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GILL PARASITES ASSOCIATED WITH *Clarias gariepinus* IN SANGO FISH FARMS, ILORIN, KWARA STATE, NIGERIA

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ABSTRACT

A study was carried out on gill parasites associated with *Clarias gariepinus* in Sango fish farm, in Ilorin, Kwara State. A total of Eighty-One (81) *Clarias gariepinus* were obtained from the fish farm in Ilorin. The fish were examined for gill parasites. Four different gill parasites were identified namely: *Acanthocephala spp*, *Gyrodactylus spp*, *Procamallanus spp* and *Trichodina spp*. Out of the Eighty-One (81) *C. gariepinus* examined, 23 (61.67%) were infected and were observed to harbour One Hundred and Two (102) gill parasites. Percentage of Infection was highest in fish ranging from 41.0 – 50.0 cm (100%) while fish ranging from 20.0 - 31.0 cm had the least percentage of infection (23.80%). Highest infections (100%) were observed among the fish specimens that weighed between 410.0 - 500.0g. Fewer parasites (10%) were observed among the fish specimen that weighed between 210.0- 300.0g. while fish specimen that weighed between 100.0 — 200.0 g had intermediate numbers of parasites (32.72%). Percentage and Index of infection (31.11% and 16.17) were more significant in males than in females (30.56% and 15.27) respectively. Statistical relationship between the fish farm and gill parasites infestation was highly significant. High statistical relationship was also observed between gill parasite infestation, sex and size of *C. gariepinus*. The study concluded that there were gill parasites in *C. gariepinus* species from Sango in Ilorin. The study recommends regular treatment of cultures *Clarias gariepinus* fish for maximum yield, aquaculture health and general well-being.

Key words: Gill, parasites, *Clarias gariepinus*, fish farm and Ilorin.

INTRODUCTION

Fish is an important source of protein containing calcium, lipids, minerals, vitamins and oils (Udechukwu et al., 2018). Fish production through aquaculture has become the fastest and acceptable substitute to wild life (Agatha, 2012). The African catfish, *Clarias gariepinus* was described as one of the most important freshwater fish in Africa by Bishai and Khalil (1997). This African catfish are found in Africa, inhabiting tropical swamps, lakes and rivers (Olufemi, 1991). Clay (1979) considered *Clarias gariepinus* to be one of the omnivore that feed on aquatic insects, fish and higher plant debris. They can also feed on terrestrial insects, mollusk and fruit (Barton, 1979). *Clarias gariepinus* and *Clarias angularis* are the most common fish available in Nigeria (Udechukwu et al., 2018). The need for animal protein has brought tremendous increase in the development of fish farming (Bichi and Yelwa, 2010). However, like any other organism, fishes are also affected by parasites (Okanem et al., 2011). Fish are susceptible to the same types of agents that affects animals, including viruses, bacteria, fungi, and parasites (Abubakar, 2016). Parasite is an organism that lives in or on another organism at the expense of its host (Abubakar, 2016), an organism that lives in or on another organism at the expense of its host is a parasite. There are many different kinds of parasites- both internal and external ranging from very small (8/25,000 inch = 8 microns) to some that are 5-6 inches long. Most problems are caused by protozoans (single-cell animal) that live on the gills (Abubakar, 2016). In this view, protozoans are single-celled organisms, many of which are free-living in the aquatic environment. Typically, no intermediate host is required for the parasite to reproduce (direct life cycle). They can build up to very high numbers when fish are crowded, as it is common in culture systems, causing weight loss, debilitation, and mortality (Nnadi, 2012). Fish parasites cause damage to their host immune system thus increasing their susceptibility to secondary infections and subsequent economic losses (Onyedineke et al., 2010). Many protozoa can be identified in histological sections, but others can be detached from skin or gills (Abubakar, 2016).

Monogenetic trematodes as ectoparasites of fish often cause a variety of problems such as high mortalities and stressful conditions in fish leading to chronic dermatitis of their fins and body surface (Barker et al., 2002). Furthermore, the monogenean trematode has high affinity for the gill tissues used for gaseous exchange; damages to these tissues usually lead to asphyxiation and death (Tchokote and Olufemi, 2012). Abubakar (2016) emphasized that *Gyrodactylus cichlidarum* and *Dactylogyrus diplectanum* infect the fish in a big way; *Gyrodactylus* infects skin and gills while *Dactylogyrus* affects only the gills. Ayanda (2009) reported the occurrence of Cestodes; *Procamallanus* and *Paracamallanus* species of nematodes and *Neoechinor hynchus* species of acanthocephalans in wild and cultured *Clarias gariepinus* in Ilorin, North central Nigeria; while Hussein et al. (2012) reported *Capillaria*, *Contracaecum* and Cestodes in *Clarias gariepinus* caught from Lake Hawassa, Ethiopia.

Abubakar (2016) stated that the prevalence and magnitude of pathogenic infections in fishes are closely related to the sanitary condition prevalent in the water as well as the general health of the fishes themselves. However, occurrence of parasites in the gills of *Clarias gariepinus* may be as a result of pollutants in water or the health status of the fish which may affect the fish populations negatively (Abubakar et al., 2019). Therefore, the aim of this study was to investigate and examine the occurrence of gill parasite in *C. gariepinus* from some selected fish farms within Ilorin metropolis and to assess the effects on fish health.

This aim of the study was to investigate the occurrence and presence of gill parasites in *Clarias gariepinus* obtained from Sango fish farm in Ilorin, kwara State, Nigeria.

MATERIALS AND METHODS

The Study Area

The study took place in Ilorin South Local Government Area of Kwara State. Ilorin is located on latitude 8°30' and 8°50'N and longitude 4°20' and 4°35'E of the equator. Ilorin city occupies an area of about 468sqkm and it is situated in the transitional zone within the forest and the guinea savannah regions of Nigeria. It is about 300 kilometers away from Lagos and 500 kilometers away from Abuja the federal capital of Nigeria. The climate of Ilorin is tropical under the influence of the two trade winds prevailing over the country. Ilorin metropolis experiences two climatic seasons i.e. rainy and dry season. The rainy season is between March and November and the annual rainfall varies from 1000 mm to 1500 mm, with the peak between September and early October. Also, the mean monthly temperature is generally high throughout the year. The daily average temperatures are in January with 25 °C, May 27.5 °C and September 22.5 °C (Abubakar et al., 2019)

Sample Collection and Treatment

81 fresh samples of *Clarias gariepinus* with sizes ranging from 120g to 500g were purchased from Sango fish farm; an artificial fish farm in Ilorin South Local Government Area. Twenty-Seven (27) samples of *Clarias gariepinus* were obtained from the Sango fish farm fortnightly-three times-from March to April 2019. The fish were carefully harvested into sterile polythene containers and taken to the Biology laboratory unit of the Department of Zoology, University of Ilorin, Ilorin for cultural examination of the skin and gills.

Sex Determination

Sex determination of the fish samples were carried out using the method of Imam and Dewu, (2010). Examination of genital apparatus shows that male has a pointed genital papilla while the females has roundish opening.

Measurement of the Length and Weight of the fish samples

Measurement of the total and standard length of the fish samples were carried out adopting the method of Abubakar (2018) using a measuring board. The total length was taken as the length of the fish from the top of the snout to the end of the caudal fin while the standard length was taken as the distance between the top of the snout and the caudal peduncle. The weights of the fish samples were measured in gram with a weighing balance (Model: J180517660).

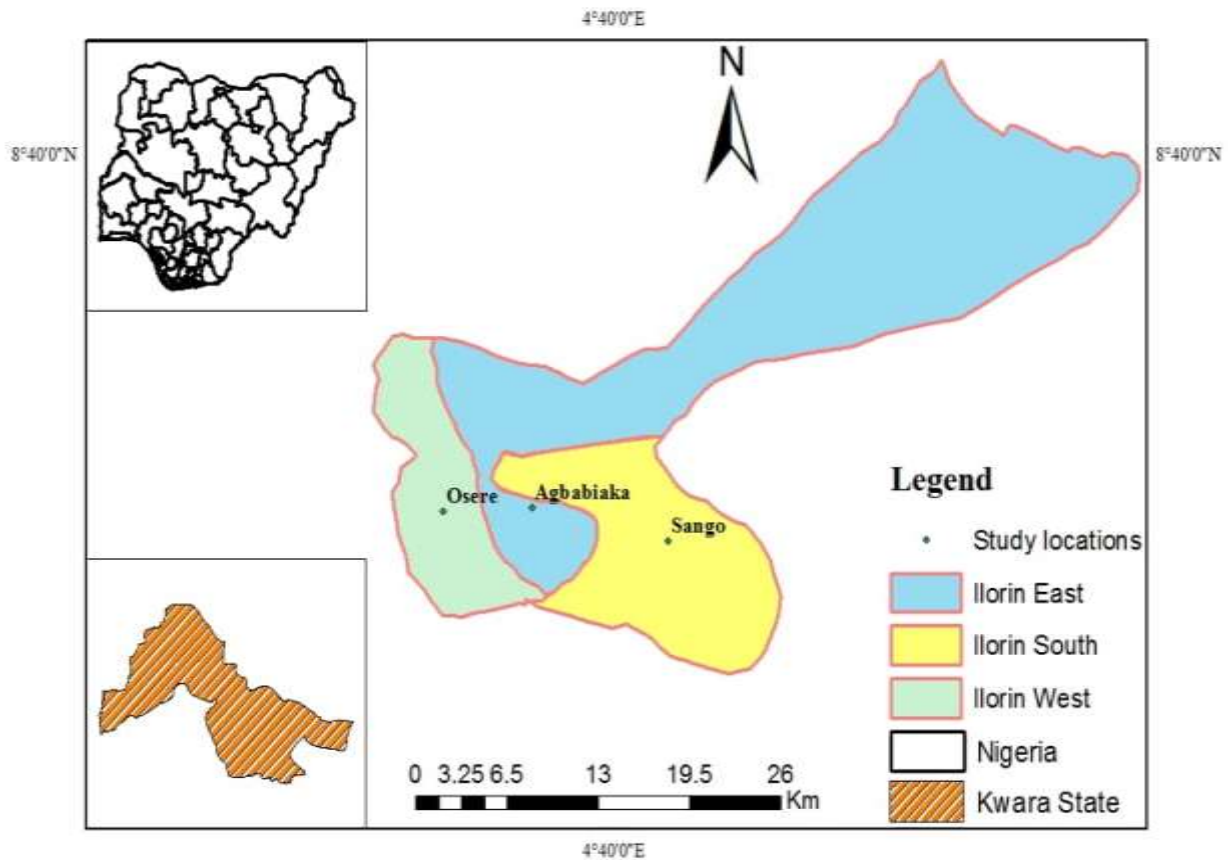


Figure 1: Map of Ilorin showing the site of fish collection (•)

Examination and Identification of Gill Parasites

The procedures for Gill parasites examination carried out were as described by various authors including Emere and Egbe (2006); Bichi and Ibrahim (2009) and Omeji, *et al* (2010). The fresh fish samples were dissected and examined under the stereoscopic microscope. Gill rakers were extended apart by a needle and were examined for parasites under stereoscopic microscope. The gills were cut with scissors, placed in a Petri-dish while gill filaments were dissected using anatomical needle and examined under the microscope. Gill scrapes were placed on glass slide; two drops of water were added. The glass slide was then covered with cover slide. They were examined under the microscope first on the low power objective (x10) followed by high objective (x40). Parasites were identified using the manual and pictorial guides of Mackiewicz and Beverly-Burton (1967), Paperna (1996), Noga (2000) and Pouder *et al.*, (2005) respectively. The pictures of parasites observed were taken using a digital camera and were later transferred into a laptop.

Statistical Analyses

The difference between sex, size and gill parasites were analyzed with one-way analyses of variance (ANOVA) and Duncan's multiple range test with a Statistical package program (SSPS version 16.0) for $p < 0.05$. Percentage of infection, density of infection, intensity of infection and index of infection were calculated using the method of Adeshnia *et al.*, (2016):

$$\text{Percentage of infection} = \frac{\text{infected sample}}{\text{Total sample examined}} \times 100 \quad (1)$$

$$\text{Density of infection} = \frac{\text{number of parasite collected}}{\text{Total sample examined}} \quad (2)$$

$$\text{Intensity of infection} = \frac{\text{number of parasite collected}}{\text{number of infected sample}} \quad (3)$$

$$\text{Index of infection} = \frac{\text{Number of infected sample} \times \text{number of parasite collected}}{\text{total sample examined}} \quad (4)$$

RESULTS

The results of gill parasites on *Clarias gariepinus* from Sango fish farm in Ilorin metropolis are present Table 1.

Gill Parasites infestation in relation to Sex in the fish farm from Sango

Table 1: Sex of *C. gariepinus* and associated gill parasites from Sango fish farm .

<i>C. gariepinus</i>	Female	Male
<i>C. gariepinus</i> examined	14	13
<i>C. gariepinus</i> infected	3	2
Parasite isolated	16	16
Percentage of infection (%)	21.42	15.38
Intensity of infection	5.33	8
Index of infection	3.46	2.46
Density of infection	1.14	1.23

Relationship between Sex of *C. gariepinus* and their gill infestation were not significant at $P > 0.05$.

The results from Table 1 showed that from 14 females and 13 males of *C. gariepinus* that were examined for gill parasites only 3 females (21.42%) and 2 males (15.38%) were infected respectively. The percentage and Index of infection were higher in females (21.42 and 3.46 respectively) than in males (15.38 and 2.46 respectively). However, intensity of the infection was higher in males (8.00) than in females (5.33).

There was no significant different in the infection rate of female and male fish at $p > 0.05$

Gill parasite infestation relative to Standard length of *C.gariepinus* obtained from Sango fish farm

Table 2: Standard length distribution and gill parasite infestation in *C. gariepinus* from Sango fish farm (Relative to standard length)

<i>C. gariepinus</i> 's size (cm)	20 – 30	31 – 40
<i>C. gariepinus</i> examined	22	5
<i>C. gariepinus</i> infected	2	3
Parasites isolated	11	21
Percentage of infection (%)	9.09	60.00
Intensity of infection	5.5	7
Index of infection	1	12.60
Density of infection	0.50	4.2

Relationship between gill infestation and standard length of *C. gariepinus* at $P > 0.05$

The results revealed that out of the 27 *C. gariepinus* examined based on standard length, only 5 were infested with gill parasites.

Gill parasite infestation relative to Total length of *C. gariepinus* obtained from Sango fish farm

Table 3: Total length distribution and gill parasite infestation in *C. gariepinus* from Sango fish farm (Relative to total length)

<i>C. gariepinus</i> 's size (cm)	20 – 30	31 – 40	41 – 50
<i>C. gariepinus</i> examined	13	11	3
<i>C. gariepinus</i> infected	1	1	3
Parasites isolated	3	8	21
Percentage of infection (%)	7.69	9.09	100
Intensity of infection	3	8	7
Index of infection	0.23	0.72	21
Density of infection	0.23	0.72	7

Relationship between gill infestation and Total length of *C. gariepinus* at $P > 0.05$

The results showed that out of the 27 *C. gariepinus* examined based on Total length, only 5 were also infected with gill parasites.

Gill parasite infestation relative to Total weight of *C. gariepinus* obtained from Sango fish farm

Table 4: Total weight distribution and gill parasite infestation in *C. gariepinus* from Sango fish farm (Relative to total weight)

<i>C. gariepinus</i> 's size (gm)	100 – 200	210 – 300	410 – 500
<i>C. gariepinus</i> examined	17	7	3
<i>C. gariepinus</i> infected	1	1	3
Parasites isolated	3	8	21
Percentage of infection (%)	5.88	14.28	100
Intensity of infection	3	8	7
Index of infection	0.17	1.14	21
Density of infection	0.17	1.14	7

Relationship between gill parasite infestation and Total weight of *C. gariepinus* at $P > 0.05$

The results indicated that from 27 *C. gariepinus* examined based on Total weight, only 5 was also infested with gill parasites.

Table 5: Occurrence of gill parasite relative to sex in *Clarias gariepinus* obtained from Sango fish farms

<i>C. gariepinus</i> 's sex	Female	Male	Total	P-value
<i>C. gariepinus</i> examined	36	45	81	
<i>C. gariepinus</i> infected	11	14	23	
Parasites isolated	50	52	102	
Percentage of infection (%)	30.56	31.11	61.67	0.00
Intensity of infection	4.54	3.71	8.25	0.00
Index of infection	15.27	16.17	31.44	0.00
Density of infection	1.38	1.15	2.53	0.21

Relationship between gill parasite infestation and sex of *C. gariepinus* at $P > 0.00$

The results revealed that out of 36 females and 45 males *C. gariepinus* that were examined for gill parasites, 11 females (30.56%) and 14 males (31.11%) were infested with gill parasites respectively. However, percentage and Index of infection were higher in males (31.11 and 16.17) than females (30.56 and 15.27) while intensity of the infection were higher in females (4.54) than males (3.71). There were statistically significant differences in the percentage of infection, intensity of infection and index of infection between females and males

Gill parasite infestation in relation to Standard length from Sango fish farm

Table 6: Standard length distribution and gill parasite infestation relative to P-value in *C. gariepinus* obtained from Sango fish farm

<i>C. gariepinus</i> 's size (cm)	20 – 30	31 – 40	41 – 50	P-Values
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<i>C. gariepinus</i> examined	42	34	5	
<i>C. gariepinus</i> infected	10	9	5	
Parasites isolated	40	42	25	
Percentage of infection (%)	23.80	26.47	100	0.00
Intensity of infection	4	4.67	5	0.76
Index of infection	9.25	11.11	25	0.02
Density of infection	0.95	1.23	5	0.04

Relationship between gill parasite infestation and standard length of *C. gariepinus* relative to P-Value.

The results showed that out of 81 *C. gariepinus* that were examined on Standard length: 20 – 30, 31 – 40 and 41 – 50cm, only 24 were infected. There were statistical differences in percentage, intensity, index and density of infection at $p \leq 0.00$, $p \leq 0.76$, $p \leq 0.02$ and $p \leq 0.04$ respectively

Gill parasite infestation in relation to Total length from Sango fish farm

Table 7: Total length distribution and gill parasite infestation relative to P-Value in *C. gariepinus* obtained from Sango fish farm

<i>C. gariepinus</i> 's size (cm)	20 – 30	31 – 40	41 – 50	P-Values
<i>C. gariepinus</i> examined	44	32	5	
<i>C. gariepinus</i> infected	10	9	5	
Parasites isolated	40	42	26	
Percentage of infection (%)	22.72	28.12	100	0.19
Intensity of infection	4	4.67	5.20	0.65
Index of infection	9.09	11.81	26.00	0.22
Density of infection	0.90	1.31	5.20	0.02

Relationship between gill parasite infestation and Total length of *C. gariepinus* relative to P-Value.

The results showed that out of 81 *C. gariepinus* that were examined on Total length: 20 – 30, 31 – 40 and 41 – 50cm, only 24 were infected. There were statistical differences in percentage, intensity, index and density of infection at $p \leq 0.19$, $p \leq 0.65$, $p \leq 0.22$ and $p \leq 0.02$ respectively

Gill parasite infestation in relation to Total weight from Sango fish farms

Table 8: Total weight distribution and gill parasite infestation relative to P-Value in *C. gariepinus* obtained from Sango fish farm

<i>C. gariepinus</i> 's size (g)	100 – 200	210 – 300	410 – 500	P-Values
<i>C. gariepinus</i> examined	55	20	6	
<i>C. gariepinus</i> infected	18	2	6	
Parasites isolated	66	15	27	
Percentage of infection (%)	32.72	10	100	0.01
Intensity of infection	3.67	7.5	4.5	0.16
Index of infection	21.6	1.5	27	0.00

Density of infection	1.2	0.75	4.5	0.10
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Table 8 showed that 81 fish were examined based on their Total weight ranging from 100 – 200, 210 – 300 and 410 – 500g out of which only 26 were infected. There were statistically significant differences in the percentage and index of infection between the various sizes ($p \leq 0.01$ and $p \leq 0.00$ respectively) while the intensity and density of infection was not statistically significant at $P \geq 0.16$ and 0.10 respectively)

Gill parasites isolated from *C. gariepinus* obtained from Sango fish farm

Table 9: Gill parasite isolated from *C. gariepinus* from Sango fish farm

Gill parasite species isolated	No of <i>C. gariepinus</i> examined	No of <i>C.gariepinus</i> infected	No. of parasites
<i>Trichodina spp</i> 13.58	81	11	73
<i>Procamallanus spp</i> 4.93	81	4	9
<i>Gyrodactylus spp</i> 2.46	81	2	6
<i>Acanthocephala spp</i> 9.87	81	8	16
Nematodes 2.46	81	2	3

Table 9 shows that out of the 81 *C. gariepinus* examined, 11(13.58%), 4(4.93%), 2(2.46%), 8(9.87) and 2(2.46%), were infected with *Trichodina spp*, *Procamallanus spp*, *Gyrodactylus spp*, *Acanthocephala spp* and Nematodes respectively. The total number of *Trichodina spp*, *Procamallanus spp*, *Gyrodactylus spp*, *Acanthocephala spp* and Nematodes recovered from infected fish were 73, 9, 6, 16 and 3 respectively.

Photomicrographs of isolated gill parasites from *Clarias gariepinus* are shown in Plates 1, 2, 3, 4 and 5.



Plate 1: Photomicrograph of *Acanthocephalan* spp from scraped gill of *C. gariepinus* (MG x40)



Plate 2: Photomicrograph of *Gyrodactylus spp* attached to the gill of *Clarias gariepinus* (MG x 100)



Plate 3: Photomicrograph of Nematode from scraped gill of *C. gariepinus* (MG x40).



Plate 3: Photomicrograph of *Procamlanus* spp attached to the gill of *Clarias gariepinus* (MG x40)



Plate 4: Photomicrograph of *Trichodina* spp from gill of *Clarias gariepinus* (MG x20)

DISCUSSION

A total of four gill parasites were isolated (*Acanthocephalan spp*, *Gyrodactylus spp*, *Trichodina spp* and *Procamallanus spp*) from infected *Clarias gariepinus* from Sango fish farm in Ilorin. This result is similar to the works of Shotter (1977) and Aken'Ova (1999) who reported the occurrence and isolations of gill parasites from infected fish samples in Northern Nigeria. The removal of *Gyrodactylus spp* from the infected fish's skin and gill agreed with the observation of Abubakar, (2016). Oniye et al. (2004) also reported occurrence of gill parasites from some infected fish samples in Zaria. Yakubu et al. (2002) documented occurrence of gill parasites in infected fish in Jos. Iyanda (2009) also isolated gill parasites from some infected fish samples in Ilorin.

Majority of the parasites which are protozoans cause numerous external and internal diseases on the affected fish. This is in agreement with the work of Abubakar (2016) who observed large scale spoilage of fish by *Gyrodactylus* in a Minna fish farm. The descriptions and culture of the parasites agreed with those given by Noga (1995), Paperna (1969) and Thurston (1970).

The high percentage of *Trichodina spp* isolated from gills of *C.gariepinus* in this study might be due to its convergent habitation and bluish coating of the water which agreed with the work of Obiekezie and Ekanem (1995) who reported that *Trichodina spp* are parasites of cultured *Clarias* species in Southern part of Nigeria. Van As and Basson (1992) documented information on infection of *Trichodina spp* on Clariidae in Africa. Paperna (1980) described *Trichodina indica* as a parasite of fish that feeds on its host gill tissue. Obiekezie and Ekanem (1995) reported lesions accompanied with cellular invasion on gill tissues of *Heterobranchus longifilis* infected with *Trichodina maritinkae*. Paperna (1980) observed morbid epizootics with severe losses due to *Trichodina* infestation among newly hatched fry of channel catfish in warm-water ponds in Thailand. The fading colour and excess mucus on caudal penduncle of the infected fish were associated with the presence of *Gyrodactylus spp* which were isolated from the fish's gill. This might be due to their free access to fish gill filaments. This agreed with the report of Abubakar (2016). Obiekezie and Taege (1991) observed severe mortality in a population of *C. gariepinus* infected with *Gyrodactylus groschafti*. Peggy et al., (2009) stated that *Gyrodactylus* species generally preferred the gill filaments. *Acanthocephalan* isolated from the infected fish might be due to the presence of an infected fish from the wild that has contacted the water. Paperna (1996) associated *Acanthocephalans* with epithelial mucosa with damage proportional to the depth of penetration of the proboscis. *Procamallanus spp* isolated from the infected fish might also be due to their highly equipped mouth. Oniye et al. (2004) reported that *Procamallanus laevionchus* is equipped with an alimentary canal with which it roams around the intestine tract and graze on nutrients. Onwuliri and Mgbemen (1987) stated that *Procamallanus laevionchus* is widely distributed in several fish hosts in Russia, Europe and Africa. Paperna (1996) reviewed that *Clarias gariepinus* species were infected by gill parasites because their gills are continuously exposed to any potential infective stages of parasites in water that infect their gills directly. Aken'Ova (1999) placed the occurrence of parasites on availability of infective stages of the gill parasites.

The overall percentage of gill parasites infestation in this study was high (61.67%) which can be attributed to the total spoilage and bluish colour of the water which is in contrast with the work of Oniye et al, (2004) who recorded 19.17%. Bich and Yelwa, (2010) recorded 17.7% in Bagauda fish farm Kano. Emere and Egbe (2006) reported prevalence of 12.17% in river Kaduna. However, the findings of this study is similar with the work of Anosike et al., (1992)

who recorded 52.0% and Onwuliri and Mgbemena (1987) that documented 59.8% in cultured populations of *C. gariepinus*. Williams and Jones (1994) noted that parasitism varies from one aquatic ecosystem to the other which is influenced by the interplay of mixed biotic and abiotic factors. The high prevalence of parasites in this study is due to increase in contact between the host and the parasite in the culture system due to overcrowding of the fish in the pond. This is in agreement with the work of Anosike *et al.* (1992).

The results in size (weight and length) of *Clarias gariepinus* in this study in which the percentage of infection increased with increase in size is similar to the observations made by Iyanda (2009) and Olurin and Samorin, (2006) that the longer and heavier the fish, the greater the susceptibility to parasite infection. This is in agreement with the works of Omeji, *et al.*, (2010) who reported higher rate of protozoan parasites in bigger *C. gariepinus* and *Heterobranchus longifilis* than the smaller ones and Emere and Egbe (2006) who also reported higher rate of protozoan parasites in bigger *Synodontis clarias* than the smaller ones. These observations could be attributed to the fact that bigger fish provides larger surface area for the infection to multiply in numbers than in smaller ones; also changes in diet from phytoplankton and zooplankton to insects, larvae, snails, worms and crustaceans for food, smaller fishes grow into bigger ones (Obano *et al.*, 2010). This is in contrast with the work of Salawu *et al.*, (2013) who reported no significant prevalence difference among the host size in relation to helminth parasites recorded from Ogun River and Asejire Dam fish. Lagler *et al.* (1979) reported correlation between parasitic infection and fish length which also corresponds to fish age. Akinsanya *et al.* (2007) attributed this to the random selection and the low level of immunity in the smaller sized fish. Differences in the percentage of gill parasite infestation observed between the male and female *C. gariepinus* species may be due to differential feeding quantity and quality. Similar observation was made by Emere (2000). Higher percentage of infestation recorded in male *C. gariepinus* than in the females might be due to the fact that male *C. gariepinus* are known to engage in more physical activities such as swimming and can also explore available food resources that can easily be parasitized than in the females which might expose them to more parasitic infestation than in their female male *C. gariepinus* counterparts (Abubakar, 2018). This is contrary to the findings of Iyanda, (2009) who reported higher parasitic infestation in female *C. gariepinus* than in the male *C. gariepinus* as a result of their quest for survival. Omeji, *et al.*, (2010) and Emere and Egbe (2006) reported that due to physiological state of the female *C. gariepinus*, most gravid females could have reduced resistance to infestation by parasites. High significant difference in gill parasite infestation between male and female *C. gariepinus* recorded in this study is in agreement with finding of Iman and Dewu (2010) who reported significant difference in parasitic infestations between the sexes of *Clarias* species sold at Galadima Fish Market, Kano Metropolis.

CONCLUSION

The study concluded that there were gill parasites in *C. gariepinus* species from Sango fish farm in Ilorin.

RECOMMENDATIONS

The study recommends regular treatment of cultures *Clarias gariepinus* fish for maximum yield, aquaculture health and general well-being.

REFERENCES

Abubakar M. I. (2016). Fish Pathology in perspective. First published (2016) Boga Press Ibadan, Oyo State, Nigeria, ISBN-978-978-54270-9-7 Pp: 91-127

Abubakar, M.I. (2018): Effect of sublethal concentration of sniper 1000EC on weight and length growth of *Oreochromis niloticus* obtained from Asa dam under laboratory conditions. Centrepoint: Science Edition. 24(2); 21-38, [http:// www.unilorin.edu.ng/centrepoin](http://www.unilorin.edu.ng/centrepoin)

Abubakar, M.I., Adeshina, I., Ayelaja, A.A. & Abdulraheem, I. (2019): Induction of Normocytic-Normochromic Anaemia in *Clarias gariepinus* (Burchell, 1822) exposed to acute concentration of 1,1-dimethyl 4,4-bipiridillium (Paraquat) under laboratory conditions. *FuLafia Journal of Science and Technology* 5 (2); 46-52,

Abubakar, M.I., Adeshina, I., Abdullahi, A.M., Hamza, F.O., Abdulraheem, I & Musa, A.M. (2019): Occurrence of Fungi in Frozen Titus (*Scomberscombrus*) sold in some areas in Ilorin Metropolis. *Journal of Agriculture and Environment* 15(2); 117-131, Published by Faculty of Agriculture, Usman Danfodiyo University, Sokoto. <http://jae.udusok.edu.ng/index.php>.

Agatha A. Nwabueze (2012). Disease Status of *Clarias gariepinus* (Burchell, 1822) and some fish ponds in Asaba, Nigeria Department of Fisheries, Delta State University, Asaba Campus, Asaba. Email: arimiche@yahoo.com *Int'l Journal Of Agric. And Rural Dev. Saat Futo 2012 Volume 15 (3):1216-1222, 2012 Page 1216*

Aken'Ova, T. O. L. (1999). Helminth infection of the gills of *Clarias* species in Zaria. *The Nigerian Journal of Parasitology*, 20: 113-121

Akinsanya B. Hassan A. A, Otubanjo OA (2007). A comparative study of the parasitic helminth fauna of *Gymnarchus niloticus* (Gymnarchidae) and *Heterotis niloticus* (Osteoglossidae) from Lekki Lagoon Lagos, Nigeria. *Pakistan J. Biol. Sci.* 10(3):427-432.

Anosike, J. C. , Omoregie, E. , Ofojekwu, P. C. and Nweke, I. E. (1992). A survey of helminth parasites of *Clarias gariepinus* in Plateau state, Nigeria. *Journal of Aquatic Sciences* 7:39 — 43.

Barker, D.; Cone D. and M. Burt (2002): *Trichodinamurmanica* (Ciliophora) and *Gyrodactylus pleuronecti* (Monogenea) parasitizing hatchery reared winter flounder, *Pseudopleuronectes americanus* (Walbaum): effects on host growth and assessment of parasite interaction. *Journal of fish diseases*, vol. 25, 81-89.

Bishai, H.M., Khalil M.T. (1997). Freshwater Fishes of Egypt. *Publication of the National Biodiversity Unit No. 9, Department of Natural Protectorate, Egyptian Environmental Affairs Agency, Egypt*. Bichi, A. H and Ibrahim, A.A (2009). A survey of ecto and intestinal parasites of

tilapia zillii (gervias) in tiga lake, Kano, Northern Nigeria. *Bayero Journal of Pure and Applied Sciences*, 2(1): 79 – 82

Bichi, A.H. and Yelwa, S.I. (2010). Incidence Of Piscine Parasites On The Gills And Gastrointestinal Tract of *Clarias Gariepinus* (Teugels) At Bagauda Fish Farm, Kano. *Bayero Journal of Pure and Applied Sciences*, 3(1): 104 - 107 Received: January, 2010 Accepted: March, 2010

Bruton, M. N. (1979). The food and feeding behaviour of *Clariasgariepinus* species: Claridae in lake Sibaya, South Africa, with its emphasis on its role as predator of cichlids. *Trans Zool. Soc.London*: vol. 35, 47-114. *International, Wallingford, UK*, pp 391-416.

Clay, D. (1979). Population biology, growth and feeding of the African catfish *Clariasgariepinus* with special reference to juveniles and their importance in fish culture. *Arch. Hydrobiol*, vol. 87, 453-482

Nnadi, E. I. (2012). A Study of Parasitic Infections of *Clariasgariepinus* In Natural Waters of Owerri, Imo State, Nigeria. *Int'l Journal Of Agric. and rural dev. saatfuto 2012 volume 15 (2): 976 – 981, 2012 page 976*

Emere, M. C. and Egbe, N. E. L. (2006). Protozoan Parasites of *Clarias spp* (A freshwater fish). *Best Journal* 3(3): 58-64.

Hussein, A., Tefera, M. and Astrate, S. (2012). Gastrointestinal helminth parasites of *Clarias gariepinus* (catfish) in Lake Hawassa, Ethiopia. *Scientific Journal of Animal Science* 1(4):131 – 136.

Imam T.S and Dewu RA, (2010). Survey of Piscine ecto and intestinal parasites of *Clarias* species sold at Galadima road fish market, Kano metropolis, Nigeria. *BioscienceResearch communication*. 2010; 22(4): 209pp.

Iyanda, O.I. (2009). Comparative parasitic helminth infection between cultured and wild species of *Clarias gariepinus* in Ilorin, North Central Nigeria. *Scientific Research and Essay*, 4(1): 18-21.

Kwara state government (2017). <https://kwarastate.gov.ng/geography/>

Lagler KF, Bardachi JE, Miller RR (1979). *Ichthyology*. John Wiley, New York. p. 882

Mackiewicz, J. and Beverley-Burton, M. (1967) Monobothrioides woodland (Cestoidea: Caryophyllidea) from *Clarias mellandi* Boulenger (Cypriniformes: Clariidae) in Zambia, Africa. *Proceedings of the Helminthological Society of Washington*, 34(2): 125-128.

Micha, J. C. (1973). *Eludedis populations piscicoles de I. vbanguiettentatives de selection et d'adaptators de quelques especes a l'etany de piscicultue-Nogent-san-Marine, Centre Technique Forestier Tropical*. 110.

- Noga, E. J. (1995). Fish Disease: diagnosis and treatment. Mosby St. Louis, Missouri. 367pp
- Obano, E.E., Ezeri, G.N.O. and Aniyie, UK. (2010). Studies on the parasitic infections in fishes in Ovia River at Ikoro, Ovia-South West Local Government Area of Edo State, Nigeria. Bioscience Research Communications. 22(3):137.
- Obiekezic, A. 1., and Tacge, N. 1. (1991): Mortality in hatchery reared fry of the African Catfish *Clarias gariepinus* (Burchell) caused by *Gyrodactylus Groschafti* Ergcns, 1973. Dull. Em. Ass. Fish Pathol. 11(2), 82-8
- Obiekczie, R. and Ekanem, D. (1995): Experimental infection or *Heterobranchus longifilis* (Tclcostei. Clariidae) with '*Irishodina maritinkae* (Ciliophora, Peritrichida). Aquat, LIVING Resource, 439-44
- Okaeme., A. N., Obiekezie, A . I., Rehmen., J. and Mark, O. A. (1998). Parasites and diseases of cultured fish of lake Kainji area Nigeria. Fish Biology Journal 32: 479- 481.
- Olufemi, B. E. A. D. a. A. S. (1991): Aerobic bacterial pathogens isolated from the African catfish *Clarias gariepinus*. Trop. Vet., 9, 177-180.
- Omeji, S. Solomon, S.G and Obande, R.A. (2010). A comparative study of the common protozoan parasites of *Heterobranchus longifilis* from the wild and pond environments in Benue State. Pakistan Journal of Nutrition 9 (9): 865-872.
- Omeji, S., Solomon, S. G. and Idoga, E. S. (2011). Study of the common Protozoan Parasites of *Clarias gariepinus* from Wild and Cultured Environments in Benue State, Nigeria. *Journal of Parasitology Research*, 2: 1-8.
- Oniye, S.J., Adebote, D.A and Ayanda O. I. (2004). Helminth parasites of *Clarias gariepinus* in Zaria, Nigeria. *Journal of Aquatic. Sciences*, 19(2):71-76.
- Onyedineke NE, Obi U, Ofoegbu PU, Ukogo I (2010). Helminth Parasites of some Freshwater Fish from River Niger at Illushi, Edo State, Nigeria. *Journal of Animal. Science*. 6(3): 16-21
- Onwuliri, C. O. E. and Mgbemena, M. O. (1987). The parasitic fauna of some fresh water fish from Jos Plateau, Nigeria, *Nigerian Journal of Applied Fisheries and Hydrobiology* 2: 33-37
- Paperna, I. (1996) Monogenetic trematodes of fish of the volta basin and South Ghana *bulletin de l'Institut fundamental d'Afrique Noir*, 31:840-880.
- Paperna, L (1980): Parasites, Infections and Diseases of Fish in Africa. CIFA Tecb. Paper No 7. 216
- Peggy, R., Frank, R. and Kenneth, R. E. (2009). A series of fisheries and Aquatic Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. 10pp.

Pouder, D. B. Curtis, E. W. and Yanong, R. P. E, (2005). Common, Freshwater Parasite Pictorial Guide Edis (online) [Http://edis.ifas.ufl.edu](http://edis.ifas.ufl.edu).

Salawu M. T.1, Morenikeji O. A. Sowunmi A. A and Odaibo A. B (2013). Comparative survey of helminth parasites of *Clarias gariepinus* (Burchell, 1822) and *Clarias pachynema* (Boulenger, 1903) from the Ogun River and Asejire Dam in south-west Nigeria. *International Journal of Fisheries and Aquaculture* Vol. 5(1), pp. 7-11, <http://www.academicjournals.org/IJFA> DOI: 10.5897/IJFA12.072 ISSN 2006-9839

Shotter, R. A. (1977). Copepod parasites of fishes from Northern Nigeria. *Bulletin de l'Institut Fondamental de l'Afrique Noire, Ser A*, 39 (3): 583-600.

Tchokote E. Y. and Olufemi B. E. (2012) The monogenean trematode, *Gyrodactylus*, a major constraint to african catfish (*clarias gariepinus*), production: a case study of small scale fish farms in Ibadan, Nigeria. Dept Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria Dept Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria Bull. Anim. Hlth. Prod. Afr., (2012), 60, 57-62

Thurston, J. (1970). The Incidence of Monogenea and Parasitic Crustacea on the gills of Fish in Uganda. *Revue de Zoologie et de Botanique Africaine*, 82:111-130

VanAs, 1. G. and Basson, L. (1992): Trichodinid ectoparasites (Ciliophora: Peritrichida) of freshwater fishes of the Zambezi River system, with a reappraisal of host specificity. *System, Parasitol.* 22, 81-10

Williams, H. and Jones, A. (1994). Parasitic worms of fish. Taylor and Francis, Bristol, UK, 593pp.

Yakubu, D. P., Omoregie, E., and Wade, J. W. (2002). A Comparative study of gut helminths of *Tilapia zilli* and *Clarias gariepinus* from River Uke, Plateau State, Nigeria. *Journal of Aquatic Sciences*, 17(2):137-139

TOXICOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF ETHANOL EXTRACT OF *CLERODENDRUM VOLUBILE* IN RODENTS

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ABSTRACT

Clerodendrum volubile is commonly known and consumed among other leafy vegetables in south-west and south-south Nigeria. This study was designed to evaluate the toxicological, biochemical and histopathological effects of *Clerodendrum volubile* ethanol extract in rodents. The toxicity profile of the ethanol extract of *Clerodendrum volubile* by determining its effects after acute and sub-chronic oral administration in mice and rats respectively, using standard methods. No death and signs of toxicity and no negative behavioral changes were observed in the extract at 5000 mg/kg in mice during the acute toxicity test. In rats, single oral alternate daily doses of *Clerodendrum volubile* extract (250, 500 and 1000 mg/kg), given for 30 days, resulted in increased body weights in treated rats compared to controls. No significant changes in organ weights were observed. There was no significant difference in the plasma activity of ALT and ALP in the treated rats compared with the control group. However, AST levels were significantly lower in the higher dose levels (500 and 1000 mg/kg) compared with the control group. The administration of the extract did not alter significantly AST and ALT activities in the liver. Kidney uric acid and creatinine concentrations revealed the extract had no deleterious effect on the kidney. No treatment-related histoarchitectural changes were observed in the liver and kidney. *Clerodendrum volubile* is safe for human consumption. However, it is advisable to apply some caution with its chronic use.

Keywords: *Clerodendrum volubile*, liver enzymes, kidney parameters, sub-chronic toxicity

INTRODUCTION

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Okigbo *et al.*, 2008). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Medicinal plants are still an indispensable source of medicaments in the contemporary health care delivery system because they are still being used for both conventional and traditional medicine. Examples of conventional medicines developed or derived from plants include aspirin (first obtained from willow), Paclitaxel (extracted from yew) and digitoxin obtained from foxglove (Newman and Cragg, 2007). Other outstanding drugs which have been developed from the ethnomedicinal uses of plants include vinblastine and vincristine from *Catharanthus roseus* (the periwinkle). Vinblastine is used for the treatment of Hodgkins, choriocarcinoma, non-hodgkins lymphomas, leukemia in children, testicular and neck cancer while vincristine is recommended for acute lymphocytic leukemia in childhood advanced stages of Hodgkins, lymphosarcoma, cervical and breast cancer (Barrales-Cureño, 2015). Additionally, reserpine from *Rauwolfia serpentina* (Indian snake root) is used for treating hypertension, aspirin from *Salix purpurea* (willow) is used for treating inflammation, pain and thrombosis and quinine from *Cinchona pubescens* (cinchona) used for treating malaria (Williams, 2006). Currently used modern drugs such as quinine, artemisinin derivatives, digoxin glycosides, tubocurarine, aspirin etc. are good examples of drugs from plant medicine. Medicinal plants in Africa are used to cure common ailments like malaria, fever, headache, burns, ulcer, cough, snake and scorpion poison, rheumatism, infertility etc. Scientific evaluation and validation of traditional medicinal plants is however, essential for their possible utilization or in elimination of harmful practices (Makonnen et al., 2003).

Clerodendrum volubile is commonly known among other leafy vegetables in South west and South-south Nigeria, because of its nutritional values. It is commonly known among the Urhobo and Itsekiri tribes of the Niger-Delta as Obenetete and among the Yoruba people of the south-west Nigeria as Dagba. It is a climbing shrub reaching up to 3 m high, with inflorescences of numerous flowers, (1½ cm long), greenish-white, yellow colouration plant spread in the deciduous forest and secondary jungle, across the West African region, from Senegal to Fernando Po (Erukainure et al., 2011).

Clerodendrum volubile has been used in the treatment of different forms of ailments such as arthritis, rheumatism, dropsy, swellings, oedema and gout. It is also used as analgesic, pregnancy tonic, anti-abortion, sedative and to aid labour in pregnant women (Burkill, 1985; Erukainure *et al.*, 2011a, b; Olanrewaju *et al.*, 2018). This plant has a reputable usage in traditional folkloric treatment of ailments especially in Southern Nigeria. It is also used as spices or cooked with other vegetables such as *Telfaria occidentalis* and *Amaranthus* (Adefegha and Oboh, 2011; Happy, 2016). The management of some neurodegenerative diseases with *C.volubile* has been reported in folklore medicine (Oboh et al., 2017).

The most extensive study on *C. volubile* is its action on diabetes (Molehin, *et al*; 2018, Molehin and Oloyede 2018 and Erukainure, *et al*; 2018). It also elicited wide range of biological activities such as antidiabetic, antihyperlipidemic, anti-hypertension, antioxidant, anticancer, anti-inflammatory, hepatoprotective, antimicrobial, and analgesic. (Ajao *et al.*, 2018). Furthermore, this plant is regarded as an underutilized indigenous vegetable of utmost nutritive and pharmacological importance (Ajao *et al.*, 2018). Although the acute toxicity and sub-acute toxicity of aqueous extract of *C. volubile* leaves have been reported (Akinpelu *et al.*, 2016; Molehin and Oloyede, 2018), the scientific information on its toxicity profile is not adequate considering its extent of use and relevance in folkloric medicine. Thus, the evaluation of the safety of short and long term consumption of *C. volubile* is necessary. This study was therefore designed to investigate the toxicological and biochemical indices of *C. volubile* ethanol extract after sub-chronic administration in albino rats to predict the safety of its consumption in inflammatory and chronic conditions.

METHODS

Plant material and authentication

Fresh *Clerodendrum volubile* leaves were collected from Ogudu in Ife East Local Government Area of Osun State, Nigeria. The plant was identified and authenticated at Department of Botany Herbarium, Obafemi Awolowo University, Ile-Ife, Nigeria. A voucher specimen (Ife-7673) was deposited in the herbarium. The leaves were air-dried for twenty one (21) days at room temperature.

Preparation of extract for administration

Five hundred gram (500 g) of *Clerodendrum volubile* leaves was extracted by maceration with 70 % ethanol in ratio of 1:5. The mixture was filtered using muslin cloth followed by Whatman filter paper. The resultant filtrate was evaporated to dryness under reduced pressure using a rotary evaporator at 40⁰ C to give a dark brown extract. The resulting extract was put in desiccators containing a self-indicating silica gel. The drug was prepared by constituting the plants extracts in distilled water.

Experimental animals

Albino Wistar rats (20) of both sexes weighing between 150-200 g and fifteen albino mice weighing between 18- 22 g were used for toxicology studies. They were obtained from Animal House Department of Pharmacology, Obafemi Awolowo University, Ile-Ife, Osun State and they were given standard feed (Bendel flour mills, broilers mash) and water *ad libitum*. They were allowed to acclimatize with the environment for a period of two weeks. All animal experiments complied with the Guide for the Care and Use of Laboratory Animals and the 'Principles of Laboratory Animal Care' (NIH publication No. 82-83, revised in 2010).

Acute toxicity (LD₅₀ determination)

Acute toxicity study of the leaves of *Clerodendrum volubile* was determined using the method of Lorke, (1983). The study was carried out in two phases, in the first phase, nine mice according to their body weight were divided into three groups of three mice per group, each group was given 10, 100 and 1000 mg extract/kg body weight orally (via a cannula), respectively. The mice were observed for signs of toxicity and mortality for 24 h and then for 14 days. From the results of the phase one study, the procedure was repeated using another set of six mice divided into three groups of two mice each, given 1600, 2900 and 5000 mg extract/kg body weight respectively. These mice were also observed for signs of toxicity which include but not limited to paw-licking, salivation, stretching, rubbing of nose on the floor and on wall of cage and death for the first critical four hours and there after daily for fourteen days. The oral median lethal dose was calculated as the geometric mean of doses that caused 0 and 100% mortality respectively.

Evaluation of Sub-chronic toxicity

Sub-chronic toxicity study was evaluated in Wistar rats using the method of Salawu *et al.*, 2010. Twenty rats of either sex were divided into four groups of five rats each, based on their body weight. The extract was orally administered every other day for 30 days. Group one which served as the control received distilled water, while Groups two, three and four were given 250, 500 and 1000 mg/kg of the extract respectively. The body weights were recorded at the beginning, then once every three days during the study. Water and food consumption were noted daily and recorded throughout the experimental period. At the end of the treatment period, blood samples were collected by ocular puncture for biochemical analyses before the animals were sacrificed by cervical dislocation 24 h after the last dose. Also liver and kidney were harvested for biochemical and histopathological analyses.

Biochemical Analyses

Blood samples from each animal, collected into lithium heparin bottles, were centrifuged at 3000 rpm for 15 min in Denley BS-400 centrifuge at room temperature. The plasma samples were collected into sterile sample bottles and kept in the freezer at -4 °C for further analyses.

One gram (1 g) of liver was homogenized in 10 ml of 0.01mM phosphate buffer with pKa of 6.86 and pH 7.2 and centrifuged at 4000 rpm for 15 min at room temperature. The supernatant was collected into clean sterile bottles, labeled and kept in the freezer at -4 °C for further analysis.

Enzyme assays

Determination of Alanine aminotransferase activity (ALT) and Aspartate transaminase activity (AST)

The plasma and liver homogenate alanine aminotransferase activity and aspartate aminotransferase activity were estimated as described by Reitman and Frankel (1957) using the Randox kit. The assay mixture consisted of plasma each (0.1 ml) and 0.5 ml R1 buffer (phosphate buffer, L-aspartate and α -oxoglutarate). The mixture was incubated at 37⁰ in a water bath for exactly 30 min. The reaction was terminated by adding 0.5 ml of solution R2 (2, 4-dinitrophenylhydrazine) and allowed to stand for 20 min at room temperature. The colour was

developed by adding 0.4 mol/l NaOH (5.0 ml) and allowed to stand for 5 min; absorbance was read at 546 nm. The reagent blank was prepared similarly but contained 0.1 ml distilled water in place of plasma or liver homogenate in the assay mixture.

Determination of Alkaline Phosphatase Assay (ALP)

Alkaline phosphatase (ALP) in plasma and liver homogenates was estimated using assay kits obtained from Randox Laboratories Limited, Antrim, United Kingdom.

Estimation of plasma total bilirubin

The plasma and liver total bilirubin was estimated as described by Jendrassik and Grof (1938).

The assay mixture contain R1 (Sulphanilic acid and Hydrochloric acid) 200 µl, R2 (Sodium nitrite) 50 µl, R3 (Caffeine and Sodium benzoate) 1000 µl, plasma each 200 µl. The mixture was incubated for 10 min at room temperature. The colour was developed by the addition of reagent R4 (Tartrate and Sodium hydroxide) 1000 µl. The mixture was further incubated for 5-30 min. The absorbance was read at 578 nm. The blank was prepared similarly but did not contain R2 (sodium nitrite) as contain in the assay mixture.

Estimation of total protein concentration in the plasma and liver homogenate.

The total protein concentration in plasma and liver was estimated by Biuret method as modified by Randox Laboratories (United Kingdom).

Estimation of kidney function indices

Plasma and kidney creatinine

The plasma and kidney creatinine activities were estimated as described by Bartels and Bohmer, (1972) using the Randox kit.

Plasma uric acid

The plasma uric acid activities was estimated as described by Fossati *et al.*, (1980) using the Randox kit.

Histopathological analysis

After rats were sacrificed, their liver and kidney were carefully excised and weighed. Sections were immediately taken from the organs and fixed in 10% neutral buffered formalin. Tissue sections were stained with hematoxylin and eosin (H & E) for light microscopic examination.

Statistical analysis

Data were expressed as mean $\pm$ SEM. Statistical analysis was performed by one way ANOVA followed by Turkey-Kramer multiple comparison test to ascertain differences between treatment groups. All analysis was performed using Graph Pad InStat 3 (version 1.1, 2007). Differences were considered to be significant if $p \leq 0.05$.

RESULTS

Acute toxicity study

Table 1: Acute toxicity study of *C. volubile* ethanol extract in mice

Groups	Dose(mg/kg)	No. of Dead rats	% Mortality
Stage1			
1	10	0/3	0.00
2	100	0/3	0.00
3	1000	0/3	0.00
Stage 2			
1	1600	0/2	0.00
2	2900	0/2	0.00
3	5000	0/2	0.00

No death and signs of toxicity were observed in the ethanol extract at 5000 mg/kg (Table 1). Acute toxicity testing at 5000 mg/kg body weight of ethanol extract of *C. volubile* did not produced any gross negative behavioral changes such as excitement, restlessness, convulsions and coma in the treated mice.

Sub-chronic toxicity study of *C. volubile* ethanol extract in rats**Table 2:** Effect of sub-chronic administration of *Clerodendrum volubile* ethanol extract on body weight of rats

Groups	Doses(mg/kg)	Body weight(g) before treatment	Body weight (g) after treatment
Control	-	125.30±5.94	152.96±3.35
<i>C. volubile</i>	250	132.02±2.28	155.80±5.48
	500	141.62±2.46	169.44±4.02
	1000	130.35±4.54	156.18±4.15

Data are expressed as mean ± SEM. Not significant when compared to control $p > 0.05$ n = 5.

The rats exposed to ethanol leaf extract of *C. volubile* for thirty (30) days experienced increase in their growth and relative body weights comparable to their corresponding control groups (Table 2). The relative organ weight of rats is presented in Table 3. There is no significant ($p > 0.05$) change in the relative weight of the liver and kidney when compared with control

Table 3: Effect of *C. volubile* ethanol extract on relative organ weight in rats.

Organ	Control	250mg/kg	500mg/kg	1000mg/kg
Liver	0.018±0.006	0.020±0.007	0.018±0.0010	0.02±0.001
Kidney	0.59±0.01	0.58±0.02	0.59±0.02	0.58±0.01

Data are expressed as mean ± SEM of average relative weights of both the liver and kidney of rats subjected to 30 days administration of ethanol leaf extract of *C. volubile*

Table 4: Effect of *Clerodendrum volubile* ethanol extract on Biochemical Parameters in Plasma of Rats

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Total Protein (g/dl)	8.26±0.12	8.14±0.22	7.95 ± 1.10	7.52 ± 0.83
AST (U/I)	69.9±7.2	48.7±9.7	38.8 ± 1.9*	38.8 ± 4.2*
ALT (U/I)	26.52±2.21	21.28±2.68	24.78 ± 2.49	27.55 ± 4.17
ALP (U/I)	3.37±0.00	3.66±0.00	3.71 ± 0.00	3.01 ± 0.00
Total Bilirubin (µmol/l)	3.37±0.86	2.96±0.32	5.99 ± 3.29	3.42 ± 0.92
Creatinine (µmol/l)	20.22±1.74	19.94±2.143	19.28 ± 0.60	23.66 ± 2.8

Values are expressed as mean ±SEM (standard error of mean) for five animals in each group.

Values with * are statistically significant at $p < 0.05$ from the normal control group as compared.

AST - Aspartate aminotransferase

ALT - Alanine aminotransferase

ALP - Alkaline phosphatase

The effect of the extract on biochemical parameters are shown in Table 4. There was no significant ($P > 0.05$) difference in the plasma total protein and total bilirubin of treated animals when compared with the control group. There was no significant ($p > 0.05$) difference in the plasma activity of liver function marker enzymes ALT and ALP in the treated rats when compared with the control group. However, AST levels were significantly ($p < 0.05$) lower in the higher dose levels (500 and 1000 mg/kg) compared with the control group. Plasma creatinine levels were higher in 1000 mg/kg group but the difference was not significant ($p > 0.05$).

Table 5: Effect of *Clerodendrum volubile* ethanol extract on Biochemical Parameters in Liver of Rats.

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Total Protein(g/dl)	4.49±0.40	4.75±0.75	1.48±0.78*	0.94±0.16**
AST (U/I)	234.15±15.44	226.20±24.67	203.30±4.31	194.44±3.56
ALT (U/I)	122.92±5.57	123.94±6.75	126.74±2.51	134.08±5.41
ALP (U/I)	0.83±0.00	3.82±0.00	1.36±0.00	0.60±0.00

Values are expressed as mean ±SEM (standard error of mean) for five animals in each group.

* $p < 0.05$, ** $p < 0.01$ compared with control..

AST - Aspartate aminotransferase

ALT - Alanine aminotransferase

ALP - Alkaline phosphatase

In the liver, administration of the extract caused a dose-dependent decrease and a dose-dependent increase in the activities of AST and ALT respectively but these differences were not statistically significant ($p > 0.05$) (Table 5). A significant ($p < 0.05$) decrease in total protein concentration occurred at the higher doses.

Table 6: Effect of ethanol extract of *Clerodendrum volubile* on Biochemical Parameters in Kidney of Rats.

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Uric Acid (mg/dl)	8.53±2.24	6.71±0.98	6.46±1.29	5.97±1.13
Creatinine (μmol/l)	9.87±2.52	3.08±0.22 ^{**}	2.46±0.27 ^{**}	1.52±0.22 ^{**}

Values are expressed as mean ±SEM (standard error of mean) for five animals in each group.

Values with ^{**} are statistically significant at $p < 0.01$ from the normal control group as compared.

A significant ($p < 0.05$) decrease in creatinine levels was seen in the extract treated groups compared with the control group (Table 6). A decrease in uric acid concentration was observed in the treated groups although this difference was not significant ($p > 0.05$).

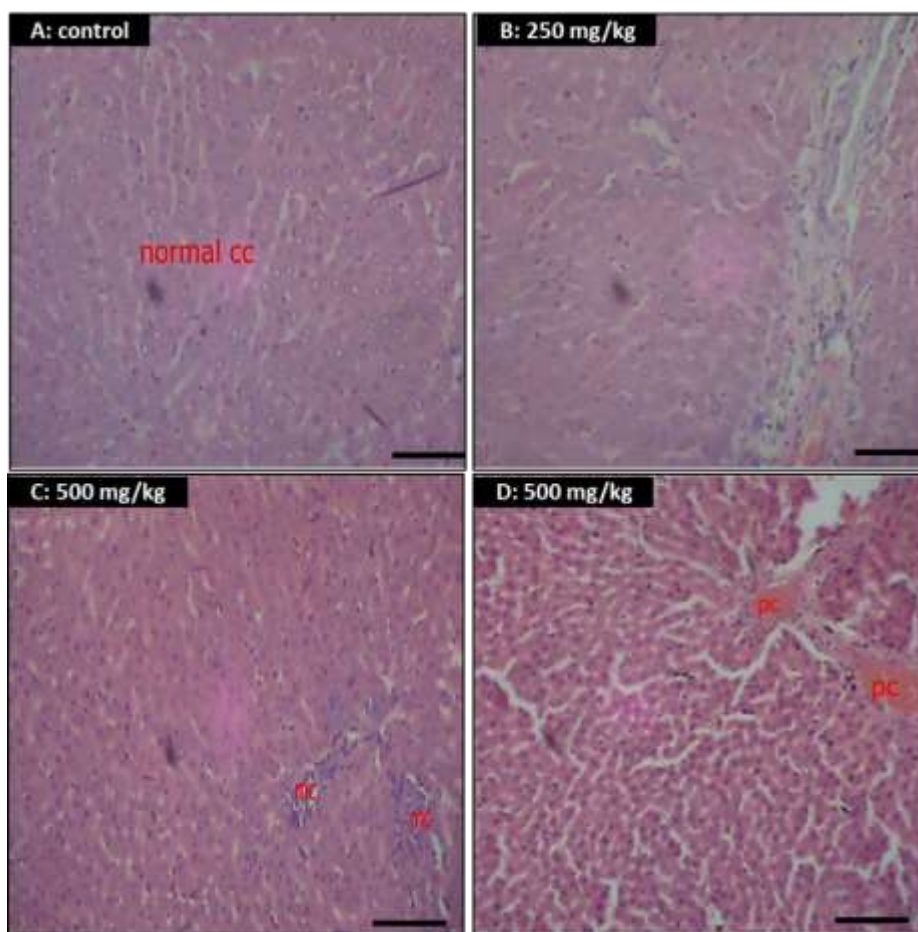


Figure 1: Effect of *Clerodendrum volubile* ethanol extract on liver of rats. Tissue sections were stained with hematoxylin and eosin (H & E) for light microscopic examination.

A. Control showing well-outlined normal cytoarchitecture of hepatocytes B. normal cytoarchitecture of hepatocytes C. showing normal hepatocytes with areas of mild to moderate cellular infiltration (mc) by mononuclear cells. D. Liver section showing otherwise normal hepatocytes with areas of portal congestion (pc) of red cells.

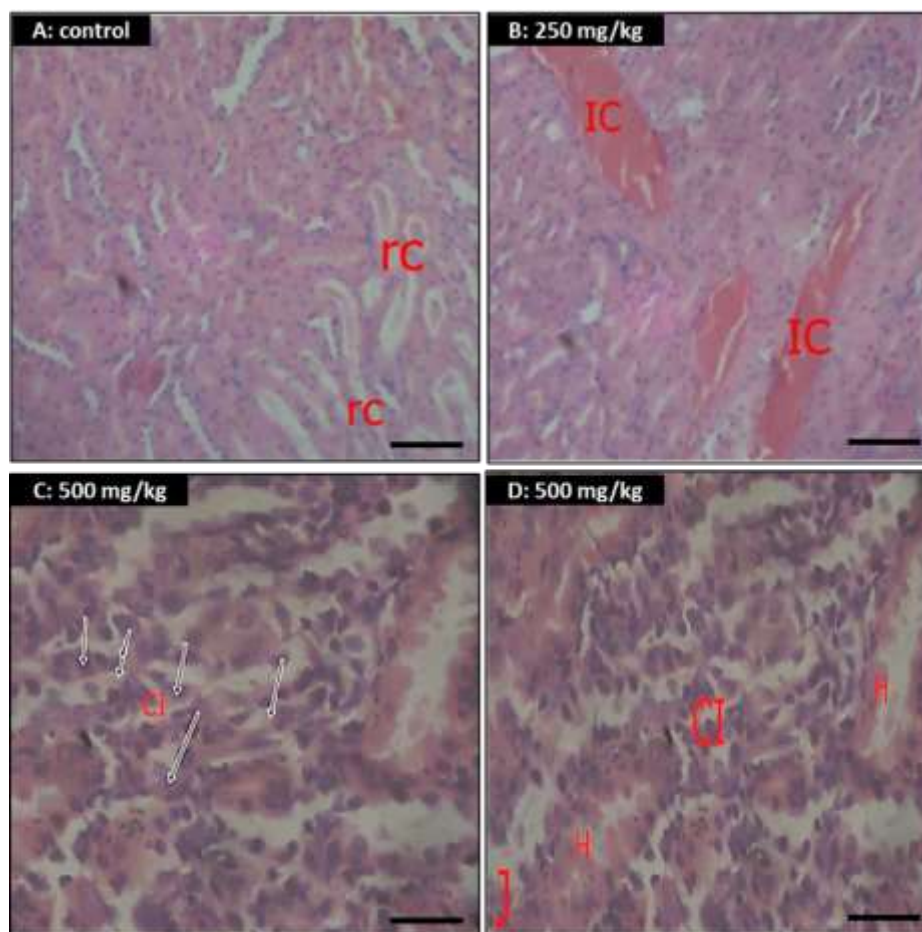


Figure 2: Effect of *Clerodendrum volubile* ethanol extract on kidney of rats. Tissue sections were stained with hematoxylin and eosin (H & E) for light microscopic examination.

A- Control shows normal cellular architecture of the kidney with the renal corpuscles (rc). B - moderate interstitial congestion is seen within the cortex (IC). C-shows areas of mononuclear cellular infiltration (CI) accompanied with mild hemorrhage at a higher concentration. D-areas of mononuclear cellular infiltration (CI), accompanied with mild hemorrhage (H) at a higher concentration, are evident.

Histological analysis of the liver revealed moderate cellular infiltration of mononuclear cells at 500 mg/kg and a congestion of red blood cells at 1000 mg/kg (Figure 1). Cellular infiltration and interstitial congestion was observed at higher doses in the histological examination of the kidney (Figure 2).

DISCUSSION

Diabetes is an inflammatory disease. The antidiabetic and other pharmacological effects of this plant have been reported (Molehin, *et al*; 2018, Molehin and Oloyede 2018 and Erukainure, *et al*; 2018). The ethanol extract of *Clerodendrum volubile* exhibited anti-inflammatory and anti-hyperlipidaemic effects (Akinpelu, *et al*; 2016, Olarenwaju *et al* 2018). The toxicological evaluation of *C. volubile* therefore is very useful and necessary for its safe use for medicinal purposes and herbal medicine production. This is expected to reduce the high cost of obtaining health care since the plant is readily available and the cost of preparation is relatively cheaper. This study was carried out to investigate the possible toxic effects of *C. volubile* leaf ethanol extract in mice and adult albino rats. Overexposure to toxic substances and an inability to properly metabolize them can impact negatively on one's health, producing a wide variety of symptoms such as headaches, muscle and joint pain, fatigue, and allergy or flu-like symptoms.

Extraction with alcohol in this research is in consonance with folkloric practices (Ubolom *et al.*, 2011). In addition, a polar organic solvent (e.g. ethanol, methanol, or an aqueous alcoholic mixture) is employed in an attempt to extract as many compounds as possible. This is based on the ability of alcoholic solvents to increase cell wall permeability, facilitating the efficient extraction of large amounts of polar and medium to low polarity constituents (Lavie *et al.*, 1987; Seidel, 2005). Thus, the extraction with aqueous ethanol would have captured more constituents than the aqueous extraction.

Many health problems are solved using medicinal plants, however, a number of these plants may be toxic if not properly prepared or dispensed (Nasri and Shirzad, 1997). A scientific approach was taken to evaluate the safety of *C. volubile* plant based on the fact that it is used in managing ailments especially in developing countries where the level of literacy is low, the status of health management is poor and about 80% of the population patronize herbal drugs (Ekor, 2014). Furthermore, there is a general misconception that natural products are not toxic and are devoid of adverse effects. This leads to improper use and unrestrained intake and this has also resulted in severe poisoning and acute health problems (UNESCO, 2013).

The lack of mortality with oral treatment at 5000 mg/kg body weight suggests that the LD₅₀ value is more than 5000 mg/kg and the ethanol leaf extract of *C. volubile* is practically non-toxic with acute administration (Abid and Mahmood, 2019). This corroborates the reports of Akinpelu *et al* (2016) and Molehin and Oloyede (2018) that the ethanol and aqueous leaf extracts of *C. volubile* are safe. It could be due to wide margin of safety that *C. volubile* is consumed as a non-conventional food in various parts of South-south of Nigeria. It is therefore safe for acute ethno-therapeutic management of acute conditions ailments (Burkill, 1985). The considerable weight gain observed in animals treated with the extracts indicates that the extract does interfere with growth processes and may have promoted growth by stimulating the synthesis of body proteins. Kluwe *et al* (1981) documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxicants and thereafter defined nephrotoxicity as increased kidney weight either absolute or relative. The ethanol leaf extract of *C. volubile* did not induce any toxic effect on the organs tested going by this

indicator since the relative weight of the organs is not significantly different from control values.

The plasma biochemical parameters were studied to evaluate the effects of *C. volubile* ethanol extract on hepatic and renal functions. Alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) are well known biomarkers predicting possible toxicity thus they are good indicators of liver function (El-Hilaly and Lyoussi 2002, Ramaiah 2007). The higher the activities of these enzymes the greater the degree of liver damage (Cheesbrough, 1991; Gatising *et al.*, 2005; Garba *et al.*, 2006). Elevation in the levels of serum transaminase enzymes is highly indicative of hepatic impairment in animals (Alvarez and Mukherjee 2011) and there is a good correlation between its level and severity of hepatic necrosis (Lu, 1996). These enzymes are only released in significant amounts into the bloodstream from the cytosol and subcellular organelles when hepatic injuries occur (Lu, 1996). Increase in ALP level is observed in bone disorders involving osteoblastic activity. However, an elevation in ALP activity between 2 and 8 fold the normal value usually is the first clue to intra hepatic and extra hepatic cholestasis (biliary obstruction) (Moss, 1987, Young and Holland, 1995). High levels of AST and ALT indicate liver damage, cardiac infraction and muscle injury. Thus, the significant decrease in the activities of plasma AST levels at 500 and 1000 mg/kg compared with that of control suggests the extract does not have toxic effects on the liver and other organs having significant quantities of this enzyme. ALT is more hepato-specific than AST because it is more sensitive to hepatic damage (Herfindal and Gourley, 2000). This extract did not alter the ALT levels in the treated group even at higher doses, which further confirms it is not harmful to the liver. This

supports the claim that methanol leaf extract of *C. volubile* is non-toxic (Molehin 2020).

A significant increase in serum or plasma total protein is an indication of tissue injury (Ekam and Udosen, 2012). In this study, *C. volubile* ethanol extract did not alter the total protein level, this further confirms its safety. Total bilirubin, exhibited no significant alterations as compared with the normal control. It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence the effects of ethanol extract of *C. volubile* on kidney function were assessed by evaluating plasma biochemical parameters such as uric acid and creatinine. There was no significant difference between uric acid levels in treated and control animals. The measurement of serum creatinine concentration was a useful index for the diagnosis of chronic kidney disease and when serum creatinine level was higher than the normal value, renal failure was most likely a possible outcome (Edmund and David, 2006). Elevated serum creatinine level has been reported as an indicator of possible kidney dysfunction (Gross *et al.*, 2005, Atangwho *et al.*, 2013). Thus, the highly significant reduction in creatinine concentration in all the extract-treated groups, in this study, is a likely indication that the extract does not exert deleterious effect on renal function. *C. volubile* extract may be a useful remedy for lowering creatinine concentrations in medical conditions associated with raised serum creatinine levels or in drug-induced high serum creatinine levels. In the light of the foregoing it appears that *C. volubile* ethanol leaf extract at the doses used may have beneficial effects on the liver and kidney. These observations were further confirmed by the histological assessment of the organs.

Conclusion

This study provided valuable data on acute and sub-chronic oral toxicity profiles of *C. volubile* that should be useful for future *in vivo* and clinical evaluation of this plant. The sub-chronic oral administration of *C. volubile* ethanol extract was non-toxic. However, chronic administration and consumption of this extract at high doses should be used with caution.

REFERENCES

- Abid, R. and Mahmood, R. (2019) Acute and sub-acute oral toxicity of ethanol extract of *Cassia fistula* fruit in male rats. *Avicenna J Phytomed* 9(2): 117–125.
- Adefegha, S.A. and Oboh, G. (2011) Enhancement of total phenolics and antioxidant properties of some tropical green leafy vegetables by steam cooking. *Journal of Food Processing and Preservation* 35 (5): 615-622.
- Akinpelu, B. A. Apata, J. T. Iwalewa, E. O. and Oyedapo, O. O. (2016) Evaluation of anti-hyperlipidemic potential of ethanol leaf extract of *Clerodendrum volubile* P. beauv. *Ife Journal of Science* 18(3):789 – 800.
- Ajao, A.A., Oseni, O.M., Oladipo, O.T. Adams, Y.A., Mukaila, Y.O. Ajao, A.A. (2018). *Clerodendrum volubile* P. Beauv (Lamiaceae), an underutilized indigenous vegetable of utmost nutritive and pharmacological importance *Beni-Suef University Journal of Basic and Applied Sciences* 7(4): 606 – 611.
- Alvarez, A.M. and Mukherjee D. (2011) Liver abnormalities in cardiac disease and heart failure. *Int J Angiol* 20:135–142.
- Atangwho, I.J., E.E. Edet, G.E. Egbung, D.E. Uti and P.E. Ebong, 2013. Effect of *Vernonia amygdalina* supplemented diet on selected

- tissues function in diet-induced obese rats. *J. Med. Plants Res* 7: 1825-1832.
- Barrales-Cureño, H.J. (2015) Applications and production of *C. roseus* alkaloids *Biotechnología Aplicada* 32(1): 1101 -1110.
- Bartels, H. and Bohmer, M. (1972) Quantitative Determination of Creatinine. *Clinica Chimica Acta* 37: 193.
- Burkill, H.M., 1985. The Useful Plants of West Tropical Africa, vol. 1, Royal Botanic Gardens, Kew, pp. 319.
- Cheesbrough, M. (1991) Medical Laboratory Manual for Tropical Countries Microbiology. ELBS Edition, Cambridge, 196-205.
- Edmund, L. and David, J. (2006) Kidney Function Tests. In: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, Carl, A.B., R. Edward and E. David (Eds.). 4th Edn., Elsevier, New Delhi, India, pp: 797-808.
- Ehrenkranz, R.R.L., Lewis, N.G., Kahn, C.R., Roth, J., (2005). Phlorizin: A review. *Diabetes Metab. Res. Rev.* 21, 31–38.
- Ekam, V.S. and Udosen, E.O. (2012) Total protein, albumin, and globulin levels following the administration of activity directed fractions of *Vernonia amygdalina* during acetaminophen-induced hepatotoxicity in Wistar albino rats. *Global Journal of Pure and Applied Sciences* 18(1&2): 25 -29.
- Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology/Ethnopharmacology* 4 (177): 1 - 10.
- El-Hilaly, J.E. and Lyoussi, B. (2002) Hypoglycaemic effect of the lyophilized aqueous extract of *Ajugaiva* in normal and streptozotocin diabetic rats. *J. Ethnopharmacol* 80:109–113.
- Erukainure, O.L., Oke, O.V., Adeboyejo, F., Kayode, F.O., Ateyoju, O., 2011a. Nutritional evaluations of *Clerodendrum volubile*, a tropical non-conventional vegetable as sole dietary protein source in rats. *Int. J. Biol. Med. Res.* 2 (1), 374–377.

- Erukainure, O.L., Oke, O.V., Ajiboye, A.J., Okafor, O.Y., 2011b. Nutritional qualities and phytochemical constituents of *Clerodendrum volubile*, a tropical non-conventional vegetable. *Int. Food Res. J.* 18 (4), 1393–1399.
- Erukainure, Ochuko L; Rahman M. Hafizur, Nurul Kabir, M. Iqbal Choudhary, Olubunmi Atolani, Priyanka Banerjee, Robert Preissner, Chika I. Chukwuma, Aliyu Muhammad, Eric O. Amonsou, and Md. Shahidul Islam (2018) Suppressive Effects of *Clerodendrum volubile* P Beauv. [Labiatae] Methanolic Extract and Its Fractions on Type 2 Diabetes and Its Complications *Frontier in Pharmacol* 9: 8
- Fossati, P., Prencipe, L. and Berti, G. (1980) Use of 3,5-Dichloro-2-Hydroxybenzenesulfonic Acid/4-Aminophenazone Chromogenic System in Direct Enzymic Assay of Uric Acid in Serum and Urine. *Clinical Chemistry* 26: 227-231.
- Garba, I.H., Gatsing, D. and Ubom, G. (2006) Elevated total and isoenzyme forms of acid phosphatase in falciparum malaria. *Comptes Rendus Biologies*, 329(2): 75 -78.
- Gatsing, D., R. Aliyu, J.R. Kuate, I.H. Garba and K.H. Jaryum et al., (2005). Toxicological evaluation of the aqueous extract of *Allium sativum* bulbs on laboratory mice and rats. *Cameroon J. Exp. Biol.* 1: 39-45.
- Gross, J.L., M.J. de Azevedo, S.P. Silveiro, L.H. Canani, M.L. Caramori and T. Zelmanovitz, (2005). Diabetic nephropathy: Diagnosis, prevention and treatment. *Diabetes Care* 28: 164-176.
- Happy, O.T. (2016). Comparative qualitative analysis of phytochemicals in *Baphia pubescens* and *Clerodendrum volubile* leaves. *Asian Journal of Ethnopharmacology and Medicinal Foods* 02 (01): 18-21.
- Herfindal, E.T., Gourley, D R H, Gourley, G.K. and Holt, J.M. In: Textbook of Therapeutics : Drug and Disease management... Publisher: Philadelphia : Lippincott Williams & Wilkins, 2000.
- Jendrassik, L. and Scott, J. E. (1938). Vereinfachte photometrische methoden zur bestimmung des bilirubins, *Biochemistry zeitschrift*, 297: 81.
- Kluwe WM, McNish R, Smithson K, Hook JB (1981) Depletion by 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, tris(2,3-dibromopropyl) phosphate, and hexachloro -1,3-butadiene of reduced non-protein sulfhydryl groups in target and non-target organs. *Biochem Pharmacol* 30: 2265–2271.

- Lavie, D., Bessalle, R., Pestchanker, M. J., Gottlieb, H. E., Frolow, F., and Giordano, O. S. (1987). Trechonolide A, a new withanolide type from *Trechonaetes laciniata*. *Phytochemistry* 26, 1791–1795.
- Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Arch Toxicology*. 54:275-287.
- Lu, F.C. (1996). Basic Toxicology Fundamentals, Target Organs, and Risk Assessment. 3rd Ed.
- Makonnen, E., Debella, A., Abebe, D., Teka, F. (2003). Analgesic properties of some Ethiopian medicinal plants in different models of nociception in mice. *Phytotherapy Research*.17:1108-1112.
- Molehin OR and Oloyede O (2018) In vitro Antioxidant and Sub-acute toxicity studies of Aqueous extract of white butterfly (*Clerodendrum volubile*) leaves. *International Journal of Plant Research* 31: 92 - 101.
- Molehin OR, Oloyede OI and Adefegha SA. (2018) Streptozotocin-induced diabetes in rats: effects of White Butterfly (*Clerodendrum volubile*) leaves on blood glucose levels, lipid profile and antioxidant status. *Toxicology Mechanism and Methods*. 28(8):573-586.
- Molehin, O. R. and Oloyede O. I. (2018) Attenuation of oxidative stress and hepatic damage by white butterfly (*Clerodendrum volubile*) leaves in streptozotocin-induced diabetes in rats. *Journal of Basic and Clinical Physiology and Pharmacology* 30(1): 81–89.
- Molehin, O.R. (2020) Alleviation of doxorubicin-induced nephrotoxicity by *Clerodendrum volubile* leaf extract in Wistar rats: A preliminary study. *J Herbmed Pharmacol* 9(2): 138-144.
- Moss, D.W. (1987). Diagnostic aspect of Alkaline Phosphatase and its isoenzymes. *Clinical Biochemistry*. 20: 225-230.
- Nasri, H. and Shirzad, H. (2013) Toxicity and safety of medicinal plants. *J HerbMed Pharmacol* 2(2): 21-22.
- Newman, D.J., Cragg, G.M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*, 70 (3): 461–77.
- Oboh, G., Ogunraku, O.O., Oyeleye, S.I., Olasehinde, T.A., Ademosun, A.O., Boligon, A.A
(2017) Phenolic Extracts from *Clerodendrum volubile* Leaves Inhibit Cholinergic and Monoaminergic Enzymes Relevant to the Management of Some Neurodegenerative Diseases. *Journal of Dietary Supplements* 14(3): 358 - 371.

- Okigbo, R.N., Eme, U.E., Ogbogu, S. (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. *Biotechnol. Molecular Biology. Rev.* 3(6): 127-134.
- Olarenwaju, O. Apata, J.T; Akinpelu, B.A, Akomolafe, R.O., Oyemitan, I.A., Asaolu F.T., Ologe M.O., and Iwalewa, E.O. (2018) Anti-inflammatory potentials, membrane stabilizing and xanthine oxidase inhibitory activities of *Clerodendrum volubile* ethanolic leaf extract on carrageenan-induced inflammation in rats. *International Journal of Pharmacology and Toxicology*, 6(1): 7-11.
- Ramaiah SK (2007) A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food Chem Toxicol* 45 (9): 1551–1557.
- Reitman, S., Frankel, S. (1957). A calorimetric method for the determination of serum glutamic oxaloacetic acid and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28:56-63. Watson, D. (1967). *Clinical Chemistry*, 7: 603-625.
- Salawu, A.O., Tijani, Y.A., James, A.A. and Oga, E.F. (2010). Acute and subacute toxicity study of ethanolic extract of the stem bark of *Faidherbia albida* (Mimosoidae) in rats. *African Journal of Economic and Taxonomic Botany*, 8(2): 291-300.
- Seidel V. Initial and Bulk Extraction (2005) In: From: Methods in Biotechnology, Vol. 20, Natural Products Isolation, 2nd ed. Edited by: S. D. Sarker, Z. Latif, and A. I. Gray. Humana Press Inc., Totowa, NJ, pp 27 - 46.
- Ubulom, P., Akpabio, E., Udobi, C.E. and Jeminiwa, R, (2011). Antifungal activity of aqueous and ethanolic extracts of *Picralimanitida* seeds on *Aspergillus flavus*, *Candida albicans* and *Microsporum canis*. *Research in Pharmaceutical Biotechnology*, 3(5): 57-60.
- UNESCO (1996). Terminal Report: Promotion of Ethnobotany and the sustainable use of Plant Resources in Africa. p.60.

Williams, L.A.D. (2006). Ethnomedicine. *The West Indian medical journal* 55(4):215-6.

Young, L.Y., Holland, E.G. (1995). Applied Therapeutics: The Clinical use of Drugs. 6th ed, *Applied Therapeutics*, Inc. pp. 14-20.

HISTOPATHOLOGY OF GILLS AND LIVERS OF JUVENILE *Clarias gariepinus* (BURCHELL, 1822) EXPOSED TO AQUEOUS EXTRACTS OF *Carica papaya* DRY SEEDS UNDER LABORATORY CONDITIONS

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Abstract

African Sharptooth, *C.gariepinus* (mean weight 11.81g±0.23g and mean length 9.8cm ± 0.2) were exposed to graded concentration (0, 150, and 180, 210 and 240mgL⁻¹) of aqueous extract of *C. papaya* dry seeds in triplicates for 4 days. They were fed a 25% crude protein diet at 1% biomass half at 800 and 1600h. Experimental design was a completely randomized type. The control fish's gill histology revealed normal histomorphology with intact blood vessels, gill slits and supporting cartilage apparent. Exposed fish showed degeneration of the gill filament, hypertrophy, hyperchondrial of the gill arch and lesion. Normal tubules, haematopoietic structures, and a portal triad with a dilated portal vein were found in the livers of control fish. The exposed fish livers showed vacuolation, steatosis, necrosis and degeneration of hepatocyte degeneration. The findings suggest that pathology of *Clarias gariepinus*' gill and liver could be useful biomarker for assessing aqueous extract of *Carica papaya* dry seeds toxicosis.

Keywords: Gill, liver, histopathology, *Carica papaya* and hepatocytes

Introduction

Botanical biocides contaminations of natural water are widely reported in Nigeria because of their usage by locals. Piscicidal plants such as *Blighia sapida*, *Kigelia Africana*, *Tetrapleura tetraptera*, *Raphia vinifera*, *Parkia biglobosa* and *Tephrosia vogelii* are widely used by local fisherforks (Ojogu et al., 2017). Extracts of plants such as *Deris elliptica*, *D. trifoliata*, *Adeniace ssamploides*, *B. aegyptiaca*, *Carica papaya*, *Tephrosia candida*, *T. vogelii*, *Parkia folicoides* contain biocides which are used to catch fish in different parts of the world (Ekpendu et al., 2016). Adaramoye et al. (2010) reported that the presence of (tspace.library.utoronto.ca) macerated plant materials in rivers and streams stupefy fish for easy collection. Desai (2009) observed that the alkaloid contents of the leaves and stem of *Croton zambesicus* is active against microorganism and that the essential oil found in the leaves also contain P-cymene, linalool and beta-caryophyllene. Ajani and Ayoola (2010) added that most of the plants contain chemicals which are traditionally used to harvest fish in almost all part of the world.

Carica papaya is a perennial tree with a crown of very large palmate leaves at the base of which the fruits are clustered. Its fruits are palatable feedstuff as well as its leaves that are widely used by the locals (Acquaye et al., 2000). *C.papaya* seed contain piscicidal and anti fertility properties which are widely used as fish poison for controlling the excessive breeding of tilapia in some part of in Nigeria (Akah et al., 2001). The active ingredients of *carica papaya* are carpine, chymopapain, papain, bactericidal a glycone of lucotropaeolin benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosin and carpasemine and are concentrated are in the black seeds (Akah et al., 2001). However, the presence of these botanicals in high concentrations may have adverse effects on aquatic organisms.

The sharptooth catfish (tspace.library.utoronto.ca) (*Clarias gariepinus*) is an ecologically important and commercially valued fish in Nigeria (Abubakar, 2013). These catfish are frequently and widely culture in fish farms all over Nigeria (Ojogu et al., 2017). The demand for this fish by large population of Nigerian has necessitated its harvest with the use of poisons by the locals (Abubakar et al., 2019).

In spite of the wide uses of *C. papaya* seeds as biocides in aquaculture, little toxicological studies have been done on its effects on the culture fishes. The mode in which the various organs respond to xenobiotic exposure has proven their useful as a tool in determining the health condition of fish, since histopathological biomarkers are considered the primary indicators of exposure to pollutants and show little temporal variation (Abubakar *et al.*, 2019). One of the great advantages of using histopathological biomarkers in environmental studies is that this category of biomarkers allows the examination of the target organs (Idi-Ogede *et al.* 2016). Furthermore, the alterations found in these organs are clearer than functional ones (Fanta *et al.*, 2003). It also reveals signs of damage to animal health (Hinton and Lauren, 2016). The gills being a respiratory organ of fish (Abubakar *et al.*, 2019) are frequently in contact with external environment and thus vulnerable to aquatic toxicants (Abubakar, 2013). The gills are particularly sensitive and can also be responsible maintaining the optimal osmotic pressure and acid-base balance of body fluids. Due to the high level of vascularization present in the gills, they are highly vulnerable to mechanical injury (Abubakar *et al.*, 2019). They play an important role in excreting unnecessary and harmful metabolic products and in supporting food drainage (Genten *et al.*, 2009). Because their large surface area contacts the external environment, the gills are sensitive to even minor chemical or physical changes in the surroundings and are the target organ for many contaminants in water (Hinton *et al.*, 2016). Water flowing through the gills exposes the organ to constant contact with potentially harmful chemical and biological substances, which is reflected in morphological changes in the organ and impairment of its functions. Moreover, even minor mechanical damage to the structure of gills may be dangerous to fish. Lesions resulting from any of these influences may be a direct cause of increased mortality in reared fish (Abubakar *et al.*, 2019). Studies have been conducted on histopathological changes in the gills and liver of fish exposed to various substances (Auta, 2001). The liver of fish can be considered as a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health (Kolbasi *et al.*, 2009) and the effects of a wide variety of environmental pollutants. The liver plays a major role in complex enzymatic processes of tetraiodothyronine (thyroxine)-triiodothyronine (T_4 - T_3) conversion (Kolbasi *et al.*, 2009). The metabolic rate of hepatocytes is certainly modulated by thyroid hormones. Thyroid dysfunction may perturb liver function, and liver disease effects thyroid hormone metabolism (Kolbasi *et al.*, 2009). Hence, the structure, anatomy, physiology,

morphology and health condition of fish gills and liver need to be studied more closely. The aim of this study was to examine the histological alterations in the gills and liver of *C.gariepinus* after being exposed to an aqueous extract of *C. papaya* dry seeds.

Materials and methods

Procurement of *Carica papaya* seed

Pawpaw (*Carica papaya*) fruits were obtained from Gammon market in Ifelodun, Kwara State. The seeds were extracted from the fruits using Ayotunde and Ofem (2005) methods which involved cutting the fruits into half with a sterilized knife and rinsing the seeds in distilled water to remove the adhering fruit membrane. After that, the seed were spread out on a paper and allowed to air dry at room temperature in an enclosed environment. Using a mortar and pestle, the dried seed was ground into a coarse powder, weighed and placed into a container, stored in a cold, dry area until needed.

Extract preparation

Aqueous extraction of the coarsely grinded *C. papaya* seed powder was obtained by crushing the seeds into coarsely powdery form using mortar and pestle, 1200g powdered *C. papaya* seeds was soaked in 4800ml of distilled water for 48hours in closed container at room temperature with occasional shaking, then filtered through muslin cloth and finally the filtrate was water bath for 6 hours till the filtrate dried. The weight of extract obtained after subjecting to water bath was 34.05g. A stock solution was prepared with the extract obtained by adding 1.0g of the *C. papaya* extract in 1Litre of distilled water using the method of Olaifa *et al.* (2003).

Collection of fish and Acclimatization

Two hundred and fifty (250) African sharptooth catfish juveniles with mean weight of $11.81\text{g} \pm 0.23\text{g}$ and mean length $9.8\text{cm} \pm 0.2$ were purchased from National Centre for Agriculture and Mechanization Integrated Farm Project

(NIFAP), Idofian, Ifelodun Local Government, Kwara State. They were transported early in the morning inside a black 50Litres capacity jerry-can. They were held in a 1000Litres capacity plastic tank for 14 days for acclimation. The tank was filled up to 25 Litres level with borehole water. They were fed a 25% crude protein diet at 1% biomass half at 800 and 1600h, respectively. The water in the acclimation tank was replenished at 2days interval. Feeding of fish was stopped 24hours prior to exposure to acute toxicity bioassay media (Abubakar (2016)).

Bioassay

The following concentrations of aqueous extract of *C. papaya* dry seed were prepared using the method of Abubakar (2016).

1 Litre = 1000g;

1ml = 1mg;

1500ml of the toxicant in 10 litres of water = 150mg/L

1800ml of the toxicant in 10 litres of water = 180mg/L

2100ml of the toxicant in 10 litres of water = 210mg/L

2400ml of the toxicant in 10 litres of water = 240mg/L

Preliminary 24 hours range finding test was conducted for the juvenile catfish (*C. gariepinus*) following static bioassay procedures described by Abubakar (2016) to determine the toxic range of aqueous extracts of *C. papaya* seeds on juvenile catfish.

Ten (10) juvenile fish were selected at random into five (5) plastic aquaria with holding capacity of 20Litres.

It was filled with 10Litres of water. Only test fish was put at a time and observed to determine the time mortality would occur. The concentrations from which serial dilutions will begin were arrived at after the survival of fish beyond 2 hours on exposure to the toxicant.

The toxicants were introduced in a single dose. The mortality of the test fishes in the aquaria were monitored and recorded every five (5)minutes for the first hour, every fifteen (15) minutes for the next second hour, once, every thirty (30) minutes for the next three (3) hours and every four hours for the twenty-four(24) hours period.

Experimental design

Completely randomized design was used, consisting of three triplicate treatments including a control, 15 plastic aquaria of 20 litres capacity containing 10 Litres of water each was used. Three plastic aquaria serve as control while the other twelve serve as treatments for the test fish. Ten juveniles of African sharp tooth catfish were randomly distributed to each of the plastic aquaria and impound with 10 litres of borehole water.

Acute bioassay test

In order to estimate lethal concentrations (LC50), a 96- hour static acute toxicity bioassay was performed in the Central Research laboratory, Faculty of Agriculture, University of Ilorin using the APHA (1998) method. African sharp tooth juvenile mean weight $11.81\text{g} \pm 0.23\text{g}$ and mean length $9.8\text{cm} \pm 0.2\text{cm}$ were exposed to different concentrations of aqueous extract of *C. papaya* dry seed. The test fish were exposed to four concentrations of the toxicant (aqueous extract of *C. papaya* dry seed) and the fifth had no toxicant which served as the control. The nominal concentrations were 150ml, 180ml, 210ml, 240ml and a control with no toxicant. Each concentration level was in triplicate. The desired stock solution was measured using 50ml calibrated beaker and introduced into 10Litres of borehole water inside the plastic aquaria. The mixture was allowed to stand for 30 minutes for proper mixing before randomly introducing test fishes. One hundred and fifty (150) juveniles of African sharptooth were randomly distributed into the toxicant concentrations to give a stocking density of 10 fish per plastic aquaria using the method of Abubakar (2013). Each plastic tank was covered with nylon mesh screen to prevent the fish from leaping out of the tank. Feeding of fish stopped 24hours prior to and during the 96h exposure period. This was to prevent interference with the absorption and metabolism of the extract by wastes in reconstituted extracts. The exposed and control fish were observed for signs of toxicity with prompt recordings at 24h (0h, 3h, 6h and 12h) 48h, 72h and 96h exposure period.

Histopathological Analysis

One dead fish from each exposed group was chosen at random and their gills and livers were removed using a dissecting set according to Abubakar (2016).

One live fish from each control group was chosen at random and euthanized according to the method of Abubakar *et al.* (2019). The gills and liver were sectioned 1-2mm, in thickness and fixed in 10% formalin (doi.org) and processed according to standard paraffin procedures. Five micrometer thick paraffin sections were placed on glass slides and stained with (doi.org) haematoxylin and eosin. Approximately 2-4 paraffin sections of each fish were histopathologically analyzed. Photomicrographs were then taken at low power ($\times 100$) objective with a digital camera (Olympus C4-0 AB-4). Photomicrography of control groups were compared with those of exposed groups under the guidance of a pathologist from the Veterinary Medicine Department, University of Ilorin, Kwara State, Nigeria.

Results

Histopathological changes observed in the gills and liver of experimental fish showed different lesions, which ranged from desquamation of epithelia lining of cartilage of the gills to degeneration of the hepatocytes and necrosis of the liver of the exposed fish. The observed histological changes were as a result of various clinical factors. The higher the concentrations of aqueous extract of *C. papaya*, the more severe the degree of damage to fish gill and liver. The control gills and livers had normal morphology and internal arrangement components (Plates 1 to 10) as revealed in their structures.

A. HISTOPATHOLOGICAL STUDY OF GILLS



Plate 1: Photomicrograph of the gill of a control *C.gariepinuss* showing normal gill histomorphology with intact blood vessels, gill slits, normal vascular secondary lamella and supporting cartilage apparent stained with H&E Mag. X100.The SL, CA and BV inside the image stand for:

SL- Secondary lamella,
CA-Cartilage apparent
BV-Blood vessel

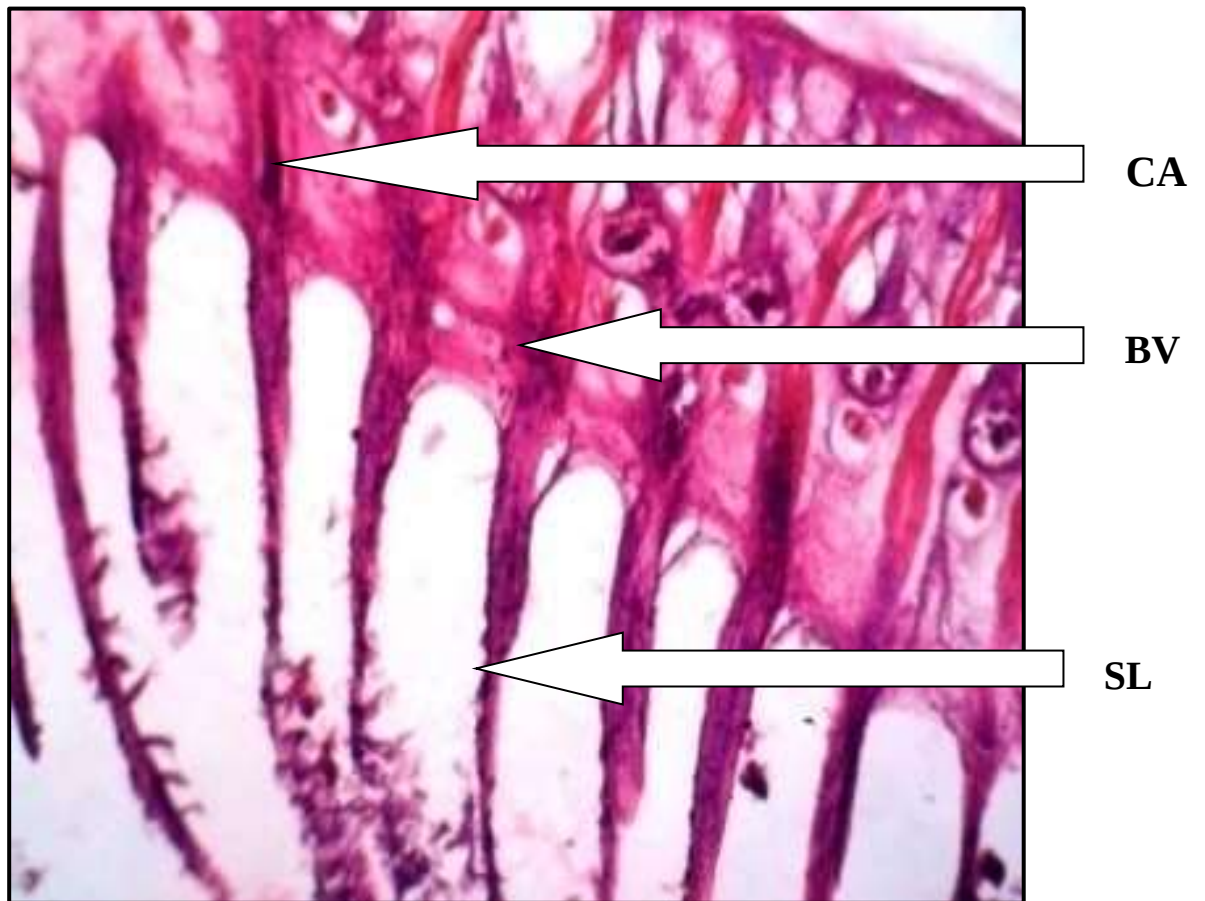


Plate2: Photomicrograph of the gill of *C. gariepinus* exposed to 150 mg/L^{-1} of aqueous extract of *C. papaya* dry seed showing desquamation of the epithelial lining of supporting cartilage (CA), secondary lamellae (SL) and blood vessel (BV) stained with H&E Mag . X100. The SL, CA and BV inside the image stand for:

SL- Secondary lamella,
CA-Cartilage apparent
BV-Blood vessel

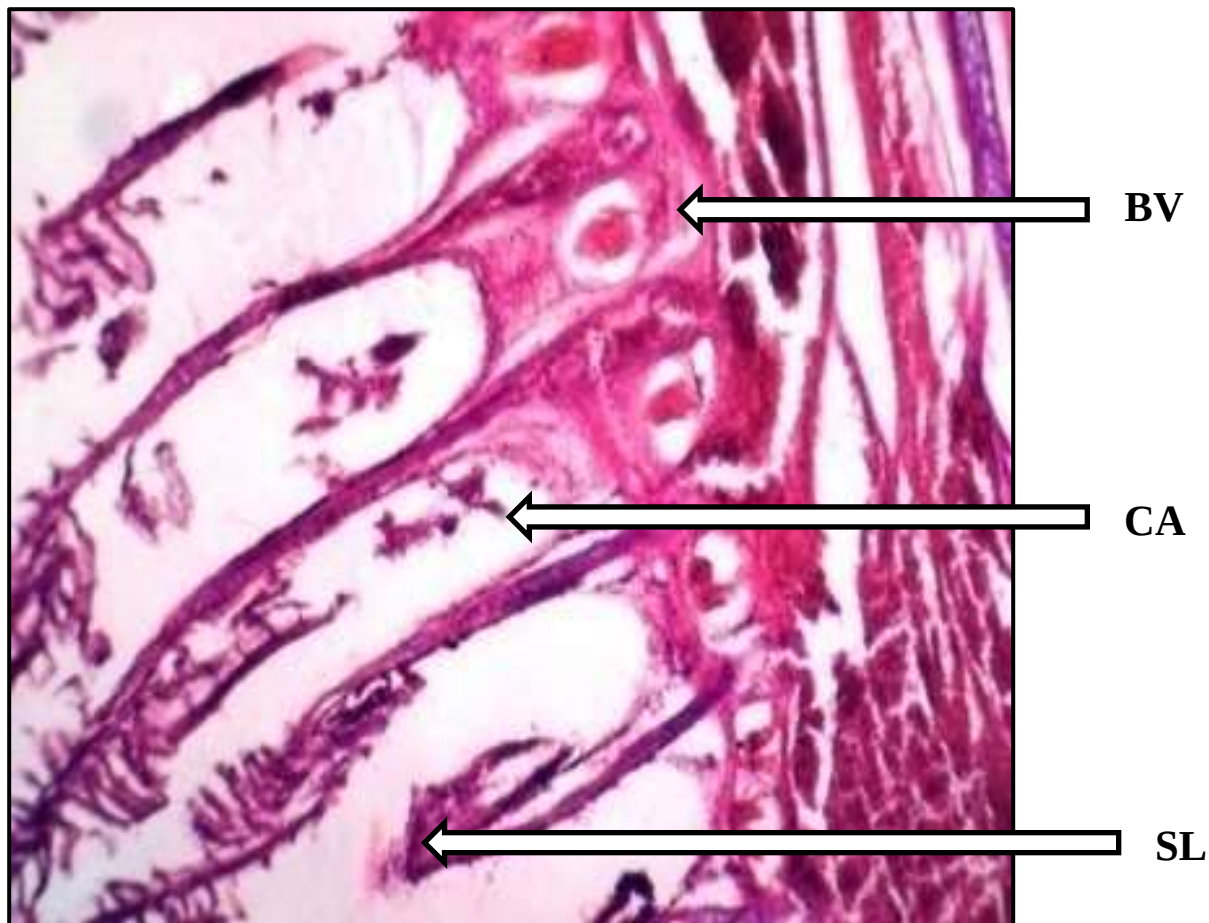


Plate 3: Photomicrograph of the gill of *C. gariepinus* juvenile exposed to 180 mg/L^{-1} of aqueous extract of *C. papaya* dry seed showing hypertrophy of blood vessel (BV), supporting cartilage (CA) and secondary lamellae (SL) stained with H&E Mag . X100. The SL, CA and BV inside the image stand for:
SL- Secondary lamella,
CA-Cartilage apparent
BV-Blood vessel

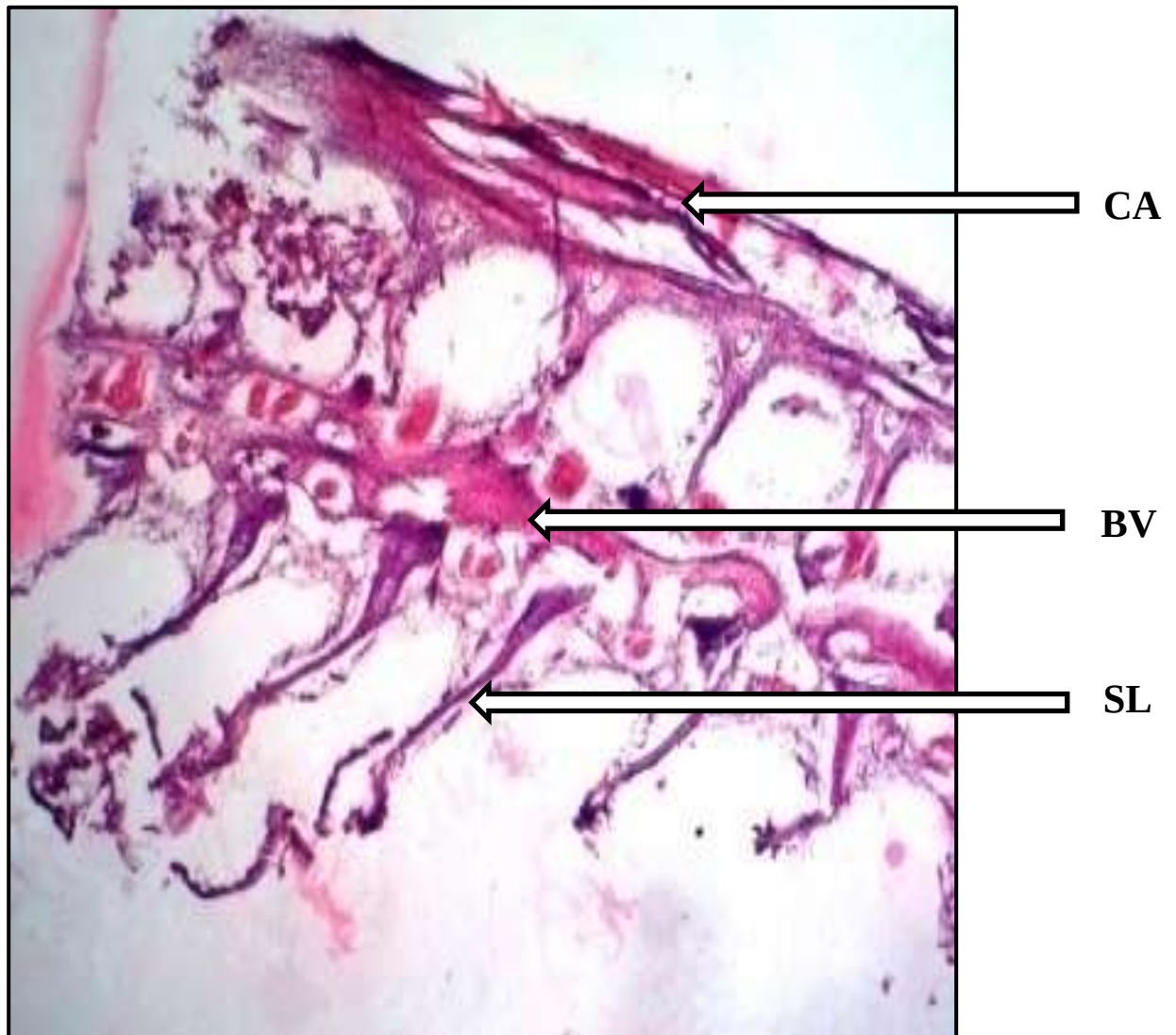


Plate4: Photomicrograph of the gills of *C. gariepinus* juvenile exposed to 210 mg/L⁻¹ of aqueous extract of *C. papayadry* seed showing less vascularized secondary lamella in form of hyperchondrial and

hyperthrophy of the gill arch stained with H&E Mag . X100. The SL,
CA and BV inside the image stand for:
SL- Secondary lamella,
CA-Cartilage apparent
BV-Blood vessel

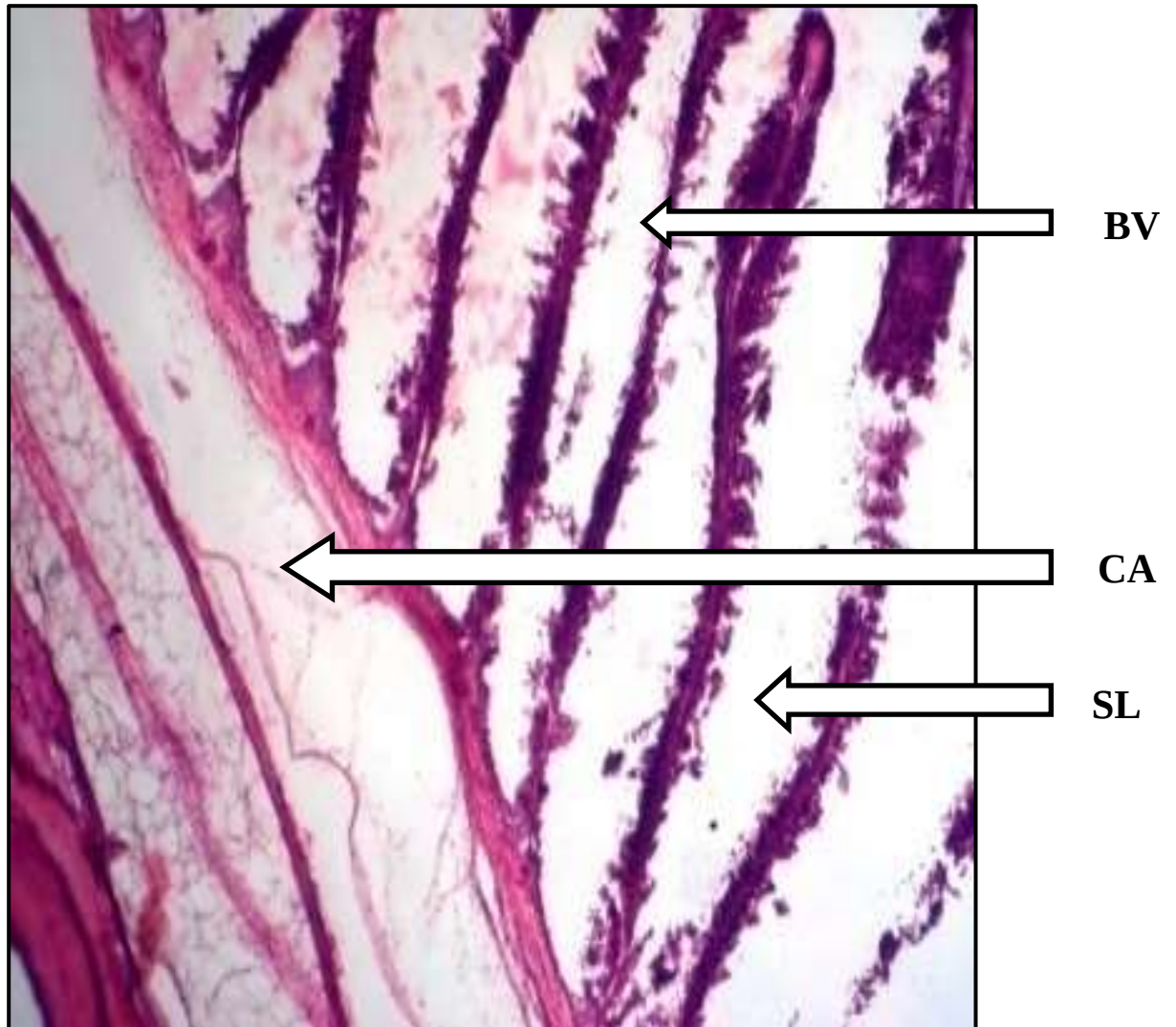


Plate 5: Photomicrograph of the gill of *C. gariepinus* exposed to 240 mgL^{-1} of aqueous extract of *C. papayadry* seed showing alterations as the secondary lamella appears hyperchondrial and less vascularised. High degenerated lesion observed at the gill arch, filament, and lamellae stained with H&E Mag . X100. The SL, CA and BV inside the image stand for:
SL- Secondary lamella,
CA-Cartilage apparent
BV-Blood vessel

B. Histopathological study of livers

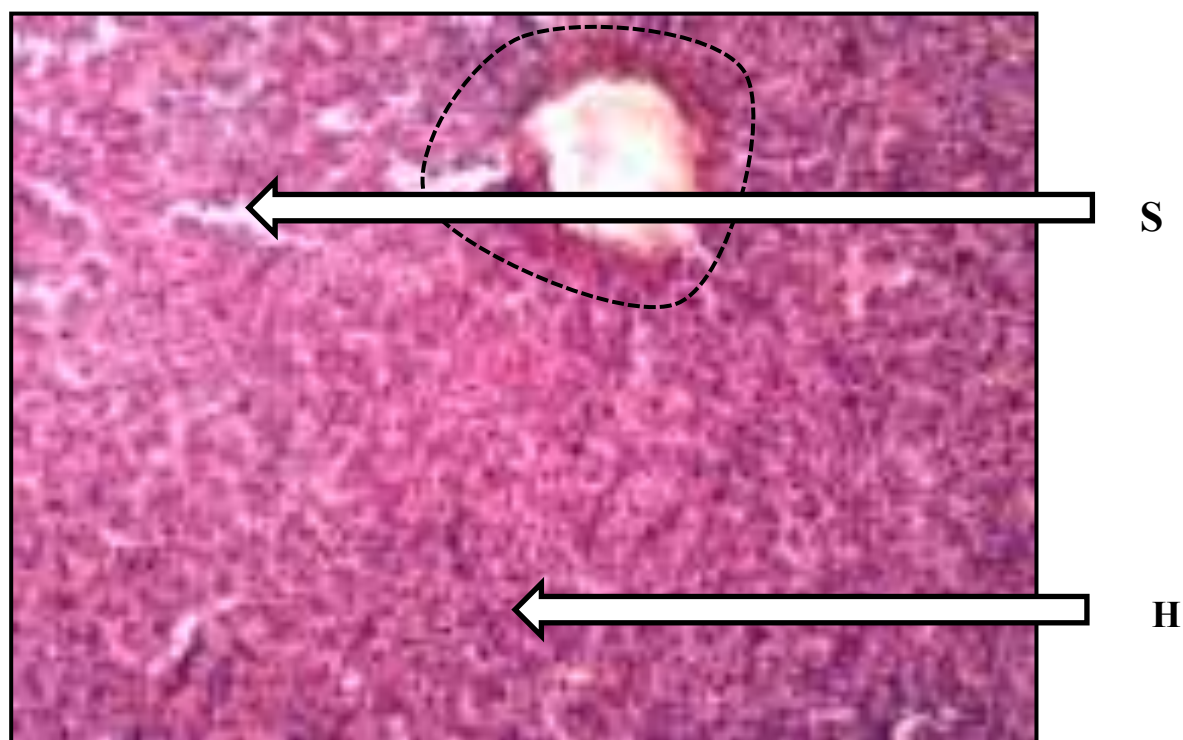


Plate 6: Photomicrograph of the liver from a control *C. gariepinus* showing typical sized spaced central vein surrounded by densely distributed

hepatocytes in cords. Mild sinusoidal dilated apparent space stained with H&E Mag . X100. The S and H inside the image stand for:
H- Hepatocyte
S-Sinusoidal

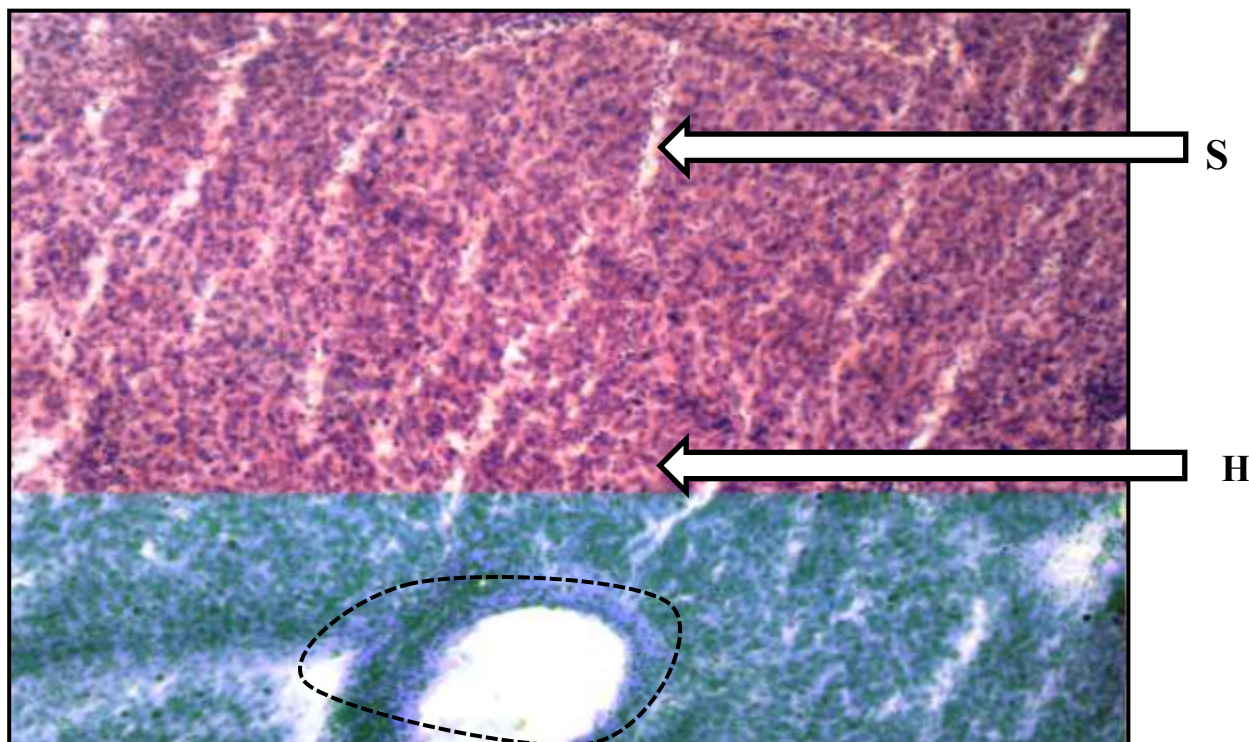


Plate 7: Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 150 mgL^{-1} of aqueous extract of *C. papaya* dry seed showing vacoulation of hepatocyte stained with H&E Mag . X100. The S and H inside the image stand for:
H- Hepatocyte
S-Sinusoidal

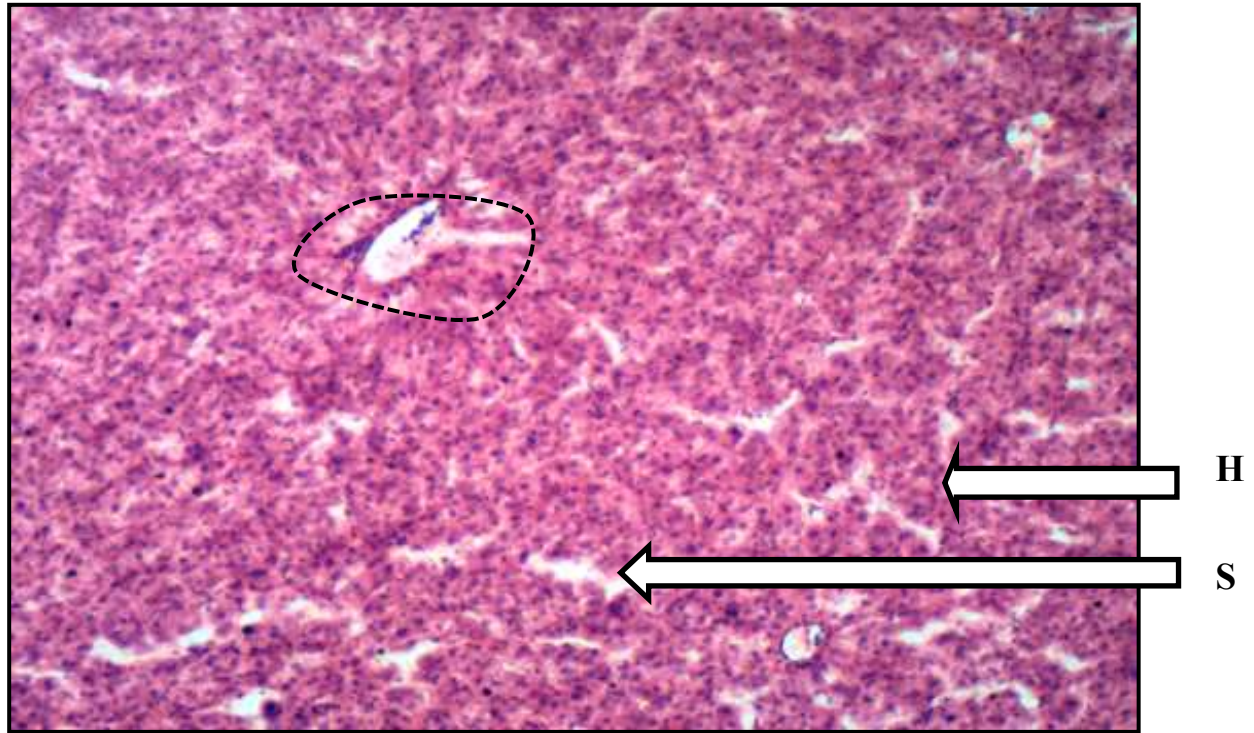


Plate 8: Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 180 mgL^{-1} of aqueous extract of *C. papaya* dry seed showing hyperplasia and necrosis of hepatocyte stained with H&E Mag . X100. The S and H inside the image stand for:
H- Hepatocyte
S-Sinusoidal

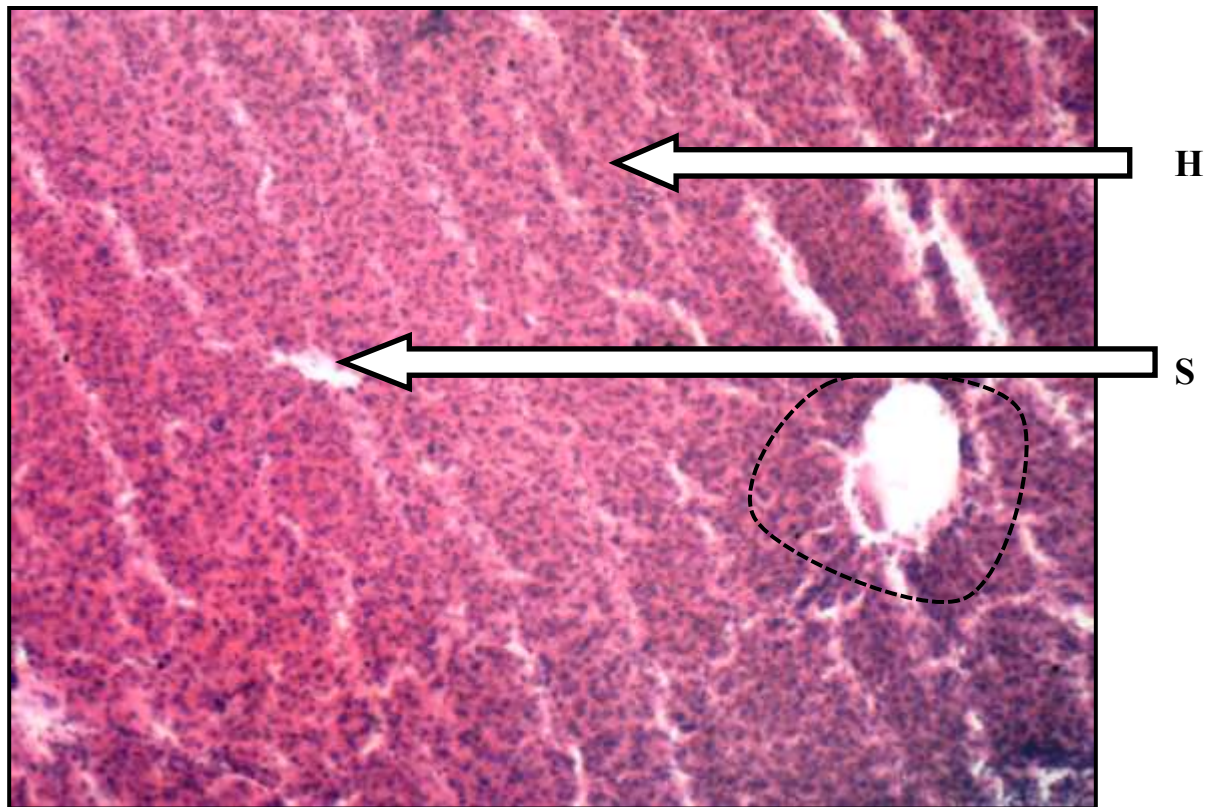


Plate 9: Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 210 mgL^{-1} of aqueous extract of *C. papaya* dry seed showing hyperplasia, degeneration of hepatocyte and necrosis stained with H&E Mag . X100. The S and H inside the image stand for:
H- Hepatocyte
S-Sinusoidal

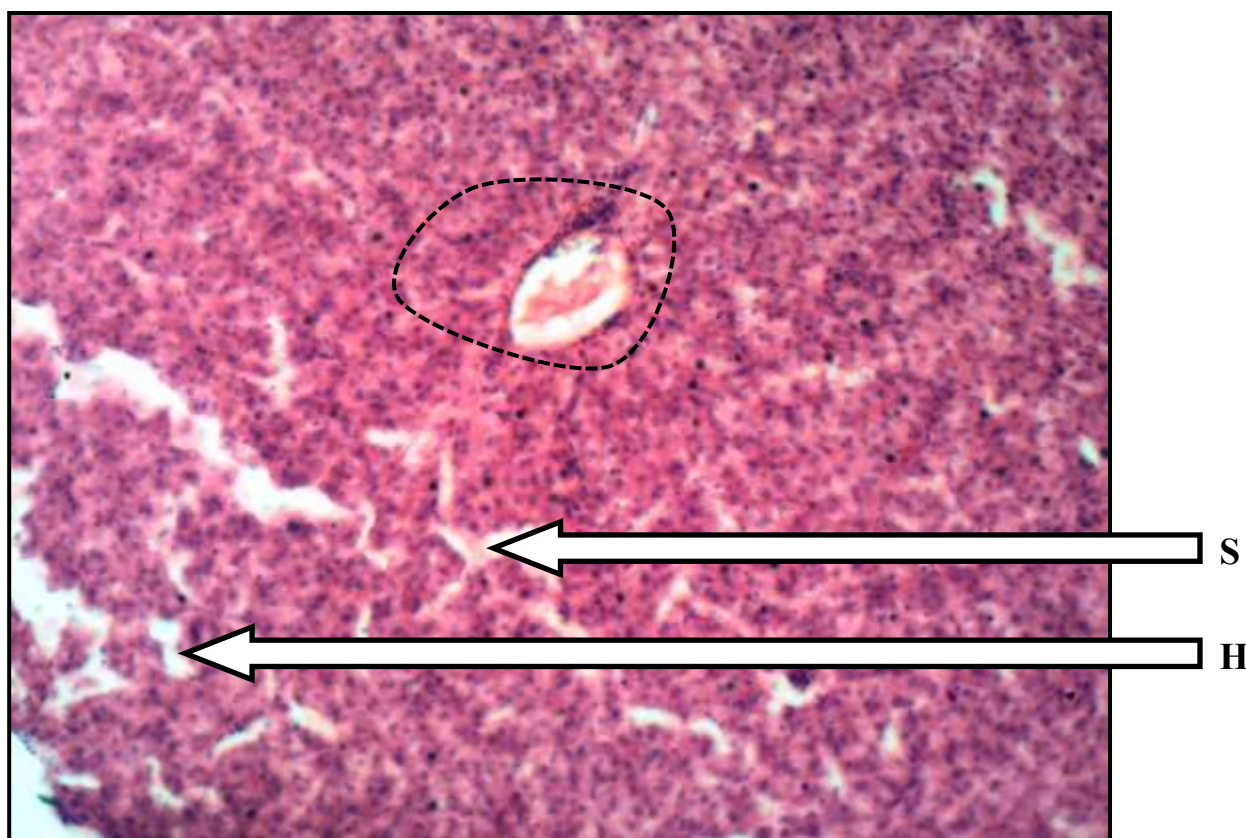


Plate 10: Photomicrograph of the liver of *C. gariepinus* juvenile exposed to 240 mgL^{-1} of aqueous extract of *C. papaya* dry seed showing steatosis, vacuolation, necrosis and degeneration of hepatocyte stained with H&E Mag . X100. The S and H inside the image stand for: H- Hepatocyte; S-Sinusoidal

Discussion.

Histological study of the gills shows a typical structural organization of the lamella in the (tspace library.utoronto.ca) control fish while the exposed fish however show progressive architectural distortion(tspace library.utoronto.ca) (hyperplasia and hypertrophy of secondary lamella, degeneration of gill filament, hyperchondrial of gill arch and lesions) at various concentration levels depicts a dose-dependent distortion especially in the higher

concentration of the extract. The functional implication of these lesions portends serious threat to respiration more specifically the obstruction of the interlamellar space (water channel) which has a direct effect on gaseous exchange across the lamellar epithelium of the gill (Camargo and Martinez, 2007; Kumar *et al.*, 2010). This might be due to the potent and toxicological efficacy of the phytochemicals (such as alkaloids, carpaine, tannins, papains) present in *Carica papaya* extracts. This corroborates with the observation of Abubakar (2013) who reported that *C. gariepinus* accumulate toxicants through the lamella surface. Accumulation in the gills is considerable because of their external location and necessarily intimate contact with the water that allows for dissolved or suspended materials in the water to be absorbed through the delicate epithelium. The lamella epithelial lining reacts to the toxicants creating tissue osmoregulatory imbalance. The change in physiological property is evident in shrinkage observed at the lamella thus suggesting that extract of *carica papaya* intake mostly occurs via the gills. Prolonged exposure results in marked degeneracy of the epithelium followed by (tspace library.utoronto.ca) reduction in lamellae. Respiratory distress caused by the degeneration of gills is a sign that the total (tspace library.utoronto.ca) surface area of the gill has been drastically reduced. Asphyxiation occasioned by insufficient gaseous exchange was responsible for the death (tspace library.utoronto.ca) observed at the highest concentrations of the *carica papaya* extracts. The gill histopathology alterations recorded by *C. papaya* extracts in this study are similar to the works of Ajani and Ayoola (2010) who documented gill lesions as a result of the effect of *Adenia cissampeloides* on the gill of tilapia. Abalaka *et al.* (2010) also reported lesions on African catfish as a result of toxicity of extracts of *P. biglobosa*. It is also similar to the findings of Idi-Ogede *et al.* (2016) who studied the effects of sniper 1000EC on the histology of *Oreochromis niloticus* under laboratory conditions. The 240 mg/L⁻¹ concentration of aqueous extract of *C. papaya* dry seed was observed to alter the secondary lamella of the gill and thus, made it appeared hyperchondrial and less vascularised. This result is in agreement with the study of Olurin *et al.* (2006) who reported fused secondary lamellae, hyperplasia and oedema in the gills of *C. gariepinus* (academicjournals.org) exposed to glyphosate. Hyperplasia in some situations represents adaptations (academicjournals.org) by the organism to protect underlying tissues from toxicants (Abubakar *et al.*, 2019). However, increase in thickness of epithelial layers (academicjournals.org) and fusion of adjacent secondary lamellae as a result of hyperplasia will not only decrease

the surface area available for oxygen extraction, it will also increase the oxygen diffusion distance between water and blood (Idi-Ogede *et al.*, 2016). Thus, as hyperplasia might indeed have a protective role, it may also inhibit the respiratory, secretory and excretory functions of the gills (doi.org). It lowers circulation at the gills, widens the blood spaces and thus contracts the pillar cells (Idi-Ogede *et al.*, 2016). Epithelial and Hyperplasia were also observed by Abubakar (2013). Variations in the epithelial surface of gills show important physiological adaptations relating to the area available for increased gaseous exchange (Olurin *et al.*, (2006). The vulnerability of the gill is considered because of their external location and (academicjournals.org) intimate contact with water which made them liable to damage by toxicants. The lamellar reduction of the treated fish must have been caused by respiratory stress. Fused lamellae can disrupt the circulation of the deoxygenated blood via the branchial arteries into the secondary lamellae in a direction opposite to that of water flow. As a result, oxygen uptake is hampered (Olurin *et al.*, 2006). This can cause asphyxiation, tissue necrosis and final death. Apparent lamellae oedematous changes were observed and were probably due to increased capillary permeability which could lead to difficulty in respiration and osmo regulation stress may occur (Olurin *et al.*, 2006).

The liver of fish may not show the diversity (academicjournals.org) of pathology as seen in higher animals due to lack of Kupffer cells in the liver sinusoid (academicjournals.org) (Abubakar, 2013). However, its susceptibility to a number of toxicants cannot be overemphasized (Olurin *et al.*, 2006). The high proportion of fibrotic tissue within the lobules and peribiliary connective tissue of the treated fish showed hepatic cirrhosis. It is thus believed that the most dramatic cirrhosis found in fish is the peribiliary cirrhosis of the hepatorenal syndrome associated with dietary toxicity (Abubakar, 2006). The most frequent of the degeneration was hepatocytes enlargement with large vacuoles and sinusoid congestion (Abubakar, 2013). The shrinkage of the hepatic cells can result in cirrhosis - the contracting of the blood vessels thereby greatly impeding the portal flow through the liver (academicjournals.org) (Olurin *et al.*, 2006). The functions of the liver such as the conversion of glucose to glycogen for storage, regulation of lipids and deamination of amino acids are impaired. The blockage of the sinusoids makes the blood flow from the hepatic artery and veins into the central vein rather difficult (Olurin *et al.*, 2006). The sinusoids widened to make up the right volume of blood in the central vein. The function of the canaliculi that forms the bile duct is

hampered and as such, bile secreted from gall bladder cannot adequately get into them. The exposure of *C. gariepinus* to glyphosate caused histopathological alterations both in gills and liver tissues (academicjournals.org). The high accumulation of *C. papaya* extract in the liver of the fish signifies a key role in accumulation and detoxification. Although agent such as metallothionein in fishes when bioaccumulate in the liver tissue may reach a proportion in which the function of the liver may be impeded, thus resulting in gradual degeneration of the liver cells (Idi-Ogede *et al.*, 2016). The surface area of the liver cell is also decreased which may be due to the increase in the intrabiliary fibre-connective tissue. The intercellular spaces seen are zones of total cell degeneration. Thus cirrhosis, the outcome of prolonged hepato-cellular injury is manifested by fibrosis of hepatic cords and peribiliary connective tissue. Oxygen required to support the intense metabolic activity of the liver is supplied in arterial blood via the hepatic artery (Abubakar *et al.*, 2019) which results in necrosis of parenchyma cells. Sinusoids clog (doi.org) up thereby blocking the blood from the hepatic artery and the interbiliary portal vein which will have to pass through the sinusoids (tspace.library.utoronto.ca) to get to the central vein. The inability of blood to get to the central vein on time makes the liver pump blood harder resulting in liver stress (Olurin *et al.*, 2006). The (tspace.library.utoronto.ca) kupffer cells are attached to the walls of the sinusoids (phagocytic cells of the liver which participate (with the spleen) in the removal of spent erythrocytes and other particulate debris from circulation. The function of the kupffer cells will be impeded due to the clogging up of the sinusoids (Olurin *et al.*, 2006). This makes old erythrocytes to accumulate in the blood (tspace.library.utoronto.ca). These erythrocytes are weak and their binding power to oxygen is seriously reduced such that less oxygen is taken into the body system (doi.org). The canaliculi surrounding the liver cells are also clogged by the syncytial arrangement of the degenerated liver cells and bile secretion into the canaliculi is impaired. Also lipid regulations by the liver are affected.

Conclusion

In conclusion, aqueous extract of *Carica papaya* dry seed brought distortion in structure, anatomy, physiology, morphology and health condition of exposed fish' gills and livers. It was recommended that continual introduction of (tspace.library.utoronto.ca) botanical extracts into the streams, rivers and ponds should be checked order to sustain the life of aquatic biotas.

References

- Abubakar, M.I. (2013). Toxicity of 2,3-dichlorovinyl dimethyl phosphate (sniper 1000EC) on *Clarias gariepinus* (Burchell, 1822) and *Oreochromis niloticus* (Trewava, 1983) under laboratory conditions. PhD Theses. Federal University of Agriculture, Abeokuta, Nigeria (doi.org). 184pp
- Abubakar, M.I (2016): Fish pathology in perspective. Ibadan. Published by Boga Press, Mokola-Ibadan, Oyo State. Pp 178.
- Abubakar, M. I., Adeshina, I., and Abdulraheem, I. (2019). Histopathology of the gills, livers and kidney of *Clarias gariepinus* (Burchell , 1822) exposed to sniper 1000EC under laboratory conditions. 19–30. <https://doi.org/10.18276/ab.2019.26-02>
- Acquaye, D., Smith, M., Letchamo, W. and Simon, J. (2000). A snopp: The miracle tree: Center for new use agriculture and natural plant product. Rutgers Uni., Cook College, 59 Dudley Road, Foran Hall, New Brunswick. Acute toxicity of the root extract of Jimson's Weed, *Datura innoxia* to the African catfish (*Clarias gariepinus*) fingerlings (www.eajournals.org). *Journal of Aquatic Science*, 17(2):131-133.
- Adaramoye, O., Achem, J., Akintayo, O. and Fafunso, M. A. (2010). Lipid lowering effect of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol (doi.org) *Atherosclerosis*, 2: 17 – 108.
- Ajani, A. A. and Ayoola, S. O. (2010). Acute toxicity of (www.eajournals.org) piscicidal plant extracts (*Adenia cissampeloides*) on tilapia (*Sarotherodon galilaeus*) juveniles. *Iranian Journal of Energy and Environment* , 1(3): 246 – 254.

- Akah PA, Oli AN, Enwerem NM, Gamaniel K. (2001). Preliminary studies on purgative effect of *Carica papaya* root extract. *Fitoterapia* (doi.org) 1997; 68(4):327-331. 2.
- Auta, J. (2001). Toxicity of Dimethoate to Juveniles of *Oreochromis niloticus* (Trewavas) and *Clarias gariepinus* (Tengels). Ph.D. Thesis. Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria.
- Ayotunde E.O, Offem B.O (2005). Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to Nile Tilapia *Oreochromis niloticus* (Linne 1757), Fingerlings. *Journal of Agricultural Technology and Environment*. 2005;1: 1- 4 (www.journaljfar.com)
- Camargo M.M and Martinez C. B. (2007). Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. *Neotrop. Ichthyol*(doi.org) 2007; 5:327-336.
- Desai, S.D., Desai, D.G. and Kaur, H. (2009). Saponins and their biological activities. *Pharma Times*, 41: 13–16.
- Ekpendu, E. A., Saliu, J. K., & Otitolaju, A. A. (2016). The joint action effects of the binary mixtures of some botanical piscicides on post juvenile *Clarias gariepinus*. 8 (February), 20–27. <https://doi.org/10.5897/IJFA2015.0513>.
- Fanta E, Saint Anna Rios F, Romao S, Vianna A, Fieberger S (2003). Histopathology of the fish *Corydoras paleatus* contaminated with sublethal levels of organophosphorus in water and food. *Ecotoxicol. Environ. (doi.org)Safety* 54:119-130.

- Genten, F., Danguy, A., and Terwinghe, E. (2009). Atlas of Fish Histology (1st ed.) CRC Press.
- Hinton, D.E., Baumann, P.C., Gardner, G.R. Hawkins, W.E., Hendricks, J.D., Murchelano, R.A., Okihiro, M.S., (2016). Biochemical, Physiological and Histological Markers of Anthropogenic Stress, *Lewis Publishers, Boca Raton*. Pp (doi.org) 155-209
- Idi-Ogede, A.M., Ezeri, G.N.O., Omoniyi, I.T., and Ikililu, A. (2016). Effects of sniper 1000EC on body weight, liver and kidney histology of *Oreochromis niloticus* under laboratory conditions. *Gashua Journal of Irrigation and Desertification studies*. 2(2). Pp 214-223
- Kolbasi, T.B., Burcu, I., Ucuncu, S., Onen, O. (2009). The effects of sodium perchlorate on the liver of Molly fish (*Poeciliasphenops*, Cyprinidae, Teleostei). *African Journal of Biotechnology*, 8, 2640–2644(doi.org).
- Kumar, M., Prasad, M. R., Srivastva, K., Tripathi, S., and Srivastva, A. K (2010). Branchial histopathological study of catfish *Heteropneustes fossilis* following exposure to purified neem extract, azadirachtin. *World Journal of Zoology*, 5(4): 239 – 243.
- Ojogu, N.A., Annune, P.A and Okaji, G.R (2017). Toxicological effects of aqueous extract of *Piptadenas triumaficanumbak* on *Clarias gariepinus* juvenile, *Banat's Journal of Biotechnology*. 8(15): 123-135.
- Olaifa, F.E., Olaifa, A.K. and Lewis, O.O. (2003). Toxic stress of lead on *Clarias gariepinus* (African catfish) fingerlings. *Afr. J. Biomed Res.*, 6:101-104.
- Olurin, K.B., Olojo, E.A.A., Mbaka, G.O and Akindele, A.T. (2006). Histopathological

responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate. *African Journal of Biotechnology* (repository.ipb.ac.id) 5(24). Pp. 2480-2487.

Synthesis, Characterization, and Antimicrobial Studies of 3,3-thiodipropionic Acid Sulfathiazole, Sulfamethoxazole, and Salicylaldehyde Schiff-Base Metal Complexes

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Abstract

Schiff- bases, as obtained from sulfa drugs, act as ligands capable of effectively coordinating with metal ions. Complexes of mixed sulphathiazole and 3,3-thiodipropionic acid ligand, and alkaline earth metal complexes of sulfamethoxazole [4-amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide] and salicylaldehyde Schiff-base were synthesized by refluxing technique. The metal complexes obtained were characterized by elemental analysis, spectroscopic techniques (Fourier-transform infrared spectroscopy), and powder X-ray diffraction techniques. The Schiff-base ligand was observed to bind as a bidentate coordinating via the with N- and O- groups. Fourier-transform infrared study of the Schiff-base showed a strong band at 3379cm^{-1} due to phenolic $\nu(\text{O-H})$ which is seen to shift to 3471 and 3454cm^{-1} in the Ca(II) and Mg(II) complexes respectively, while the $\nu(\text{M-O})$ and $\nu(\text{M-N})$ were seen at 565 and 450cm^{-1} respectively in the $[\text{Ca}(\text{SMX-L})]$ complex, and at 560 and 460cm^{-1} respectively in the $[\text{Mg}(\text{SMX-L})]$ complex. Antimicrobial behaviour of the mixed sulphathiazole and 3,3-thiodipropionic acid metal

complexes showed activity against the *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*Klebsiella spp*) *Pseudomonas* (*Pseudo*), *Escherichia coli* (*E. coli*) Microbes.

Keywords: Schiff base, metal complexes, antimicrobial, alkaline earth metals

Introduction

In recent times, therapeutic medicines/drugs have been invented having improved properties (toxicological and pharmacological) in complexes and Schiff-base ligands. Schiff bases possess versatile imine (C=N) group which have been reported to have broad spectrum of biological activities and the incorporation of metals ions into these ligands forming metal complexes have also been investigated and found to show some degree of improved antibacterial, antifungal, antitumor, and anti-inflammatory activities [Gupta *et al.*,1998]. Schiff bases, first reported in 1864 by Hugo Schiff, are condensation products of ketones (or) aldehydes (aldehyde and ketones) with primary amines [Worku *et al.*, 2003].

The Sulfonamides, considered as efficient antibacterial group, are part of the first sets of antimicrobial drugs used, which paved the way for the antibiotic revolution in medicine. It has been intensively manufactured since the 1940s [Tilles 2001] and sulfamethoxazole (SMZ or SMX) is one of such classed as a sulfonamide bacteriostatic antibiotic. It is an antibacterial drug which has been used since the 1960s in the treatment of various systemic infections, and also against gonorrhoea, meningitis, and serious respiratory tract infections

(*Pneumocystis carinii*)[Ma *et al.*, 2007]. Sulphathiazole, is a 4-amino-N-(1,3-thiazol-2-yl)-benzenesulfonamide commonly utilized as a chemotherapeutic agent. Metal chelates of sulfanilamides have been reported to exhibit interesting physiological and pharmacological properties possessing more bacteriostatic activity than the sulfa drug itself [Bethi *et al.*, 2011].

Studies on the chelates of sulfanilamide have been relevant physiologically and pharmacologically due to the broad bacteriostatic effect of the metal chelates of these sulfa-drugs than the parent-drugs themselves [Bethi *et al.*, 2011]. It is reported that sulfonamide derivatives can exhibit a wider variety of pharmacological activities by the presence of entities such as metal ions of the different functional groups without a modification of the structural-S(O)₂N(H)- features/ functional group [Owa *et al.*, 1999]. The possibility of obtaining enhanced antimicrobial properties in metal complexes of heterocyclic ligands containing nitrogen, sulfur, and/or oxygen atoms has attracted increased attention due to the quest for better drugs [Johnson and Yardily, 2019].

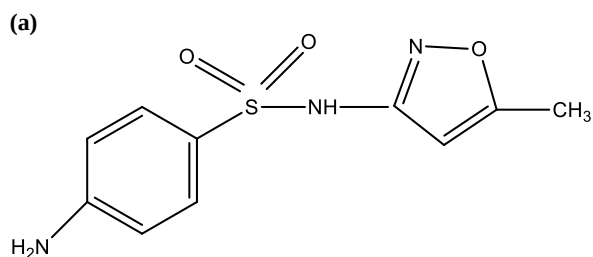




Figure 1: Structure of (a) Sulphamethoxazole; (b) Sulphanilamide (c) p-Amino Benzoic Acid

The antimicrobial activity of these drugs is believed to arise from the structural resemblance of the sulphanilamide group with the p-amino benzoic acid where the sulpha drug acts in like manner with this metabolite by blocking folic acid synthesis in the bacteria resulting in the death of the cells [Mukta and Nehra, 2002]. The presence of the SO_2NH moiety in sulpha- drugs such as sulphadiazine, sulphamethoxazole, and sulphathiazole imparts the important toxophoric function [Mukta and Nehra, 2002] which enables several drugs containing this functionality to be in extensive clinical use, including as antihypertensive agent [Kanda *et al.*, 2011], antibacterial [Stokes *et al.*, 2012], antiprotozoal, antifungal [Rahavi *et al.*, 2008], anti-inflammatory [Kennedy *et al.*, 1999], nonpeptidic vasopressin receptor antagonists [Serradeil-Le Gal, 2001], and translation initiation inhibitors [Natarajan *et al.*, 2004].

Studies conducted by Bharti *et al.*, (2013) suggested that Schiff bases derived from sulpha drugs are good ligands which are able to coordinate to transition metal ions forming metal complexes due to these ligands having several coordination modes. The ability of sulpha drugs to concentrate in the urine

before being excreted enables their utilization in the treatment of urinary tract infections[Bharti *et al.*, 2013].

There is limited literature report on the preparation as well as antimicrobial studies of alkaline-earth metal complexes of sulfamethoxazole Schiff base, and the transition metal complexes of mixed 3,3-thiodipropionic acid and sulphathiazole ligands. Report of this mixed-ligand complexes is also limited. We herein report the synthesis of Schiff base ligands using sulfamethoxazole and salicylaldehyde, and the preparation of the metal complexes of the prepared Schiff base ligands. We also report the preparation of metal complexes of mixed 3,3-thiodipropionic acid and sulphathiazole ligands, and their antimicrobial studies.

Materials and Method

The sulfa drugs, sulphamethoxazole (SMX) and sulphathiazole (STZ), were obtained locally from Sam Pharmaceuticals Ltd, Ilorin, Kwara State, Nigeria, while the salicylaldehyde (98 %) and 3,3-thiodipropionic acid (TDP, 99 %) ligand were purchased from Sigma Aldrich Co., Germany. Melting point measurements were carried out using an Electrothermal 1A9000 Series digital melting point apparatus, elemental analysis (CHN) was carried out using a Perkin Elmer 204C microanalyser at Medac Ltd., Surrey, UK, and X-ray diffraction analysis was carried out using a Nonous Kappa CCD diffractometer in the 2θ range of $4 - 40^\circ$ using $\text{Cu K}\alpha$ ($\lambda = 1.5406\text{\AA}$). Fourier transform infrared (FTIR) spectra was obtained on a Shimadzu 8400 spectrum FT-IR instrument over a wavelength range of $500 - 4000\text{ cm}^{-1}$.

Synthesis of the Schiff-base (SMX-SL)

A solution of 0.253g of sulphamethoxazole (1mmol) in ethanol (30ml) was slowly added to a solution of 0.1ml (1mmol) of salicylaldehyde in ethanol (20ml). Four drops of glacial acetic acid were added to the resulting mixture and the mixture was refluxed for 6 hours at 80 °C. Yellow coloured crystals were obtained and isolated from the resulting clear solution after 7 days.

Synthesis of the Ca(II) Metal Complex of the Schiff Base [Ca(SMX-SL)]

The Schiff-base ligand prepared and the metal salt were reacted in a 1:1 mole ratio. The Schiff base (0.143g, 0.4 mmol) was weighed and dissolved in 7ml of hot ethanol, 0.0944g (0.4 mmol) of calcium(II) nitrate tetrahydrate was also weighed and dissolved separately in 7ml of ethanol. The two solutions were then mixed together giving a pale-yellow coloured mixture which was refluxed for 6 hours at 80°C. The solution obtained was left standing and orange crystalline solids were obtained and isolated after 14 days.

Synthesis of the Mg(II) Metal Complex of the Schiff base [Mg(SMX-SL)]

The Schiff-base ligand prepared and the metal salt were reacted in a 1:1 mole ratio. The Schiff base (0.0714 g, 0.2 mmol) was weighed and dissolved in 6 ml of hot ethanol, 0.0512 g (0.2 mmol) of magnesium(II) nitrate hexahydrate was also weighed and dissolved separately in 6 ml of ethanol. The two solutions were then mixed together giving a pale-yellow coloured mixture which was refluxed for 6 hours at 80 °C. The solution obtained was left

standing and deep-yellow crystalline solids were obtained and isolated after 10 days.

Synthesis of the Transition Metal Mixed Ligand Complexes

Sulphathiazole (0.255 g, 1 mmol) was weighed and dissolved in 10ml ethanol and added to an ethanol solution of 3,3-thiodipropionic acid (0.178 g, 1 mmol). To this mixture, 0.1704g (1 mmol) of CuSO₄ dissolved in 10ml distilled water was added. The mixture was then refluxed for 2 hours at 100 °C. A clear solution was obtained thereafter and kept in a conical flask covered with perforated aluminum foil and left standing. Precipitates were formed after three weeks, filtered, dried at room temperature, and stored in a sample bottle. Similar procedures were used for the preparation of the Ni(II) and Fe(II) metal complexes.

Antimicrobial Screening Test

The disk-diffusion method employed for the antimicrobial screening of the mixed sulphathiazole and 3,3-thiodipropionic acid metal complexes against the *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*Klebsiella spp*) *Pseudomonas* (*Pseudo*), *Escherichia coli* (*E. coli*) Microbes.

Results and Discussion

Melting Point and Elemental Analysis Results

The [Ca(SMX-SL)] and [Mg(SMX-SL)] complexes of the Schiff base prepared showed melting points of 215 °C and 205 °C respectively which was observed to be higher than the melting point (198 °C) of the Schiff base (SMX-SL). This is attributed to the binding of the metal atom to the Schiff-

base thereby increasing the melting point. Elemental analysis of the complexes presented in Table 1 indicated the successful coordination of the Schiff base ligand to Ca(II) and Mg(II) ions [Ca(SMX-SL)].

Table 1: Melting Points and Elemental Analysis of the Schiff base and Complexes

Compound	Colour	Melting point (°C)	Elemental Analysis (% found (calc.))		
			C	H	N
SMX-SL	Pale-Yellow	198	57.14 (57.14)	4.13 (4.20)	11.82 (11.76)
Ca(SMX-SL)]	Orange	215	56.82 (56.02)	4.13(3.87)	11.79 (11.53)
Mg(SMX-SL)]	Deep yellow	205	56.87 (56.11)	4.07 (3.98)	11.64 (11.42)

FTIR Spectroscopy Results

FTIR spectra of the metal complexes showed a broad band within the range of 3471-3454 cm^{-1} which was assigned to the ν (OH) absorption band due to the presence of coordinated water while the Schiff base SMX-SL showed strong band at 3379 cm^{-1} due to phenolic -OH group which was observed to be absent in the complexes indicating the involvement of this group in coordination with the metal ion. The bidentate mode of coordination of the Schiff base was observed in the sharp absorption bands at 468 and 550 cm^{-1} attributed to the ν (M-N) and ν (M-O), in the [Ca(SMX-SL)], and at 451 and 569 cm^{-1} attributed to the ν (M-N) and ν (M-O), in the [Mg(SMX-SL)]. The $\nu(\text{SO}_2)$ of the ligand and the complexes showed no appreciable difference which indicates that the group was not involved in coordination. The relevant absorption bands for the Schiff base and its complexes are presented in Table 2.

FTIR absorption bands of the mixed sulphathiazole & 3,3-thiodipropionic acid Cu(II) complex, [Cu(STZ)(TDP)SO₄], shows the $\nu(\text{M-O})$ absorption bands at 561 cm^{-1} while the $\nu(\text{M-N})$ band was observed at 485 cm^{-1} . The

[Fe(STZ)(TDP)Cl₂] complex showed absorption bands for the $\nu(\text{M-O})$ at 501 cm^{-1} while the $\nu(\text{M-N})$ was observed at 469 cm^{-1} . The $\nu(\text{M-O})$ absorption band for the [Ni(STZ)(TDP)Cl₂] was observed at 558 cm^{-1} while the $\nu(\text{M-N})$ was observed at 479 cm^{-1} . These absorption bands indicate that the two ligands are coordinated to the metal ions in the complexes. Table 3 presents the absorption bands observed in the FTIR spectra of the mixed-ligand complexes.

Table 2: FTIR Absorption Bands of the Schiff base and Complexes

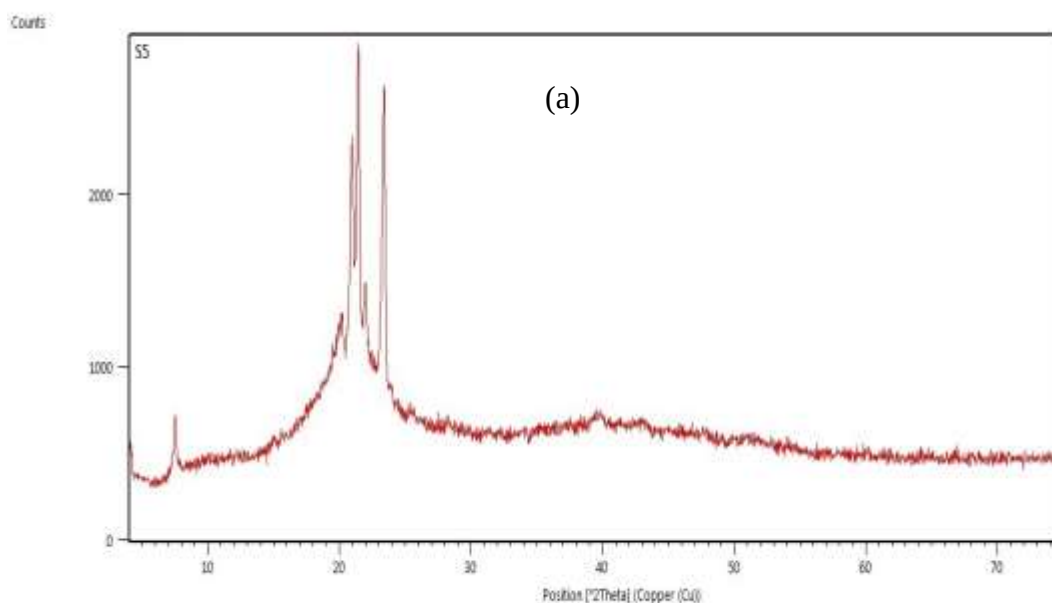
COMPOUND	$\nu(\text{OH})$ cm^{-1}	$\nu(\text{SO}_2)$ cm^{-1}	$\nu(\text{NH})$ cm^{-1}	$\nu(\text{C=N})$ cm^{-1}	$\nu(\text{C-O})$ cm^{-1}	$\nu(\text{M-N})$ cm^{-1}	$\nu(\text{M-O})$ cm^{-1}
SMX	3300.31	1267.27	3379.40	-	1157.33	-	-
SMX-SL	3379.40	1344.43	3379.40	1693.56	1163.11	-	-
[Ca(SMX-SL)]	3471.98	1338.64	3471.98	1685	1166.97	550	468
[Mg(SMX-SL)]	3454.62	1336.71	3454.62	1693.56	1166.97	569	451

Table 3: FTIR Absorption Bands of the Mixed-ligand Complexes

Ligand/complexes	$\nu(\text{O-H})$ cm^{-1}	$\nu(\text{C-O})$ cm^{-1}	$\nu(\text{M-O})$ cm^{-1}	$\nu(\text{M-N})$ cm^{-1}	$\nu(\text{C=O})$ cm^{-1}	$\nu(\text{C=N})$ cm^{-1}	$\nu(\text{C-N})$ cm^{-1}
Sulphathiazole	3588	1144	-	-	1717	1676	1419
3,3-thiodipropionic acid	3480	1194	-	-	1723	-	-
[Cu(STZ)(TDP)SO ₄]	3568	1140	567	485	1715	1659	1409
[Fe(STZ)(TDP)Cl ₂]	3553	1153	501	469	1707	1682	1403
[Ni(STZ)(TDP)Cl ₂]	3572	1151	558	479	1714	1678	1411

Powder X-ray Diffraction (PXRD) Analysis Results

PXRD analysis of the $[\text{Fe}(\text{STZ})(\text{TDP})\text{Cl}_2]$ complex showed prominent peaks at 2θ values of 9° , 20° , 21° , and 25° , while the $[\text{Cu}(\text{STZ})(\text{TDP})\text{SO}_4]$ complex showed a prominent peak at 2θ value of 20° with the pattern indicating the amorphous nature of the compound [Adimula *et al.*, 2017; 2018]. The PXRD patterns of the $[\text{Fe}(\text{STZ})(\text{TDP})\text{Cl}_2]$ and $[\text{Cu}(\text{STZ})(\text{TDP})\text{SO}_4]$ are shown in Figure 2(a & b) respectively.



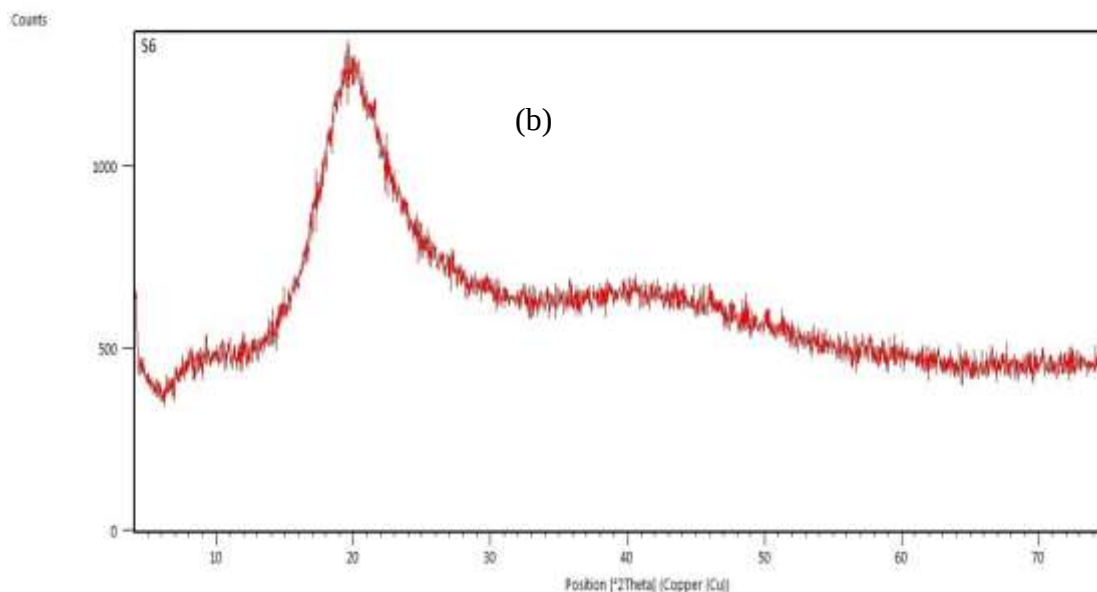


Figure 2: PXRD pattern of the (a) $[\text{Fe}(\text{STZ})(\text{TDP})\text{Cl}_2]$ complex; and (b) $[\text{Cu}(\text{STZ})(\text{TDP})\text{SO}_4]$ complex

Antimicrobial Screening Results

The mixed ligand complexes were tested against some disease-causing microorganisms and were found to be active only against the *Klebsiella spp* microbe. The $[\text{Ni}(\text{STZ})(\text{TDP})\text{Cl}_2]$ was observed to have the highest activity amongst the metal complexes synthesized against the *Klebsiella spp* microbe. This activity was observed to be higher than that of the sulphathiazole parent drug. This is attributed to the presence of the metal ion in the complex. Table 4 presents the antimicrobial screening results of the mixed sulphathiazole-3,3-thiodipropionic acid metal complexes, the activity shown by the metal complexes was seen to be higher than that exhibited by the ligands.

Table 4: Zone of Inhibition of the Organisms in the presence of the complexes

Organisms	Zone inhibition (mm)				
	TDP	STZ	[Ni(STZ)(TDP)Cl ₂]	[Fe(STZ)(TDP)Cl ₂]	[Cu(STZ)(TDP)SO ₄]
<i>Klebsiella spp</i>	0.00	20.00	27.00	29.00	25.00
<i>S. aureus</i>	0.00	0.00	0.00	0.00	0.00
<i>Pseudo</i>	0.00	0.00	0.00	0.00	0.00
<i>E. coli</i>	0.00	0.00	0.00	0.00	0.00

Conclusion

Mixed-ligand metal complexes of Fe(II), Ni(II), and Cu(II) ions were prepared, characterized, and tested against some disease causing microorganisms. Antimicrobial screening results showed that these complexes are active against the *Klebsiella spp* microbe and showed better activity than the parent sulphathiazole drug. Elemental analysis of the Schiff-base metal complexes showed that the Schiff-base successfully coordinates with the Ca(II) and Mg(II) ions separately, and FTIR analysis showed the bidentate mode of these coordination.

References

Adimula, V. O., Tella, A. C., Udejaja, I. C., Durodoye, A. T., and Lawal, A. (2018). Coordination polymers based on mixed carboxylate ligands: synthesis and thermal studies. Commun. Fac. Sci. Univ. Ank. Series B. 60(2): 1-16.

Adimula, V.O., Tella, A.C., Shamle, N. and Lawal, A. (2017). Solid-state synthesis and characterization of Cu (II) complexes of 3,4,5-trihydroxybenzoic acid. *Ilorin Journal of Science*. 4(1): 151 – 160.

Bethi, S., Vidyasagar, M., Rajamanohar, K., Venkateshwar, R. J. and Gummudavelly, S. (2011). Synthesis and pharmacological evaluation of new benzimidazole derivatives. *Der. Chem. Sinica.*, 2(1)P: 84-90.

Bharti, J., Suman, M., Neha, S., and Shrikant, S. (2013). Synthesis, Characterization and Antibacterial Studies of Co (II) and Fe (II) Complexes with Sulfamethoxazole Schiff Base. *Asian Journal of Biochemical and Pharmaceutical Research*. 3(3): 152- 158.

Gupta, R., Kumar, R., and Gupta V. (1998). Four-Membered Heterocycles. In: *Heterocyclic Chemistry*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-72276-9_7.

Jain, M., Sampat, N., Trivedi, P. C., and Singh, R. V. (2002), Nematicidal, Fungicidal and Bactericidal Activities of Manganese (II) complexes with Heterocyclic Sulphonamide Imine. *Metal-Based Drugs*, 9: 1-2.

Johnson, J. and Yardily, A. (2019). Chalconoid metal chelates: spectral, biological and catalytic applications *Journal of Coordination Chemistry*. 72: 2437-2488.

Kanda, Y., Kawanishi, Y., Oda, K., Sakata, T., Mihara, S., Asakura, K., Kanemasa, T., Ninomiya, M., Fujimoto, M., and Kanoike T. (2011). Synthesis

and structure-activity relationships of potent and orally active sulfonamide ETB selective antagonists. *Bioorg. & Med. Chem.* 9: 897-907.

Kennedy, J.F. and Thorley, M. (1999). *Pharmaceutical Substances*, 3rd Ed, A. Kleeman, J. Engel, B. Kutscher & D. Reichert George Thieme Verlag, Stuttgart/New York, 1999, pp.2286.<https://doi.org/10.1023/A:1008114712553>.

Ma, M., Cheng, Y., Xu, Z.; Xu, P., Qu, H., Fang, Y., Xu, T., and Wen, L. (2007). *Evaluation of polyamidoamine (PAMAM) dendrimers as drug carriers of anti-bacterial drugs using sulfamethoxazole (SMZ) as a model drug. European journal of medicinal chemistry* 42 (1): 93–98.

Natarajan, A., Guo, Y., Harbinski F., Fan Y.-H., Chen H., Luus L., Diercks J., Aktas H., Chorev M., and Halperin J. A. (2004). Novel arylsulfoanilide–oxindole hybrid as an anticancer agent that inhibits translation initiation. *J. Med. Chem.* 47: 4979.

Owa. T., Yoshino, H., Okauchi, T., Yoshimatsu, K., Ozawa, Y., Hata, S. N., Nagasu. T., Koyanagi, N. and Kitoh, K. (1999). Discovery of novel antitumor sulfonamides targeting G1 phase of the cell cycle. *J. Med. Chem.* 42, 3789-3799.

Ezabadi, I. R., Camoutsis, C., Zoumpoulakis, P., Geronikaki, A., Soković, M., Glamočilić, J., and Čirić A. (2008). Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: Synthesis, biological evaluation, lipophilicity and conformational studies. *Bioorg. & Med. Chem.* 16, 1150-1161.

Serradeil-Le, G. C. (2001): An overview of SR121463, a selective non-peptide vasopressin V2 receptor antagonist. *Cardiovascular Drug Rev.* 19: 201-214.

Stokes, S. S., Albert, R., Buurman, E. T., Andrews, B., Shapiro, A. B., Green, O. M., McKenzie, A. R., and Otterbein, L. R. (2012). Inhibitors of the acetyltransferase domain of N-acetylglucosamine-1-phosphate-uridylyltransferase/glucosamine-1-phosphate acetyltransferase (GlmU). Part 2: Optimization of physical properties leading to antibacterial aryl sulfonamides. *Bioorg. & Med. Chem. Lett.* 22, 7019-7023.

Tilles, S. A. (2001). Practical issues in the management of hypersensitivity reactions: Sulfonamides. *South Med. J.* 94: 817-824.

Worku, D., Negussie, M., Raju, V. J. T., Theodros, S., and Jonsson, J.A. (2003), Studies on transition metal complexes of herbicidal compounds. II: Transition metal complexes of derivatized 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) *Bull. Chem. Soc. Ethiop.* 17(1): 35-43.