



Thirty days oral *Aframomum melegueta* extract elicited analgesic effect but influenced cytochrome p4501BI, cardiac troponin T, testicular alfa-fetoprotein and other biomarkers in rats

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ABSTRACT

Ethnopharmacological relevance: Pain is the commonest symptom of a disease and the percentage of persons manifesting one form of pain is growing globally. *Aframomum melegueta* (AM) is commonly used by traditional doctors as medication for many ailments such as body pains and rheumatism because it possesses anti-inflammatory, anti-allergenic, antiviral, anti-ageing and anti-tumour phytochemical agents.

Aim of the study: Traditionally a botanical remedy in the management of pain was assessed. A common tropical plant *Aframomum melegueta* (AM) was evaluated for the amelioration of pain. For further pharmacologic understanding sensitive marker were used to assess the effect of the extract on the organ as a multifaceted approach to the evaluation of safety and analgesic efficacy.

Materials and method: Sensitive biomarkers such as troponin-T (CTnT), cardiac troponin-I (CTnI), interleukin-beta (IL- β), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) were evaluated using the enzyme-linked immunosorbent assay (ELISA) method and electrocardiographic parameters were also evaluated. The dynamics of concentrations of the various subfamilies of cytochrome were also assessed using ELISA in the evaluation of thirty-day oral AM, while histopathological changes of organs were also observed.

Results: Thirty-day oral AM doses 40 mg/kg and 80 mg/kg showed analgesic potential but influenced IL-6 level, IL-1 β , TNF- α and P-LCR. Electrocardiographic parameters showed the extract had arrhythmogenic effects the other cardiac parameters influenced was CTnT. The testicular alfa-fetoprotein and prostate specific antigen were also influenced. There were also some histopathological changes.

Conclusions: The extract showed analgesic, anti-oxidant and anti-inflammatory potential with possible adverse effects consistent with testicular and prostate cancers, cardiovascular complication, hepatic congestion and cholestasis.

1. Introduction

Major adverse effects confronted in the management of pain using the non-steroidal anti-inflammatory drugs (NSAIDs) include; metabolic and hormonal disorders, ulcers, hepatotoxicity, nephrotoxicity and a number of other drug idiosyncrasies in human and veterinary

therapeutics (Harirforoosh et al., 2014). Literature had shown *Aframomum melegueta* (AM) is commonly used by traditional doctors as medications for concoctions in various treatments of many ailments among which include body pains and rheumatism (Mohammed et al., 2017; Moret, 2013; Osuntokun, 2020). It possesses various active phytochemical principles which could elicit anti-inflammatory,

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anti-allergenic, antiviral, anti-ageing and anti-tumour effects (Onoja et al., 2015). Some of its African vernacular names are *ose oji*, *ataare* and *cittááin* Nigeria, *Fam-wisa*, *wisa* in Ghana and *nengrekondre peprein* Liberia (Ilic et al., 2014; Osuntokun, 2020; Umukoro and Ashorobi, 2007). It is a perennial herb commonly cultivated for its medicinal and nutritional value in the Tropical zones and other parts of the world. It was observed in previous research that it possesses hepatoprotective effects which was attributed to cytoprotective potentials that mitigates xenobiotics (El-Halawany et al., 2015). This property is due to the array phytoactive substances which in tandem confers antidotal tendency (El-Halawany et al., 2015; Omoboyowa et al., 2017). These phytoactive substances justify reasons for the multi-systematic effects of its seeds in human and animals which makes relevant in many traditional poly-herbal compilations. In a previous research conducted it was observed that methanol-chloroform extracts of AM significantly played a protective role in CCl₄-induced acute liver injury which might be due to its antioxidative potentials which, ameliorated the inflammatory responses and apoptosis of liver tissue (El-Halawany et al., 2015).

This study focuses on revalidation of the folkloric claims therapeutic and safety using sensitive biomarkers. This would further unveil more medicinal potentials and ensure caution being taken to avoid adverse effects. There are claims that AM used to be administered for a relative longer time ranging in concoctions by traditional healers to achieve treatment (Kemka Nguimatio et al., 2019).

A previous review buttressed the fact that traditional herbal practitioners extensively use AM in treatment and prevention of ailments in Africa and other parts of the world (Osuntokun, 2020). AM could be administered to their patients for weeks and sometimes months. Many scientific findings previously conducted were aimed at assessment of its haematological, biochemical and pharmacological potentials. The continuous administration of extracts of AM by oral administration or other routes could result in the bioavailability of active principles reaching levels in the bio-phase that might predispose to toxicity and adverse effects (Ilic et al., 2014; Inegbenebor et al., 2010). In the same vein, many conventional drugs such as NSAIDs have a number of side effects among which are ulcer, decreased rate of platelets aggregation, abortion, hepatotoxicity and nephrotoxicity among others (Harirforoosh et al., 2014). These adverse effects prompted this scientific work to further assess its pharmacologic efficacy using an analgesimeter and carry out other molecular biomarkers in understanding the effect of AM. This study evaluates the effect of the methanolic extract of AM orally administered for 30 days on haematological, biochemical and other sensitive biomarkers such as troponin-T (CTnT), cardiac troponin-I (CTnI), interleukin-beta (IL-β), interleukin-6 (IL-6), tumor necrosis factor-alfa (TNF-α) and electrocardiographic parameters. This study also determines the dynamics of concentrations of the various subfamilies of CYP450 involved in the metabolism of the AM such as CYP 450 1A2, CYP 450 2C19, CYP 450 3A4, CYP 450 3A5 and CYP 450 1B1. The histopathological effects of the AM on the parenchymal tissues were also observed.

2. Materials and method

2.1. Ethical approval

Ethical approval was sought from the Faculty of Veterinary Medicine, University of Ilorin and the proposal was subjected to screening to ensure adequate care to the experimental animals. The committee also ensured that monitoring of procedures was carried out to ensure standard in conformity to approved and recommended laid down rules according to the National Institutes of Health (NIH) guideline for animal care and use (Albus, 2012) after which Ethical Approval Number FVMERC/007/2018 was assigned.

2.2. Plant description, procurement and the ethanolic extraction

Aframomum melegueta (AM) commonly known as Grains of Paradise, which belongs to Kingdom *plantae*, Phylum *Tracheophyta*, Class *Liliopsida*, Order *Zingiberales*, Genus *Aframomum*, Species *Aframomum melegueta* was checked with was checked with <http://www.theplantlist.org> and the plant's description was carried out to ensure easy identification while adhering to standard practices in plant research (Chan et al., 2012). A Voucher Specimen Number 1511 was assigned to specimen at the herbarium of Department of Biological Sciences, Ahmed Bello University, Zaria, Nigeria where the plant was authenticated at the botanical unit. Dried AM fruits were locally purchased from Oja Oba market, Olorunsogo, Igbeti City, longitude 8° 45' 11.3558"N and Latitude 4° 8' 9.6828"E and elevation 378 '310, Oyo State, Nigeria. 500 g of seeds were unsheathed from the fruit, air dried and the dried seeds later powdered using mortar and pestle (Moulinex, China). The sample was pulverized then subjected to extraction using a Soxhlet extractor (UGO basile®, Italy) at a temperature range of 60 °C–80 °C where it was soaked in n-hexane for 72 h, stirred at intervals before it was filtered and after which the filtrate was soaked in methanol for another 24 h with intermittent agitation. This extract was filtered, and then the filtrate subjected to in vacuo evaporation and concentrated using a rotary evaporator (UGO basile®, Italy) as previously conducted by Ilic et al. (2014) to produce semi-solid methanolic extract of *A. melegueta* with 2.67% yield, which was used for this experiment.

2.3. Experimental animals

Adult male Wistar rats of weight range 245–290 g were procured from the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ilorin, Nigeria. The animals were housed using the standard laboratory cage specifications. Animals were managed in conformity with internationally accepted standards for laboratory animal use and care as stated by the Canadian Council on Animal Care, (CCAC, 1993). They were fed with pelletized feed (Top feed®, Nigeria) and were given water *ad libitum*. Animal room temperature and relative humidity were 19–22 °C and 30–70%, respectively, and there was a 12 h light/dark cycle. The rats were allowed to acclimatize for 21 days prior to commencement of experiment in accordance with the guidelines for laboratory animal care and use (Institute for laboratory animal research, 2011).

2.4. Experimental design

A total of 24 Wistar albino rats were randomized into four groups of 6 rats each and includes a control given distilled water at 2.5 ml/kg and three other test groups that were administered 40 mg/kg and 80 mg/kg of methanolic extract of AM orally for thirty days and the group administered oral diclofenac (Tuyil® Nigeria) at a dose of 2 mg/kg. The conventional drug was administered 2 h prior to induction of pain using an analgesimeter, and after calming the rats, heart assessment was carried out. The dose selection was in the range of previous published therapeutic studies (Derry et al., 2015). The dose per body weight was calculated based on using standard procedure and manufacturer's instructions.

2.5. Assessment of analgesic potentials of *Aframomum melegueta* and electrocardiography

A paw pressure analgesimeter (UGO basile®, Italy) of model 37,216 was used in the induction of pain (İkiz Gediz et al., 2015; Kumar et al., 2012). The hindlimbs and the forelimbs were used in this assessment. The force to induce pain was measured on the scale of 10 g steps per calibrated mark, so 11 mark calibrated would be considered 110 g. It was considered that the model used in this study has a lower pressure of 50%. The induction of pain was observed and reading was taken after

resentment and withdrawal of the limb by the animal subjected to the load using standard technique. The electrocardiographic parameters such as HR (Heart Rate), P (atrial contraction), PR (delay at Atrioventricular bundle), QRS (ventricular contraction), QT (time from ventricular contraction to ventricular relaxation), QT_c (QT corrected) were measured using an EDAN® - VE 1010 digital veterinary electrocardiographic machine as previously described by Azeez et al. (2017).

2.6. Blood collection and haematological analysis

After induction of pains, the animals were rested for a week before blood samples were collected by cardiac puncture. The rats were euthanized by humane decapitation and samples were collected into sterile lithium heparinized tubes (NIH Office of Animal Care and Use Animal Research Advisory Committee, 2005; van Herck et al., 2001). An automated analyzer Cell Dyne®, model 331,430 (USA) was used to carry out the following haematological analysis; packed cell volume (PCV), haemoglobin concentration (HGB), red blood cell (RBC) count, white blood cell (WBC) count, mean haemoglobin concentration (MHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) as well as platelets (PLT) count, PLT distribution weight, PLT large cell ratio. White blood cells and differential cells count were also analyzed using the automated analyzer Cell Dyne®, model 331,430 (USA).

2.7. Biochemical and serum chemistry analysis

The biochemical analysis was conducted at Central Research and Diagnostic Laboratory, Ilorin, Nigeria registration number 1225407. Samples collected for biochemical analysis and plasma were decanted by centrifuging at 4500 rpm. The biochemical parameters analyzed were ALT (Alanine Aminotransferase), ALP (Alkaline Phosphatase), AST (Aspartate Aminotransferase), Ca⁺⁺ (Calcium ion), K⁺ (Potassium ion) and Na⁺ (Sodium ion), using commercial test kits Randox® (United Kingdom) using the previously principles of Tietz (1976). ABCAM® test kit (USA) for rats, was used to assay for Cytochromes (CYP 450 1A2, CYP 450 2C19, CYP 450 3A4, CYP 450 3A5 and CYP 450 1B1) in samples from liver homogenates of experimental animals in accordance with the manufacturer's instructions. Stress markers such as superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), catalase (CAT), glutathione-reductase (G1-R), glutathione peroxidase (G1-P), glutathione-S-transferase (GSH) were assayed from the homogenized liver and heart tissues respectively using ABCAM® test kit (USA). Other parameters analyzed were Na⁺-K⁺ATPase (Sodium-Potassium Adenosine Triphosphatase), Ca⁺⁺ATPase (Calcium Adenosine Triphosphatase) from red blood cells, Interleukins (IL-β and IL-6), tumor necrosis factor-α (TNF-α), while the cardiac tissue was homogenized for the assay of cardiac troponin I (CTnI), cardiac troponin T (CTnT) Creatinine-kinase (CK) in serum, creatinine-kinase in the testis, serum α-fetoprotein, testicular α-fetoprotein, prostate specific antigen, serum alkaline phosphatase and testicular alkaline phosphatase were also assayed using standard procedure in the ABCAM® test kit manual using enzyme linked immunosorbent assay (ELISA) using a standard procedure.

2.8. Histopathology

Liver tissue was harvested from each experimental rat in the groups. They were preserved in 10% neutral buffered formalin for minimum a day before fixing in wax and a microtome was used to prepare of slides. The tissues were dehydrated by washing in ascending concentration of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned with a microtome, stained with Hematoxylin and Eosin (H and E) and mounted on glass slides. All sections were examined under light microscope (x100) magnification. Photographs of the lesions were taken with an Olympus® photomicroscope (Germany) for observation of histopathological lesions.

2.9. Statistical analysis

The values of various parameters, expressed as mean ± standard deviation (Mean ± SD) were compared using the Analysis of variance (ANOVA) in consonance with Tukey's multiple comparison *post-hoc* test using GraphPad Prism version 5.03. P < 0.05 was considered as level of significance (Hedges and Rhoads, 2010).

3. Results

3.1. Effects of methanolic extract of *Aframomum melegueta* on response to pains

Table 1 shows the analgesic effect of *Aframomum melegueta* when orally administered for 30 days. The AM (40 mg/kg) group had significantly higher weight bearing (P < 0.05) at pain induction by analgesimeter response (higher threshold to pain) and higher analgesic potency than AM (80 mg/kg) group and the control. It was comparatively (P < 0.05) similar in magnitude to the threshold in the group administered with the conventional drug (diclofenac) in the hind limb readings of the rats. However, in the forelimb readings of the AM (40 mg/kg) and (80 mg/kg) groups are comparatively (P < 0.05) similar, while the group administered with the conventional drug (diclofenac) had the highest (P < 0.05) weight bearing (highest threshold to pain and highest analgesic potency) than the control and other test groups.

3.2. Effect of methanolic extract of *Aframomum melegueta* on electrocardiographic parameters

Table 2 shows effect of methanolic extract of *Aframomum melegueta* on electrocardiographic parameters of male Wistar rats. There was a significant (P < 0.05) increase in amplitude of P wave in the AM (80 mg/kg) test group this was followed by the conventional drug group when compared to the other groups.

3.3. Effects of methanolic extract of *Aframomum melegueta* on haematological parameters

The haematological effect of *Aframomum melegueta* when orally administered for 30 days as shown in Table 3 below in which white blood cell and platelet large cell ratio were significantly higher (P < 0.05) in the AM (40 mg/kg) and (80 mg/kg) treated groups when compared to the other test groups. The monocytes count was significantly higher (P < 0.05) for the AM (80 mg/kg) test groups. In the same vein the group administered with the conventional drug was significantly higher (P < 0.05) than the AM (40 mg/kg).

3.4. Effects of methanolic extract of *Aframomum melegueta* on some biochemical parameters

Table 4 shows the biochemical effect of *Aframomum melegueta* when orally administered for 30 days in the following K⁺, Na⁺, Ca⁺⁺, ALT,

Table 1
Effects of Methanolic Extract of *Aframomum melegueta* on response to pains.

Part Assessed	Groups (n = 24)			
	Control	AM (40 mg/kg)	AM (80 mg/kg)	Diclofenac
Hind Limb	11.38 ± 3.26 ^c	14.42 ± 2.76 ^b	10.33 ± 0.97 ^c	25.00 ± 0.00 ^a
Fore Limb	10.38 ± 2.07 ^c	22.40 ± 2.60 ^b	21.21 ± 7.24 ^b	27.15 ± 4.60 ^a

Values are expressed as Mean ± SD. *Aframomum melegueta* (AM). Values bearing different superscripts a, b, c along the same row differ significantly (P < 0.05).

Table 2Effect of methanolic extract of *Aframomum melegueta* on electrocardiographic parameters.

(n = 24) Parameters	Control	AM (40 mg/ kg)	AM (80 mg/ kg)	diclofenac
HR (bpm)	210.00 ± 3.50	199.60 ± 6.30	224.60 ± 15.9	202.60 ± 19.90
P (ms)	39.40 ± 5.50 ^a b	40.20 ± 3.80 ^a b	66.40 ± 16.40 ^a	57.00 ± 20.00 ^b
PR (ms)	57.80 ± 6.00	57.00 ± 3.30	71.40 ± 12.00	69.80 ± 25.20
QRS (ms)	28.80 ± 6.20	24.40 ± 2.60	19.40 ± 3.70	19.40 ± 3.80
QT (ms)	121.80 ± 49.60	133.00 ± 51.70	157.60 ± 30.80	123.80 ± 42.10
QTc (ms)	253.80 ± 7.50	233.20 ± 69.80	292.40 ± 54.80	222.40 ± 84.9

Values are expressed as Mean ± SD. *Aframomum melegueta* (AM). Values bearing different superscripts a, b, c along the same row differ significantly (P < 0.05). HR (Heart Rate); P (Atrial Depolarization); PR (Atrioventricular Bundle Delay); QRS (Ventricular Depolarization); QT (Ventricular Repolarization); QTc (Corrected QT). bpm (Beats per minute); ms (Milliseconds).

Table 3Effects of methanolic extract of *Aframomum melegueta* on haematological parameters.

Parameters	Control	Groups (n = 24) AM (40 mg/ kg)	AM (80 mg/ kg)	Diclofenac
WBC X 10 ⁹ /L	3.78 ± 0.04 ^c	8.48 ± 2.45 ^a	10.99 ± 2.97 ^a	5.96 ± 1.87 ^b
Mono X 10 ⁹ /L	0.73 ± 0.09 ^c	0.74 ± 0.05 ^c	1.90 ± 0.32 ^a	0.91 ± 0.06 ^b
Lym X 10 ⁹ /L	5.25 ± 0.71	7.46 ± 2.37	4.42 ± 1.63	4.12 ± 1.72
Gran X 10 ⁹ /L	0.56 ± 0.05	1.90 ± 0.92	2.70 ± 0.81	1.20 ± 0.23
Lym (%)	69.00 ± 2.20	56.00 ± 6.80	63.00 ± 6.40	55.00 ± 6.20
Mono (%)	18.10 ± 1.89	15.93 ± 2.66	13.30 ± 2.96	21.97 ± 4.69
Gran (%)	13.73 ± 0.53	28.10 ± 5.48	24.08 ± 5.24	21.10 ± 2.60
RBC X 10 ¹² /L	7.57 ± 0.76	8.49 ± 0.16	8.10 ± 0.46	8.68 ± 0.26
HGB (g/dl)	12.8 ± 1.17	13.82 ± 0.22	13.75 ± 0.65	13.33 ± 0.20
PCV (%)	40.69 ± 3.48	44.89 ± 0.66	43.20 ± 2.30	45.72 ± 1.35
MCV (fl)	53.17 ± 0.31	54.17 ± 1.10	54.00 ± 0.82	52.83 ± 0.75
MCH (pg)	17.08 ± 0.28	16.43 ± 0.17	17.20 ± 0.69	16.08 ± 0.21
MCHC (g/dl)	32.10 ± 0.61	32.02 ± 0.63	30.95 ± 0.53	30.96 ± 0.34
RDW (%)	20.18 ± 0.44	21.42 ± 0.56	22.40 ± 0.52	22.15 ± 0.71
PLT X 10 ⁹ /L	611.00 ± 60.40	633.55 ± 109.50	678.50 ± 70.31	676.00 ± 85.56
PLT (%)	0.49 ± 0.05	0.52 ± 0.09	0.52 ± 0.09	0.48 ± 0.07
PLT-L X 10 ⁹ /L	97.00 ± 21.00 ^b	117.0 ± 15.21 ^a	109.40 ± 22.04 ^a	89.25 ± 19.14 ^c

Values are expressed as Mean ± SD. *Aframomum melegueta* (AM). Values bearing different superscripts a, b, c along the same row differ significantly (P < 0.05). WBC (White Blood Cells); Mono (Monocytes); Lym (Lymphocytes); Gran (Granulocytes); RBC (Red Blood Cells); HGB (Haemoglobin Concentration); PCV (Packed Cell Volume); MCV (Mean Corpuscular Volume); MCH (Mean Corpuscular Haemoglobin); MCHC (Mean Corpuscular Haemoglobin Concentration); RDW (Red Blood Cell Distribution Width); PLT (Platelets); PLT-LCR (Platelet-Large Cell Ratio).

AST, ALP in plasma and, Na⁺-K⁺ATPase, and Ca⁺⁺ATPase of the RBC.

3.5. Effects of methanolic extract of *Aframomum melegueta* on tissue antioxidative enzymes

Table 5 shows the effect of *Aframomum melegueta* when orally administered for 30 days on some antioxidative enzymes. There was a significant decrease (P < 0.05) in MDA in the AM (40 mg/kg) and (80 mg/kg) groups when compared to the other test groups in the heart and liver tissues. The glutathione reductase level in the heart and liver tissues were significantly (P < 0.05) higher in the AM (80 mg/kg) test group than other test groups, although (40 mg/kg) was also significantly high (P < 0.05) in the heart tissue than the control.

Table 4Effects of Methanolic Extract of *Aframomum melegueta* on some Biochemical Parameters.

Parameters	Control	Groups (n = 24) AM (40 mg/ kg)	AM (80 mg/kg)	Diclofenac
ALT (U/l)	45.98 ± 20.07	62.01 ± 1.99	57.65 ± 0.24	42.48 ± 1.81
AST (U/l)	110.8 ± 55.42	149.30 ± 1.44	153.70 ± 6.97	157.00 ± 3.40
ALP (U/l)	46.00 ± 2.30	45.77 ± 13.71	57.50 ± 4.24	24.84 ± 5.16
Ca ⁺⁺ (mg/dl)	4.02 ± 0.27	4.19 ± 0.24	3.51 ± 0.034	3.85 ± 0.21
K ⁺ (PPM)	1.83 ± 0.24	1.93 ± 0.24	1.45 ± 0.02	1.70 ± 0.09
Na ⁺ (PPM)	53.26 ± 0.42	52.52 ± 0.54	53.32 ± 0.46	53.65 ± 0.85
Na ⁺ -K ⁺ ATPase (ngiP/min/l)	30.95 ± 6.84	47.71 ± 5.01	45.63 ± 3.09	37.99 ± 1.31
Ca ⁺⁺ ATPase (ngiP/min/l)	3.20 ± 0.42	3.76 ± 0.09	4.63 ± 0.39	4.28 ± 0.65

Values are expressed as Mean ± SD. *Aframomum melegueta* (AM). Values bearing different superscripts a, b, c along the same row differ significantly (P < 0.05). ALT (Alanine Aminotransferase); AST (Aspartate Aminotransferase); ALP (Alkaline Phosphatase); Ca⁺⁺ (Calcium ion); K⁺ (Potassium ion); Na⁺ (Sodium ion); Na⁺-K⁺ATPase (Sodium-Potassium Adenosine Triphosphatase); Ca⁺⁺ATPase (Calcium Adenosine Triphosphatase).

3.6. Effects of methanolic extract of *Aframomum melegueta* on some cytokines and other sensitive biomarkers

Table 6 shows significantly higher (P < 0.05) IL-β level in the AM (80 mg/kg) group when compared to the control and other test group. The group treated with the conventional drug (diclofenac) had a significantly higher (P < 0.05) of IL-6 level than other test groups. AM (40 mg/kg) and (80 mg/kg) test groups had significantly (P < 0.05) higher IL-6 level when compared to the control group. The TNF-α was significantly higher (P < 0.05) in the control group than other test groups. TAFP (testicular alfa-fetoprotein and PSA (prostate specific antigen) had significantly (P < 0.05) higher values in the AM (80 mg/kg) and the AM (40 mg/kg) groups when compared to the control and the conventional treated group. The AM (80 mg/kg) group had the significantly (P < 0.05) higher value, this was followed by the AM (40 mg/kg). The trend of significance in the TAFP and PSA are similar when compared to other groups and are comparatively (P < 0.05) similar. CTnT had highest significant (P < 0.05) level in the AM (80 mg/kg) group, this was followed AM (40 mg/kg) group when compared to the other test groups. CTnI had no significant changes in the experimental groups.

3.7. Effects of methanolic extract of *Aframomum melegueta* on concentration of cytochrome enzymes

Table 7 shows the effects of methanolic extract of *Aframomum melegueta* on the activity of cytochrome enzymes involved in metabolism in male Wistar rats had no significant differences in the levels of CYP subfamilies, the only subfamily had significantly (P < 0.05) higher levels was the CYP 4501B1 in the (80 mg/kg) and (40 mg/kg) groups compared to the control and the conventional drug (diclofenac) treated groups.

3.8. Effects of methanolic extract of *Aframomum melegueta* on the parameters of electrocardiography

Figs. 1–4 show the specific effect of methanolic extract of *Aframomum melegueta* on PQRS and T waves in Wistar rats. Fig. 1 is the normal (non-treated control). Fig. 2: *Aframomum melegueta* (40 mg/kg) resulted

Table 5Effects of methanolic extract of *Aframomum melegueta* on tissue antioxidative enzymes.

	Liver	Tissue		Groups (n = 24)		Heart	Tissue	
Parameters	Control	AM (40 mg/kg)	AM (80 mg/kg)	Diclofenac	Control	AM (40 mg/kg)	AM (80 mg/kg)	Diclofenac
SOD (U/I)	55.97 ± 21.54	59.08 ± 27.77	65.30 ± 25.83	171.6 ± 93.36	121.30 ± 10.60	212.70 ± 84.40	192.40 ± 60.85	82.09 ± 40.70
MDA (U/I)	8.61 ± 0.79 ^b	1.98 ± 1.54 ^d	2.58 ± 1.46 ^c	9.30 ± 0.41 ^a	5.50 ± 4.26 ^a	1.04 ± 0.22 ^d	1.08 ± 0.03 ^d	1.26 ± 0.15 ^c
GSH (U/I)	126.80 ± 1.22	125.40 ± 1.03	128.60 ± 1.28	124.20 ± 1.21	127.30 ± 2.12	126.70 ± 2.08	129.30 ± 1.13	126.70 ± 3.45
CAT (U/I)	473.60 ± 177.0	209.70 ± 72.17	277.20 ± 36.29	350.0 ± 96.78	606.30 ± 156.00	729.90 ± 71.66	497.40 ± 141.50	710.80 ± 33.45
Gl-R (U/mg)	7.79 ± 3.12 ^c	6.19 ± 2.13 ^d	8.19 ± 1.07 ^b	16.37 ± 2.23 ^d	17.86 ± 4.65 ^d	21.55 ± 2.12 ^c	54.32 ± 9.45 ^a	23.12 ± 1.48 ^b
Gl- P (U/I)	2.71 ± 1.88	3.08 ± 0.41	4.51 ± 0.54	2.75 ± 0.68	2.64 ± 1.21	3.19 ± 0.35	4.44 ± 0.57	2.83 ± 0.61
GST (nmole)	0.096 ± 0.00	0.21 ± 0.07	0.11 ± 0.02	0.16 ± 0.013	0.12 ± 0.07	0.071 ± 0.032	0.08 ± 0.02	0.10 ± 0.03

Values are expressed as Mean ± SD. *Aframomum melegueta* (AM). Values bearing different superscripts a, b, c along the same row differ significantly (P < 0.05). SOD (Superoxide dismutase); MDA (Malondialdehyde); GSH (Glutathione); CAT (Catalase); Gl-R (Glutathione-reductase); Gl-P (Glutathione peroxidase); GSH (Glutathione-S-transferase).

Table 6Effects of Methanolic Extract of *Aframomum melegueta* on some Cytokines and other Sensitive Biomarkers.

Parameters	Groups (n = 24)			
	Control	AM (40 mg/kg)	AM (80 mg/kg)	Diclofenac
IL-1β (ng/l)	0.00 ± 0.00	120.70 ± 19.44 ^{a,b}	129.40 ± 16.52 ^a	121.20 ± 29.64 ^{a,b}
IL-6 (ng/l)	31.96 ± 1.60 ^c	52.25 ± 8.11 ^{a,b}	54.77 ± 8.26 ^{a,b}	66.04 ± 10.51 ^a
TNF-α (ng/l)	45.53 ± 6.23 ^a	27.85 ± 6.33 ^b	31.20 ± 1.70 ^b	25.85 ± 2.66 ^b
CTnT (ng/ml)	0.55 ± 0.15 ^b	0.99 ± 0.42 ^{a,b}	1.40 ± 0.41 ^a	0.25 ± 0.00 ^b
CTnI (ng/ml)	0.36 ± 0.07	0.38 ± 0.08	0.39 ± 0.07	0.45 ± 0.00
SCK-MB (ng/ml)	0.75 ± 0.02	0.77 ± 0.03	0.74 ± 0.02	0.77 ± 0.01
HCK-MB (ng/ml)	2.72 ± 0.03	2.70 ± 0.01	2.72 ± 0.01	2.75 ± 0.04
SAFP (pg/ml)	3.24 ± 0.060	3.36 ± 0.16	3.36 ± 0.09	3.50 ± 0.16
TAFP (pg/ml)	3.67 ± 0.05 ^b	5.10 ± 0.04 ^{a,b}	12.56 ± 0.06 ^a	3.72 ± 0.05 ^b
PSA (pg/ml)	4.73 ± 0.64 ^b	5.56 ± 0.25 ^b	6.43 ± 0.98 ^a	3.95 ± 0.39 ^b
SALP (U/L)	1.11 ± 0.07	1.41 ± 0.26	1.23 ± 0.09	0.77 ± 0.05
TALP (U/L)	289.90 ± 264.90	591.40 ± 534	381.8 ± 425.00	400.20 ± 289.00

Values are expressed as Mean ± SD. *Aframomum melegueta* (AM). Values bearing different superscripts a, b, c along the same row differ significantly (P < 0.05). IL-β (Interleukin-beta); IL-6 (Interleukin-6); TNF-α (Tumor necrosis factor-α); CTnT (Cardiac troponin-T); CTnI (Cardiac troponin-I) SCK-MB (Creatinine-kinase in serum); TCK-MB (Creatinine-kinase in the testis); SAFP (Serum α-fetoprotein); TAFP (Testicular α-fetoprotein); PSA (Plasma prostate specific antigen); SALP (Serum alkaline phosphatase); TALP (Testicular alkaline phosphatase).

in the flattening of the T wave; *Aframomum melegueta* (80 mg/kg) also had the same pattern of flattening of the T waves. In Fig. 3, *Aframomum melegueta* (80 mg/kg) increased the amplitude of QRS and resulted in the widening of QRS and T wave when compared to normal in the control. Fig. 4 had normal pattern of PQRS and T.

3.9. Effects of methanolic extract of *Aframomum melegueta* on histopathological changes of liver tissue

Plates 1-8 show the histopathological changes in various test groups with no microscopic changes in the control group. The conventional group also had no lesion in the tissues. There were microscopic changes manifested as lesions in the AM (40 mg/kg) and (80 mg/kg) test groups lesions. The AM (40 mg/kg) test group had mild cholestatic central vein in the liver tissue and no lesions in the heart tissue, while in the (80 mg/kg) test group there was mild congestion in the liver tissue and no lesions in the heart tissue sequel to administration of AM in (40 mg/kg)

Table 7Effects of methanolic extract of *Aframomum melegueta* on concentration of cytochrome enzymes.

Concentration CYP 450	Groups (n = 24)			
	Control	AM (40 mg/kg)	AM (80 mg/kg)	Diclofenac
CYP 450 1A2 (pmole/ml)	7.74 ± 0.56	8.48 ± 1.87	9.66 ± 2.91	7.82 ± 2.11
CYP 450 1B1 (pmole/ml)	3.72 ± 0.36 ^c	4.07 ± 0.19 ^b	5.68 ± 1.00 ^a	3.54 ± 0.19 ^c
CYP 450 2C19 (pmole/ml)	3.98 ± 0.59	3.33 ± 0.97	4.34 ± 1.67	4.02 ± 0.31
CYP 450 3A4 (pmole/ml)	2.69 ± 0.02	2.70 ± 0.04	2.72 ± 0.06	2.66 ± 0.05
CYP 450 3A5 (pmole/ml)	0.79 ± 0.01	0.79 ± 0.02	0.80 ± 0.02	0.79 ± 0.01

Values are expressed as Mean ± SD. *Aframomum melegueta* (AM). Values bearing different superscripts a, b, c along the same row differ significantly (P < 0.05). Cytochrome Enzymes (CYP 450 1A2; CYP 450 1B1; CYP 450 2C19; CYP 450 3A4; CYP 450 3A5).

and (80 mg/kg) for 30 days.

4. Discussion

The blood cells increase by the doses of 40 mg/kg and 80 mg/kg of AM extract in this study is due to the trace elements such as copper and iron and other metabolites on the haematopoietic sites. There was also immune boosting effect of AM which is associated with improvement of the cell mediated. The methanolic AM extract at 40 mg/kg and 80 mg/kg administered for 30 days caused a high concentration due to the cumulative effect of the constituents resulted to an attainment of a steady state of active phytochemical principles and secondary metabolites such as flavonoids, tannins, alkaloids and the nutritional components as iron, copper and vitamin B complex as observed by Omoboyowa et al. (2017). This would result to an advantageous therapeutic effect which reflected on haematological parameters due to its haematinic potentials. This haematinic effect improved the white blood cell count and would elicit high proliferation of the cell mediated components of the blood.

The iron, vitamins and flavonoids also improved the platelet large cell ratio (PLT-LCR) which is an important parameter for the assessment of blood clotting. The meritorious therapeutic tendency of these parameters infers that in contrast to the conventional NSAIDs which cause a decline in thromboxane - a major eicosanoid important in platelet aggregation. AM markedly improved the PLT-LCR; that indicates improvement of thromboxane concentrated in the PLT. The observation on platelets in this work is comparable to the observations of Toh et al. (2019) and Faggio et al. (2017). This finding in this study was not in agreement with another study in which there was a significant

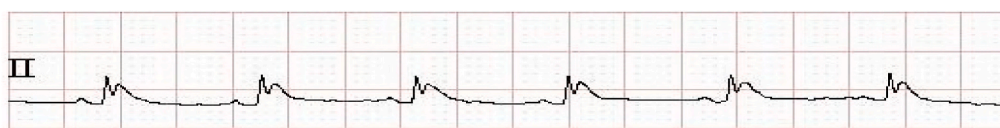


Fig. 1. Control not treated had a normal pattern with PQR and T waves.

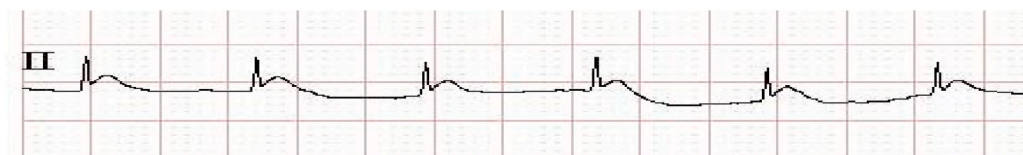


Fig. 2. *Aframomum melegueta* 40 mg/kg showing flattening of the T wave.

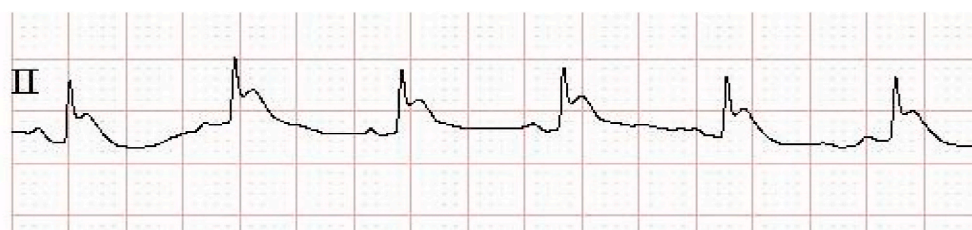


Fig. 3. *Aframomum melegueta* 80 mg/kg showing increased amplitude of QRS and widening of QRS and T wave ending on the baseline.

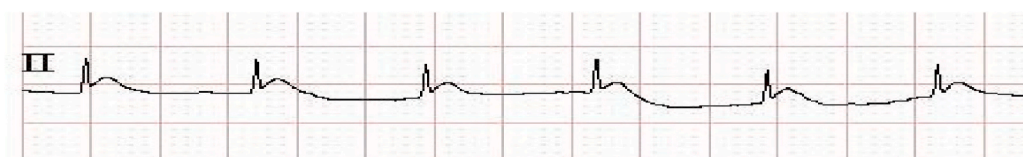


Fig. 4. Diclofenac showing QRS complex and T wave similar to Group B.

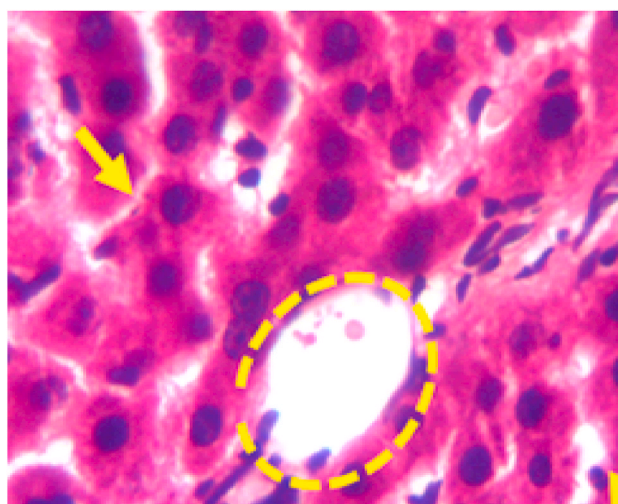


Plate 1. Normal halo-spaced central surrounded by densely packed hepatocytes. The nuclei of the hepatocytes are intensively stained. There was no histopathological alteration. In the control group X400 using H and E stain.

thrombocytopenia after administration of AM (Omoboyowa et al., 2017). The difference in these findings of the two studies could be due to the variation in doses of AM extract. The previous research had relatively higher doses in contrast to this study which was carried out using lower doses administered for 30 days. Another cause in the difference of

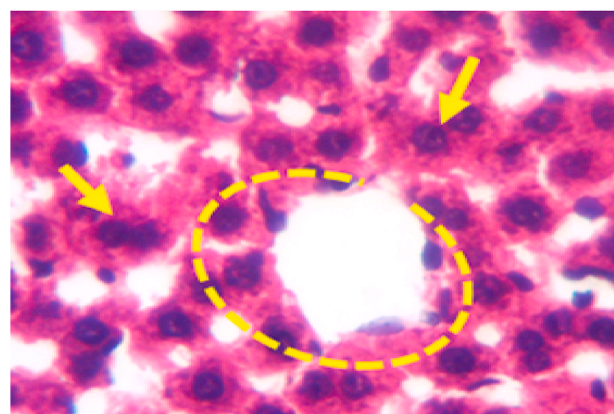


Plate 2. normal halo-spaced central surrounded by densely packed hepatocytes. The nuclei of the hepatocytes are intensively stained. There is no histopathological alteration in the group administered 40 mg/kg of *Aframomum melegueta* x400 using H and E stain.

result is due to the use of 2, 4-dinitrophenylhydrazine in the induction of anaemia in the previous study which would have caused a significant change in the haematological components. This would have reflected on the haemopoietic center and haematological chemical mediators. Also, the duration of administration of AM extract in this study was 30 days and was different from the previous.

This study gives a strong insight that the AM acts in a similar way as

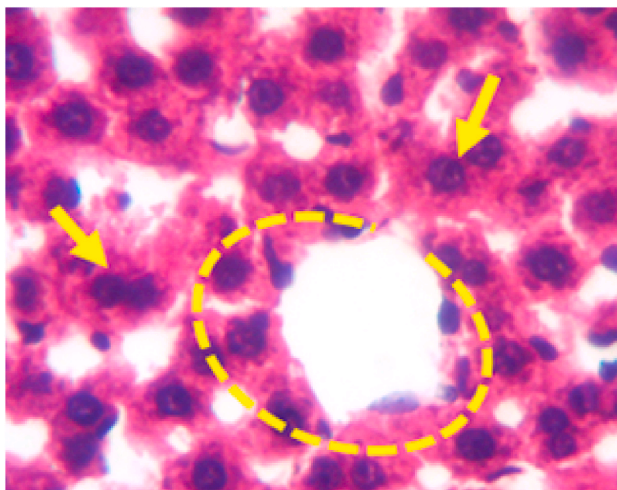


Plate 3. Mild cholestatic central vein with characteristically normal hepatocytes distribution and staining intensity in the group administered 40 mg/kg of *Aframomum melegueta* x400 using H and E stain.

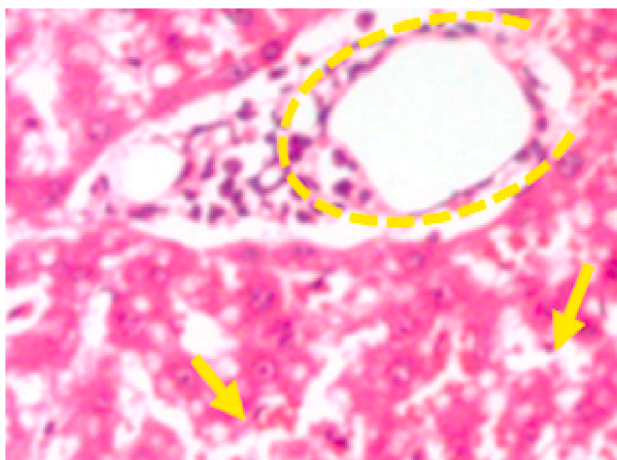


Plate 4. normal halo-spaced central surrounded by densely packed hepatocytes. The nuclei of the hepatocytes are intensively stained. There is no histopathological alteration in the group administered 80 mg/kg of *Aframomum melegueta* x400 using H and E stain.

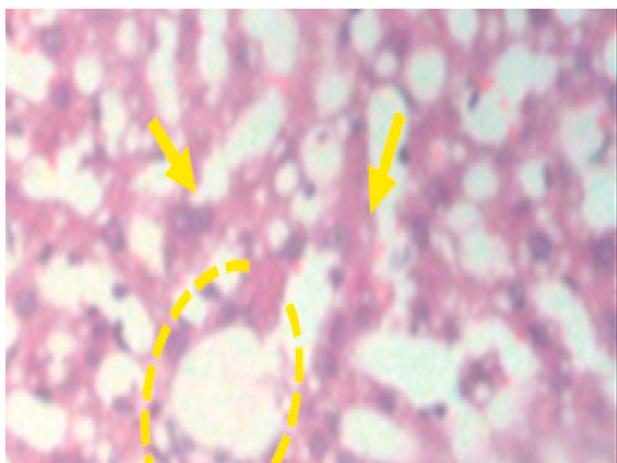


Plate 5. This shows mild congestion of the liver tissue in the group administer 80 mg/kg of *Aframomum melegueta* x400 using H and E stain.

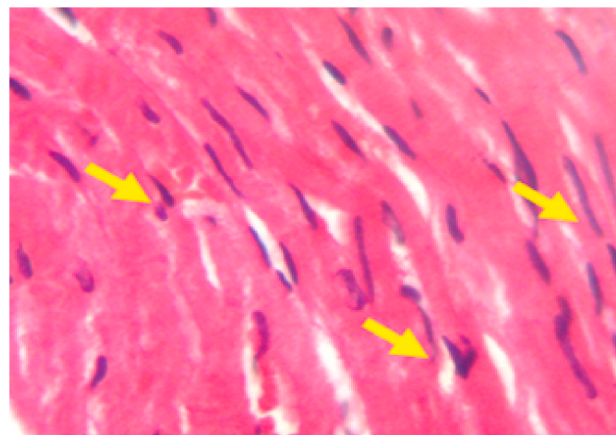


Plate 6. There appear to be numerous cardiac myocytes as indicated by the densely distributed nuclei. There is no apparent histopathological alteration in the group administered 40 mg/kg of *Aframomum melegueta* x400 using H and E stain.

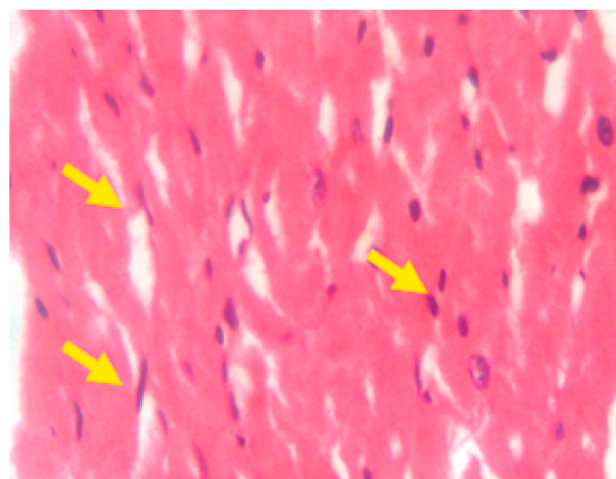


Plate 7. The nuclei appear large and characteristically normal with no histopathological alteration in the heart muscles group administered 80 mg/kg of *Aframomum melegueta* x400 using H and E stain.

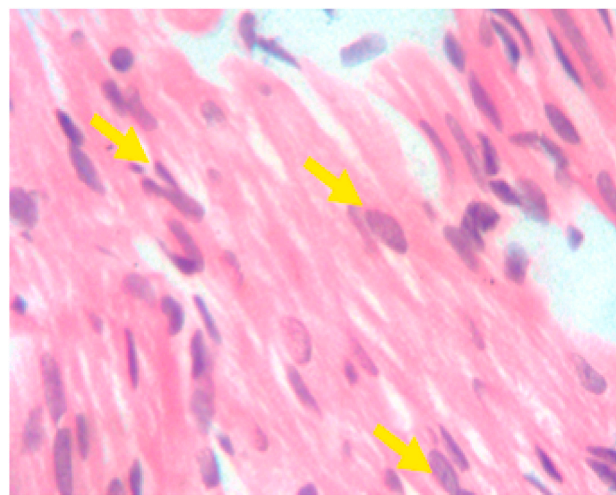


Plate 8. The nuclei appear large and characteristically normal with no histopathological alteration in the group administered 80 mg/kg of *Aframomum melegueta* x400 using H and E stain.

the NSAIDs observed that the ethanolic extract of AM has the potential to produce anti-inflammatory action by inhibition of COX-2 enzyme activity and inhibition of pro-inflammatory genes expression (Ilic et al., 2010, 2014). The finding in this study could corroborate with the scientific fact of presence of compounds which could be associated with the pharmacologic efficacy as an anti-inflammatory remedy.

In this study AM altered the dynamics of the cytokines assessed by decreasing the TNF- α when compared to the control. It is a well-known fact that it might be due to down-regulation in the gene expression in the cells of inflammation and is also associated with induction of expression of selectin (Ilic et al., 2014). It implies that there was decrease in the mediators associated with macrophage inflammatory proteins, which are chemokines that attract macrophages to site of inflammation. Chemokines could also be associated to tumour formation, another perspective to explain this is endogenous production of TNF- α in the tissues could be associated with anticancer activity during non-physiologic regulation which was seen in the control group. This assumption is in line with the previous observation of van Horssen et al. (2006). The AM extract at the doses of 40 mg/kg and 80 mg/kg administered for 30 days decreased the level of TNF- α , therefore, it could be suggested that the extract influenced these chemokines. This infers that there would be less likelihood of cellular apoptosis of target cells of the tissues and this was a reflectance in the parenchymatous tissues including the liver and heart tissue in this research. It also implies that administration of 40 mg/kg and 80 mg/kg administered for 30 days could decrease the tendency to the development of tumor. It is evident from this study that 40 mg/kg and 80 mg/kg administered for 30 days influenced the dynamics of the cytokines IL-1 β and IL-6 in a similar trend. This could be due to the anti-inflammatory effect of the cytokine IL-6. This work therefore strongly gives credence to the fact that the extract could elicit its analgesic effect through influencing the production of IL-6, causing its direct influence on the soluble IL-6 receptors and decrease of pro-inflammatory cytokine impression causing a direct effect on the IL-1 β and most likely its soluble receptors. This is also buttressed by the significant increase or up-regulation in the test group administered with conventional drug (diclofenac). IL-6 therefore, is influenced here to act in eliciting an anti-inflammatory interplay to counteract the pro-inflammatory cytokines to regulate cellular homeostasis.

The analgesic effect of AM at 40 mg/kg administered for 30 days had a significantly higher threshold to pain when compared to the control. This buttressed the fact that the AM extract had analgesic potential. It was suggested in a previous research that AM extract has anti-stress effect and elicits effects on the nerves (Yuan et al., 2017). There is high tendency that the naked nerve endings might be influenced and this might affect the pain pathway thus eliciting it analgesic potential. In the same vein the influence of the extract on the cytokines as IL-6 and IL-1 β might explain its analgesic potential but this is not fully understood. The advantage of this extract over the NSAIDs is its anti-stress and cytoprotective effects. IL-6 is also associated with nervous tissues function in tandem with the peripheral and somatic nervous system especially as occasioned by stress. This scientific elucidation was given credence to by the analgesimeter observations in this work, which showed that the analgesic potentials sequel to induction of pains in the AM extract dose of 40 mg/kg. The AM extract in this study also increased the anti-oxidative enzyme glutathione reductase at a dose dependent extend with the 40 mg/kg at a lower and 80 mg/kg to a higher extent in the liver and heart tissues. This could be directly associated with the anti-oxidative property of the extract due to its rich flavonoid. The AM also manifested high cytoprotective property by decreasing the MDA in the 40 mg/kg and 80 mg/kg by preventing lipid peroxidation which could cause a compromise in the integrity of the cells of tissues. Explanation for antioxidative property is due to the presence of other principles such as capsaicin, dihydrocapsaicin, delphinidin, limonene and linalool that possess potential (Mohammed et al., 2017; Odetunde et al., 2015; Omoboyowa et al., 2017; Onoja et al., 2015). This was also proven

by the non-elevation in the ALT, AST, ALP enzymes due to cellular integrity maintenance by mopping and scavenging reactive associated with induction of pains or exposure to xenobiotic.

The CYP 4501B1 was significantly elevated among the CYP 450 enzymes involved in the metabolism of AM in this study others assessed were the CYP 450 1A2, CYP 4502C19, CYP 4503A4, CYP 450 3A5 whose concentrations were not influenced. This also had been incriminated in cancer because an increase of CYP1B1 was associated with proliferation of the prostate cells and hormonal mediated carcinogenesis in a previous literature (Sissung, 2006). It had also been associated with initiation and progression of carcinogenesis of other tissues. The involvement of this subfamily of CYP1B1 gives an insight of a very high tendency of AM seed extract causing cancer if administered for 30 days.

The CTnT is a sensitive regulatory protein and a marker for myocardial degenerative changes was observed in AM 80 mg/kg group to be significantly elevated compared to the other groups. This is indicative of the effect of extract on the myofibrils of the heart musculature. This study also shows that the level of CTnT in the blood was also dose dependent. This is attributed to the fact that higher concentration of extract could predispose to myocardial infarction. There is also another perspective pertaining to a higher dose of 80 mg/kg when administered for 30 days causing cardiotoxicity. The elevated level of CTnT was reflected on the electrocardiographic changes in this study. The P wave in the 80 mg/kg group altitude indicates the effect of the extract on the sinoatrial node of the heart. The associated increased level phytochemicals including vitamins and electrolytes including sodium, potassium and calcium in the AM extract. AM also had cardiac glycosides that are most likely possess positive inotropic property. Cardiac glycosides could also predispose to cardiotoxicity which manifested as arrhythmias. The presence of cardiac glycosides was a previous finding of another study of Odetunde et al. (2015). This caused an irregularity in the cardiac tone resulting to flattening and widening of T and QRS complex which is an indicative of the changes occurring predominantly in the ventricular region. In the same vein, the pace of depolarization for the onset of another cardiac cycle was also distorted. The widening of the QRS complex could be associated with predisposition to ectopia resulting to bradycardia due to the high dose of 80 mg/kg for 30-days of AM extract. The effects of AM extract administered for 30 days on the electrocardiographic parameters was not common but an assumption could be made by the recent finding that cardiac glycosides could cause the effects in this study as in previous research works (Odetunde et al., 2015; Osuntokun, 2020).

The elevated testicular alpha fetoprotein (TAFP) in the 80 mg/kg AM oral administration for 30-days could be translated to the extract predisposing to carcinogenesis of testicular tissue. This could be associated with the fact that MA had effect on the increase in the surge of testosterone which in turn triggered an androgenic induced carcinogenesis. The scientific evidence could be substantiated further by the elevated level of PSA in the similar trend as the TAFP in the experimental animals. This findings could also be linked with the elevation of CYP 450 1B1 which also was incriminated in a hormonal mediated tumour of prostate Sissung et al. (2006). There is dearth of information on the effect of AM on PSA but speculation could be made that a dose of AM extract at 80 mg/kg for 30 days could predispose to testicular cancer. Previous research had shown that some phytochemical constituents and secondary metabolites could cause a down-regulation of PSA (Chen et al., 2008; Ibrahim et al., 2017). However, our observation in this study was different from the previous studies with an elevation of PSA which could be linked to the cumulative effect of AM secondary metabolites and phytochemical constituents on the receptors in the prostate and the antigen. Also, the difference in the phytochemical constituents in AM could also be an explanation to the variation in effect on the PSA. The dose of 80 mg/kg of AM for 30-day might interfere with the architecture of DNA and resulted to changes in cellular physiology and cellular factors thus causing cellular responses which culminate to carcinogenesis.

5. Conclusions

Methanolic extract of AM abolished pain, accentuated innate antioxidant activities, facilitated hepatic clearance, and induced inflammatory response at both molecular and cellular levels. However, testicular cancer, prostate tumor, cardiovascular and coronary heart diseases, cholestasis and hepatic congestion were potential complications consistent with the administration of methanolic extract of AM for 30 days in rats.

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Author contributions

Biobaku, K. Talha conceptualized and designed this study. He was responsible for collection of *Aframomum melegueta*. In conjunction with Asogwa T Nnaemeka, he did the ethanolic extraction. He also did the entire writeup of this manuscript before it was reviewed by Amid, S. Adetayo for final submission.

Azeez, O. Mistura is a cardiovascular physiologist who is adept in EDAN Electrocardiography. She was responsible for recording and interpreting the ECG in this present study.

Amid, S. Adetayo's contributions were consistent with pain measurement using the analgesimeter as well as creation of graphical abstract and the review of this manuscript for final submission.

Asogwa, T. Nnaemeka was involved in haematology, serum chemistry analyses and Enzyme Linked immunosorbent Assays. He also conducted the statistical analysis of the results.

Abdullahi, A. Abubakar took part in blood sample collection for haematology and serum chemistry analyses. He joined Abdulhamid, J. Atata in the collection of liver tissue sample for histopathological analysis.

Raji, O. Lukman focused on the Enzyme Linked Immunosorbent Assay of the testicular alfa-fetoprotein, prostate specific antigen and testicular alkaline phosphatase. He also reviewed the manuscript before final submission.

Abdulhamid, J. Atata collected liver tissue samples for histopathological analysis and interpretation using an Olympus® photomicroscope.

Declaration of competing interest

The authors declare no conflict.

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