher Gleason scores are likely to be observed in patients with higher PSA levels among patients being evaluated for prostate tumors.

Keywords: Adenocarcinoma; prostate cancer; histomorphology

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INTRODUCTION

Prostate cancer is the second common cancer diagnosis made in men behind skin cancer. Globally, prostate cancer is the fifth leading cause of death and may be asymptomatic at the early stage with an indolent course (Rawla, 2019). Diagnosis of prostate malignancy is made on the underpinning of urinary tract symptoms and elevated PSA levels (>10ng/mL) which then prompts the need for biopsy to confirm the diagnosis. There has been global shift to genetically modified diet and generally people use high energy foods in form of fast foods which according to various researches can alter prostate molecules and be molecular determinants of prostate cancer variation. The molecular basis of carcinogenesis in the prostate cancer are

emerging due to the fact that alterations in molecules that regulate the cell cycle and apoptosis contribute to the pathogenesis of prostate cancer. While studies done in the western world have demonstrated that acinar adenocarcinoma is common, variations due to changing lifestyle, fast foods and genetics among African men cannot be ignored. There is scarce information on prostate biology and histological characteristics of prostate tumors among men in western part of Kenya who are all exposed to factors that can alter the biology of prostate gland thus predisposing to prostate cancer variants that may be different from the conventional adenocarcinoma. Such scarcity of information could lead to assumptions that all prostate tumors are adenocarcinoma. Such assumptions can cause delayed decision making in care. In addition, geographical and racial factors are documented by American society of urology as independent risk factors for prostate tumors. Men in western part of Kenya could have different prostate histological characteristics given different geography and race. Such knowledge will guide the aggressiveness in pursuit of treatment for patients whose prostate specimens are reported positive for cancer. The study sought to determine histomorphological characteristics of prostate specimens analyzed and processed at JOOTRH between 2017 and 2022.

MATERIALS AND METHODS

Study area

The study was conducted at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) which is a level five hospital located in Kisumu Kenya and has a well-established pathology laboratory with adequate staffing and necessary equipment to analyze histology specimens.

Study design

The current study was a descriptive cross sectional retrospective study design that utilized prostate specimen histology reported in a pathology laboratory as reported between 2018 and 2022. Random sampling was used to get the specimen as reported. A sampling frame consisted of pathology register of the histopathological reports of the prostate specimens analyzed in the laboratory. Each name in the register was assigned serial numbers. All the numbers were fed in a computer program (randomizer application) to randomly sample (determined by Yamane Taro formula) 80 names out of those so that each had an equal chance of getting selected.

Sampling methods

Systematic random sampling was used. A sampling frame consisted of pathology register of the histopathological reports of the prostate specimens analyzed at JOOTRH. Each name in the register was assigned serial numbers. All the numbers were fed in a computer program (randomizer application) to randomly sample 80 names out of those so that each had an equal chance of getting selected. Only samples reported with PSA levels were included in the study.

Data collection

The researcher collected the data from hospital pathology laboratory with the help of two research assistants who were laboratory technicians working in the pathology laboratory and conversant with retrieving soft copy data from the storage site. The data from each prostate pathology report was then transferred into each data extraction form for each patient profile: the age, clinical notes including PSA levels, microscopic and macroscopic examination, and conclusion was extracted and recorded in the research data extraction form. Photographs of slides mounted on a

microscope were also taken for prostate tissues with prostate cancer and with benign prostatic hypertrophy.

Data analysis

Descriptive and inferential statistics were done with SPSS version 29 for Windows. One sample chi-square was used to test the hypothesis that there is no variation in specimen colour and surface. One sample t test was used to assess if the specimen size differed significantly from the mean. ANOVA was used to check whether microscopic features differed significantly. A post hoc analysis based on the Levine statistic was achieved by Dunnett's 3 analysis to check which group contributed to differences. A p value of <0.05 was considered statistically significant at 95% CI.

Ethical approval

This study was licensed by National commission of science, technology and innovation (NACOSTI) vide license number NACOSTI/P/23/22845. The study was approved by JOOTRH ethics committee vide letter reference number ISERC/JOOTRH/659/22. The data collection was allowed by the JOOTRH hospital CEO vide letter reference number GEN/21A. No patient identifiers were collected during the study.

RESULTS

Gross morphology of prostate specimens

Specimen color

A total of 80 prostate histology reports for prostate specimens were retrieved. The prostate specimen colors reported were categorized as white, tan/white, tan/brown, and tan/grey. Prostate color was not reported in eight reports. Of the 72 specimens observed, 50 (69.4%) were reported to be white, 12 (16.7%) were reported to be tan/white, 9 (12.5%) were tan brown, and 1 (1.4%) was tan grey (Table 1).

Table 1: Prostate tissue color

	Frequency	Percent
White	50	62.5
Tan/white	12	15.0
Tan/Brown	9	11.3
Tan/grey	1	1.3
No colour reported	8	10.0
Total	**80**	**100.0**

One sample chi-square was used to test the hypothesis that the categories of prostate specimen color occur with equal chances. One sample Chi-square statistic was used to examine this hypothesis. There was statistically significant variation (X^2=79.444, p=0.001) in prostate specimen color as reported in prostate histology.

Specimen surface

The surface of prostate specimens was described in clinical categories: coarse, shrunken, or nodular (Figure 2). Of the 80 specimens, (47; 58.75%) had prostate biopsy surfaces that were coarse, followed by nodulated surfaces (16; 20.00%) and shrunken surfaces (17; 21.25%); Figure 2. The study sought to establish whether the texture reported was due to chance.

Table 2: Chi-square test of fitness output

Test Statistics	
	Surface texture
Chi-Square	23.275[a]
df	2
Asymp. Sig.	.000

One sample chi-square test of fitness was used to test the hypothesis that prostate surfaces as reported occurred by chance. The researcher could not demonstrate that the prostate specimen surfaces occur with equal changes (p<0.05, X^2=23.275, df=2, 95% CI) (Table 2). Hence H1 was supported.

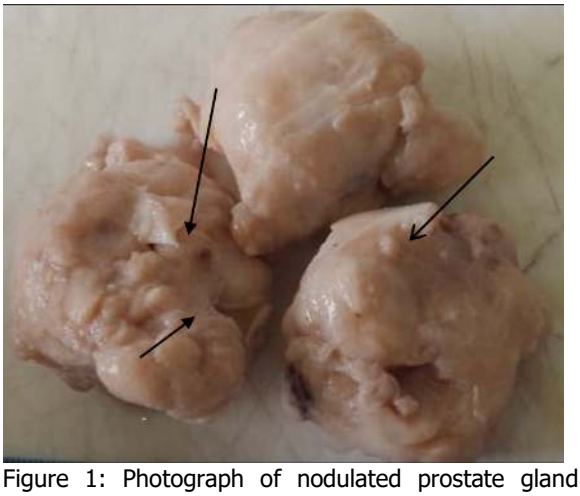


Figure 1: Photograph of nodulated prostate gland (arrow indicate specific nodules)

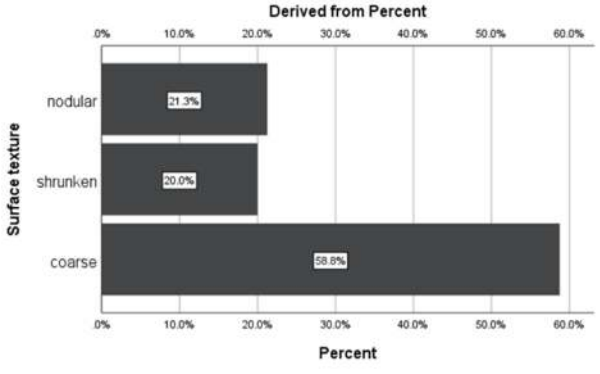


Figure 2: Frequency distribution of Prostate surface texture

Specimen size

Majority of the biopsy specimens 55 (68.8%) measured between 0-29 mm, 12 (15%) measured 30-59 mm, 7 (8.8%) measured 60-89, 3 (3.8%) measured 90-119, 2 (2.5%) measured >150 mm and 1 (1.3%) measured 120-149 mm (Figure 3).

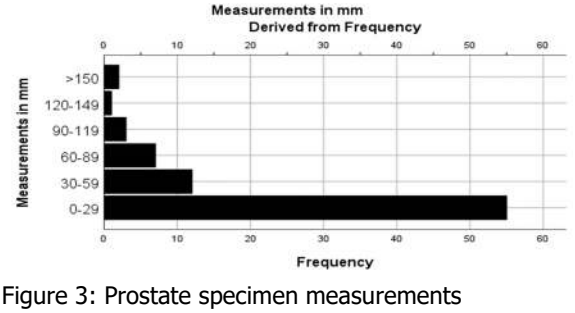


Figure 3: Prostate specimen measurements

One sample t test was run to assess if the specimen sizes differed significantly in comparison to the mean prostate biopsy size. The descriptive statistics showed that prostate biopsy size had a mean of 1.61, with a standard deviation of 1.142. The results reveal that there is no statistically significant difference in the prostate biopsy sizes in comparison to the mean (p=0.984, t=0.020, 95% CI) (Table 3).

Table 3: One sample t test on specimen measurement in mm

One-Sample Test

	Test Value = 1.61					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Measurements in mm	.020	79	.984	.002	-.25	.26

Microscopic morphology

The study sought to establish the microscopic features of prostate specimens as reported. Majority 43 (54%) of the specimens had BPH/no cancer. The microscopic features of 37 (46%) prostates specimens were reported as either having atypical findings, high grade prostatic intraepithelial neoplasia (HGPIN), or prostate cancer. The majority of specimens analyzed were labelled as having prostate cancer in 20 (25%), followed by those labelled as atypical in 15 (18.75%), and high-grade PIN in at least 2 (2.5%) (Figure 4).

Gleason scores

The specimens were further characterized in terms of Gleason or group scores. (Table 4).

Table 4: Gleason scores, PSA crosstabulation

		PSA (ng/ml)					Total
		0-4	5-10	11-49	50-99	>100	
Gleason Scores	Gleason<6/Group 1	0	2	3	2	1	8
		0.0%	66.7%	60.0%	33.3%	4.8%	22.2%
	Gleason 7/Group 2	1	0	1	1	6	9
		100.0%	0.0%	20.0%	16.7%	28.6%	25.0%
	Gleason 7/Group 3	0	1	1	1	5	8
		0.0%	33.3%	20.0%	16.7%	23.8%	22.2%
	Gleason 8/Group 4	0	0	0	2	4	6
		0.0%	0.0%	0.0%	33.3%	19.0%	16.7%
	Gleason 9or10/Group 5	0	0	0	0	5	5
		0.0%	0.0%	0.0%	0.0%	23.8%	13.9%
Total		1	3	5	6	21	36
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Prostate microscopic features and age

The hypothesis tested if the microscopic features differed across different age groups. Prostate histology reports were divided into

four groups based on the age of the patient (Group 1: 40–49 years, Group 2: 50–59 years, Group 3: 60–79 years, and Group 4: above 80 years) (Figure 4). The ANOVA results suggest that the microscopic features of the groups did not differ significantly (F2, 34 = 1.469, p = 0.244, 95% CI). Since Levine's statistic for the mean is significant (p=0.001), an equal variance was not assumed.

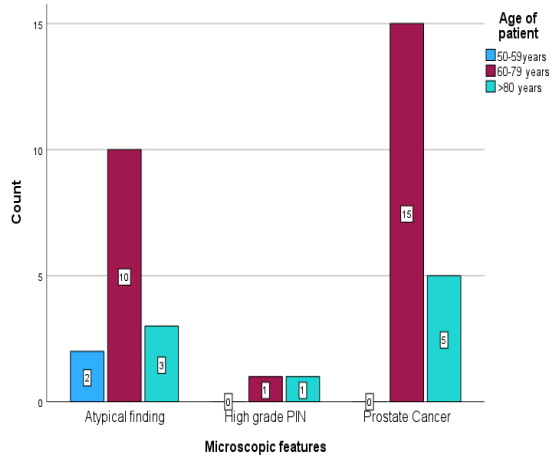


Figure 4: Microscopic features

To check for individual differences between groups, post-hoc comparisons were assessed using Dunnett's T3. The test indicated that the mean microscopic features for ages 50–59 years (M = 1.00, SD = 0.000) were significantly different from those for ages 60–79 years (M = 2.19, SD = 0.981). The mean differences were significant at the 0.05 level. However, no significant differences were detected between the other groups.

Gleason score

Majority 6 (28.6%) of patients who presented with PSA levels greater than 100 ng/ml contributed most to group 2 Gleason score (25%) (Table 4). The study sought to establish the correlation between Gleason scores and PSA levels.

Table 5: Pearson PSA and Gleason scores correlation

Correlations

		PSA (ng/ml)	Gleason Scores
PSA (ng/ml)	Pearson Correlation	1	.474**
	Sig. (2-tailed)		.004
	N	80	36
Gleason Scores	Pearson Correlation	.474**	1
	Sig. (2-tailed)	.004	
	N	36	36

**. Correlation is significant at the 0.01 level (2-tailed).

Pearson correlation statistics was used to examine the correlation between Gleason's scores and PSA levels. There is a statistically significant positive correlation between Gleason scores and PSA levels (p = 0.004, r = 0.474) (Table 5). Therefore, the null hypothesis was not supported.

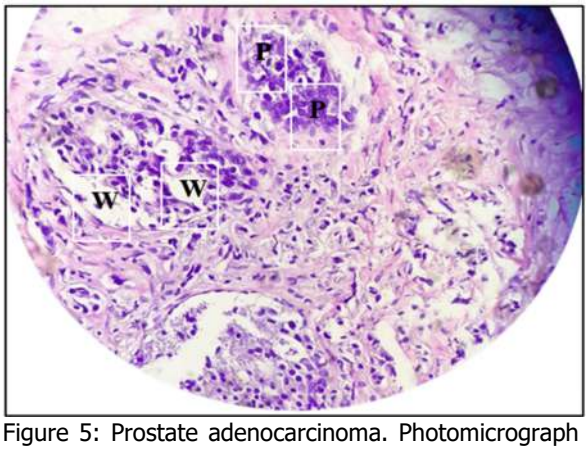


Figure 5: Prostate adenocarcinoma. Photomicrograph showing large nuclear, prominent nucleoli and collagenous micronodules infiltration. P: Prominent nucleoli, W: collagenous micronodules infiltration

Figure 5 prominent nucleoli and micronodule infiltration is notable which is tandem with prostate adenocarcinoma. Histological characteristics of prostate cancer requires any of the three: the presence of circumferential perineural infiltration, collagenous micronodules and glomerulation.

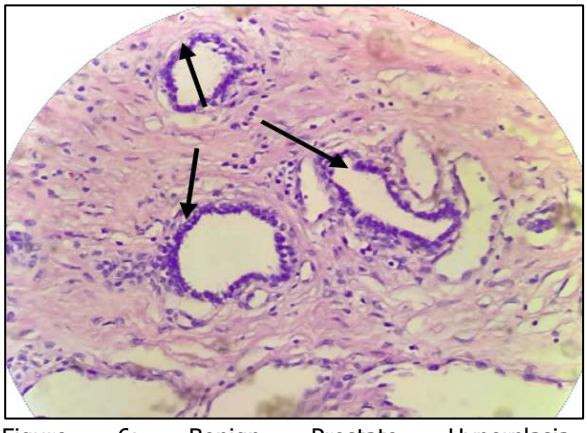


Figure 6: Benign Prostate Hyperplasia. Photomicrograph showing solid nests. Arrow points to nested cells

In Figure 6 the arrow shows nested cells in two layers of epithelial cells which is characteristic of benign prostatic hyperplasia. Benign prostatic hyperplasia is characterized by infoldings, two layers of epithelial tissues giving it a nested cells appearance (arrows). It is also characterized by hyperplasia of smooth muscles of prostate, enlarged glands and enlarged fibrous tissue.

DISCUSSION

The results from the current study suggest that there is significant variation in prostate specimen color, with the majority being white (50%), tan/white (12%), tan/brown (9%) and tan gray (1%) (Table 1). It should be kept in mind that a normal prostate gland appears white on gross examination and therefore one possible interpretation of results shown in Table 1 is that color variation occurs due to pathologic changes or biopsy technique. Lindh et al. (2018), found that white, tan, yellow, and orange tumors made up the majority of those that could be definitively diagnosed as prostate tumor. In the current study, there was no documentation of color as yellow or orange, a major contradiction from findings by Lindh et al. (2018). This could be because of variation in pathologist coding of colors due to non-standardization of prostate color reporting. Another possible explanation could be that the biopsy technique or the stage of prostate disease, distort the gross prostate histologic features to induce gross changes in prostate appearance.

The current study revealed that there is no difference in the prostate biopsy sizes in comparison to the mean of 14 mm. ($p = 0.984$). The findings shown in Figure 3 are similar to that of Obek et al. (2012) who indicated that mean prostate core biopsy size is 12 mm and Fiset et al. (2013) who found biopsy size to be 13 mm. One possible interpretation of these results is that needle core length could be an important morphometric parameter of transrectal prostate biopsies that directly influences the biopsy size and thus the cancer detection rate. The results of the current study agree with other authors (Fiset et al., 2013; Öbek et al., 2012) and this could be explained by the fact that there could be guidelines on the standard prostate biopsy technique in which core biopsy needle is used. It could also be argued that perhaps core biopsies are the common technique of sample collection for prostate specimens.

The current study found that prostate microscopic features of the groups differ significantly with age and that microscopic features for age 50–59 years were significantly different from 60–79 years, with the majority of the pathological changes (HGPIN and prostate cancer) observed more in the age bracket 60–79 years compared to atypical changes at 50–59 years. (Figure 4). The results are in agreement with other authors (Kopp et al., 2011; Liu et al., 2020), whose data suggest that patients aged 60–79 have an increased likelihood of developing BPH and prostate cancer. One possible explanation of the results could be that perhaps alterations in the prostate histological profile are pronounced at 60–79 years of age. The current study found that prostate atypical findings were mainly

observed in men aged 50–59, which agrees with Matti et al. (2022) and Miyai et al. (2014). The implications of this finding are that men aged 50–59 need screening for prostate diseases, possibly because they have an increased risk for prostate disease.

In the current study, 18.5% of patients whose samples were analyzed had atypical findings (Figure 4), compared to 30.6% and 25% in Yanez & So (2015) and Kopp et al. (2011), respectively. It should be noted however that the Yanez & So (2015) study was a 2-year prospective study carried out in the Philippines, which is a different geographical region, and thus racial differences could be an explanation of the variation, indicating that perhaps atypical prostate findings are more prevalent among men aged 50-59 in western countries compared to other parts of the world. Another possible interpretation is that most men present for prostate evaluation at ages 50-59 in sub-Saharan Africa.

The current study found that 5.4% of the samples analyzed had high-grade intraepithelial neoplasia, which is in contradiction to studies such as Kim et al.'s (2021) 70.4% and Pierorazio et al.'s (2007) 88.4%. The significant variation could suggest that either reporting for HGPIN is inadequately reported or perhaps the sample size in the current study was small compared to Pierorazio et al.'s (2007) study of 3460 subjects, which covered an 18-year period between 1988 and 2006. Another possible interpretation could be that, given that the studies by Fitet et al. (2013) and Pierorazio et al. (2007) were done in developed countries where an aging population forms a larger proportion of the population, it is more likely to have prostate pathologies reported compared to sub-Saharan Africa, where there are fewer aged men likely to present for evaluation of prostate disease. It is also possible that economic implications can make fewer men present for prostate evaluation in sub-Saharan hospitals.

Chi-square statistics were used to examine the association between Gleason's scores and PSA levels. The current study found a statistically significant correlation between Gleason scores and PSA levels ($p = 0.004$, $r = 0.474$) (Table 5). The current study results agree with Cihan et al.'s (2019) finding that Gleason score of patients was significantly and positively correlated with age and PSA levels. It should be noted, however, that although the findings agree, Cihan et al. (2019) carried out a prospective study, and their reporting would likely have been better compared to a retrospective study. Similarly, Gündodu et al. (2020) found that Gleason scores correlate positively with PSA levels in a prospective study of patients who underwent radical prostatectomy. The current findings, however, are in contradiction with Sanli et al. (2017). This could perhaps be due to the fact that Sanli et al. (2017) focused on patients who were on treatment follow-up, and therefore the relationship could have been confounded by treatment.

The histology reports analyzed were all from the hospital pathology laboratory computer data base, and this study did not establish whether there were any errors in data entry. More research on prostate histology among patients on treatment is required. Further research is needed to determine the changes in prostate histological features following treatment for prostate cancer.

CONCLUSION

These results suggest that there is significant variation in prostate biopsy specimen color in different men, but the majority of prostate specimens are white. Variations in colors such as tan and yellow could signify the effect of a disease process on the prostate that induces color alterations. The study found positive correlation between Gleason scores and PSA levels, implying that high Gleason scores are observed in patients with high PSA levels. Patients whose prostate specimens were reported to have HGPIN or

atypical findings need a repeat histology as soon as possible since these are premalignant findings or lesions that mimic prostate carcinoma. Based on these conclusions, the following recommendations are made: There is significant variation in histomorphology of prostate specimens in relation to color and surface. Therefore, pathologists need to correlate the specimen color with extend of disease using tools such as Gleason scores. Men aged 50-59 may have atypical findings on histology.

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ORIGINAL ARTICLE

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ANTI-CANCER AND TISSUE-NEUROPROTECTIVE POTENTIALS OF MORINGA OLEIFERA AND MUSA SAPIENTUM AGAINST IN-VIVO CADMIUM CHLORIDE-INDUCED SKIN TOXICITY

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ABSTRACT

Background: Cadmium is an established carcinogen. Cadmium exposure resulted in skin carcinogenesis in rats. Furthermore, neurotransmitter-cancer interaction, tissue innervation, and angiogenesis influence cancer prognosis, metastasis, and survival. In this study, we evaluated the anticancer and neuro-protective potentials of MO11 (isolated from *Moringa oleifera* leaves) and MS06 (isolated from *Musa sapientum* suckers) against *in-vivo* Cadmium Chloride ($CdCl_2$)-induced skin toxicity. **Methods:** Twenty-four adult male wistar rats were randomly divided into six groups (n = 4). Group 1 was control. Groups 2-4 and 6 received a single intraperitoneal administration of 1.5 mg/kg $CdCl_2$ on Day 1. Thereafter, Groups 3, 4, and 6 were post-treated with 15 mg/kg MO11, 15 mg/kg MO11 + 7 mg/kg MS06, and 3.35 mg/kg Doxorubicin respectively (Days 1-17). Group 5 received an olive oil dose (vehicle) (Days 1-17). Skin histopathology and tissue-enzyme-linked-immunosorbent assays (Tissue-ELISA) of neurotransmitters (Dopamine and Glutamate), biomarkers of myelination (Myelin Basic Protein), drug metabolism and carcinogenesis (Cytochrome-p450), apoptosis (Caspase-3 and p53), proliferation (Ki67), and angiogenesis (sVEGFR) were evaluated in skin homogenates. Data were statistically analyzed using the Mann-Whitney-U test at $P \leq 0.05$. **Results:** Histopathological analyses revealed normal skin histology (Groups 3 and 4), mild skin histo-alterations (Group 6), and gross skin histopathological alterations (Group 2). Tissue-ELISA analyses showed upregulations of dopamine, Glutamate and Cytochrome-p450, but downregulations of Myelin Basic Protein, Caspase-3, Ki67, p53, and sVEGFR in groups 3, 4, and 6, compared with group 2. MO11 and MS06 achieved better anticancer effects than doxorubicin. **Conclusion:** Overall, MO11 and MS06 possess histo-protective, neuro-protective, re-myelination, anti-proliferation, anti-angiogenesis, and anti-cancer potentials.

Keywords: anticancer potentials; anti-toxic principles; cadmium; *moringa oleifera; musa sapientum;* skin carcinogenesis

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INTRODUCTION

Cadmium (Cd) is employed industrially in the production of products such as lasers, television screens and batteries (Huff et al., 2007; Wang and Du, 2013; Andjelkovic et al., 2019). Cd is classified as a human carcinogen, and is one of the 10 chemicals or groups of chemicals of concern for human health according to the World Health Organization (Huff et al., 2007; Wang and Du, 2013; Andjelkovic et al., 2019). Cd-exposure (at 1.0, 0.1, and 0.01% weight/volume for 10 days) resulted in alopecia, hyperkeratosis, acanthosis, ulceration, and increased mitotic index in the skin of rats and mice (Lansdown and Sampson, 1996). In addition, exposure of surgically induced skin wounds to 1.0% $CdCl_2$ results in sustained edema, epidermal hyperplasia, inflammatory cell infiltration, and significantly increased skin metallothionein content in rats (Lansdown et al., 2001). Furthermore, human skin penetration of 12.7% (water) and 8.8% (soil) of 5 microliters/cm^2 of Cd dissolved in water were reported (Wester et al., 1992). Significant increase in blood Cd content in humans correlated with severe psoriasis following adjustment for covariates and control of confounding factors (Liaw et al., 2017). The mechanism of Cd-induced skin carcinogenesis in animal models, however, remains poorly understood.

Cancer cells comprise cancer stem cells, macrophages, and vascular endothelial cells, which aid metastasis (Lan et al., 2017; Akinlolu et al., 2021). Cancer cells interact with neighboring cells and extracellular components such as nerve fibers, secretory elements of nerve fibers, stroma cells, immune cells, and endothelial cells, which collectively exert a strong influence on cancer prognosis, metastasis, and survival (Lan et al., 2017). Dopamine is a neurotransmitter involved in regulation of processes such as motor control, arousal, motivation and reward (Noori et al., 2015). Induction of Dopamine causes apoptosis via the Cytochrome C/Caspase-dependent pathway and inhibition of tumor growth (Lan et al., 2017). In contrast, dopamine levels are downregulated in tumors (Lan et al., 2015; Kuol et al., 2018). Glutamate is the major excitatory neurotransmitter of the central nervous system which is involved in regulation of metabolism, and whose levels must be rightly maintained (Zhou and Danbolt, 2014). Metabolism-related genes are mutated in cancers, making them glutamate-dependent. Hence, dysregulation of glutamate levels promotes tumor growth (Yi et al., 2019).

The roles of Dopamine, Glutamate and neuropeptides released by nerve fibers within the tumor microenvironment in cancer prognosis, metastasis, and survival via complex neuro-cancer interactions have led neurotransmitters to be considered as targets for therapeutic interventions (Lan et al., 2017). In addition, adequate myelination of nerve fibers is needed for the proper functioning of nerve fiber-supplying tissues (Akinlolu et al., 2020a). Hence, neurobiological examinations of tissue innervation is of importance in understanding metastasis.

Moringa oleifera (MO) and *Musa sapientum* (MS) are ethno-medicinal plants and dietary supplements which are well grown and widely available in Nigeria (Akinlolu et al., 2021). MOF6, a sub-fraction of ethanolic extracts of MO leaves showed significant neuroprotective potentials against cuprizone-induced cerebellar damage (Omotoso et al., 2018a) and dysregulated acetylcholinesterase concentrations in sodium arsenite-induced neurotoxicity (Akinlolu et al., 2020b) in rats. MOF6 equally showed hepatoprotective, anti-proliferation, and anti-drug resistance potentials in 7,12-Dimethylbenz(a)anthracene-induced hepatotoxicity in rats (Akinlolu et al., 2021). Furthermore, MSF1, which was fractionated from MS suckers, showed hepatoprotective,

anti-proliferation, and anti-drug resistance potentials in 7,12-Dimethylbenz(a)anthracene-induced hepatotoxicity in rats (Akinlolu et al., 2021).

Therefore, in-order to further determine their anticancer and tissue-neuroprotective potentials, this study evaluated the effects of MO11 (isolated from *Moringa oleifera* leaves) and MS06 (isolated from *Musa sapientum* suckers) on skin concentrations of neurotransmitters (Dopamine and Glutamate), biomarkers of myelination (Myelin Basic Protein), drug metabolism and carcinogenesis (cytochrome p450), apoptosis (Caspase-3 and p53), proliferation (Ki67), and angiogenesis (sVEGFR) in $CdCl_2$-induced skin toxicity in adult male Wistar rats.

MATERIALS AND METHODS

Ethical approval and compliance with ethical standards

Ethical approval for this study was obtained from the Ethical Review Committee of the University of Ilorin, Nigeria. Appropriate measures were taken to ensure minimal pain and discomfort in the rats used in this study. The ethical approval number is UERC/ASN/2018/1161. Furthermore, this research study was conducted in accordance with the internationally accepted principles for laboratory animal use and care as provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC), the Directive 2010/63/Eu of the European Parliament and of the Council of September 22, 2010, on the protection of animals used for scientific purposes, and the Guidelines of the U.S. Public Health Service and NIH regarding the care and use of animals for experimentation (NIH publication #85-23, revised in 985).

Collection, authentication and deposition of MO leaves and MS suckers

MO leaves and MS suckers were obtained locally and freshly from forest reserves in Ilorin, Kwara State, Nigeria. The obtained plant samples were authenticated, deposited, and assigned Herbarium Identification Numbers UILH/001/1249 and UILH/002/1182, respectively, at the herbarium of the Department of Botany, University of Ilorin, Nigeria.

Evaluations of antioxidant and anti-microbial activities of MO and MS fractions

The antioxidant activities of the plant extracts were evaluated using the modified 2,2- diphenyl-1-picrylhydrazyl method as previously described by Chaves et al. (2020). In addition, the anti-microbial activities of the plant extracts were evaluated by testing the cytotoxic potential of each fraction against E. coli and Salmonella typhimurium, as previously described by Elisha et al. (2017).

Extraction, partitioning of fractions and isolations of MO11 from MO leaves and MS06 from MS suckers

MO11 and MS06 were isolated from *Moringa oleifera* leaves and *Musa sapientum* suckers, respectively, following a series of antioxidant analyses (2,2-diphenyl-1-picrylhydrazyl method), anti-microbial analyses (anti-Escherichia-coli and anti-Salmonella-tiphimurium tests), chromatography, and spectroscopic fractionation techniques, as described previously (Akinlolu et al., 2022a; Ameen and Akinlolu, 2023).

Animal care and feeding

24 adult male Wistar rats (average weight, 155 g; age, 2 months) were purchased from a colony breed at Badagry in Lagos State, Nigeria. The rats were randomly divided into six groups with four rats per group. The rats were acclimatized for a week at the animal house of the Faculty of Pharmacy of Olabisi Onabanjo University, Nigeria, before

beginning the experimental procedures. The rats were maintained under standard conditions and permitted unlimited access to food and drinking water ad libitum. The body weights of the rats were computed on a daily basis using an electronic compact scale (SF-400C weighs in grams) (Valid Enterprise, Kalbadevi, Mumbai, India).

Grouping of rats and extracts/drugs administration

MO11 and MS06 were dissolved in Olive Oil (the vehicle). Rats in Control Group 1 (Baseline Control) received physiological saline for only 17 Days (Days 1-17). Each rat in Experimental Groups 2-4 and 6 received a single intraperitoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ (Sigma-Aldrich, Japan Co.) on Day 1. Rats in group 2 (Negative Control) were left untreated throughout the experimental procedure for 17 Days (Days 1-17). Thereafter, rats in group 3 were post-treated with oral administration of 15 mg/kg body weight of MO11 for 17 Days (Days 1-17). Rats in group 4 were post-treated with oral administration of a combined mixture of 15 mg/kg body weight of MO11 and 7 mg/kg bodyweight of MS06 for 17 Days (Days 1-17). Rats in group 5 received only oral administration of Olive Oil (vehicle, 1 mL/kg body weight) for 17 Days (Days 1-17). Rats in Group 6 were post-treated with tail intravenous administration of 3.35 mg/Kg bodyweight of doxorubicin (standard anticancer drug – Positive Control) for 17 Days (Days 1-17).

Completion of experimental procedures

Twenty-four hours after the last day of drug and extract administration on day 17, the experimental procedures were completed and the rats were sacrificed on day 18 by cervical dislocation, as previously described by Omotoso et al. (2018b). For animal sacrifice, no anesthesia was used, as approved by the University Ethical Review Committee, after their evaluation of the experimental protocol based on the fact that the biomarkers to be examined include enzymes such as Caspase-3 and metabolic agents such as cytochrome p450, which may be endogenously altered by anesthetic agents requiring post-experimental control of confounding factors.

Histo-pathological evaluations of the skin

The excised normal morphological skin portions (Groups 1 and 3 – 6) and alopecia (hairless) skin portions (Group 2) of rats were processed for light microscopy using conventional histological procedures. Histopathological evaluations of the skin were conducted using the Hematoxylin and Eosin technique, as previously described by Akinlolu et al. (2021 and 2022b). Photomicrographs of slides were captured with the aid of Olympus binocular microscope (Tokyo, Japan) which was connected to a 5.0 megapixel Amscope camera (Amscope Inc., Irvine, CA, USA). Tissue sections were photographed at 100X magnification.

Tissue-Enzyme Linked Immunosorbent Assays (Tissue-ELISA) of concentrations of biomarkers in skin homogenates of rats

Normal morphological skin portions (Groups 1 and 3 – 6) and hairless skin portions (Group 2) of the back of all rats were excised and isolated. Thereafter, 1 g of the excised skin portion of each rat was thoroughly homogenized using a porcelain mortar and pestle in 4 ml of ice-cold 0.25 M sucrose solution. The tissue homogenates were additionally filled up to 5 ml with sucrose in a 5 ml serum bottle. Skin homogenates were centrifuged at 3000 rpm for 15 min (Model 90-1). The supernatant was collected with Pasteur pipettes, placed in a freezer at -20 °C, and assayed for concentrations of Dopamine, Glutamate, Myelin Basic Protein (MBP), cytochrome p450, Caspase-3, Ki67, p53, and sVEGFR in the skin of all rats of

Control Group 1 and Experimental Groups 2 – 6 using ELISA, as previously described by Akinlolu et al., 2021.

Statistical analysis

The computed data of the concentrations of each biomarker are expressed as arithmetic means ± standard deviation. The Mann-Whitney U test (Wilcoxon-Mann-Whitney Test, 2016) was used for statistical comparison of the concentration of each biomarker between the two groups, because the sample size of 24 was less than 30. Significant difference was confirmed at 95% confidence interval, with associated p - value of less than 0.05 ($P \leq 0.05$).

RESULTS

Morphological observations of the skin

No alopecia was noted in the skin of rats in Groups 1 and 3 – 6 (Figures 1a and 1c – 1f). However, morphological observations showed severe skin alopecia in rats in $CdCl_2$-only treated group 2 (Figure 1b).

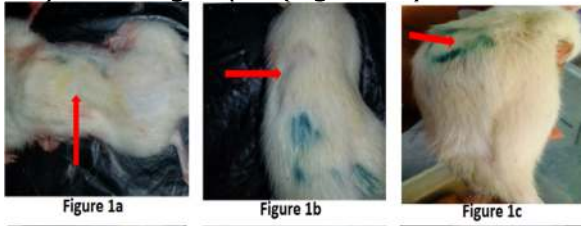


Figure 1a: Photomicrograph of the skin of the back of rats of Group 1 which received physiological saline. Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 1.

Figure 1b: Photomicrograph of the skin of the back of rats of Group 2 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ (Negative Control). Red solid arrow indicates site of skin alopecia of the back of rat. Morphological observations showed morphological anomaly indicated by skin alopecia of the skin of back of rats of Group 2.

Figure 1c: Photomicrograph of the skin of the back of rats of Group 3 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ and were post-treated with 15 mg/Kg bodyweight of MO11. Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 3.

Figure 1d: Photomicrograph of the skin of the back of rats of Group 4 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ and were post-treated with 15 mg/Kg bodyweight of MO11 and 7 mg/Kg bodyweight of MS06. Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 4.

Figure 1e: Photomicrograph of the skin of the back of rats of Group 5 which received only 1 ml/Kg bodyweight of Olive Oil (vehicle). Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 5.

Figure 1f: Photomicrograph of the skin of the back of rats of Group 6 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ and were post-treated with 3.35 mg/Kg bodyweight of Doxorubicin (standard anticancer drug – Positive Control). Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 6.

Histo-pathological evaluations of the skin

Histopathological evaluations showed normal skin histo-architecture in rats of Groups 1 (Figure 2a) and 3-5 (Figures 2c – 2e). In contrast, histopathological evaluations showed depletion of keratinocytes of the stratum corneum of epidermal layer in the hairless skin of rats of $CdCl_2$-only treated Group 2 (Figure 2b). In addition, histopathological analyses showed mild depletion of the stratum corneum of the epidermal layer of the hairless skin of rats in Group 6 (Figure 2f).

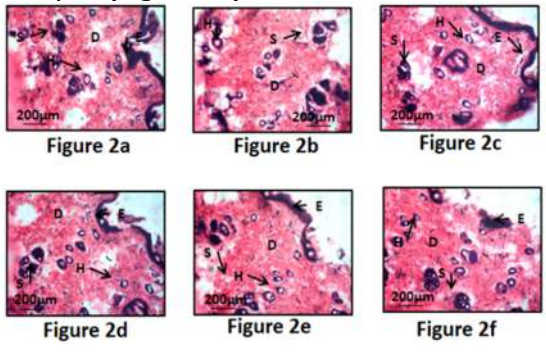


Figure 2a: Representative photomicrograph of the Skin of rats of Group 1 which received physiological saline. Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Staining intensity, cellular and morphological delineation appear normal. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2b: Representative photomicrograph of the Skin of rats of Group 2 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride. Haematoxylin and Eosin. Histological analyses show anomalies of the histoarchitecture of the Skin components such as the stratum corneum, epidermal layer, hair follicle (H) and sebaceous gland (S). Distortion of the stratum corneum and epidermal layer is apparent. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2c: Representative photomicrograph of the Skin of rats of Group 3 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride and were post-treated with 15 mg/Kg bodyweight of *Moringa oleifera*. Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Staining intensity, cellular and morphological delineation appear normal. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2d: Representative photomicrograph of the Skin of rats of Group 4 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride and were post-treated with 15 mg/Kg bodyweight of *Moringa oleifera* and 7mg/Kg bodyweight of *Musa sapientium* (dissolved in 1 ml of Olive oil). Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Staining intensity, cellular and morphological delineation appear normal. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2e: Representative photomicrograph of the Skin of rats of Group 5 which received 1 ml/Kg bodyweight of Olive oil only. Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Mild distortion of the epidermal layer is apparent. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2f: Representative photomicrograph of the Skin of rats of Group 6 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride and were post-treated with 3.35 mg/Kg bodyweight of Doxorubicin. Haematoxyline and Eosin. Histological analyses show mild anomalies of the histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Mild distortion of the stratum corneum and epidermal layer is apparent. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Concentrations of Dopamine, Glutamate and Myelin Basic Protein (MBP) in skin of rats: Group 2 versus Group 1

The results showed significantly lower ($P \leq 0.05$) levels of Dopamine and Glutamate, but significantly higher ($P \leq 0.05$) levels of MBP in homogenates of skin of rats in Group 2, when compared with Control Group 1 (Table 1).

Concentrations of Dopamine, Glutamate and MBP in skin of rats: Group 2 versus Groups 3 and 4

The results showed statistically significant lower ($P \leq 0.05$) levels of dopamine in the skin homogenates of Group 2 rats than in Groups 3 and 4, as presented in Table 1. In addition, the results showed statistically significantly lower ($P \leq 0.05$) levels of glutamate, but statistically non-significant lower ($P \geq 0.05$) levels of glutamate in the homogenates of skin of rats in Group 2, compared with Groups 3 and 4, respectively, as presented in Table 1. Furthermore, the results showed statistically significant higher ($P \leq 0.05$) levels of MBP in homogenates of the skin of rats in Group 2 than in Groups 3 and 4, as presented in Table 1.

Concentrations of Dopamine, Glutamate and MBP in skin of rats: Group 2 versus Group 6

The results showed statistically significant lower ($P \leq 0.05$) levels of dopamine in the skin homogenates of Group 2 rats than in Group 6 rats, as presented in Table 1. In addition, the results showed statistically non-significant lower ($P \geq 0.05$) levels of glutamate, but statistically non-significant higher ($P \geq 0.05$) levels of MBP in homogenates of rat skin in Group 2 than in Group 6, as presented in Table 1.

Concentrations of Caspase-3, Ki67, p53, Cytochrome p450 and sVEGFR in skin of rats: Group 2 versus Groups 1, 3 and 4

The results showed statistically significant lower ($P \leq 0.05$) levels of Caspase-3, Ki67, p53, cytochrome p450, and sVEGFR in the skin homogenates of rats in Group 2 when compared with Control Group 1 and Groups 3 and 4, as presented in Table 2.

Concentrations of Caspase-3, Ki67, p53, Cytochrome p450 and sVEGFR in skin of rats: Group 2 versus Group 6

The results showed statistically significant lower ($P \leq 0.05$) levels of Caspase-3, Ki67, Cytochrome p450, and sVEGFR in the skin homogenates of rats in Group 2 than in Group 6, as presented in Table 2. However, the results showed statistically non-significant lower ($P \geq 0.05$) levels of p53 in homogenates of the skin of rats in Group 2 compared with Group 6, as presented in Table 2.

Table 1: Concentrations of Dopamine, Glutamate and MBP in skin of rats.

Drug/Extract →	**Normal Saline only Group 1**	**$CdCl_2$ only Group 2**	**$CdCl_2$-exposure + MO11 post-treated Group 3**	**$CdCl_2$-exposure + MO11 + MS06 post-treated Group 4**	**Olive Oil only Group 5**	**$CdCl_2$-exposure + Doxorubicin post-treated Group 6**
Dopamine (pg/ml)	14.86±0.67	5.97±0.11	11.44±0.21	12.68±0.16	10.95±0.38	10.94±0.27
P – value	*P* < 0.01**		*P* < 0.01**	*P* < 0.01**	*P* < 0.01**	*P* < 0.01**
Glutamate (ng/ml)	41.59±3.03	16.28±1.22	22.81±1.26	33.35±0.28	23.60±0.32	23.55±1.87
P – value	*P* < 0.01**		0.29	*P* < 0.01**	0.19	0.13
Myelin Basic Protein (ng/ml)	4.65±0.02	5.45±0.07	4.47±0.01	3.44±0.34	5.09±0.02	5.05±0.03
P – value	0.05		*P* < 0.01*	*P* < 0.01*	0.47	0.28

P - value at $P \leq 0.05$: Group 2 versus Groups 1 and 3 – 6; ** = significant increase; * = significant decrease.

Table 2: Concentrations of Caspase-3, Ki67, p53, Cytochrome p450 and sVEGFR in skin of rats.

Drug/Extract →	**Normal Saline only Group 1**	**$CdCl_2$ only Group 2**	**$CdCl_2$-exposure + MO11 post-treated Group 3**	**$CdCl_2$-exposure + MO11 + MS06 post-treated Group 4**	**Olive Oil only Group 5**	**$CdCl_2$-exposure + Doxorubicin post-treated Group 6**
Caspase-3 (ng/ml)	169.69 ±1.56	356.89 ±26.88	101.04 ±10.21	45.21±1.99	147.81 ±1.56	293.44±2.19
P – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	*P* < 0.01*
Ki67 (ng/ml)	6.21 ±0.03	14.82±0.47	2.73±0.33	2.05±0.04	4.40±0.19	10.07±0.40
p – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	*P* < 0.01*
p53 (ng/ml)	26.42 ±0.56	37.68±1.04	28.08 ±0.28	23.22±1.25	30.86 ±0.01	34.09±1.56
P – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	0.41
Cytochrome p450 (ng/ml)	121.88 ±1.40	41.65±0.93	96.85 ±1.73	118.28 ±2.20	80.32 ±3.20	78.52±3.00
P – value	*P* < 0.01**		*P* < 0.01**	*P* < 0.01**	*P* < 0.01**	*P* < 0.01**

sVEGFR (ng/ml)	362.92 ±1.25	578.33 ±17.50	302.50 ±1.67	288.89 ±2.22	418.33 ±5.83	498.33±7.50
P – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	*P* < 0.01*

P - value at *P* ≤ 0.05: Group 2 versus Groups 1 and 3 – 6; ** = significant increase; * = significant decrease).

DISCUSSION

The observed skin alopecia (Figure 1b) and histopathological alterations in rats of $CdCl_2$-only treated group 2 (Figure 2b) confirmed $CdCl_2$-induction of skin carcinogenesis, necrosis, and inflammation, as reported (Akinlolu et al., 2022b). These observations are in agreement with those of previous studies (Lansdown and Sampson, 1992; Lansdown et al., 2001), which reported Cd induction of skin alopecia, ulceration, epidermal hyperplasia, inflammatory cell infiltration, and sustained edema in rats and mice. Furthermore, the observations of this study showed amelioration of Cd-induction of skin alopecia and histo-alteration in rats post-treated with MO11 and MS06 (Figures 2c and 2d) as reported (Akinlolu et al., 2022b). In contrast, post-treatment with doxorubicin (Figure 2f) resulted in a lower degree of amelioration of Cd-induced skin histo-alterations in rats, as reported (Akinlolu et al., 2022b).

The findings of this study showed significant downregulations of Dopamine and Glutamate in $CdCl_2$-only treated group 2 when compared with Normal Saline-only Control Group 1, as presented in Table 1. These results confirmed the dysregulation of Dopamine and Glutamate concentrations in the hairless skin area. These observations are in agreement with the previously reported Cd-induced downregulations of dopamine (Gupta et al., 2018) and glutamate (Lafuente et al., 2002) in rats. In addition, Dopamine levels are downregulated in tumors (Lan et al., 2015; Kuol et al., 2018), while dysregulation of glutamate levels aids tumor growth (Yi et al., 2019). Hence, the observed downregulations of Dopamine and Glutamate in rats of Group 2 indicate that these neurotransmitters are involved in neuro-cancer interaction mechanism of $CdCl_2$-induced carcinogenesis.

Post-treatments with MO11, MO11+MS06 and Doxorubicin in Groups 3, 4, and 6 resulted in the upregulations of Dopamine and Glutamate when compared with $CdCl_2$-only treated Group 2 (Table 1), suggesting that MO11, MS06 and Doxorubicin ameliorated $CdCl_2$-induced adverse effects on neurotransmitter-cancer interactions and possess anticancer potential.

In addition, the results of this study showed significant upregulation of MBP levels in the skin of $CdCl_2$-only treated Group 2 when compared with Groups 1, 3, and 4, as presented in Table 1. MBP is a membrane actin-binding protein (Muller et al., 2013) which is upregulated in response to demyelination, axonal degeneration and oxidative stress (Akinlolu et al., 2020a; Afifi and Embaby, 2016). Hence, observed MBP-upregulation suggests possible Cd-induction of de-myelination of nerve fibers supplying the hairless skin area of rats in Group 2.

Post-treatments with MO11 and MO11+MS06 in Groups 3 and 4 resulted in significant downregulation of MBP levels when compared with $CdCl_2$-only treated Group 2, as presented in Table 1. However, post-treatment with doxorubicin resulted in non-significant MBP downregulation in group 6 compared with that in group 2. These observations suggest that MO11, MS06 and Doxorubicin ameliorated $CdCl_2$-induced de-myelination of nerve fibers in the skin of rats, although MO11and MS06 possess better re-myelination potential than doxorubicin.

Furthermore, the results of this study showed significant downregulation of cytochrome p450 levels in the homogenates of hairless skin of $CdCl_2$-only treated Group 2 when compared with normal hairy skin tissues of Groups 1, 3, 4, and 6, as presented

in Table 2. Cytochrome p450 is part of monooxygenases that oxidize fatty acids, steroids, and xenobiotics, thereby enhancing water solubility and expulsion of foreign compounds, and further clearance of drugs/compounds (Rodriguez-Antona et al. 2006; Afifi and Embaby, 2016; Manikandan and Nagini, 2018). Therefore, our observations suggest a possible decrease in clearance and detoxification of Cd content of the skin in rats of Group 2. In addition, these observations suggest that MO11, MS06 and Doxorubicin ameliorated the $CdCl_2$-induced decreased clearance and detoxification of Cd in the skin in the skin of rats of Groups 3, 4 and 6.

It must be noted that there is a significant increase in blood Cd content in humans with severe psoriasis (Liaw et al., 2017). Hence, the observed increase in cytochrome p450 concentrations in $CdCl_2$-exposed rats post-treated with MO11 and MS06 implied that these drug compounds could be considered for further evaluations in the treatment of psoriasis in humans suspected of significant cadmium exposure.

The findings of this study showed significant upregulations of Caspase-3, Ki67, p53, and sVEGFR in hairless skin of $CdCl_2$-only treated Group 2, when compared with Groups 1, 3, and 4 (Table 2). The significant upregulation of Caspase-3 is associated with the activation of apoptosis following induction of necrosis (Akinlolu et al., 2020a), and it is also positively correlated with p53-induction of apoptosis towards resolution of induced necrosis (Akinlolu et al., 2020a). Ki67 is an established biomarker of proliferation, which is significantly upregulated in hyperplasia (Akinlolu et al., 2020a), while VEGF is an established angiogenic factor (Mahoney et al., 2021). Therefore, our observations suggest $CdCl_2$-induction of hyperplasia, necrosis, and angiogenesis in the hairless skin of rats in Group 2.

Post-treatments with MO11 and MO11+MS06 in Groups 3 and 4 resulted in significant downregulations of Caspase-3, Ki67, p53, and sVEGFR levels, respectively, when compared with $CdCl_2$-only treatment in Group 2, as presented in Table 2. In addition, post-treatment with doxorubicin in Group 6 resulted in significant downregulations of Caspase-3, Ki67, and sVEGFR levels, but non-significant p53 downregulation when compared with $CdCl_2$-only treatment in Group 2, as presented in Table 2. These observations suggest that MO11, MS06 and Doxorubicin ameliorated $CdCl_2$-induced skin hyperplasia, necrosis, and angiogenesis and possess anticancer and anti-angiogenic potentials. However, MO11 and MS06 possess better anticancer potentials than doxorubicin.

LC-MS and spectroscopic analyses confirmed the presence of Guanine, Glutamic acid, Leucine and Phenylalanine, and other anticancer compounds in MO11 and MS06 isolates, as reported by Ameen and Akinlolu (2023). Guanine, Glutamic acid, and phenylalanine are established anticancer, antioxidant, and anti-inflammatory compounds (Dutta, Ray, and Nagaran, 2013; Dasari and Tchounwou, 2014; Lee et al. 2017; Lieu et al. 2020; Ziyu et al. 2020). Therefore, the significant and better anticancer potentials of MO11 (Group 3) and MO11+MS06 (Group 4) than doxorubicin (Group 6) following $CdCl_2$-induced skin carcinogenesis (Table 1 and Table 2) could have been due to the presence of Guanine, Glutamic acid, Leucine and Phenylalanine, and other anticancer compounds in MO11 and MS06 isolates.

CONCLUSIONS

Overall, the findings of this study suggest that post-treatments with MO11, MS06 and Doxorubicin conferred cyto-/histo-protection against $CdCl_2$-induced toxicity, carcinogenesis (alopecia, necrosis, angiogenesis and neuro-cancer interaction) and demyelination via the upregulations of

Dopamine, Glutamate and Cytochrome p450, but downregulations of MBP, Ki67, p53, Caspase-3 and sVEGFR in the skin of rats. In addition, the findings of this study showed that MO11 and MS06 achieved better re-myelination, anti-necrosis, and anticancer potentials than doxorubicin. Hence, MO11 and MS06 are recommended for further evaluations as potential drug candidates and dietary supplements for the treatment of skin alopecia and cancers.

DATA AVAILABILITY STATEMENT

The datasets computed and/or analyzed in this study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST*:* Authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Conception and design: AA and MA.
Critical revision of the article for intellectual content: AA, MA, GE, NA, RA and BF.
Final approval of the article: AA, MA, GE, NA, RA and BF.
Obtaining of funding: AA, MA, GE, NA, RA and BF.
Administrative, technical or logistic support: AA, MA, GE, NA, RA and BF.
Collection and assembly of data: AA, MA, GE, NA, RA and BF.

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ORIGINAL ARTICLE

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ALTERATIONS IN HISTOMORPHOLOGY, BIOCHEMICAL PARAMETERS AND GROSS MORPHOMETRY IN LIVER OF ALBINO RATS FOLLOWING ADMINISTRATION OF RIFAMPICIN AND ISONIAZID

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ABSTRACT

Background: Rifampicin (RIF) and Isoniazid (INH) are two main medicinal drugs used as first line regimen in the treatment of Tuberculosis. These drugs have shown to induce hepatotoxicity and alteration of liver parenchyma upon their administration. The aim of this study was to investigate the gross morphometric, histomorphological and biochemical effect of INH and RIF on the liver architecture. **Methods:** Adult albino rats with an average weight of between 150g to 250g were used in the study. A total of 12 rats were randomly assigned into 2 groups each group containing 6 rats. Group one was the control group (no intervention). Group two was the experimental group, given INH (50mg/kgbwt) and RIF (50mg/kgbwt). At the end of the experiment, after 21 days, the albino rats were sacrificed humanely, liver harvested, weighed and the gross parameters measured using a ruler and caliper. Blood samples were taken for liver serum biomarkers analysis. Thereafter, the livers were processed and stained with Haematoxylin and Eosin for histological examination. **Results:** There was a significant (p value < 0.0001) decrease in gross morphometric measurements of the weight, length, width, thickness in experimental group. The three selected liver biochemical parameters (ALT, AST and ALP) were observed to be high above their normal ranges. The liver sections in positive control group showed deranged histomorphological features. **Conclusion**: co-administration of Rifampicin and Isoniazid can have effect on gross morphometric, histomorphology and liver biochemical parameters of the liver therefore, their administration should be done in caution.

Keywords: hepatotoxicity, morphometric, Isoniazid, Rifampicin, histomorphology
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INTRODUCTION

Hepatotoxicity refers to as harm to the liver or disruption of hepatic function induced by exposure to xenobiotic such as alcohol, drugs, chlorinated solvents, food additives, peroxidized fatty acids, fungal toxins, environmental toxicants and radioactive isotopes (Gulati et al., 2018). The liver is the major organ for drug elimination and metabolism (Choijamts et al., 2018). Anti-tubercular drugs such as rifampicin (RIF) and isoniazid (INH) are among the drugs contributing to liver toxicity (Mathers *et al.,* 2015; Zeinab, 2012; Shah *et al.,* 2011)

Rifampicin (RIF) and Isoniazid (INH) are the two main drugs being used to treat Tuberculosis (TB) for 4-6 months (Zeinab, 2012). INH is an anti-mycobacterial drug which has been applied clinically for about 70 years and is still currently being used for TB treatment. It is a bactericide that prevents the mycolic acids formation in the cell wall of bacterial (Tayal et al., 2007). The ability to cause liver damage and even eventual hepatic failure during INH and RIF's management forms a major challenge (Li et al., 2015; Ghosh *et al.,* 2011; Kuwabar *et al.,*

2011). Despite of hepatotoxicity caused by these drugs, they are still first line regimen in the treatment of TB because of their high level of efficacy.

The duration of manifestation of liver toxicity vary between 1-25 weeks with an average of 12 weeks. Elevation of liver biomarkers has been reported to occur as early as first week and as late as 9th month (Arif et al., 2022). The level of liver toxicity is high in cases of other risk factors such: high alcohol consumption, HIV/AIDS (Sankar et al., 2015). Additionally, the liver is prone to several diseases including hepatitis, allergic reactions, hepatic encephalopathy and non-alcoholic fatty liver disease among others (Gulati et al., 2018).

The aim of this study was to investigate the gross morphometric, histomorphological and biochemical effect of INH and RIF on the liver architecture. This will address the gap on the gross morphometric changes on the liver which is lacking in the available published literatures. The findings in the histomorphology and liver biochemical parameters will aid in confirmation of the gross morphometric changes.

MATERIALS AND METHODS

Experimental animals

Adult albino rats were bred in a condition which is microbiologically controlled for all the experimental and control groups. They were obtained from University of Nairobi. The albino rats were bred in cages which hold a maximum of 6 rats per cage. The rats were acclimatized for 1 week in an animal house (26±2°C) with 12h light and dark cycles in the animal house in zoology department, Maseno University. Animal feeds were obtained from Unga Feeds in Kisumu Town. The animals were fed with standard rodent pellets and water provided ad libitum. The albino rats were fed each morning at 0800 hours in their spacious polycarbonate cages. Animal ethical approval was obtained from Institutional Scientific Ethical Review Committee (ISERC) of University of Eastern Africa Baraton. The study research license was obtained from National Commission for Science Technology and Innovation (NACOSTI).

Inclusion criteria

- All healthy albino rats in the cage
- Animals with average weight of 150-250g were included in this study.
- Animals between 6-8 weeks of age

Exclusion criteria:

Sick animals in the cage

Animal ethics

All procedures were performed in line with the Guide for the Care and Use of Laboratory Animals *(Albus, 2012).*

Drugs and chemicals

They were obtained from Yala sub-county Referral Hospital. (Batch No. NRT2103A). Rifampicin and Isoniazid were administered at a dosage of 50mg/kg/day, which was converted to animal equivalent dosage and administered for 21 days. *(Sue et al., 2021; Rana et al., 2006)*

Grouping of the animals and drug administration.

A total of 12 rats were randomly assigned into 2 groups each group containing 6 rats. Group one was the control group (no intervention), was given food and water only. Group two was the experimental group, given INH (50mg/kgbwt) and RIF (50mg/kgbwt) orally. At the end of the experiment, after 21 days, the albino rats were sacrificed humanely, and liver harvested.

Morphometric measurement

After 21 days of experimentation, the animals were sacrificed by use of chloroform in jar, the liver morphometric measurements, which includes the weight, length, width and thickness were taken using a ruler and

caliper. The liver was then stored for histological examinations.

Biochemical analysis

Blood samples were obtained from the posterior venacava. The blood samples were centrifuged at 3000rpm for 10 minutes, sera collected and analyzed using ELISA kits.

Microscopic & Photographic Examination of Histological Section

H&E Staining. The right lobe of each liver (5 mm × 5 mm × 3 mm) was fixed with 4% formaldehyde solution and embedded in paraffin. Next, tissue slices were prepared and stained with hematoxylin (Servicebio, China) for 5 mins, differentiated by exposure to hydrochloric acid alcohol solution for 20 s, and then exposed to a weak ammonia solution (Sinopharm, Ecuador, 100021600) for 20 s. After staining with eosin (Solarbio, Turkey; G1100), the slices were dehydrated and made transparent. Finally, the pathological characteristics of the liver tissues were observed under a microscope (Olympus BP). The microscopic examination of the tissue sections prepared from the samples prepared for the study was carried out using an imaging light microscope and then photographs of the sections were taken using a compound microscope equipped with a Novel type camera.

Statistical analysis

The data was entered into excel sheet and then analysis done through SPPS version 25 (IBM). The results are expressed as a mean value ± standard error of mean (SEM). Student's t test was used to analyze differences between the two groups. Significance levels was P value less than or equal to 0.05 ($p \leq 0.05$) at 95% confidence level.

Ethical approval

The proposal was initially presented and cleared by the School of Medicine, and then the School of Graduate Studies (SGS), Maseno University (SGS=MSC/SM/00023/020). Animal ethical approval was obtained from Institutional Scientific Ethical Review Committee (ISERC) of University of Eastern Africa Baraton (UEAB/ISERC/01/09/2023). The study research license was obtained from National Commission for Science Technology and Innovation (NACOSTI-P/23/23412).

RESULTS

Gross morphometric findings.

The liver gross morphometric measurements entail weight, length, width and thickness. There was no gross anatomical changes observed on the liver between the control and experimental groups. The liver was observed to have four lobes, dark brown in colour with a smooth texture (figure 1).

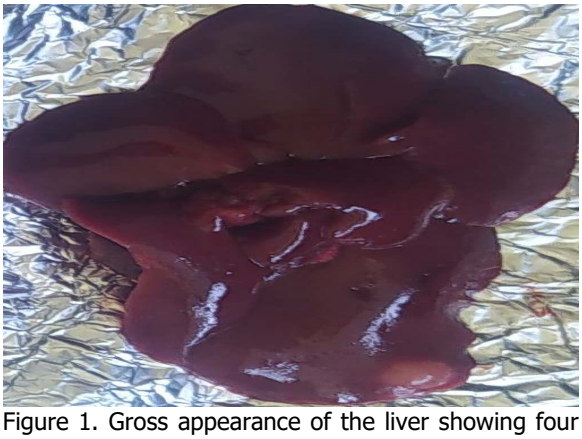


Figure 1. Gross appearance of the liver showing four lobes and dark brown in colour.

Mean liver weight in comparison with mean final body weight between experimental and control groups.

A decrease (184.78±.78) in mean final body weight was observed in experimental group (RIIH 50kg/kgbwt), compared to 245.39±.57 in control group (Food+water).

Table 1. Gross morphometric measurements between control and experimental groups.

Gross parameters	ANIMAL GROUPS		
	control group (Food+water)	Experimental group (RIIH 50mg/ Kg/bwt)	Sig.
MFBW (grams)	245.39±.57	184.78±.78**	0.0001
MLW (grams)	11.86±.20	9.197±.26**	0.0001
Length (cm)	57.00±.53	44.90±.40**	0.0001
Width (cm)	45.20±.33	36.31±.19**	0.0001
Thickness (cm)	0.53±.02	0.35±.01**	0.0001
Lobes	6	6	

All values are expressed and presented as the mean± the standard error of the mean (SEM); n=6. Data analyzed by dependent t-test. Asterisks** represents significant (p <0.0001), RIIH-Rifampicin and Isoniazid Induced Hepatotoxicity, MFBW=Mean final body weight, MLW= Mean liver weight.

Comparative mean liver length, width and thickness between positive and negative control

A decrease in the mean of liver length, width and thickness was observed in experimental group compared to control group (Food+water). The length of 44.90±.40, width of 36.31±.19 and thickness of 0.53±.02 in experimental (RIIH 50kg/kgbwt) group while length of 57.00±.53, width of 45.20±.33 and thickness of 0.35±.01 in control group. There was statistically significant difference (p≤0.0001) in experimental group as shown in Table 1.

Liver biochemical parameters changes.

This current study selected three (ALT, AST and ALP) liver biochemical indicators to assess the hepatotoxic effect of Isoniazid and Rifampicin.

All the three biochemical parameters; ALP, AST and ALT in control group were within the normal ranges with their means at 24.17±1.1, 60.43±3.4 and 74.67±5.7 respectively. In the experimental (RIIH 50kg/kgbwt) group, it was observed increase in ALT (100.4±4.7), AST (116.7±8.6) and ALP (131.1±6.3) above the normal ranges. There was statistically significant difference (p≤0.0001*) in experimental group as shown in Table 2.

Similarly, there was a decrease (9.197±.26) in mean liver weight in experimental group as compared to negative control (11.86±.20 respectively). A statistically significant difference (p≤0.0001) between experimental groups as seen in Table 1.

Liver Biochemical Parameters	control group (Food+water)	experimental group (RIIH 50mg/Kg/bwt)	sig.
ALT (μL)	24.17±1.1	100.4±4.7	0.0001**
AST (μL)	60.43±3.4	116.7±8.6	0.0001**
ALP (μL)	74.67±5.7	131.1±6.3	0.0001**

All values are expressed and presented as the mean± the standard error of the mean (SEM); n=6. Data analyzed by dependent t-test. Asterisks** represents significant (p <0.0001), RIIH-Rifampicin and Isoniazid Induced Hepatotoxicity, MFBW=Mean final body weight, MLW= Mean liver weight. AST-Aspartate Transaminase, ALT- Alanine Transaminase, ALP-Alkaline Phosphatase.

Histomorphological findings.

A normal liver histomorphological features was observed in the control group (food and water ad-libitum), where the central vein, hepatocytes, hepatic triad and capillary sinusoid appeared normal as shown in Figure 2. This was different from the experimental (RIIH 50kg/kgbwt) group that showed deranged histomorphological features, where areas of necrosis, dilatation of sinusoidal capillaries, disrupted central vein and inflamed Kupffer cells was observed as shown in figure 3.

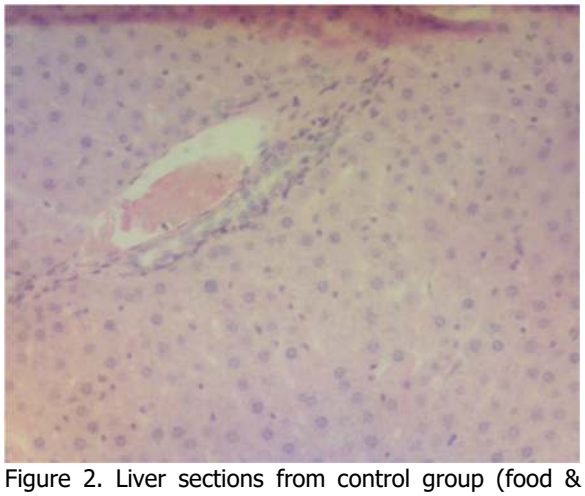


Figure 2. Liver sections from control group (food & water), showing normal histological findings; normal central vein, hepatocytes, sinusoid and Kupffer cells (X100, H&E)

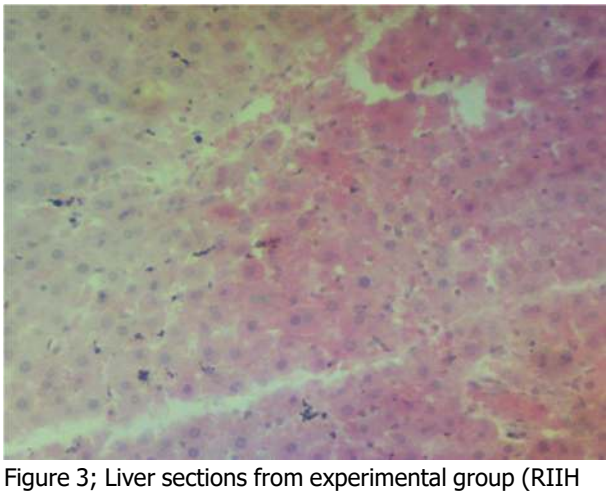


Figure 3; Liver sections from experimental group (RIIH 50mg/kgbwt) showing disrupted central vein, dilated sinusoid and necrosis**.** (X100, H&E)

DISCUSSION

Gross morphometric changes

Gross morphometric measurements of the liver include weight, length, width and thickness. The measurements are vital in determining the gross changes on the liver between control and experimental groups (Buabeid et al., 2022).

In this current study, the liver gross appearance was dark brown in colour with a smooth texture after administration of rifampicin and isoniazid. There were no obvious gross anatomical changes observed between control and experimental group. These findings are in line with (Humayun et al., 2017) who also observed similar features.

However, Shabbir *et al.,* (2020) observed a significant (p value < 0.0001) decrease in liver weight and mean terminal body weight have been recorded when antitubercular drugs were administered among albino rats (Shabbir et al., 2020). The observation by Shabbir *et al.,* (2020) was consistent with the present study findings which recorded a decrease (184.78±.78) in mean final body weight in experimental (RIIH 50kg/kgbwt) group, compared to 245.39±.57 in control (food and water ad-libitum) group. The mean liver weight was recorded at (9.197±.26) in experimental (RIIH 50kg/kgbwt) group and 11.86±.20 in control (food and water ad-libitum) group. These findings are also similar to Nawar *et al.,* (2006) and Oloo *et al.,* 2021.

The current study also recorded significant (p value < 0.0001) decrease in the length, width and thickness of the liver. Other studies also observed significant ($p \leq 0.05$) decrease in the gross morphometric measurements, after administration of paracetamol in albino rats. (Rono K Walter, 2020; Mahmood et al., 2014 and Girish *et al.,* 2009). These derangements in the length, width, thickness of the liver and terminal body weight could be attributed to hepatotoxic injury to liver parenchyma induced by rifampicin and isoniazid.

Contrary to this, a study by Jaber et al., (2018) on morphometric effect of Tegretol on liver of albino mice shows no significant ($p>0.05$) difference between control and experimental group (Jaber *et al.,* 2018). This difference in findings could be attributed to the duration of the experiment which was done for thirty days.

Changes in liver bio-chemical parameters (ALT, AST and ALP)

The three (ALT, AST and ALP) selected liver biochemical parameters were determined to assess the effect of Rifampicin and Isoniazid on liver in adult albino rats. In cases where

liver is damaged, these enzymes are usually released into the blood stream (Buabeid et al., 2022). In the present study, all the three biochemical parameters; ALP, AST and ALT in control (water and food ad-libitum) group were within the normal ranges with their means at 24.17±1.1, 60.43±3.4 and 74.67±5.7 respectively.

Contrary to this, the three parameters showed significant (p value < 0.0001) increase above their normal ranges in experimental (RIIH 50kg/kgbwt) group compared to control group with their means at 100.4±4.7, 116.7±8.6 and 131.1±6.3 respectively. Other studies observed similar findings when Isoniazid and Rifampicin were administered in mice (Xi *et al.,* 2011; Parameswari *et al.,* 2012; Sankar *et al.,* 2015; Oloo *et al* 2021; Usmani *et al.,* 2016;). The increase in enzyme activities might be attributed to rise in liver functionality induced by the drugs biochemical parameters which have toxic properties that induce injury to liver parenchyma.

Histomorphological findings

Histomorphological features of the liver include; central vein, capillary sinusoids, portal triad, lobules and Kupffer cells. The features are important in assessing injury to the liver histo-architecture (Buabeid et al., 2022).

The present study observed normal liver histomorphological features in control group (food and water ad-libitum). This observation was different from the experimental group (isoniazid and rifampicin) that showed deranged features where areas of necrosis, dilatation of sinusoidal capillaries, disrupted central vein and inflamed Kupffer cells was observed. These observations are in harmony with other studies that recorded deranged histological features on liver when they introduced Isoniazid and Rifampicin among albino rats (Salah & Abdoul, 2021; Dubiwak *et al.,* 2021; Zodape & Bhise, 2018). These findings are attributed to necrosis caused by rifampicin and isoniazid which induces oxidative stress leading to injury to hepatocytes.

Contradicting findings were observed when 40mg/kg of paracetamol was administered orally for twenty-eight days. There were no derangement in histomorphology of the liver sections. These changes were attributed to low dose of paracetamol administered. (Oloo *et al.,* 2020).

CONCLUSION

In conclusion, the results obtained indicate that co-administration of Isoniazid and Rifampicin may induce alteration in gross morphometric measurements, histomorphology and biochemical parameters on the liver. This is due to hepatotoxic properties of the two drugs which causes injury to the liver parenchyma.

RECOMMENDATION

The study recommends that co-administration of Isoniazid and Rifampicin over a long period of time should be done cautiously, with serial monitoring of the stated liver biochemical parameters so as to assess for development of hepatotoxicity. Furthermore, studies should be done on adjuvants or polyherbal formulations to be done in human patients which would be administered concurrently with Isoniazid and Rifampicin to prevent injury to the liver.

CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this article.

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ORIGINAL ARTICLE

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RENAL GROSS MORPHOLOGICAL RESTORATIVE EFFECTS OF *CURCUMA LONGA* ON SILDENAFIL INDUCED NEPHROTOXICITY AMONG MALE ALBINO RATS.

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ABSTRACT

Background: Sildenafil is a phosphodiesterase inhibitor used in the management of erectile dysfunction and management of pulmonary hypertension. *Curcuma longa* is a traditional herbal plant that is commonly used as a diet in Africa and Asian countries. It has a variety of benefits including; antioxidant, anti-cancerous, management of diabetes and respiratory diseases. The objective of this study was to evaluate the renal gross morphological changes in restorative effects of *Curcuma longa* on sildenafil induced nephrotoxicity among male albino rats. **Methodology:** A total of 25 male albino rats were used and classified control or experimental group. Simple random sampling method was used to allocate them into each group. Animals in group1 were only fed on feeds and water ad libitum, group 2 to 5 received Sildenafil 1mg/gm bwt for 15 days. Animals in group 3,4 and 5 were further subjected to *Curcuma longa* at calculated dose of low, medium and high respectively. Animals in group 2 were sacrificed 4 hours post last dose and the remaining animals were sacrificed 7 days later. On sacrificing gross morphometrics were done. **Results:** It was observed that mean weight of rat, weight and volume of kidney increased significantly (p=0.0001) in medium and high dose *Curcuma longa* as compared to Sildenafil induced nephrotoxicity group. The mean length and thickness in medium and high dose *Curcuma longa* increased significantly (p=0.0001) as compared to Sildenafil induced nephrotoxicity group. **Conclusion:** Medium and high dose *Curcuma longa* have renal gross morphological effects on sildenafil induced nephrotoxicity among male albino rats.

Keywords: Antioxidant, Anti-cancerous, Diabetes, Kidney and pulmonary hypertension.

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INTRODUCTION

The economic burden and care of patients with acute kidney injury and chronic renal injury cannot be overlooked. Nephrotoxicity still remains the leading cause of acute kidney injury accounting for 18%-27% of most case hospitalized. The cost particularly may increase especially if the patient might need renal transplant or dialysis. Most of the population affected are from African countries due to increased industrialization, poor hygiene, sedentary lifestyle and poor access to health care systems. Sildenafil is a phosphodiesterase inhibitor majorly used in management of erectile dysfunction and pulmonary hypertension(Hsu et al., 2015; Nichols et al., 2002). Overtime there is increased report of cases of individuals developing acute kidney injury secondary to overdose or prolonged use. A case of 39-year-old man who developed signs and symptoms of AKI secondary to a two-day consecutive intake of 100mg Sildenafil was admitted with deranged renal biochemical markers of blood urea and nitrogen levels and creatinine levels(Liu et al., 2018). On the other hand, *Curcuma longa* is a traditional herbal plant with wide range of benefits; antioxidant, anti-cancerous, used in management of dermatological conditions, respiratory conditions, and renal diseases(Witkin & Li, 2013). Sildenafil has been postulated to cause nephrotoxicity by

releasing radical elements like oxygen, nitrogen oxide and enzymatic activities due to presence of hemeoxygenase enzymes. These radicals destroy the glomerulus structure, increase bowman`s space, dilate kidney tubules and cause epithelial cell destruction and necrosis(Suriyakumari et al., 2016). It also causes damage to cellular permeability and vacuolations. *Curcuma longa* on the other hand might have antioxidant and anti-inflammatory benefits. It has widely been used in renal attenuation and amelioration however, there is paucity of data on the restoration capacities of *Curcuma longa*. Therefore, this study sought to evaluate the renal gross morphological changes in restorative effects of *Curcuma longa* on sildenafil induced nephrotoxicity among male albino rats.

MATERIALS AND METHODS

The present study used twenty-five male albino rats(150-250gm) of species *Rattus Norvegicus* which was calculated using modified human resource formula (Arifin & Zahiruddin, 2017). The animals were selected using systematic random sampling method having attained desired weight at commencement of study. Here every second rat was picked from a pool of 100 which the animal house could hold for a single study. The animals were put in polycarbonate cages for a period of ten days to allow acclimatization take place. The standard experimental conditions like humidity, temperature and light/dark cycle of 12 hours were maintained. Feeds and water were given under strict hygiene conditions with researcher upholding occupational animal handling procedures.

These animals were grouped as either control or experimental. The experimental group was further grouped into four subgroups as Sildenafil induced nephrotoxicity group, low dose *Curcuma longa* group, medium dose *Curcuma longa* group and high dose *Curcuma longa* group. Each group had five animals that were simple randomly selected. The four subgroups received a single dose of 1µgm/gm bwt/day Sildenafil for 15 days through gastric gavage (Suriyakumari et al., 2016). This was adopted because sildenafil has shown to be nephrotoxic when used as high dose or used for a prolonged period of time. The five animals in group2 were sacrificed four hours post last dose of sildenafil and renal function test was done to confirm toxicity while group 3, 4 and 5 were subjected to *Curcuma longa* at different doses for further studies at 38.75mg/Kg/day low dose *Curcuma longa*, 77.5mg/Kg/day medium dose *Curcuma longa* and 155mg/kg/day high dose *Curcuma longa* (França-Berthon et al., 2021; Nair & Jacob, 2016).

The renal biochemical parameters for low, medium and high dose *Curcuma longa* were determined at the end of experiment and results were analyzed using one way ANOVA and post hoc Bonferroni was adopted to determine inter group significance in assessment of restorative effects. Chloroform was used as anesthesia after which a vertical incision was made from pubic symphysis to xiphoid process so as to expose the viscera and collect the kidney samples. The kidneys were identified and excised. The fibrous capsule and adipose tissue were removed before weighing was done.

Gross morphological studies were then carried out whereby volume of the organs was determined using Archimedes principle, weight by use of electronic weighing machine whereas length, width and thickness were determined by use of a digital Vanier caliper. The following dimensions of right and left kidney were taken; Width: Measured from the medial to lateral border. Length: Measured from the upper to lower pole. Thickness: Measured from the visceral to parietal surface. The ethical approval to

carry out the study was sought from Baraton University of Eastern Africa (UEAB/ISERC/08/01/2023) and NACOSTI (NACOSTI/P/2023/23374).

RESULTS

A total of 25 adult male albino rats of between the weights of 150g and 250g were randomly selected for study. Each rat was allocated to the 5 groups as either pretreatment group or treatment group. Every group received a total of 5 rats (20%). They were treated with Sildenafil 1µg/gmbwt/day for 15 days to induce nephrotoxicity then *Curcuma longa* was introduced at varying doses for 7 days to try and restore the damage caused. The following results were observed from the study.

Behavioral changes and mortality report.

Rats in all the 5 selected groups did not show any behavior changes during routine inspection. There were no observable signs of nephrotoxicity during and after administration of drugs. No mortality was recorded during the entire process of the experiment.

Body weight

The average body weight of the 25 rats used at the commencement of the study was 183.75g with a standard error of ±3.14g.

Mean terminal body weight, mean weight of right and left kidney and mean volume right and left kidney between positive control group and experimental groups

The mean terminal body weight group four (MCL77.5mg/gm/bwt/day) and five (HCL155mg/gm/bwt/day) was statistically significantly different (P=0.0001) and (P=0.0001) respectively as compared to the positive control (Sildenafil 1µg/gmbwt/day) group. The mean weight of right kidney in group four and five was statistically significantly different (P=0.0001, P=0.0001) as compared to the positive control (Sildenafil 1µg/gmbwt/day) group whereas the mean weight of left kidney in group four and five was statistically significantly different (P=0.0001, P=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group. The mean volume of right kidney in the group four and five was statistically significantly different (P=0.0001, P=0.0001) as compared to the positive control (Sildenafil 1µg/gmbwt/day) group. The mean volume of left kidney in the group four and five was statistically significantly different (P=0.0001, P=0.0001) as compared to the positive control (Sildenafil 1µg/gmbwt/day) group respectively (Table 1).

Table 1: The mean terminal body weight, mean kidney weight and volume between positive control group and experimental groups.

Mean body weight, kidney weight and volume in gms and mls					
Groups	**Mean terminal body weight**	**Mean weight right kidney**	**Mean weight left kidney**	**Mean volume right kidney**	**Mean volume left kidney**
SIN 1µg/gmbwt/day	254.74±.81	0.94±.02	0.82±.04	1.38 ±.04	1.14 ±.02
Low *Curcuma longa* dose (38.75mg/gm/bwt/day)	270.92±2.23	0.98±.06	0.90±.03	1.39 ±.11	1.24 ±1.59
Medium *Curcuma longa* dose (77.5mg/gm/bwt/day)	285.22±1.76	1.02±.02	0.94±.02	1.66±06	1.58 ±1.61
High *Curcuma longa* dose (155mg/gm/bwt/day)	288.98±2.2	1.18±.11	0.96±.02	1.86±.11	1.82 ±.08

All values are expressed as the mean± the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5), SIN- sildenafil induced nephrotoxicity, LCL-low Curcuma longa dose, MCL- medium Curcuma longa dose and HCL-high Curcuma longa dose.

Mean terminal body weight, mean weight of right and left kidney and mean volume right and left kidney between positive group and restorative group

The mean terminal body weight of rats in restorative group (different doses of *Curcuma longa*) was statistically significantly different (*P*=0.0001) when compared to positive control (Sildenafil 1µg/gmbwt/day) group. The mean weight of right and left kidney in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to positive control (Sildenafil 1µg/gmbwt/day) group. The mean volume of right and left kidney in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to positive control (Sildenafil Iµg/gmbwt/day) group respectively (Table 2).

Table 2: The mean terminal body weight, mean kidney weight and volume between positive control group and restorative group.

Kidney gross morphometries in mm.						
Groups	**Mean right kidney length**	**Mean left kidney length**	**Mean right kidney width**	**Mean left kidney width**	**Mean right kidney thickness**	**Mean left kidney thickness**
SIN 1µg/gmbwt/day (5 rats)	14.16±.36	11.97±.58	10.27±.16	9.66±.22	3.03±.07	2.91±.02
Restorative groups (15 rats)	17.37±.17	16.95±.16	10.46±.26	10.14±.14	4.81±.17	4.38±.11
P value	**0.0001**	**0.0001**	1.000	0.264	**0.0001**	**0.0001**

All values are expressed as the mean± the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5), SIN- sildenafil induced nephrotoxicity.

Comparative mean of total kidney weight, percentage ratios kidney weights to mean body weight in controls against experimental groups.

It was observed that the mean weight percentage ratio of right kidney in positive control group (sildenafil 1µg/gmbwt/day) reduced 0.37% as compared to the negative control group (feeds + water) 0.41% whereas the mean weight percentage ratio of right kidney in high Curcuma longa dose group increased (0.41%) as compared to positive control group (sildenafil 1µg/gmbwt/day) (Table 3).

Table 3: A table indicating the total kidney weight and percentage ratios kidney weights to mean body weight in control against experimental groups.

	Gross measurements				
Groups	**Weight of rats**	**Weight of right kidney**	**Weight of left kidney**	**Mean % right kidney weight ratio**	**Mean % left kidney weight ratio**
Control (feeds+ water ab libitum)	284.78±.32	1.16±.08	0.92±.02	0.41%	0.32%
SIN 1µg/gmbwt/day	254.74±.81	0.94±.02	0.82±.04	0.37%	0.32%
Low *curcuma longa* 38.75mg/kg/day	270.92±2.23	0.98±.06	0.90±.03	0.36%	0.33%
Medium *curcuma longa* 77.5mg/kg/day	285.22±1.76	1.02±.02	0.94±.02	0.36%	0.33%
High *curcuma longa* 155mg/kg/day	288.98±2.2	1.18±.11	0.96±.02	0.41%	0.33%

KEY: All values are expressed as the mean, ± is the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5), SIN- sildenafil induced nephrotoxicity, LCL-Low curcuma Longa, MCL-Medium Curcuma Longa, HCL-High Curcuma Longa

Comparative mean width and thickness between positive control and experimental groups.

The mean length of right kidney in group 4 and 5 was statistically significant (P=0.0001 and P=0.0001) as compared to positive control group (Sildenafil1µg/gmbwt/day) whereas the left kidney of group 4 and 5 was statistically significant (P=0.0001 and P=0.0001) respectively as compared to positive control group (Sildenafil1µg/gmbwt/day) respectively. The mean thickness of right kidney in group 4 and 5 was statistically significant (P=0.0001 and P=0.0001) respectively when it was compared to positive control group (Sildenafil1µg/gmbwt/day) while the thickness of left kidney was statistically significant (P=0.0001 and P=0.0001) when in group 4 and 5 as compared to positive control group (Sildenafil1µg/gmbwt/day) respectively (Table 4).

Table 4: A table indicating mean length, width and thickness between positive controls and experimental groups.

	Kidney gross morphometries in mm.					
Groups	**Mean right kidney length**	**Mean left kidney length**	**Mean right kidney width**	**Mean left kidney width**	**Mean right kidney thickness**	**Mean left kidney thickness**
SIN 1microgram/gmbwt/day (5 rats)	14.16±.36	11.97±.58	10.27±.16	9.66±.22	3.03±.07	2.91±.02
Low curcuma longa dose (38.75mg/gm/bwt/day)	17.15±.34	16.64±.30	10.18±.70	9.99±.18	4.76±.44	4.18±.11
Medium curcuma longa dose (77.5mg/gm/bwt/day)	17.25±.20	16.75±.15	10.48±.36	10.11±.32	4.78±.23	4.36±.19
High curcuma longa dose (155mg/gm/bwt/day)	17.69±.30	17.46±.23	10.74±.24	10.31±.27	4.90±.27	4.59±.24

KEY: All values are expressed as the mean, ± is the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5), SIN- sildenafil induced nephrotoxicity, LCL-Low curcuma Longa, MCL-Medium Curcuma Longa, HCL-High Curcuma Longa

The mean length, width and thickness between positive control and restorative group.

The mean length of right and left kidney in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group. The mean thickness of right and left kidney in restorative group was statistically significantly different (R; *P*=0.0001, L; *P*=0.0001) as compared to the positive control (sildenafil 1µg/gmbwt/day) group respectively (Table 5).

Table 5: The mean length, width and thickness between positive controls and restorative group.

Kidney gross morphometries in mm.						
Groups	**Mean right kidney length**	**Mean left kidney length**	**Mean right kidney width**	**Mean left kidney width**	**Mean right kidney thickness**	**Mean left kidney thickness**
SIN 1µg/gmbwt/day (5 rats)	14.16±.36	11.97±.58	10.27±.16	9.66±.22	3.03±.07	2.91±.02
Restorative groups (15 rats)	17.37±.17	16.95±.16	10.46±.26	10.14±.14	4.81±.17	4.38±.11
P value	**0.0001**	**0.0001**	1.000	0.264	**0.0001**	**0.0001**

KEY: All values are expressed as the mean± the standard error of the mean (SEM). The test of significance was performed in rows. Values are expressed as mean ± standard error of mean (n=5), SIN- sildenafil induced nephrotoxicity

DISCUSSION

In this study the control groups were grouped into negative control (feeds+ water ad libitum) and positive control (SIN 1 microgram/gmbwt sildenafil). During gross anatomical observation after dissection, the two kidneys lay on each side of upper lumbar vertebrae within abdominal cavity, this concurred with the normal anatomical relation of Kidneys as observed by other authors (Olukole, 2021) . The superior anterior part of right kidney related to liver while left was associated with pancreas, stomach, spleen, small intestines and descending colon. These findings were similar to those recorded by (Al-Samawy, 2012) in albino rats.

On examination of borders, the lateral border was convex in shape while the medial border was concave and indented at hilus. On examination of the kidneys of rats that were subjected to sildenafil they appeared darker brown in color, shrinked and pale. The obvious shrinkage may have been due to the increased oxidative stress imposed by free reactive and nitrogen oxide radicals released by sildenafil as was observed by (Kirbas et al., 2015) who recorded same in the literature on paracetamol effect on kidney and significant reduction in growth of collagen fibers in kidneys as an effect of sildenafil administration and (Hegazy et al., 2021) on effects of paracetamol on kidney since the two drugs exhibit a similar nephrotoxicity model.

The current study noted a significant (P=0.0001) reduction in weight of the rats on treatment with sildenafil as compared to control group, in addition, the mean terminal or live weight of control group was 284.78±.32gm, this was slightly higher as compared with previous studies carried out in Nigeria (Onyeanusi et al., 2009) of 140.625±3.07gm and similar to(Olukole, 2021) as seen in domesticated African great cane rat. The high weight observed in control group might have been due to the long duration of study and no stress factors that

could interfere with eating habits and weight gain.

The mean weight of rats in SIN group was 254.74±.81gm in the present study, this was also higher as compared to a study done by(Sivasankaran et al., 2007) who noted a mean weight of 183.16±3.76gm after administration of sildenafil**.** Similar trend in reduction of weight of rats when exposed to sildenafil were also noted by(Mohamed Yousry et al., 2016; Ngulde et al., 2016). The reduction in body weight might suggest that nephrotoxicity process was taking place as a result of administration of sildenafil. Nephrotoxicity leads to accumulation of toxins in the blood associated with reduced metabolism and loss of appetite, the decrease in production of erythropoietin might also lead to anaemia thus causing fatigue, loss of appetite and finally weight loss. The weight of right kidney was higher than left kidney representing 0.41% and 0.32% respectively which was similar to wistar rats and domesticated African great cane rats in the savanna zones of Nigeria (Onyeanusi et al., 2009)**.** However, this was lower than 0.76% results obtained by(Hebel & Stromberg, 1976). This difference could be due to the variations in breed, environmental factors and age.

It was observed that the mean weight of experimental groups increased steadily as compared to sildenafil induced nephrotoxicity group. This increase in weight might have been due to Curcumin which is an active component in *Curcuma longa* that serves as a supplementary diet thus improves growth and weight gain rate. Previous studies in Turkey showed similar trends in weight increase whereby rats that were subjected to Curcumin gained more weight as compared to those that were exposed to aflatoxin(Hatipoglu & Keskin, 2022) . The mean weight of right and left kidney significantly(*P*=0.0001) increased on high *Curcuma longa* dose as compared to positive control group. The noted increased kidney weight might have been due to reduced destruction of renal collagen fibers, reduced inflammatory process and increased kidney histoarchitecture restructuring due to remodeling nature of *Curcuma longa* in nephrotoxicity**.**

The mean volume, length and thickness of kidney in experimental groups increased significantly(*P*=0.0001) as compared to positive control group. The previous studies observed that these changes might have been due to antioxidant and anti-inflammatory effects of *Curcuma longa* as it helps in improving or protecting the kidney(Akinyemi & Adeniyi, 2018). The increase in volume might have also been due to reduced destruction of epithelial cells by nitric oxide, vasodilation of glomerulus and proximal convoluted tubules as a result of reduced infiltration and increased glomerular filtration rate.

CONCLUSION

It can be concluded that medium and high dose *Curcuma longa* has kidney gross morphological restorative benefits in Sildenafil induced nephrotoxicity among male albino rats. Therefore, *Curcuma longa* can be used to mitigate the nephrotoxic effects of Sildenafil in the future or be used as a component in drugs that can be useful in management of Sildenafil induced nephrotoxicity.

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ORIGINAL ARTICLE

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VARIATIONS IN THE ORIGIN OF THE CORONARY ARTERY IN BLACK AFRICAN POPULATION: A CADAVERIC STUDY IN WESTERN KENYA

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ABSTRACT

Background: The coronary arteries which originate from the right and left aortic orifices are the main blood vessels to the myocardium. Variations are anomalies which could affect the overall perfusion of the myocardium causing conditions like ischemic heart diseases leading to sudden unpredicted death. Variants can also cause confusion to cardiologists and heart surgeons leading to challenges in patient management. This study sought to determine these variations among black African population in Western Kenya by assessing the variations in origin of the coronary artery. **Methods:** A cross-sectional study design was used in the Maseno University, Uzima University and Masinde Muliro University Human anatomy laboratories. Out of a total of 89 cadavers,72 samples were identified using Cochran's formula. Data sheets were used to capture the variables while descriptive statistics was used to calculate the frequency and percentage of the variation in the origin of the coronary arteries while Pearson's correlation test was used to analyze the relationship between the variables and variations $P=\leq 0.05$ considered as significant. All ethical approval was issued via license number: NACOSTI /P/22/21905.**Results:** Dual aortic origin was the most common occurrence at 55.6% while 44.4% were variations with more than one orifice on the aortic cusps. **Conclusions**: There were variations which could be fatal during surgical procedures, but also important alternate pathway for the perfusion of the myocardium. There is need for early routine imaging of the heart for effective management and to avoid mortalities and morbidity associated with the variations.

Keywords: Origin, Cadaver, Coronary Artery, Variation.

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INTRODUCTION

The coronary arteries are the first blood vessels from the ascending aorta that supplies the muscles of the heart with oxygen and nutrients. The principal branches of coronary artery are the right coronary artery (RCA) and the left coronary artery (LCA) (Rehman *et al.,* 2011). Variations in the origin of coronary arteries are thought to be responsible for the changes in the coronary blood vessels. This changes could either be compensatory, collateral or a duplication of another vessel which could have positive or negative attributes depending on their location, distribution and function (Olabu *et al.,* 2007).

Joshi *et al.,* (2010) notes that the most common causes of coronary artery variations is the anomalous origin which is important in the clinical presentation like sudden death. These variants are the second commonest cause of sudden unpredicted death worldwide especially in the young professional athletes due to the changes they cause in the coronary blood vessels. The American heart association highlights that approximately 19% of fatal heart conditions are caused by coronary artery variations due to the differences in coronary blood flow which affects the perfusion of the myocardium (Angelini *et al.,* 2002; Gać *et al.,* 2022).

Procedures like cardiac catheterization, coronary bypass procedures done by cardiologist heart surgeons require uttermost keenness and accuracy to avoid fatal mistakes. The difference caused by these variants can have a severe effect on the perfusion of the myocardium complicating the management of heart diseases (Olabu *et al.,*2007).

In Africa there is little literature on variations in the origin of coronary artery. However, its effects can be highly felt by the increase in cardiovascular mortality and morbidity with increased hospitalization and revascularization procedures (Khan *et al.,* 2020). A study by Olabu *et al.,* (2007) is among the few studies done in Africa and specifically in Kenya on these variations. The study asserts the prevalence of the third coronary artery and its role in the acceleration and extent of myocardial infarction (MI) in cardiac surgery but also notes its significance in the formation of collateral circulation and anastomosis.

Ogeng'o *et al.,* (2013) studied variation in luminal diameter of the left anterior descending artery a branch of the left coronary artery and its significance as a cause of stenosis among different ethnic communities. He noted that variations were important markers in the development of atherosclerosis. Similarly, variations in the origin of the coronary arteries are important in relation to the overall perfusion of the myocardium, cardiac surgeries and coronary bypass procedures to enhance awareness and improved management to the patients among the healthcare providers. Asiki *et al.,* (2018) in the Kenya national guidelines for cardiovascular diseases notes that 25% of Kenyan population admission and 13% of deaths are due to cardiovascular diseases. There has been a steady increase in the prevalence of ischemic heart diseases in Western Kenya caused by non-communicable diseases like diabetes in the population (Chege, 2016). The aim of this study was to assess and report the variations in the origin of the coronary artery among black African population. This finding will play an important role to health providers in everting possible injuries due to mistakes.

MATERIALS AND METHODS

This was a cross sectional study in which convenient sampling was used to select cadavers from three Anatomy laboratories in Western Kenya. The study adopted mixed methods to select the desired sample size. The study area was stratified into the existing 3 Universities (Maseno, Masinde Muliro and Uzima universities) according to the number of cadavers available in each laboratory. The cadavers were grouped into gender (male or female) after which the samples for each laboratory were randomly selected.

To ensure equal distribution of samples, sampling was done proportionately with the strata population by location, where the sample size per laboratory was calculated by dividing laboratory cadaver (*d*) by total location population (n), multiplied by the desired sample size (m); $n = (d/N)\mathrm{m}$. As a result, 72 cadavers were acquired as the total actual sample size using the Cochran's formula (Cochran 1967) Ahmad & Halim (2017) from a total available specimen of 89 cadavers (Table 1). The cadavers were dissected to expose the thoracic cavity.

With the help of trained laboratory technicians, the visceral pericardium was removed and the coronary artery was observed and reflected to visualize the coronary artery and its variation. The observations were recorded in a data sheet and images were taken where variations were observed. The collected data was subjected to statistical tool and descriptive statistics was used to record the incidence of variations from normal while Pearson's correlation test was used to analyze the

relationship between the variations at a $P=\leq 0.05$ considered as significant. The collected data was stored and processed in confidentially and was only be used for the current research. Approval was obtained from School of Graduate Studies Scientific review committee. Ethical review was sought from Maseno University Ethics and Review Committee (MUERC) and National commission for Science Technology and Innovation (NACOSTI) via license number: NACOSTI /P/22/21905.

RESULTS

Of the total 72 specimens, 40 hearts 55.6% had dual aortic origin, one on the right and the other on the left for the coronary arteries while 24 hearts 33.3% had an orifice in the left and 2 orifices in the right (Figure 2). Five hearts (6.9%) had an orifice in the left and 3 in the right (Table 2). Among the 40.2% (29) cases which had an orifice in the left and 2-3 in the right, 16.6% (12) of the orifices were associated to the right conus artery with 9 originating from those with an orifice in the left and 2 in the right with the remaining 3 originating from an orifice in the left and 3 in the right. The sinoatrial nodal artery had 1 of its orifice originating from the right aortic cusps with 2 openings and the other with 3 openings. The remaining 4.2% (3) hearts had orifices all the orifices on the left aortic cusps and none on the right. As a result, 55.6% of the origin were normal while the remaining 44.4% were variations.

There was no statistical significance in the variation in origin of the coronary artery at (p=0.463) (Table 3).

Table 1: Distribution of cadavers from the different universities

Laboratory location	Population in laboratory(d)	Selected sample (n)
Maseno University	45	37
Uzima University	24	19
Masinde Muliro University	20	16
TOTAL	89(N)	72(m)

Table 2: Variation in the origin of the coronary artery.

Origin of the coronary artery	Frequency	Percentage
Dual aortic origin	40	55.6
Orifice in the left and 2 Orifice in the right	24	33.3
Orifice in the left and 3 Orifice in the right	5	6.9
Orifices in the left aortic cusps	3	4.2
Total	72	100

Table 3: Test for significance in variation of origin of coronary artery

Chi-Square Tests		
	Value	df
Pearson Chi-Square	4.630a	5
Likelihood Ratio	4.511	5
Linear-by-Linear Association	0.162	1
N of Valid Cases	150	

DISCUSSION

In the current study, 55.6% (40) hearts had normal dual aortic origin while the remaining 44.4% (32) had variations. Among the variations, 40.2% (29) had an orifice in the left and 2-3 orifices in the right, while 3 (4.2%) had orifices in the left and none in the right (Table 2). Normally, the right and left coronary arteries originate from the right and left aortic cusps (Figure 1). This enables the principal arteries to form branches

effectively that will efficiently perfuse the myocardium at all angles (Bhele *et al.,* 2017). A study done by Khwansang & Chentanez (2018) on 95 hearts (48 males and 47 female) in Thailand found a lower number in variation at 31% which were on the left and 2-3 orifices in the right aortic cusps. The lower number of variations from the previous study may have been due omission of origin of the conus artery from the 2-3 orifices as part of the variation. The current study considered any other orifice which was not of the normal dual aortic origin to be a variant and noted that any differences from the above was bound to cause imbalance in the coronary arterial pressures (Figure 2).

One of the positive aspects of the multiple variant orifice in the origin of the CA is that in cases of blockage of either of the main orifices, anastomosis from the variants could help in the perfusion of the myocardium. Notably, during surgery like cardiac catheterization and ventriculotomy the multiple orifices can cause confusion leading to injury or damage of the blood vessels (Ogeng'o *et al.,* 2013; Bhele *et al.,* 2017) (Figure 3)

Mutyal *et al.,* (2014) in his study on 60 Indian hearts found a much lower number in the variations at 13.33% (18) which were three to four orifices on the right aortic cusps. The study did not have any findings on orifices on the left aortic cusps as variants which could have led to the difference in the variations, but in the current study, three study specimens had orifices on the left aortic cusps with none in the right. This meant that blood supply to the right side of the myocardium was purely by anastomosis of the available circulation or collateral circulation from the orifices in the left.

Results of a study done by Vilallonga (2003) in Spain noted almost the same prevalence in variations of origin of the coronary artery at 40.3% (31) out of the total 211 hearts dissected, this is in line with the current study, where the variant orifices were associated to the origin of the conus and the sinoatrial nodal artery. It was also noted that the presence of the variants could seriously jeopardize the functioning of the coronary arteries due to the alteration of different pressures that maintain coronary arterial circulation to the myocardium.

However, unlike Vilallonga's (2003) findings, there was a slight difference in specific variations where 16.6% (12) of the variations were found to be the origin of the conus artery while 2.7% (2) were from the origin of the SANA. This artery had one of its orifice originating from the right aortic cusps with 2 openings and the other with 3 openings. The conus artery and the sinoatrial nodal artery having their own orifice could be a saving effect to the heart such that in case of any damage or injury to the RCA with a patent aorta, the myocardium can be saved from myocardial infarction and ischaemia.

This is important especially with the current increase in non-communicable diseases, these variants can ensure proper perfusion after anastomosis and collateral circulation. However, they could also make management complicated during surgeries and even radiology. The success of delicate procedures like ventriculotomy highly depend on radiological features of the arteries before the surgery. In case of the multiple orifice and lack of opacification during radiology, fatal mistakes can be made during surgery leading to catheterization of the wrong artery. This could deny the heart muscles significant blood supply therefore predisposing it to multiple damages, injury and inability of the heart to compensate (Mutyal *et al.,* 2014).

Of importance to note is that the 44.4% variations in the origin of CA in the present study could have a serious circulatory effect on the myocardium which could easily predispose the heart to myocardial infarction and probably cardiac death. Khwansang &

Chentanez, (2018) therefore advices that these variations should be keenly noted when looking at the overall perfusion of the myocardium and more so during coronary angiographies and revascularization procedures. This is because they can cause confusion thus fatal mistakes.

Pearson's correlation test was used to analyze the relationship between the variables and variations $P=\leq 0.05$ was not significant at (p=0.463). Meaning that there was no correlation between the variations in origin and the gender.

CONCLUSIONS

There were variations in the origin of coronary arteries, with most of the variant orifices occurring on the right side of the aortic cusps.

Furthermore, the variations can be beneficial or non-beneficial to the perfusion of the myocardium leading to the development of ischemic heart diseases.

Recommendations

Training of the health care providers. This is important for all the levels of healthcare provision so that they can be aware of the variations, their effect and how to manage them. Cardiologists and heart surgeons should also be continuously informed on the changes caused by these variants on the myocardium

Early routine imaging of the heart. This will note any changes that are occurring on the heart early enough and interventions can be easily put in place to avoid morbidity and mortality.

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ORIGINAL ARTICLE

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ANATOMICAL VARIATIONS IN MORPHOLOGY OF RIGHT AND LEFT BRACHIAL PLEXUS AMONG BLACK AFRICAN POPULATION; A CADAVERIC STUDY IN WESTERN KENYA

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ABSTRACT

Background: Brachial plexus (Bp) is a network of bundles of nerves of spinal cord around neck and axilla in the body formed by union of anterior primary rami of C5 to C8, and T1. Technical expertise required for clinical and surgical procedures remains challenging for the beginners. Variations may be due to trauma, irradiation, neoplasm, infection, and autoimmune inflammatory disease. The purpose of this study was to determine the difference in variation of right and left Bp of the study population. **Methods:** Cross sectional descriptive study was used in Maseno, Uzima and Masinde Muliro Universities anatomy laboratory to get sample size of 35male and 35 female cadavers. Chi- square test was set at a p= 0.05. Ethical consideration was observed. **Results:** Statistically significant difference (p=0.009) between the right and the left upper limb in the branching of musculocutaneous nerve was recorded. A total of 17.8% variations in origin and segments of Bp, majority 9.9% were from left while 7.9% from right. Highest frequency of variation occurred at the prefixed root of Bp with 8.7% occurring on left upper limb and 12.13% on right upper limb. Right upper limbs had highest incidence of variations in branching patterns of Bp at 43% whilst left had incidence of 23.5%. Highest frequency of main terminal variation was of musculocutaneous nerve at 18.6% with 13.6% occurring on right side and 5% on left side. **Conclusion:** Knowledge of variations is essential in evaluation of unexplained sensory and motor loss after trauma and surgical interventions to the upper limb.

Keywords: Anatomical variations; Brachial plexus; cadaver; difference in laterality of right and left side

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INTRODUCTION

Brachial plexus is a network of nerves of spinal cord at the brachium (humerus) (Rasulic *et al.*, 2020). It carries motor, sensory, innervation to extrinsic thoracic muscles and sympathetic impulses from spinal cord to the arms and travels through inter-scalene muscle between the anterior scalene and middle scalene muscle then via posterior triangle of the neck (Vaid & Vaid, 2015). The union of C5, C6, C7, C8 and T1 forms it. There are different sections thus the roots, trunks, divisions and cords (M. Emamhadi *et al.*, 2016).

Studies done indicated that it is found in the upper limbs in human body with embryological origin history being the initial differentiation of mesenchyme that occurs in the fourth week of embryology(Standring, 2016). Primordial of dorsal nerves reach at the length of distal stretch of the humerus inside the sheath of the growing primordial arm muscle nerves as from C5 to T1 that begin to extend on day 32 of human life, where the nerves merge and form brachial plexus (Bala *et al.*, 2014; Rasulic *et al.*, 2020).The networks of nerves conducts signals from the spine to the shoulder, arm and hands. Brachial plexus injuries are caused by damage of those nerves (Sachar *et al.*, 2020). Usually, injuries are caused by trauma to the neck and shoulder. Symptoms may include a limp or paralyzed arm, lack of

muscle control in the arm, hand, or wrist and lack of sensation in the arms or hand(M. Emamhadi *et al.*, 2016).
Pizzo and others (2019) found that it's very significant in neurosurgeons, anatomists and anesthesiologists to grasp the technical awareness of anatomy of human neuron complex interconnecting network leading to a good outcome in critical and emergency patient care and better prognosis. The greatest significant challenges in human anatomy are nerve variations particularly in extreme is found in the upper limb (Pizzo *et al.*, 2019). Due to sports, car accidents or motorcycle, falls, medical conditions and physical activities the upper limbs are vulnerable to traumas (Hardcastle *et al.*, 2020; Verma *et al.*, 2020).It's all important to note that brachial plexus anatomical variations are very common. Most cadaveric anatomical studies of human nerves network is reported to manifest from the brachial plexus (Pizzo *et al.*, 2019).

Professor Ogeng'o (2013) and others indicated that brachial plexus injuries can occur during the delivery of newborns when after the delivery of the head, the anterior shoulder of the infant cannot pass below the pubic symphysis without manipulation (Bahm *et al.*, 2020; El Falougy *et al.*, 2013). This manipulation can cause the baby's shoulder to stretch, which can damage the brachial plexus to varying degrees (Feigl *et al.*, 2020; Singh *et al.*, 2020). This type of injury is referred to as shoulder dystocia. Shoulder dystocia can cause obstetric brachial plexus palsy (OBPP), which is the actual injury to the brachial plexus (Tay *et al.*, 2021). The incidence of OBPP in the United States is 1.5 per 1000 births, while it is lower in the United Kingdom and the Republic of Ireland (0.42 per 1000 births). While there are no known risk factors for OBPP, if a newborn does have shoulder dystocia it increases their risk for OBPP 100-fold (Menticoglou, 2018) Nerve damage has been connected to birth weight with larger newborns being more susceptible to the injury but it also has to do with the delivery methods. Although very hard to prevent during live birth, doctors must be able to deliver a newborn with precise and gentle movements to decrease chances of injuring the child (Fakoya *et al.*, 2019; Tay *et al.*, 2021).

Vajapey and associates (2021) explored human cadavers aged above 18 years where a total of 32 adult cadavers were included in the research and 64 bilateral upper extremities were finally assessed. Male cadavers showed that the brachial plexus was normal on both sides (Martin *et al.*, 2018). The median nerve was formed on the right side in distance of 45mm and on the left side in distance of 45mm away from the coracoid process. The musculocutaneous nerve entered to the coracobrachialis muscle on the right side in distance of 83mm and on the left side in distance of 105mm away from the coracoid process. While another male cadaver the brachial plexus was normal on both sides, the median nerve was formed on the right side in distance of 93mm and on the left side in distance of 99mm away from the coracoid process. The musculocutaneous nerve entered coracobrachialis on the right side in distance of 83mm and on the left side in distance of 105mm away from coracoid process (Pinto *et al.*, 2019)

According to study done by Costa and others (2019) throughout the profile regular dissection for undergraduate student, noticed was variation into emergence of the upper trunk of brachial plexus.one sided established at the right part of male adult cadaver where upper trunk established by alliance of the ventral rami in the C5 to C7 of the spinal nerve (M. P. da Costa *et al.*, 2019). Lower trunk was established by the association of the ventral branches of C8 together with T1 of spinal nerves of note was the middle trunk that was missing (Didesch & Tang, 2019). The atypical upper trunk then was cleared entirely to establish the facial correlation amid the middle and upper trunks

(El Falougy *et al.*, 2013). The noted anomaly in the upper trunk penetrated laterally interscalenus medius and scalenus anterior muscles (V. Vanaclocha *et al.*, 2015). After leaving those two trunks both nerves to subclavius together with suprascapular nerves, the trunk separated into posterior and anterior divisions in which advanced the course like as the division of the normal lower and upper trunk. Other parts of the brachial plexus were found to be within the normal range (Lague *et al.*, 2019).

According to Leape (2015), two plexuses had variation onto superior trunk both found at the right part. In the two cases, C5 with C6 roots were broken up into posterior together with the anterior division. The two anterior unite to give rise to a beginning to a" posterior superior trunk". Then these trunks unite to give a beginning of a superior trunk. Middle trunk got an association branch from C8 of the left part in a white male cadaver together with a transmitting branch as from inferior trunk on the right part of one black male cadaver, There was no variation noted on the inferior trunk amongst those cases(Leape, 2015). In the previous studies done by Vanaclocha (2015), variations at the supraclavicular side of the brachial plexus regularly were found more on the left part though in our case variation was in the right part(V. Vanaclocha *et al.*, 2015). Comprehensive understanding of the variations into the emergence of the brachial plexus is so resourceful to neurosurgeons for it would assist during surgical management of neuro fibromas, tumors and nerves sheaths as schwannomas (Pizzo *et al.*, 2019).

MATERIALS AND METHODS

The study was carried out in the anatomy laboratories of 3 medical universities where cross-sectional study design was used and selection done according to the number of cadavers in each lab. Universities were identified purposively and conveniently because of their functional human anatomy Department that have met the commission of university education standards for storage of human tissue.

To ensure equal distribution of samples, sampling was done proportionately with the strata population by location, where the sample size per laboratory was calculated by dividing laboratory cadaver (*d*) by total location population (86), then multiplying by the desired sample size (70) n= (*d**70)/86. As a result, 70 cadavers were acquired as the total actual sample size using the Yamane Taro formula (1967) from a total available specimen of 86 cadavers. Ethical clearance from the university ethical committee was obtained.

The cadavers were dissected according to the Cunningham manual of practical anatomy where they were examined as per the general principles of anatomy. They were serially numbered from 1-70 then preserved in 10% formalin. A vertical incision was made to expose the site between the triceps and biceps muscles and the following were exposed; roots which were in scalene gap, trunks that were found superior to the clavicle, divisions that sit around the posterior portion of clavicle, cords and branches which were inferior to the clavicle median, axillary, ulnar, radial, and musculocutaneous nerves were located with their origin as well. The observations were recorded in a data sheet and on digital photography.: Statistical package for social sciences (SPSS) version (26) was used for analysis. Chi square test was used to compare the means of independent variables and determine for any statistical significance. Pearson's Correlation test was used to test for linear relationship among the same pairs of variables in the population. Contingency tables were used to determine for statistical relevance between sections of brachial plexus.

RESULTS

In table 1 of the total 25 (17.8%) variations in the origin and segments of brachial plexuses, the majority 14 (9.9%) were from the left side while only 11 (7.9%) were from the right. The highest frequency of variation occurred at the prefixed root of the brachial plexus with 12 (8.7%) variations occurring on the left upper limb and 17 (12.13%) occurring at the right upper limb. The lowest occurrence of variation was at the trunk with the anterior division of the medial trunk connecting with medial nerve. This only occurred in one (0.7%) right limb of the total 140 specimens.

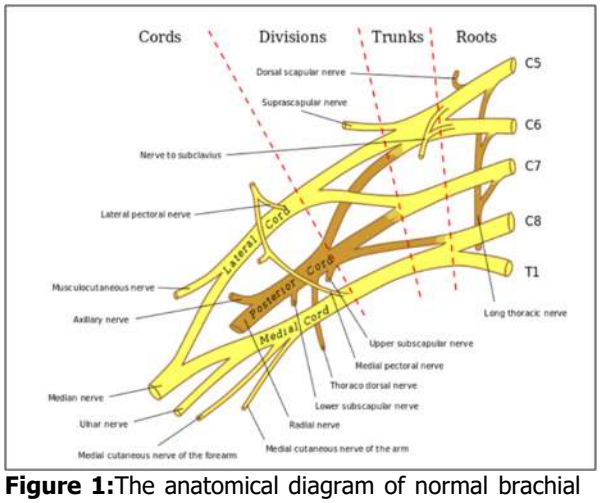


Figure 1: The anatomical diagram of normal brachial plexus (M. Emamhadi *et al.*, 2016)

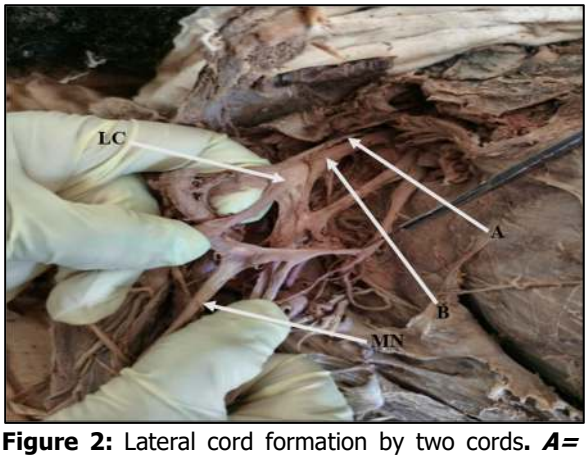


Figure 2: Lateral cord formation by two cords**.** ***A=*** *Cord one,* ***B=*** *Cord two,* ***LC=*** *Lateral cord* ***and MN=****musculocutaneous nerve.*

In table 2, there was no statistically significant difference in variations between the left and the right upper limbs in all the segments of the brachial plexus.

Illustrated in table 3 in the study, the right upper limbs had the highest incidence of variations in the branching patterns of brachial plexus at 43% (61) whilst the left had a variation incidence of only 23.5% (33). The highest frequency of variation was of musculocutaneous nerve (18.6%) with 19 (13.6%) variations occurring on the right side whilst only 7 (5%) variations occurred on the left side. Variation in origin of radial nerve had the lowest frequency with only 2 (1.42%) on the left side and 5 (3.5%) incidences on the right side.

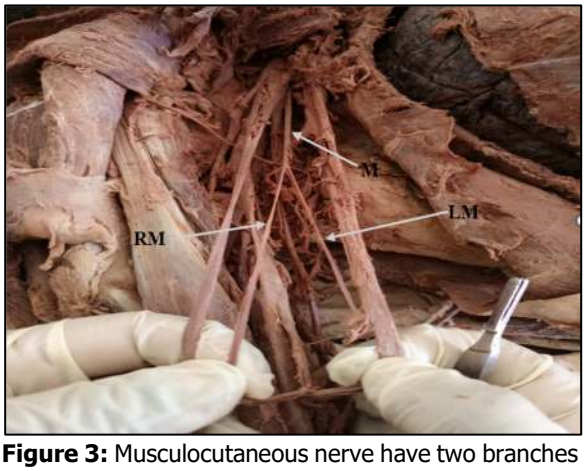


Figure 3: Musculocutaneous nerve have two branches and not piercing coracobrachialis muscle. ***M=****musculocutaneous nerve,* ***RM=*** *right branch of musculocutaneous nerve* ***LM=*** *left branch of musculocutaneous nerve.*

There was a statistically significant difference (p=0.009) between the right and the left upper limb in the branching of musculocutaneous nerve. There was no statistically significant difference in variations between the left and the right upper limbs in the remaining terminal branches of the brachial plexus (Table 4).

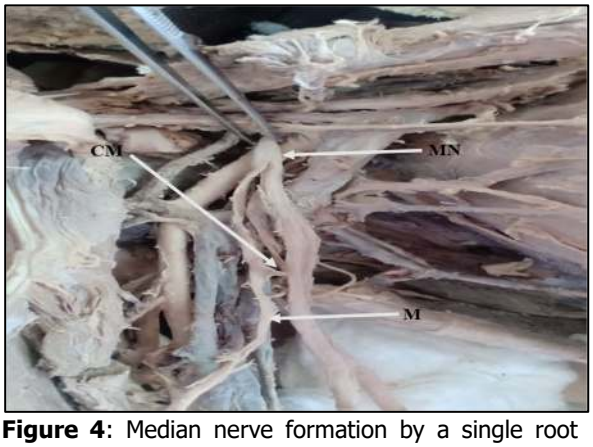


Figure 4: Median nerve formation by a single root while musculocutaneous nerve gives a communicating branch to median nerve. ***M=****Musculocutaneous nerve,* ***MN=*** *Median nerve* ***CM=*** *Communicating branch of musculocutaneous joining the median nerve.*

Table 1: Correlation between Laterality and variations in segments of brachial plexus

		Side			
		Left	Right	Total	
		Count	Count	Count	Column N %
Roots Variation	Normal	54	48	102	72.9%
	Variant Post fixed	4	5	9	**6.4%**
	Variant Prefixed	12	17	29	**20.7 %**
Trunks Variation	Anterior division of medial trunk connecting with medial nerve	0	1	1	0.7%
	Normal	64	67	131	93.6%
	Superior trunks pass midway via anterior scalene muscle	6	2	8	5.7%
Divisions Variation	Anterior division of medial trunk connecting with medial nerve	2	1	3	2.1%
	Normal	68	69	137	97.9%
Cords Variation	Normal	68	68	136	97.1%
	Variant	2	2	4	2.9%
	TOTAL VARIATION INCIDENCE	**14**	**11**	**25**	**17.8 %**

Table 2: Test for significance between Laterality and variations in sections of brachial plexus

Pearson Chi-Square Tests		
		Side
Roots Variation	Chi-square	1.326
	df	2
	Sig.	.515a
Trunks Variation	Chi-square	3.069
	df	2
	Sig.	.216a,b
Divisions Variation	Chi-square	.341
	df	1
	Sig.	.559a
Cords Variation	Chi-square	.000
	df	1
	Sig.	1.000a
Results are based on nonempty rows and columns in each innermost sub table.		

Table 3: correlation between Laterality and terminal branches of the brachial plexus

		Side			
		Left	Right	Total	
		Count	Count	Count	Column N %
Axillary Variation	Normal	62	56	118	84.3%
	Variant	8	14	22	15.7%
Radial Variation	Normal	68	65	133	95.0%
	Variant	2	5	7	5.0%
Ulnar Variation	Normal	64	58	122	87.1%
	Variant	6	12	18	12.9%

Musculocutaneous Variation	Normal	63	51	114	81.4%
	Variant	7	19	26	18.6%
Median Nerve Variation	Normal	60	59	119	85.0%
	Variant	10	11	21	15.0%
TOTAL VARIATION INCIDENCE		33	61	94	67%

Table 4: Test for significance between Laterality and variations in branching of brachial plexus

Pearson Chi-Square Tests		
		Side
Axillary Variation	Chi-square	1.941
	Df	1
	Sig.	.164
Radial Variation	Chi-square	1.353
	Df	1
	Sig.	.245
Ulnar Variation	Chi-square	2.295
	Df	1
	Sig.	.130
Musculocutaneous Variation	Chi-square	6.802
	Df	1
	Sig.	.009*
Median Nerve Variation	Chi-square	.056
	df	1
	Sig.	.813
Results are based on nonempty rows and columns in each innermost suitable.		
*. The Chi-square statistic is significant at the .05 level.		

Table 5: Frequency distribution of variations of terminal branches of brachial plexus

		Male	Female	Total	Percentage
		Frequency	Frequency	L+R UL	%
Auxiliary Nerve	Normal	61	57	118	84.2
	Variant	9	13	22	15.8
Radial Variation	Normal	68	65	113	95
	Variant	2	5	7	5
Ulnar Variation	Normal	61	61	121	86.4
	Variant	9	9	18	13.6
Musculocutaneous Variation	Normal	59	55	114	81.4
	Variant	11	15	26	18.6
Median Nerve Variation	Normal	56	63	119	85
	Variant	14	7	21	15

DISCUSSION

Difference in variation of right and left brachial plexus of the study population. It was observed that of the total 25 (17.8%) variations in the origin and segments of brachial plexuses, the majority (14) were from the left side while only 11 (7.9%) were from the right. This observation is similar to the reports (Vicente Vanaclocha *et al.*, 2015) who established that variations of the supraclavicular side of the brachial plexus were more on the left side as compared to the right side. This also concurs with reports of (Mohammadreza Emamhadi *et al.*, 2016) in Table 1 in Iranian cadavers which noted that most of suprascapular nerve which emerges from the supraclavicular zone was more on the left side than right side. This type of variation, therefore, establishes a comprehensive understanding by surgeons and neurosurgeons when conducting surgical procedures following injury or carcinomas of the brachial plexus.

It was observed that the highest frequency of variation occurred at the prefixed root of the brachial plexus with 12(8.7%) variations occurring on the left upper limb and 17 (12.13%) occurring at the right upper limb. These findings are dissimilar to (Chaudhary *et al.*, 2012) who found out that most of the variations were postfixed in females than males while assessing the conjunction or co-incidence of a four trunked brachial plexus and a post fixed brachial plexus. However, the findings in the study are similar to the reports of (Kirik *et al.*, 2017) in Turkey who found out that half of the brachial plexus were found to be prefixed, while 15% were post fixed while studying the anatomical variations of the brachial plexus in fetal cadavers. These anatomical variations noted may lead to deviations from the expected dermatological distribution and contribute to the differences in the motor innervation of muscles of the upper limb. This can further contribute to pathological changes on the upper limbs, might produce conditions that are clinically attributable to abnormal relations between the brachial plexus and ribs and altered surgical approaches of brachial plexus.

In this study, the right upper limbs had the highest incidence of variations in the branching patterns of brachial plexus at 43% (61) whilst the left had a variation incidence of only 23.5% (33). This is in agreement with observations of (Uysal *et al.*, 2003) who found out that most of the brachial plexus variations were from right (19.5%), (12%) and left (10.5%), (11.5%) among female and male respectively while assessing the brachial plexus variations in human fetuses. This study therefore postulates that although the variations are more on the right side than left side, this might interfere much with the surgical procedures at that moment and time.

Studies of Miguel Pereira da Costa *et al.* (2019) while assessing the emergence of upper and lower trunk noted that the upper trunk variation of brachial plexus were of the right part of male adult cadavers.

It was observed that the highest frequency of variation was of musculocutaneous nerve (18.6%) with 19 (13.6%) variations occurring on the right side whilst only 7 (5%) variations occurred on the left side. These results are similar to the reports of Bhattarai and Poudel (2009) in Nepal who established a 6.25% of variation of musculocutaneous nerve which was unilaterally of the right side while assessing the unusual variation in musculocutaneous nerve. Here musculocutaneous nerve variation of right side pierced the coracobrachialis muscles to run downwards between short head biceps brachii and medial intermuscular septa to join the median nerve on the medial side of the arm. In the current study Figure 3, musculocutaneous nerve branched into 2 where the right branch pierced coracobrachialis muscle while the left remained afloat.

In Figure 3, the musculocutaneous nerve gives a branch that communicates with median nerve which is an anomaly. In rare cases of variation, musculocutaneous nerve does not pierce coracobrachialis muscle however it can be observed joining the median nerve at the arm Nasrabadi *et al.* (2017).These types of variations might provide important knowledge in understanding the peripheral nerve distribution as this remains the leading cause of pain and dysfunctional injuries around the shoulder joint. On the other hand, median and musculocutaneous nerve variations can be noted during the embryonic life, and this may be due to several factors influencing mechanism of forearm muscle development. During the development of brachial plexus, as the axonal spinal fibers grow distally into the limb buds to give a dorsal and ventral division, the latter will give rise to median and ulnar nerve whereby musculocutaneous nerve later develops from the median nerve. Therefore, any developmental abnormalities

might lead to significant variation(Nasrabadi *et al.*, 2017; Schoenwolf *et al.*, 2020).

In the current study, it was observed that there was a statistically significant difference (p=0.008) in the variation of distribution of median nerve in relation to the sexes. This is in agreement with the observations of Pandey and Shukla (2007) in India where it was established that median nerve variation prevalence was 12.8% and 13.2% among male and females respectively. These changes are variant in terms of origin, location and course in relation to axillary artery. This study therefore postulates that this kind of variation are more prone during radical neck dissection and axilla surgical procedures. Formation of median nerve occur laterally to axillary artery in axilla, which is normal anatomy, however, median nerve can be associated with variations in the abnormal communication with nerves like musculocutaneous and ulnar nerves or even splitting and penetrating vessels. The following anatomical variations were observed in the reports of (Budhiraja *et al.*, 2017; Samarawickrama, 2017) in Brazil where in 22.4% of upper limbs 3 roots took part in formation of median nerve, 6.12% medial to axillary artery and 1.53% anterior to axillary artery.

In the current study Figure 4, it was observed that median nerve was formed by a single root which is abnormal rather than the normal two roots. It was also observed that the brachial artery was superficial to the median nerve which is abnormal as compared to normal anatomy brachial artery courses below the median nerve.

In this study table 5, among the variations at the terminal branches, the musculocutaneous nerve had the highest variation at 18.6% (26) while the least was the radial nerve at 5% (7). (Mohammadreza Emamhadi *et al.*, 2016)recorded 3 variations in the ulnar nerve and noted that ideally the ulnar nerve originates from the medial cord, but in their study, it received a communicating branch from the lateral cord while the median nerve received communication from the posterior cord yet it was supposed to receive communication from the lateral and medial cord. In this study, variation of the ulnar and the median nerve was higher than the previous study because the communications received by this branch were from the posterior, lateral and medial cords combined thus increasing the number of variations.

Although this may be confusing to the surgeon during treatment of neck surgeries, the communications received from other variant branches may as well have a saving effect to the parts innervated. In case of diseases, injury or tumor of specific regions affecting the functioning of nerves innervating the regions, there can be an alternate pathway for nerve transmission which will help to prevent further injury and loss of function in the affected areas. Similarly, (Aktan *et al.*, 2001; Benes *et al.*, 2021)in their study which was to determine the relationship between the terminal branches of the brachial plexus and the functioning of the arm that was done on 48 Turkish cadavers noted a lot of variations in the branches which could cause confusion to the health care providers and surgeons during assessment of nerve injuries like carpal tunnel syndrome, cubital tunnel syndrome and other neuropathies. However, they also noted that these variations in connections may also be good because they may provide sensory or motor innervation when an alternate nerve has trauma or defect and cannot function. They advised that since the variants had both positive and negative implications, it was important to identify the type of variant that an individual had so that management could be established early enough.

Another study by (Patel & Smith, 2023) on 39 previously dissected cadavers among them 20 females and 19 males observed

variations in the musculocutaneous branch where it was formed by the medial and the lateral cords and even changed its course of movement to innervate additional muscles of the anterior fore arm apart from the coracobrachialis, biceps and the brachialis. The same case applies to the present study where the musculocutaneous nerve had the highest variation which could be attributed to receiving branches from the posterior and medial cord. This could affect the conduction of plexus blockade as a component of anesthesia in the upper arm.

Further studies by (Yang *et al.*, 2009)in China on 306 cadavers observed how variations in the brachial plexus could facilitate the deterioration of the functioning of the axillary artery. The previous study found variations in 12 study subjects whose axillary nerves merged from the lateral and medial cord and ran anteromedial towards the axillary artery which brought intertwining of the artery and nerve therefore compromising the activity of the axillary artery and the other blood vessels adjacent to them. In the present study, 22 axillary nerves had variations which was higher than the previous study. These variations included originating from lateral and medial cord, or both combined which could highly interfere with the distribution of the axillary artery. This could seriously affect the functioning of the upper limb due to numbness and reduced circulatory flow.

Other variations on the branches of the brachial plexus were observed by (Guday *et al.*, 2017)) where radial nerve received communication from the inferior trunk in 7% of the cases, the ulnar nerve had communication with the lateral cord in 30% of the cases while the median nerve originated from the 2 lateral roots and the medial root in 52% of the cases. This variation from the previous studies were higher than the present study and this could be attributed to the gender and geographical location.

CONCLUSION

Musculocutaneous nerve variations are more common with most of the variation occurring on the right-side compared to left side. The multiple astonishing anatomical variations found had significant clinical correlation. This diversity suggests some level of impairment in the target muscle extreme challenge in administration of regional anesthetic injection nerve block. As in anatomical variation whereby median nerve had 3 interconnections around the axillary artery, this can lead to entrapment and vascular problems. These unfamiliar spreading of variant nerve is vulnerable to injury and can also expedite diversified scope of muscle debilitation. Clinicians and principally neuro and vascular surgeons must assert a high extent of intuition for their respective existence, if confronted with Bp variations they must be prepared in a suitable state to transform their curative and surgical intersession and to ensure excellent action design for the sufferer.

RECOMMENDATIONS

Early pre-operative radiological imaging to detect any anomaly of brachial plexus. A lot more studies should be done on the causes of anatomical variations in terms of origin and embryological characteristics and to explore more on genetics and or with environmental components.

Conflict of interest: No conflict of interest

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ORIGINAL ARTICLE

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RESTORATIVE EFFECTS OF *AZADICARACHTA INDICA ON* THE KIDNEY HISTOMORPHOMETRY IN CISPLATIN-TREATED WISTER ALBINO RATS

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ABSTRACT

Background: Cisplatin is used for treatment of soft tissue tumors and has been associated with nephrotoxicity. *Azadirachta indica* is a local herb that has been associated with attenuation and counteracting the nephrotoxic effects of cisplatin. However, there is inadequate histomorphometric evidence to support this potential. This study therefore aims to evaluate the possible Restorative Effects of *Azadirachta indica* on Kidney Histomorphometry in Cisplatin-Treated Wister Albino Rats. **Methods**: Twenty-five Wister Albino Rats of weight 250-400g were randomly selected into five groups of control or experimental with each group containing five animals. The control group only fed on water *adlibitum.* Experimental group one was administered with intraperitoneal injection 0.28mg/kg of cisplatin, other three experimental groups were given a constant dose of intraperitoneal 0.28mg/kg of cisplatin on day one followed by per oral 6.67mg/kg/bwt/day experimental group two, 5mg/kg/bwt/day experimental group three and 3.33mg/kg/bwt/day experimental group four of *azadiracta indica* on day five for seven days. On day thirteen all animals were euthanized. The kidneys were histologically prepared. Photomicrographs were taken and uploaded on stepanizer for histostereology. **Results**: The glomerulus and the Bowmans capsule of experimental group one, two and four had a significant ($P \leq 0.0001$) reduction and increased respectively, only the glomerulus space for the experimental group two had significant ($P \leq 0.002$) increase as compared to the control. **Conclusion**: Out of the three doses of *azadicracta indica*, the medium dose of 5.0mg/kg was able to restore the histomorphometry and histostereology of the kidney on administration of 0.28mg/kg of cisplatin.

Keywords: Restorative, histomorphometry, Azadirachta indica, Cisplatin, histostereology

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INTRODUCTION

Azadirachta indica is a herbal medicine used traditionally and is believed to treat several conditions such as intestinal warms, diabetes, skin condition and others (Diabaté et al., 2014). The herb is believed to have an Indian origin and is locally found abundant in Kenya (Yao et al., 2007). It's believed to have nephroprotective effects against cisplatin(Abdel Moneim et al., 2014). The therapeutic effect of *azadirachta indica* is dependent on its active components such as ascorbate, nimboline and azadirachtin responsible for the antioxidant effects (Carpenter, 2020). *Azadirachta indica* traps the free radical oxygen in the circulation hence attenuating or counteracting the effects of cisplatin (Abireh et al., 2020).

Nephrotoxicity is the process where the kidney is damaged when it has been introduced by a toxic substance.(Al-Naimi et al., 2019). Cisplatin is metabolized in the kidney causing nephrotoxicity (Miller et al., 2010).

Kidney disease is among the leading cause of death with a prevalence of 13% worldwide

(Lv & Zhang, 2019) and over 4 million of Kenyans are suffering from renal disease. Cisplatin is a cytotoxic drug used alongside other chemotherapy medications. Despite cisplatin being effective, 20-30% of patients using it develop kidney disease (Burns et al., 2021). So, cisplatin is used with precaution and sometimes restricted because of its known side effect of nephrotoxicity. Among the elderly, the incidence is high up to 65% of the patient's taking cisplatin develop nephrotoxicity (Latcha et al., 2016). Cisplatin produces free radical oxygen that damages the mitochondria at the tubules of the nephrons hence causing nephrotoxicity (Zhang et al., 2021). *Azadirachta indica* destroys the free radical oxygen in the circulation thus helping to attenuate and counteract the effects of cisplatin.

MATERIALS AND METHODS

The study was conducted at a medical institution and followed all the necessary research ethical approvals with the following reference numbers UEAB/ISERC/01/2023 and NACOSTI/P/23/23376. The sample size of twenty-five Wister albino rats was calculated using the modified human resource formulae and were randomly separated into five groups of five animals each. All animals were put in cages for seven days for acclimatization after that the control group was only fed on water *adlibitum* while other experimental groups were given a constant dose of cisplatin of 0.28mg/kg once intraperitoneally to induce nephrotoxicity. After five days, renal function test was done to confirm kidney injury for all the experimental groups. The experimental group one only received 0.28mg/kg of cisplatin, experimental group two, three and four were given a constant dose of 0.28mg/kg of cisplatin and after five days were administered with different doses of *azadirachta indica* of 6.67mk/kg/bwt/day, 5.0mg/kg/bwt/day and 3.33mg/kg/bwt/day respectively and this was adopted from (Iman et al., 2021; Jacob et al., 2022; Perše, 2021) this was given for seven days for restoration of the kidney damage and after which the rats were euthanized. The kidneys were dissected, the cortical regions were fixed, dehydrated, embedded, sectioned, stained with hematoxylin and eosin, photomicrographs were taken and reported as per the group. The photomicrographs were taken at ×100 magnification. Simple random sampling was used in selecting the photomicrographs that were further uploaded on the stepanizer stereological tool for measuring the surface area of the glomerulus space, glomerulus and the bowman's capsule.

The surface area was calculated as follows; the total number of point grid within the specified region multiplied by estimated surface area of the point grid divided by the linear magnification (Tschanz et al., 2011). The measurements of the surface areas of the glomerulus, bowman's capsule and glomerulus space obtained from the stepanizer were put into an Excel sheet and uploaded into the SPSS. One way ANOVA and post hoc test was used in comparing data from the experimental group to control group where the P-value of less or equal to 0.05 was found to be of statistical significance at 95% confidence interval.

RESULTS

Restorative histo- structural changes of the kidney on administration of cisplatin followed by different doses of Azadirachta indica

In the photomicrographs b, c and e had tubular necrosis of the distal and proximal convoluted tubules, shown by shrinking of their epithelium and necrotic tissues both in the right and the left kidneys. There also was marked renal corpuscle involvement shown by necrosis of the parietal layer of the bowman's capsules and shrinking of the

glomerulus capillaries mostly on the right kidney as compared to the left one. In the photomicrographs a and d had normal microscopic features of the renes, the renal corpuscle is normal with no necrotic or apoptotic signs in both the right and left kidney with the glomerulus having and irregular in shape. The tubules are normal with no dilatation of the epithelium in both the right and left kidney. The renal interstitial is normal with no vacuolation, necrotic, apoptotic cells and fibrosis in both the right and left kidney. (Figure 1.1)

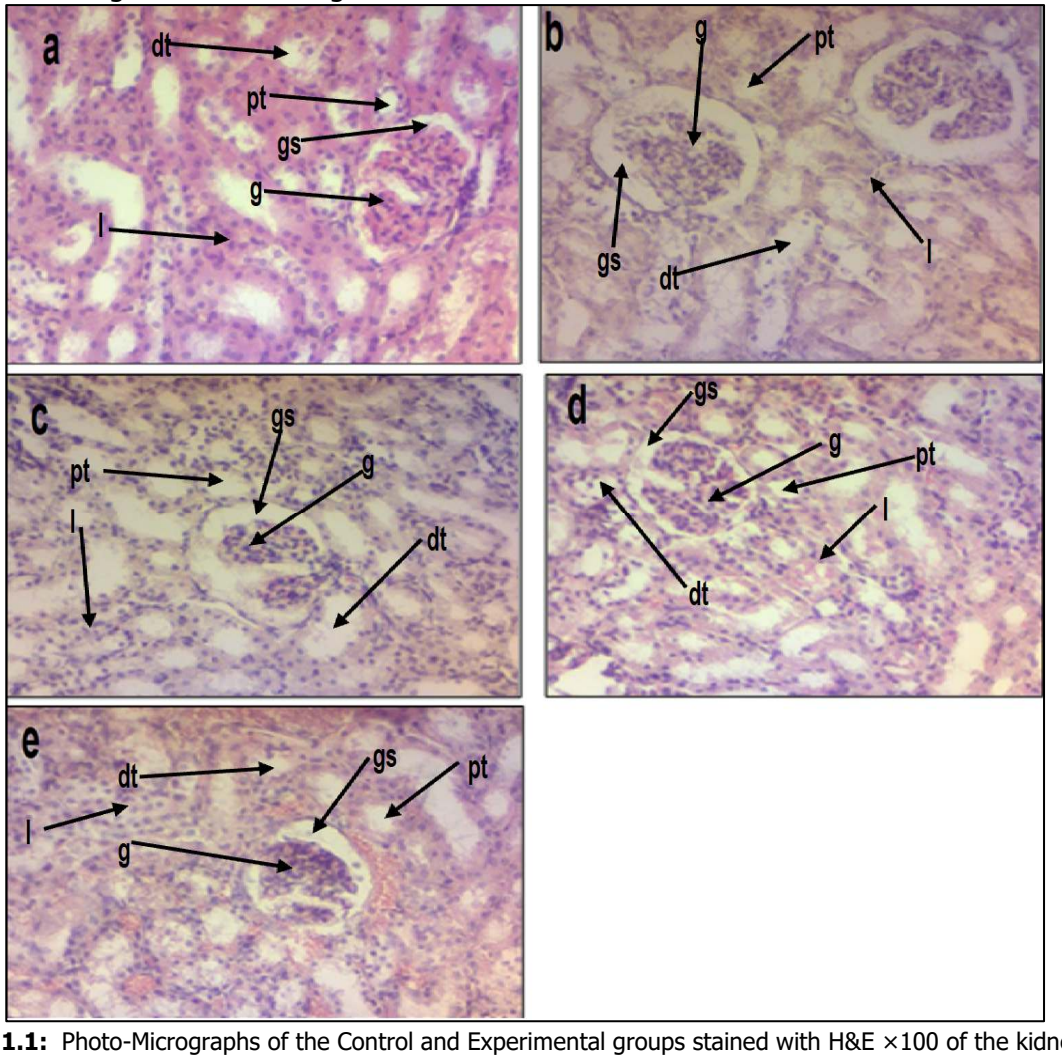


FIGURE 1.1: Photo-Micrographs of the Control and Experimental groups stained with H&E ×100 of the kidney section ***Key:*** *a- control group, b- experimental group one, c- experimental group two, d- experimental group three, e- experimental group four. g – glomerulus, gs- glomerulus space, pt- proximal convoluted tubules, dt- distal convoluted tubules and I- interstitial part.*

Histo- stereological analysis

The surface area of the renal tubules of control and experimental group could not be measured as the epithelial cells of some of the tubules in study group were disintegrated and distorted. The surface area of the glomerulus, glomerulus space and the bowman capsule were determined using the stepanizer stereological tool.

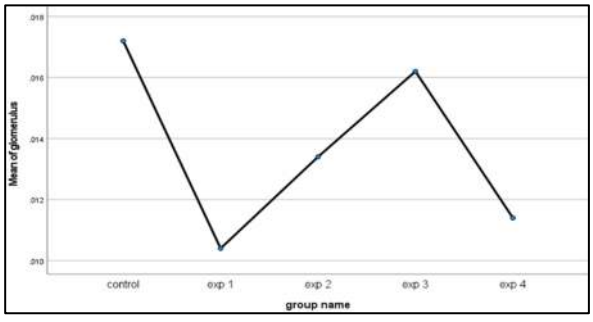


FIGURE 1.2: Mean surface area of the glomerulus of the control and the experimental groups Key: control (water+ feeds), exp 1- Experimental group one (0.28mg/kg Cisplain only), exp 2- Experimental group two 0.28mg/kg Cisplatin + Azadirachta indica 6.67mg/kg), exp 3- Experimental group three (0.28mg/kg Cisplatin + Azadirachta indica 5mg/kg), exp 4- Experimental group four (0.28mg/kg Cisplatin + Azadirachta indica 0.33mg/kg).

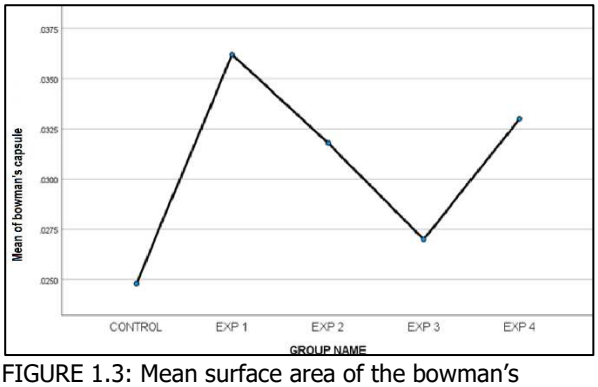


FIGURE 1.3: Mean surface area of the bowman's capsule of the control and the experimental groups.Key: control (water+ feeds), exp 1- Experimental group one (0.28mg/kg Cisplain only), exp 2- Experimental group two 0.28mg/kg Cisplatin + Azadirachta indica 6.67mg/kg), exp 3- Experimental group three (0.28mg/kg Cisplatin + Azadirachta indica 5mg/kg), exp 4- Experimental group four (0.28mg/kg Cisplatin + Azadirachta indica 0.33mg/kg).

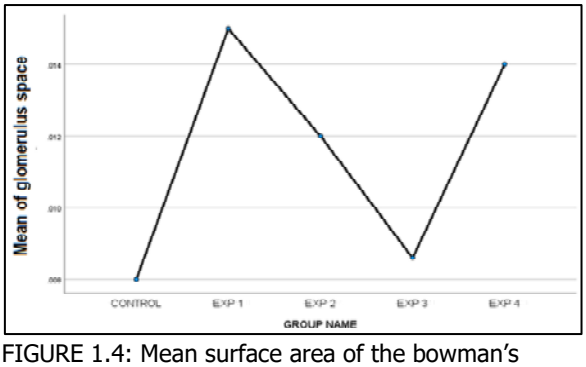


FIGURE 1.4: Mean surface area of the bowman's capsule of the control and the experimental groups. Key: control (water+ feeds), exp 1- Experimental group one (0.28mg/kg Cisplain only), exp 2- Experimental group two 0.28mg/kg Cisplatin + Azadirachta indica 6.67mg/kg), exp 3- Experimental group three (0.28mg/kg Cisplatin + Azadirachta indica 5mg/kg), exp 4- Experimental group four (0.28mg/kg Cisplatin + Azadirachta indica 0.33mg/kg).

The surface area of the glomerulus of experimental group one, two and four had a significant reduction (P <0.0001). The surface area of the of the glomerulus space and bowman's capsule significantly (P <0.0001) increase in Experimental group one, two and four, only glomerulus space of the Experimental two had a significant increase ($P \leq 0.002$). The P value was tested between the mean difference of surface area of the glomerulus, glomerulus space and the bowman's capsule for the control and Experimental one, two, three and four using one way ANOVA and post hoc test (Figure 1.2, 1.3, 1.4).

DISCUSSION

Renal histo-architectural changes are associated with nephrotoxicity. In the current study, there was a significant shrinkage of the glomerulus, and dilatation of proximal and distal convoluted tubules in the experimental group one, two and four as compared to the control group. This might be due to the circulation of free oxygen radical, endoplasmic and intracellular stress, and mitochondrial damage induced by cisplatin toxicity that led to necrosis of the parietal layer of the bowman's capsules, destruction of the basement membrane of the glomerulus, and tubular necrosis. From the literature reviewed (Al-Kahtani et al., 2014; Ibraheim & Eldamaty, 2018; Kumar Singh & Singh Karchuli, 2014) most authors recorded significant changes in the glomerulus, proximal, and distal convoluted tubules in cisplatin-induced nephrotoxicity, in line with the present findings. However, (Albalawi et al., 2023) reported contrary findings when they noted significant involvement of the interstitial part of the kidney as seen by the deposition of collagen fibrils following gentamycin-induced

nephrotoxicity. This may be due to inflammation of the intestinal part as a result of gentamycin toxicity. There was no significant change in the histo-architecture of experimental group three as compared to the control group, this perhaps demonstrated restoration of the glomerulus, distal, and proximal convoluted tubules. This study's findings concurred with those of (Abireh et al., 2020) who recorded similar results after treatment of ibuprofen-induced nephrotoxicity with *Azadirachta indica.*

The renal corpuscle, the surface area of the glomerulus, bowman's capsule and glomerulus space can be associated with the histo-stereological changes in assessing drug-induced nephrotoxicity. There was a significance increase in the surface area of the bowman's capsule in the experimental group one, two and four as compared to the control group. This may perhaps be due to the destruction of its parietal layer. The current study findings are in tandem with the results of

(Koca et al., 2013; Sasaki et al., 2018; Sobolev et al., 2021) who recorded a significant increase in the surface area of bowman's capsule after introduction of - induction of a nephrotoxic agent. There was no significance increase in the surface area of the bowman's capsule of the experimental group three as compared to the control group and this may suggest restoration. There was a significance reduction in the surface area of the glomerulus in the experimental group one, group two, four as compared to the control. This may be due to the destruction of the basement membrane or loss of appetite that led to dehydration. Results from Dixit *et al.,* (2014) also recorded a significant reduction in the surface area of the glomerulus following monosodium glutamate-induced nephrotoxicity, which he suggested a reduction in glomeruli as a result of exudation of contents of capillaries, high cellular proliferation, and hyalinization.

There was a significance increase in the surface area of glomerulus space in the experimental group one, two and four as compared to the control group. This could have been due to the shrinking of the glomerulus and dilatation of the bowman's capsule. Previous studies from the literature reviewed (Owagboriaye et al., 2022; Tobar et al., 2013) also recorded a significance increase in the enlargement of glomerulus space as a result of hyperfiltration of the glomerulus space and degeneration of the glomerulus capillaries when exposed to nephrotoxic agents. There was no significance increase in the surface area of the glomerulus space of the experimental three as compared to the control thus this may suggest reconstructive restoration.

CONCLUSION

Out of the three (Low, Medium & high) doses, the medium dose of *Azadirachta indica* was able to restore cisplatin-induced nephrotoxicity among the Wister albino rats. There was a significance increase in the surface area of the bowman's capsule, glomerulus space and reduction in glomerulus in cisplatin-induced nephrotoxicity among the Wister albino rats.

ETHICAL APPROVAL

The research ethical clearance was obtained from Baraton University of East Africa and licensed by Kenya, National commission for science, Technology and innovation (NACOSTI) with the following reference numbers UEAB/ISERC/01/2023 and NACOSTI/P/23/23376 respectively.

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ORIGINAL ARTICLE

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STEREOLOGICAL EVALUATION OF VOLUME DENSITIES OF OVARIAN FOLLICULAR COMPONENTS IN HYPERPROLACTIN RATS TREATED WITH IMMATURE COCONUT WATER.

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ABSTRACT

Background: Hyperprolactinaemia distorts ovarian structural and functional development, causing anovulation and infertility. The evaluations of volume densities (VDs) of structural components of the ovary such as; granulosa cells, theca cells and antrum have been previously used to measure folliculogenesis and determine the physiological status of ovaries. Immature coconut water (ICW) has been found to contain β-sitosterol, a compound capable of synthesizing steroid hormones *in-vivo*. Hence, this study aimed to determine the effects of ICW on VDs of follicular components in hyperprolactin rats. **Methods:** Mature female Sprague-Dawley rats were induced with hyperprolactinaemia and withdrawn to check for recovery status of an experimental induction and another set of rats were post-treated with ICW for 8, 16 and 28 days. The animals were sacrificed at the end of administration, ovarian tissues were harvested and histologically processed for the stereological evaluations of follicular components. **Results:** VDs of the antrum, granulosa cells and theca cells in the 16 and 28 days post-treated groups (19.25±7.46 ;43.22± 5.10;15.22±3.40 at16 days and 17.70±3.56; 42.75±5.23;15.10±3.60 at 28 days for the antrum, granulosa cells and theca cells respectively) were statistically comparable with the control group (19.76 ± 6.15; 43.75 ±5.71; 15.10± 3.81 for the antrum, granulosa cells and theca cells respectively) while the 16 and 28 days recovery groups (38.57±4.00*;27.20±6.32*;13.66±2.54 at 16 days and 36.58±4.00 *;24.39±5.77*;14.71±2.49 at 28 days for the antrum, granulosa cells and theca cells respectively) were not comparable with the control. **Conclusion:** These indicate that ICW stimulates follicular growth in hyperprolactin post-treated rats in the same structural pattern as the control group, which can depict a physiological approach.

Keywords: Ovary, Follicle, Immature coconut water, Hyperprolactin, Stereology, Volume densities

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INTRODUCTION

Hyperprolactinemia is defined as a persistent increase in the blood level of prolactin (PRL) >25ng/ml in non-lactating and non-pregnant people. It is a frequent endocrine contributor to reproductive issues (Thapa et al., 2021). According to reports, hyperprolactinaemia affects ovarian function, which results in irregular menstruation, anovulation and

consequently infertility. High prolactin inhibits follicular maturation which prevents the formation of mature eggs, by the decreasing the secretions of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Ramadan et al., 2021). These subsequently reduce the mitoses of granulosa cells and follicular estradiol productions by altering FSH-receptor binding sites (Misty et al., 2000). Therefore, changes in follicular components have been highlighted in the forecast of the effects of hyperprolactinaemia on folliculogenesis, hence a valuable tool in evaluating ovarian physiology and follicular growth and developmen pattern. Moreso, stereological evaluations of ovarian follicles integrated with endocrine regulations are being used to provide information on the site of action in folliculogenesis (Zahra, 2018).

Hyperprolactinaemia has been reported in disorders relating to infertility. In Nigeria, hyperprolactinaemia is a common hormonal alteration leading to ovulatory disorders and endometriosis (Olooto et al., 2012). A clinical study observed 36.2% of hyperprolactinaemia in infertile women in north-west Nigeria (Akande *et al.,* 2009).

Immature coconut water (ICW) was found to have properties similar to estrogen as estrogen levels were similar to animals with intact ovaries in postmenopausal rats (Nisaudah et al., 2009). β-sitosterol and sterols like stigmasterol, α-spinasterol and fucosterol may be accountable for the strong estrogenic effect of ICW by enhancing the biosynthesis of estrogen (Punghmatharith, 1998).

Natural non-steroid plant chemicals called phytoestrogen, often known as dietary estrogen, have both estrogenic and antiestrogenic effects. Depending on the situation, plant estrogen has been utilized as a viable alternative to hormone replacement treatment to improve fertility or not (Yildiz and Fatih, 2005). However, therapeutic approach is evolving and has proven to be quite effective (Gupta et al., 2005). Therefore, this study was designed to find out the estrogenic effects of ICW on volume densities of follicular components in hyperprolactin-induced animals using a stereological evaluation tool to measure the follicular growth and development rate.

MATERIALS AND METHODS

This study was carried out at the Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos. This study was conducted per the internationally accepted principles for laboratory guidance on animal usage and care under the Institutional Animal Care and Use Committee (IACUC) guidelines. All research protocols were conducted under the guiding principles of animal experimentation.

A total of fifty-five cyclic female Sprague-Dawley (SD) rats weighing 145-170g were obtained from a breeding stock of the Nigerian Institute of Medical Research (NIMR), Yaba Lagos, Nigeria. A taxonomist in the Department of Zoology of the University of Lagos authenticated the experimental animals. The animals were kept in standard ventilated plastic cages in the animal house of the Department of Anatomy and allowed to acclimatize for two weeks under standard laboratory conditions with a photoperiodicity of twelve hours of light alternating with twelve hours of darkness. The animals were fed with commercially available rat chow from Pfizer Nigeria Plc., Ikeja, Lagos and had free access to tap water.

Determination of the phases of estrous cycle in the animals

Puberty in the female rats was measured by estrous cyclicity. The phases of estrous cycle of the rats were established by daily histological examination of fresh vaginal smear each morning between 8:00 and

10:00 am. A small amount (approximately 0.2 ml) of normal saline was drawn into the plastic suction pipette. The tip of the pipette was pushed gently into the entrance of the vagina canal to a depth of 5mm and the fluid was flushed into the vagina canal and back up into the pipette two or three times by gently squeezing and releasing the bulb of the pipette. The smear was gently dropped onto the glass slide and viewed under a light microscope with a 40x objective lens (Marcondes et al., 2002). Staging the estrous cycle phases was done based on the cellular components of the viewed vagina smear. The metestrus phase was established with a collected smear showing leukocytes amidst remnants of large squamous cells. The diestrus phase showed a predominance of leukocytes and a few large nucleated cells. The smear with large nucleated cells with the leukocytes projections was designated as the proestrus phase. The estrus phase showed large flakes of squamous cells (McLean et al., 2012).

Hyperprolactin induction

Metoclopramide hydrochloride (MCH) was used to induce hyperprolactinaemia experimentally. The drug was purchased from Pfizer Pharmaceutical, Ikeja Lagos. MCH was administered at a dose of 0.2mg/100gbw daily for 28days. MCH administration was calculated based on the animals' weekly weights (Raffaele et al., 2009; Vinícius et al., 2013).

Collection of immature coconut fruits

The immature coconut fruits were harvested from a coconut farm owned by Chief William Kudofoke in Ajara -Topa, Badagry, Lagos, Nigeria. The fruit was authenticated at the federal institute of forestry research in Ibadan. It was assigned the ascension No FHI 109665.

Extraction and storage of the immature coconut water

The immature coconut fruits underwent washing and dehusking. Through the germinal hole, the water content (liquid endosperm of coconut fruits) was extracted. The gathered water was immediately poured into a clean, sealed bottle, kept in the refrigerator and changed every three weeks. Caution was taken in order to prevent particles from getting into the coconut water and metal contact with the water was avoided during extraction (Bustamante, 2002; FAO, 2007).

Treatment protocol

A total of fifty-five animals with approximately 4 days of cyclicity were used for this study. They were randomly divided into four major groups, 1 to 4 and further divided into eleven sub-groups of five animals each. Each rat was served with the extract according to the weight using a simple percentile faction and administration was achieved by the use of an oropharyngeal canula. The administration of substances was as shown in table 1.

Animal sacrifice and ovarian tissue collection

Sacrifices were done using cervical dislocation and incisions were made on the ventral surface of the pelvic region to extract the ovary for histological processing. The animals were sacrificed during the proestrous phase of estrous cycle following the end of administration. A midline incision on the anterior abdominal wall was made to access the abdominal cavity. The ovaries were carefully dissected out and trimmed of fat. The ovaries were weighed and fixed in 10% formal saline for histological processing.

Preparation of histological slides

The fixed tissues were transferred into ascending grades of alcohol. On day 1, the tissues were placed in 70% alcohol for 7

hours, then transferred into 90% alcohol. On day 2, the tissues were passed through three grades of absolute alcohol for one hour each and finally cleared in xylene. The tissues were then infiltrated in molten paraffin wax at 58°C and blocked out. Serial sections of 5 µm thick cut by the rotary microtome were obtained from a solid block of tissue and floated in a water bath. The sections were picked with clean slides onto which egg albumin had been coated and dried on the hot plate at 52°C. The mounted sections were dewaxed in xylene and then hydrated in descending grades of alcohol. The sections were then stained with Haematoxylin and counter-stained in Eosin and dehydrated. The sections were finally cleared in xylene and a drop of mountant was placed on the section and covered with a coverslip (Mohamed and Laurence 2011).

Stereology evaluations of follicular components using the counting grid method

The stereological evaluation of the volume densities of the antrum, granulosa cell and theca cell were carried out only on sections showing mature follicles according to the method described by Gundersen et al., 1998. A restricted artificial boundary was marked on the section limited to the boundary of the follicle. A counting grid with a set of regularly spaced points was placed on the field. All points hitting profiles (disregarding their relationship to the frame) were counted.

A point was considered a hit if a profile (including its boundary) covers the upper right corner in the cross-section where two lines cross each other. The volume density is calculated as the proportion of volume by simple percentile fraction; VD (profile counted/total specimen profile X100) (Gundersen *et al.,* 1988; Lars et al., 2020).

Statistical analysis

Results were expressed as mean ± standard deviation. Analysis was carried out using analysis of variance (ANOVA) with Scheffe's post hoc test. The level of significance was considered at $p< 0.05$

Table 1: Showing administrations and durations of test substances

GROUP/SUBGROUPS	ADMINISTRATION	DURATION (Days)
1 (Negative control)	Distilled water	28
2 (Induction and Recovery)		
a. Induction	0.2mg/100g of MCH	28
b. Withdrawal short	0.2mg/100g of MCH 28 days + withdrawal 8 days	36
c. Withdrawal medium	0.2mg/100g of MCH 28 days + withdrawal 16 days	44
d. Withdrawal long	0.2mg/100g of MCH 28 days + withdrawal 28 days	56
3 (Post-treated)		
a. Post-treated short	0.2mg/100g of MCH 28 days + 5ml/100g ICW 8 days	36
b. Post-treated medium	0.2mg/100g of MCH 28 days + 5ml/100g ICW 16 days	44
c. Post-treated long	0.2mg/100g of MCH 28 days + 5ml/100g ICW 28 days	56
4 (Positive control)		
a. ICW short	5ml/100g of ICW	8
b. ICW medium	5ml/100g of ICW	16
c. ICW long	5ml/100g of ICW	28

RESULTS

Stereological evaluations of VDs of follicular components were done only on section with mature follicles. Mature ovarian follicles were seen in the histological sections of all the ICW-treated and the 16- and 28-days recovery and post-treated groups while the induced and the 8 days withdrawal and post-treated groups did not show mature follicles in their histological sections. Stereological measurements of the follicular components in the 16 and 28 days post-treated and ICW groups(19.25±7.46;43.22±5.10;15.22±3.40

at 16 days and 17.70±3.56; 42.75±5.23;15.10±3.60 at 28 days for the antrum, granulosa cells and theca cells respectively) demonstrated comparable results in the volume densities with the control group (19.76 ± 6.15; 43.75 ±5.71; 15.10± 3.81 for the antrum, granulosa cells and theca cells respectively). This shows that immature coconut water post-treated groups demonstrated comparable stereological evaluation of volume densities of mature follicles with the control groups and the groups treated with immature coconut water only.

However, the 16 and 28 days withdrawal groups, which were the recovery groups, the volume densities (38.57 ± 4.00*;27.20 ± 6.32*; 13.66 ± 2.54 at 16 days and 36.58 ± 4.00 *;24.39 ± 5.77*;14.71 ± 2.49 at 28 days for the antrum, granulosa cells and theca cells respectively) were not statically comparable with the control group (19.76 ± 6.15; 43.75 ±5.71; 15.10± 3.81 for the antrum, granulosa cells and theca cells respectively at $p< 0.05$) even though the histological sections present mature ovarian follicles.

Table 2: Stereological evaluations of volume densities of the antrum, granulosa cells and theca cells of mature ovarian follicles of hyperprolactin rats treated with immature coconut water

GROUPS	SUB-GROUPS	ATR_{VD}(%)	$GRAC_{VD}$(%)	TAC_{VD}(%)
Control	$DSTL_{28days}$	19.76 ± 6.15	43.75 ±5.71	15.10± 3.81
Recovery	$MCH_{28\ days}$-$WD_{\ 16\ days}$	38.57± 4.00*	27.20± 6.32*	13.66± 2.54
	$MCH_{28\ days}$-$WD_{\ 28\ days}$	36.58± 4.00 *	24.39± 5.77*	14.71± 2.49
Post-treated	$MCH_{28\ days}$-ICW_{16days}	17.70± 3.56	42.75± 5.23	15.10± 3.60
	MCH_{28days}-ICW_{28days}	19.25± 7.46	43.22± 5.10	15.22± 3.40
Positive control (ICW-treated)	$ICW_{\ 8days}$	18.00 ± 4.77	49.55 ±5.19	15.13± 3.31
	$ICW_{\ 16days}$	19.09 ± 5.15	44.55 ±7.14	16.13± 3.00
	$ICW_{\ 28days}$	18.07 ± 7.70	46.55 ±5.10	16.13± 3.33

All values are expressed as mean ± standard deviation
*Significant differences; $p< 0.05$. DSTL: Distilled water; ICW: Immature coconut water; MCL: Metoclopramide hydrochloride; WD: Withdrawal; VD: Volume density; ATR: Antrum; GRAC: Granulosa cell; TAC: Theca cell.

DISCUSSION

According to mounting evidences (Lecomte et al., 1997; Susan et al., 2004; Zhong et al., 2011), PRL has a direct inhibitory effect on gonadotropin actions in the ovary and subsequently inhibits follicular development, inhibiting the formation of a mature egg.

Studies have revealed that ICW exhibits estrogen-like qualities that can control endocrine secretions. ICW was given to many groups of postmenopausal rats in a previous study, and the rats showed estrogen levels that were equivalent to those of rats that still had their ovaries (Nisaudah et al., 2009). It has been hypothesized that the presence of β-sitosterol in GCW, along with other sterols like stigmasterol, frucosterol, and β-spinasterol (plant sterols known to be involved in the synthesis of steroid hormones in-vivo), is what gives it such a potent estrogenic effect by promoting the production of endogenous estrogens that target the development of granulosa and theca cells. According to Punghmatharith (1998), the β-Sitosterol is structurally linked to animal cholesterol and may serve as a precursor to sex hormones. For measuring follicular growth and development, researchers have examined changes in microscopic components (Myers et al., 2004). An essential indicator of the process of folliculogenesis with regard to the endocrine signals and paracrine/autocrine mechanisms that control the growth and maturation of oocytes and their supporting follicular cells are accurate stereological estimations of ovarian follicles at various stages of development (West, 1990). The ovarian follicle is a three-dimensional structure, made up of theca and granulosa cells that emit hormones to regulate the

reproductive cycle and generate fertile eggs at ovulation. As follicular cells are unique in their hormone productions and modulations, with consequent indices in folliculogenesis, volume density evaluation of ovarian follicles is used to measure ovarian development and generate information on the physiological status of the ovaries (Yi-Xun et al., 2019). The stereological analyses revealed that 16 and 28 days after receiving ICW following experimental induction of hyperprolactinaemia, the mean volume densities of the antrum, granulosa and theca cells were statistically equivalent with that of the control group. The mean volume densities of the antrum, granulosa and theca cells in the recovery groups (16- and 28-days withdrawal groups), where folliculogenesis were restored following induction, were not comparable to that of the control group. Folliculogenesis is physiologically influenced by the granulosa cells. According to reports, the granulosa cells are in charge of producing estrogen and growth hormones that aid the development of oocyte (Jingqiang et al., 2021). Due to their vascular nature, theca cells communicate with the circulating blood network and give vital nutrients to the growing follicle. The fact that they create androstenedione, which the granulosa cells employ to make estradiol is more significant (Chen et al., 1993; Ashkenazi et al., 2005). In summary, the ovarian follicles will expand as a result of the granulosa and theca cells' proliferative activity and revert to abnormalities brought on by hyperprolactinaemia.

The results from this study further strengthen the effects of ICW on follicular growth and reproductive hormone profile in hyperprolactin rats (Bakare et al., 2019a; Bakare et al., 2019b). Through stereological evaluations of volume densities of follicular components, this study further revealed granulosa and theca cell proliferations as target sites of actions of ICW in hyperprolactin rat.

CONCLUSION

It is evident from this study that ICW demonstrates ameliorative effects on follicular growth and development in hyperprolactin rats by enhancing the propagation of granulosa mitoses and theca cell proliferation. This study's findings clearly depict that ICW is a promising candidate in the reversal of infertility caused by high prolactin in female Sprague-Dawley rats. The stereological evaluation employed in this study allowed for an unbiased and quantitative assessment of the changes occurring within the ovarian follicles. The use of stereology provided reliable and accurate estimations, enhancing the validity of our findings. This research outcome provides a foundation for future research and may have implications for the development of alternative treatment options for reproductive disorders. Further exploration of the underlying mechanisms and long-term effects is essential to fully understand the therapeutic potential of immature coconut water in reproductive health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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