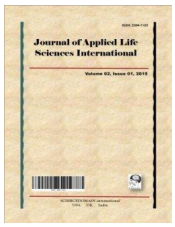

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Biosafety Assessment of Petroleum Ether Oil of *Aframomum melegueta* K. Schum in Wistar Rats

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Author's contributions

This work was carried out in collaboration among all authors. Author ACA did the literature search, designed the study, wrote the protocol and collected the data. Author SIO performed the statistical analysis and also wrote part of the manuscript. Author AAA managed the animals and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: The study was aimed at evaluating the biosafety of reported insecticidal potency of *Aframomum melegueta* petroleum extract (AMPE) used on stored products using wistar rats as a model.
Study Design: Assessment of biochemical indices on treated and control rats.
Place and Duration of Study: Department of Biology Federal University of Technology, Akure, Nigeria. April, 2015.
Methodology: *Aframomum melegueta* seeds were extracted using soxhlet extractor and the filtrate later concentrated using the rotary evaporator to obtain *A. melegueta* petroleum ether extract (AMPE). Twenty four rats were divided into four groups labeled I, II, III and IV (six rats per group). Group I served as the control group and were administered orally with Dimethyl Sulphoxide (DMSO) and normal saline, Group II were administered orally with 500 mg/kg of AMPE, Group III were administered orally with 1000 mg/kg of AMPE while Group IV were administered orally with 2000 mg/kg of AMPE all dissolved in DMSO and Normal saline. Animals were weighed, anesthetized and blood samples were collected at the end 5 days of oral administration. Blood

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samples were collected by cardiac puncture for some biochemical parameters such as Total proteins, bilirubin, cholesterol, uric acid, creatinine, Urea and Uric acid activities as well as serum, AST and ALT were determined.
Results: At the end of this research work, it was discovered that at doses higher than 500 mg/kg there were enzymatic leakages from the organs (kidney and liver) into the serum, compared to the control group.
Conclusion: The study revealed that it is advisable to use minimal dosages for the management of insect pest on products meant to be consumed by human, to prevent damage to vital organs as observed in the results at higher doses while higher doses should be for seeds meant for propagation.

Keywords: Biosafety; biomarkers; protectant; acute toxicity; cannular.

1. INTRODUCTION

Throughout history, humans have used plants to treat all kinds of illness. Plants played an important part in the health of people. They were a staple part of their diets as well as supplementary foods to improve health, and herbal remedies were used to prevent and cure diseases. Several important drugs first discovered in plants are now synthesized more cheaply than they can be extracted but the search for new ones continues especially the medicinal plants of the tropics [1]. Alligator pepper was described by K. Schum in 1904 and is a member of the ginger family (Zingiberaceae), with the scientific name "*Aframomum melegueta"* and is also known as grains of paraside, hepper pepper or mbongo spice.

The phytochemicals obtained from the seed of *A. melegueta* has been used for years in the treatment of infectious diseases and possess active ingredients that may be exploited for local development of antimicrobials and also possesses insecticidal properties [2]. Crude leaf extract of *A. melegueta* (Zingiberaceae) are used in West Africa, as an antidiabetic drug [3] and it is known to have potent antiseptic or bactericidal properties, and have therefore been used in treating wounds and preventions of infections [4]. It is also known to have potent antimicrobial activities against major pathogens. Several empirically validated studies have confirmed that this very odorous, locally available folkloric plant in Africa is a potent insect feeding deterrent, repellent and possess fumigant and/or insecticidal activities. Paradol was identified as the major insecticidal constituent of *A. melegueta* against the cowpea storage bruchid, *Callosobrochus maculatus* [5]. *A. melegueta* volatile oils also strongly repelled *Sitophilus zeamais* adults from maize grains thus highlighting the potential of using them in the protection of stored grains by resource-poor farmers with local access to these plants [6]. Since some constituents of essential oils from aromatic plants hinder the octopaminergic nervous system unique to insects, these culinary spices are hence potential sources of reduced-risk pesticides. Petroleum extracts of *A. melegueta* according to the studies reported by [7] reduced infestation by *Sitotroga cerealella* in paddy rice at low concentrations and totally prevented emergency of adult at higher concentrations. This research therefore aimed at investigating hepatoxicological effect of the petroleum extract of *A. melegueta* seed in Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Dried seeds of *A. melegueta* were purchased from Oja Oba market, Akure, Nigeria. The plant was identified taxonomically and authenticated at the Department of Crop, Soil and Pest Management Technology, (FUTA) Nigeria.

2.2 Preparation of Plant Material

The seeds were dried in the sun, and milled into powder using an electric grinding machine and put into an air tight container until needed. 100 g of the *A. melegueta* was poured in a cleaned mushin cloth and was packed into a Soxhlet extractor glass jar for oil extraction. Petroleum ether was used as solvent in ratio (1:10) for the extraction. After the extraction, it was concentrated using rotary evaporator, to recover the solvent from the oil and then the remaining solvent was left to evaporate at room temperature for 3 days. The extracted oil (AMPE) was put into bottles with air tight lid and kept in the refrigerator until needed.

2.3 Experimental Animals

Twenty- four male Albino Wistar rats (150-240 g) obtained from the laboratory animal unit of the Department of Biochemistry, Federal University of Technology, Akure, were used for this study. They were housed in aluminum cage at 6 rats per cage and were fed *ad libitum* with standard commercial pelleted feed (from Guinness Animal Foods Nig. Ltd.) with free access to clean drinking water. The animals were allowed to acclimatize for two weeks before the start of the experiment. They were kept at normal environmental temperature and natural light/darkness daily cycle. They were maintained in accordance with the recommendations of the *Guide for the care and use of laboratory animals* [8]. Each rat in a group was marked and grouped using six different colours of indelible markers for easy identification and observation in each group. The marking was done on their ears, head and body. The animal experiment protocol was approved by the Federal University of Technology, Akure animal ethics committee.

The freshly prepared plant extract (AMPE) was administered orally at different doses for 5days (every 8:00 am) by gavage method using cannular as follows:

Group I: The control group was Dimethyl Sulphoxide (DMSO) and normal saline.

Group II: 500 mg/kg of AMPE dissolved in Dimethyl Sulphoxide (DMSO) and normal saline.

Group III: 1000 mg/kg of AMPE dissolved in Dimethyl Sulphoxide (DMSO) and normal Saline

Group IV: 2000 mg/kg of AMPE dissolved in Dimethyl Sulphoxide (DMSO) and normal saline.

2.4 Experimental Procedure

2.4.1 Oral acute toxicity study

The oral acute toxicity test of AMPE was determined according to the OECD guideline no. 425 (acute oral toxicity – Up-and-Down-procedure). Briefly, the four groups of rats each were dosed orally with 500, 1000 and 2000 mg/kg of AMPE, respectively and were observed for individually during the first 30 minutes and periodically for the first 24 h for signs of toxicity and death [9]. Thereafter, they were administered the test substance following 100% for four more days. Behavioural manifestations of acute oral toxicity were also noted.

2.4.2 Serum biochemical analysis

Collected blood samples were analysed for serum total protein and albumin content was assayed using standard diagnostic kit (Randox kit, Randox Laboratories, U.K.). Assay for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were done by the method described by Reitman and Frankel [10] using Randox test kits. Uric acid activities, creatinine activities, cholesterol urea and Bilirubin (Total and direct), were assayed using standard diagnostic kit (Randox kit, Randox Laboratories, U.K).

2.5 Statistical Analysis

Data obtained were presented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA). The variant mean were separated by least significant difference (LSD) of the different groups. Significance was accepted at the level of $p<0.05$.

3. RESULTS

3.1 Acute Toxicity Study and Behavioural Changes in Experimental Animals

Changes in the behaviour and other parameters in experimental animals at the end of five days are presented in Table 1. The rats treated with 2000 mg/kg was observed to show sign of lethargy at the first 10 minutes of each dosage for five days, and this was also observed in the rats treated with 1000 mg/kg, however this was not observed in the control group and the group treated with 500 mg/kg. Aggressiveness to touch was highly observed in rats treated with 2000 mg/kg and 1000 mg/kg coupled with loud noise. However, rats in group II were less aggressive and this was also observed in the control group. Food in-take was moderate in both 1000 mg/kg and 2000 mg/kg groups; food in-take was high in group treated with 500 mg/kg and in the control group. The rate of water in-take was high in both group 1000 mg/kg and 2000 mg/kg, while in the control group and 500 mg/kg group water in-take was moderate. Furthermore, no sign of swollen eye, tremor, convulsion, fur shed, salivation and diarrhea was observed in any of the groups. Finally no death was recorded in all the treated rats after five days of administration of the test substance.

Table 1. Behavioural changes in experimental animal

	Groups			
	I	II	III	IV
Parameters				
Swollen eye	Nil	Nil	Nil	Nil
Dizziness	Nil	Nil	Nil	Nil
Food in-take	High	High	Moderate	Moderate
Aggressiveness	Nil	Nil	Moderate	High
Tremor	Nil	Nil	Nil	Nil
Convulsion	Nil	Nil	Nil	Nil
Salivation	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil
Lethargy	Nil	Nil	Minimal	High
Fur shed	Nil	Nil	Nil	Nil
Short Term outcome	Survival	Survival	Survival	Survival
Long Term outcome	Survival	Survival	Survival	Survival

Keys: *Group I: Rats treated with Dimethyl Sulphoxide (DMSO) and normal saline.*
Group II: Rats treated with 500 mg/kg (1.14 ml) of A. melegueta extract dissolved Dimethyl Sulphoxide (DMSO) and normal saline.
Group III: Rats treated with1000 mg/kg (1.68 ml) of A. melegueta extract dissolved Dimethyl Sulphoxide (DMSO) and normal saline.
Group IV: Rats treated with 2000 mg/kg (3.54 ml) of A. melegueta extract dissolved Dimethyl Sulphoxide (DMSO) and normal saline

3.2 Changes in Body Weight of Experimental Animals

Weight change in treated rats before and after exposure period is presented in Table 2. The table revealed that AMPE affected the weight of the rats. There was a decrease in the weight of the animals. Highest Percentage Weight Gain (PWG) of 5.0% was observed in the control group (I), while the group II had PWG of 3.5%, followed by the group III with 2.0% PWG. The lowest PWG was recorded in the group IV (1.6%). Increase in weight of treated rats is higher in the control group compared to other treated groups.

Table 2. Changes in the body weight of experimental animals

	Weight of animals		
Groups	**Before**	**After**	**Weight gain (%)**
I	144^{a}.2±0.02 g	150.2^{a}±0.02 g	5.0
II	159^{b}.2±0.02 g	162.7^{b}±0.05 g	3.5
II	162^{c}.6±0.03 g	164.6^{b}±0.03 g	2.0
IV	16^{d}.7±0.05 g	218.3^{c}±0.05 g	1.6

Values are means of six rats per group

3.3 Effect of AMPE on Some Biochemical Markers of Treated Rats

The effect of AMPE on total protein in the serum of treated albino rats is presented in Fig. 1. At 500 mg/kg, 1000 mg/kg and 2000 mg/kg total protein values were 10.99 g/dl, 9.63 gm/dl and 8.31 gm/dl respectively. There was significant different in the control group (7.12 gm/dl) and dosage at 2000 mg/kg. 500 mg/kg has the highest level of total protein (10.99 gm/dl). Fig. 2 showed the effect of AMPE on serum AST treated rats, comparing the level of AST in the control group with other treated groups, 10.00 U/L, 21.50 U/L and 23.75 U/L were observed in 500 mg/kg, 1000 mg/kg and 2000 mg/kg respectively. There was no significant different in the control group and the group treated with 500 mg/kg compared.

Effect of AMPE on serum ALT values in treated rats is presented in Fig. 3. Comparing with the control group, the serum ALT values were 11.66 U/L, 26.00 U/L and 30.5 U/L at 500 mg/kg, 1000 mg/kg and 2000 mg/kg respectively. The highest value of 30.5 U/L was observed in group treated with 2000 mg/kg while the lowest value of 11.66 U/L was observed in group treated with 500 mg/kg. Fig. 4 showed effect of AMPE on serum urea in treated rats. High urea value of 25.12 mmol/L and 16.96 mmol/L were observed in 2000 mg/kg and 1000 mg/kg. Lowest urea value of 10.50 mmol/L was observed in 500 mg/kg and was not significantly different from the control group which had 7.31 mmol/L.

Effect of AMPE on serum creatinine at 30 seconds and 120 seconds are presented in Fig. 5 and Fig. 6 respectively. At 30 seconds, 500 mg/kg had (85.75 mmol/L), 1000 mg/kg (105.10 mmol/L), and 2000 mg/kg (119.35 mmol/L) while at 120 seconds, 500 mg/kg had (87.35 mmol/L), 1000 mg/kg (106.25 mmol/L),

and 2000 mg/kg (121.04 mmol/L). The control group had 59.23 mmol/L and 62.81 mmol/L at 30 and 120 seconds respectively and it was significantly different from groups treated with 1000 mg/kg and 2000 mg/kg.

In Fig. 7. Effect of AMPE on serum cholesterol in treated rats, the cholesterol values in the treated rat ranged from 4.23 mmol/L in 500 mg/kg to 7.04 mmol/L in 2000 mg/kg and different from the control group (2.66 mmol/L).

Fig. 8 showed the effect of AMPE on serum uric acid in treated albino rats, At 500 mg/kg uric acid value of (356.6 mmol/L), 1000 mg/kg (525.5 mmol/L) and 2000 mg/kg (612.5 mmol/L) were observed respectively. There was a significant different in the value of the control group compared to groups treated with 1000 and 2000 mg/kg.

Fig. 9 revealed effect of AMPE on serum direct bilirubin in albino rats. At 500, 1000 and 2000 mg/kg bilirubin value obtained were 4.72 mmol/L, 6.15 mmol/L and 7.80 mmol/L respectively. There was significant different existed in the control group compared to other groups.

4. DISCUSSION

High level of toxin substances in the serum is an indication of damage to vital organs (liver and kidney) in the body of animals used, it usually result in enzymatic leakages from these organs into the serum [10]. Administration of AMPE above 500 mg/kg caused a significant increase in the level of total protein which may indicate inflammation or infections which may occur as a result of liver and kidney damage. Also administration of AMPE above 500 mg/kg is associated with increased in serum creatinine in this research and this is one of the symptoms of kidney damage. Increase in creatinine has been linked with kidney damages according to [11].

ALT is commonly measured clinically as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health. Alanine transaminase showed a marked diurnal variation [12]. The ratio of ALT to AST (Aspartate transaminase) also has clinical significance. Significantly elevated levels of ALT often suggest the existence liver damage and bile duct problems. Elevated ALT levels due to hepatocyte damage can be distinguished from bile duct problems by measuring alkaline phosphates. Also, myopathy-related ALT levels can be ruled out by measuring creatine kinase enzymes. Many drugs may elevate ALT levels, including Zileuton, omega-3-acid ethyl esters (Lovaza), anti-inflammatory drugs, antibiotics, cholesterol medications, some antipsychotics such as risperidone, and anticonvulsants.

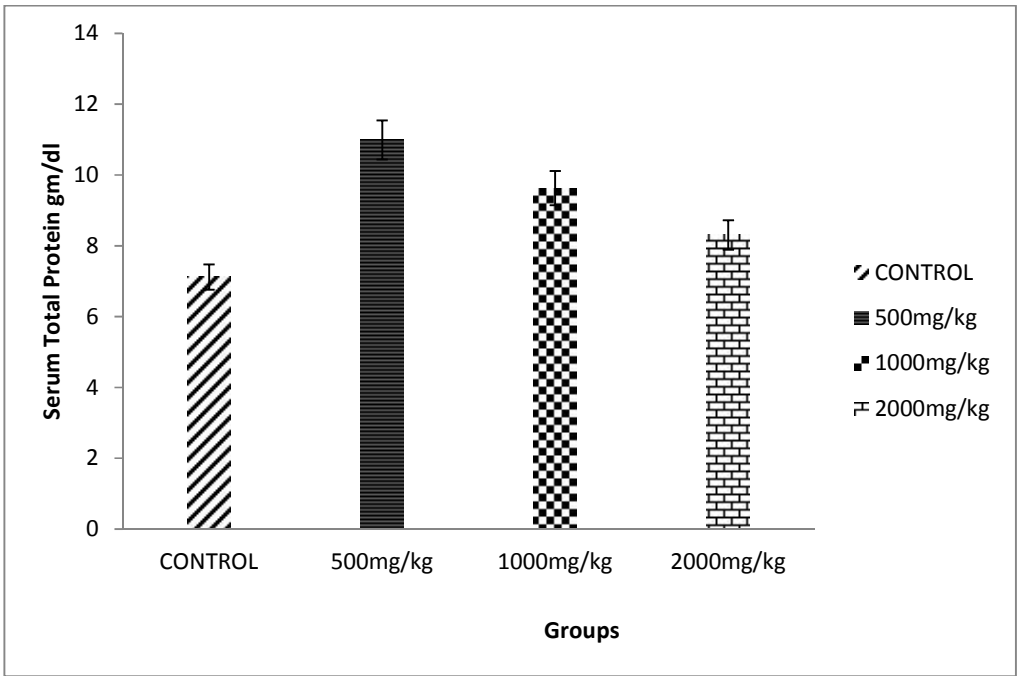

Fig. 1. Effect of *A. melegueta* petroleum ether extract on serum total protein in rats

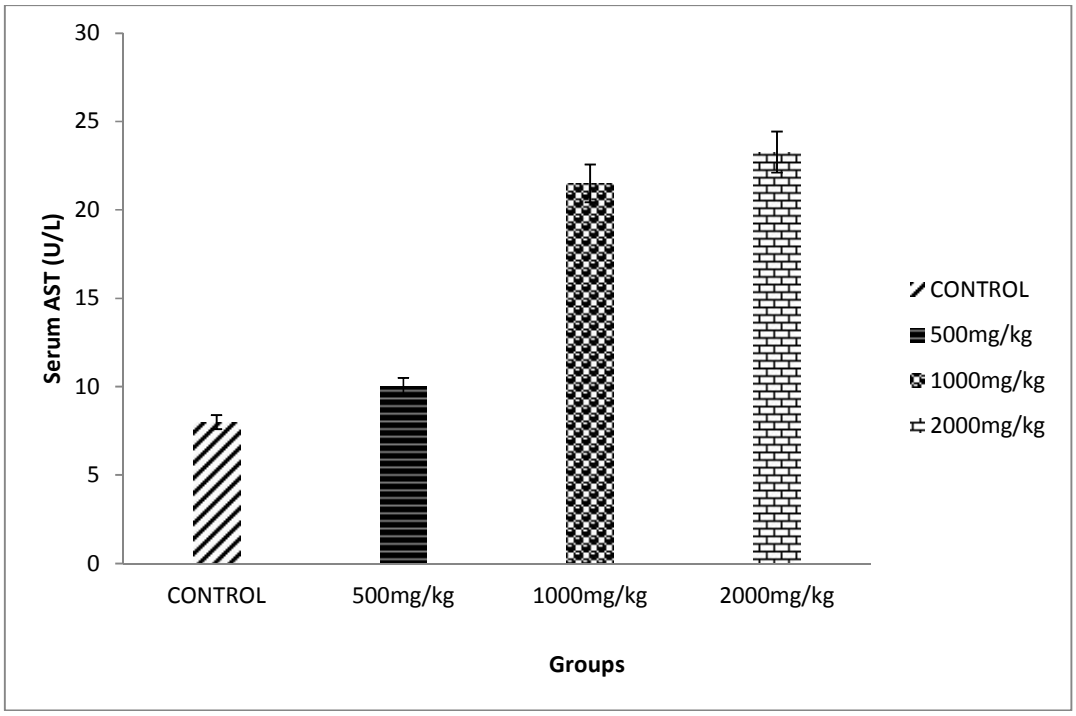

Fig. 2. Effect of *A. melegueta* petroleum ether extract on serum AST in rats

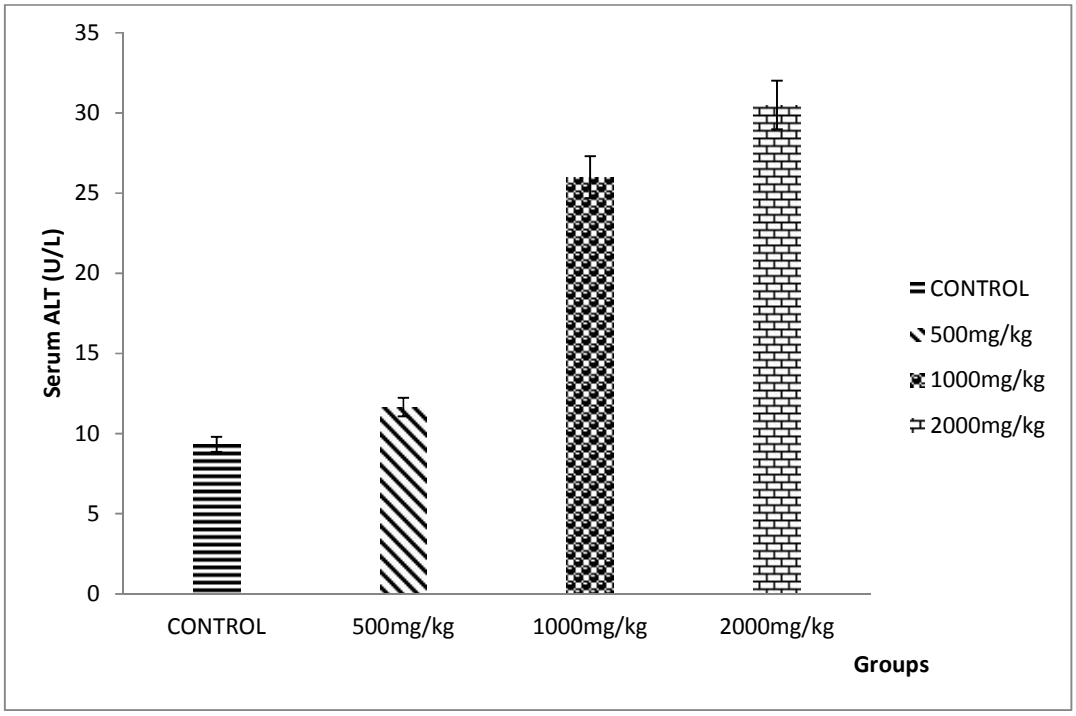

Fig. 3. Effect of *A. melegueta* petroleum ether extract on serum ALT in rats

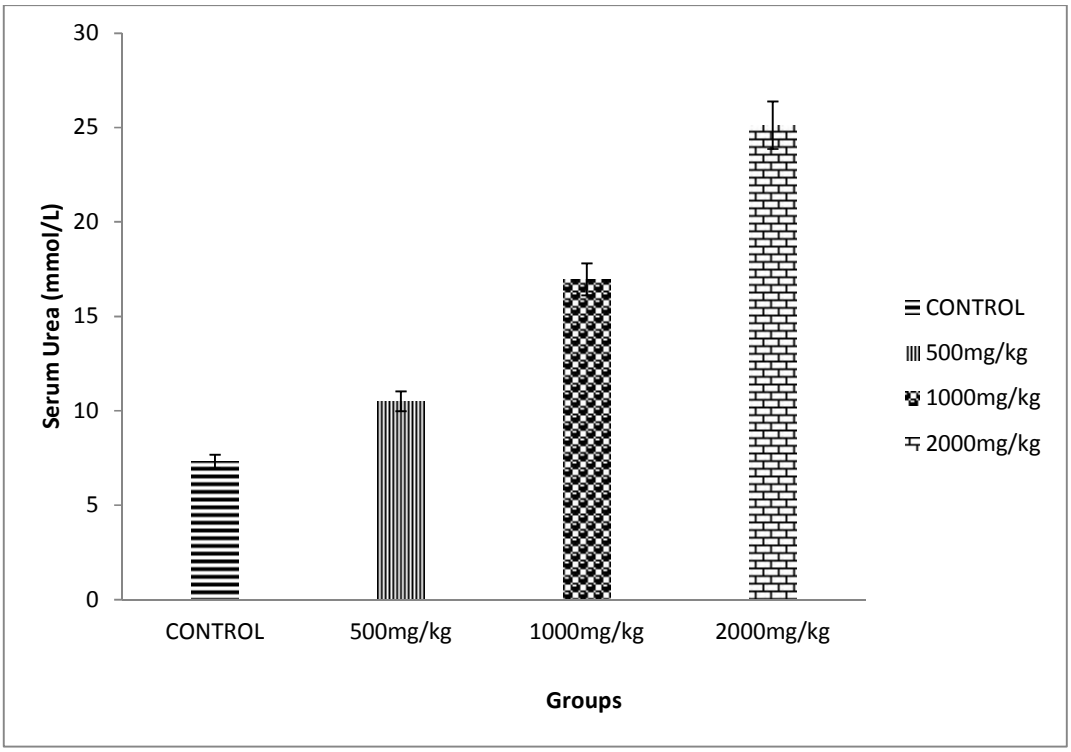

Fig. 4. Effect *A. melegueta* petroleum ether extract on serum urea in rats

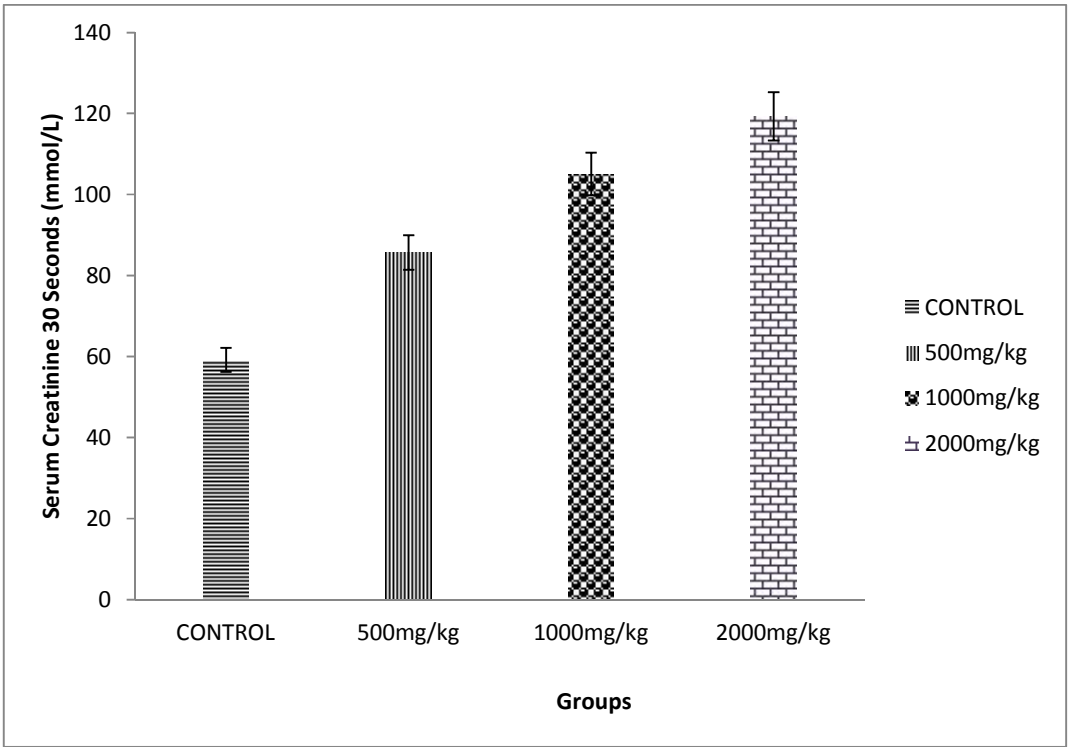

Fig. 5. Effect of *A. melegueta* petroleum ether extract on serum creatinine (30 seconds) in rats

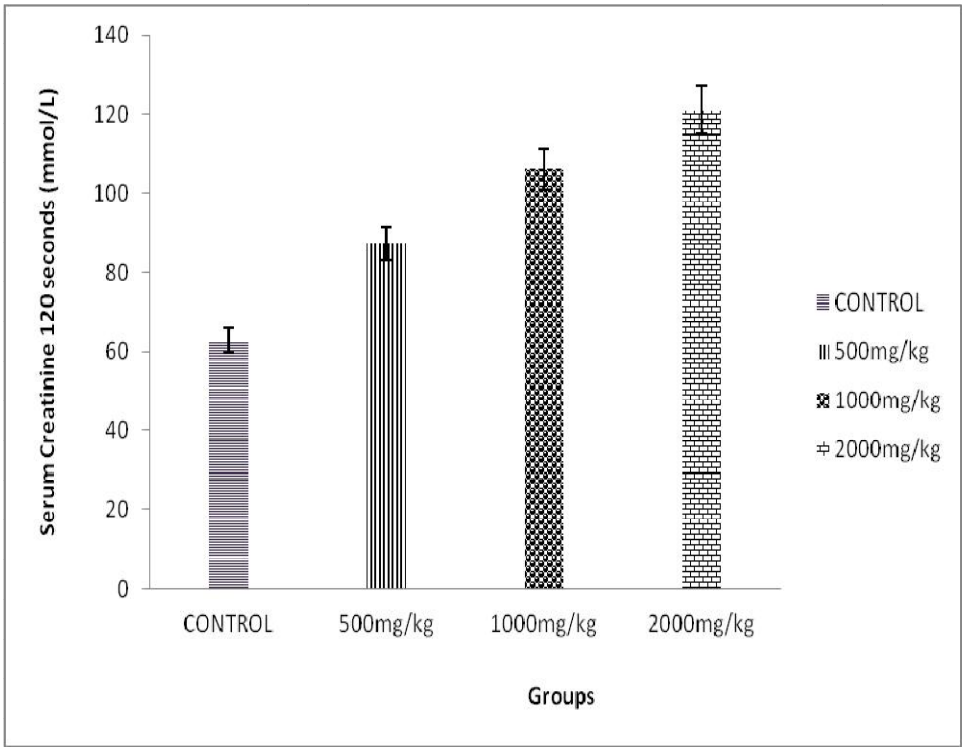

Fig. 6. Effect *A. melegueta* petroleum ether extract on serum creatinine (120 seconds) in rats

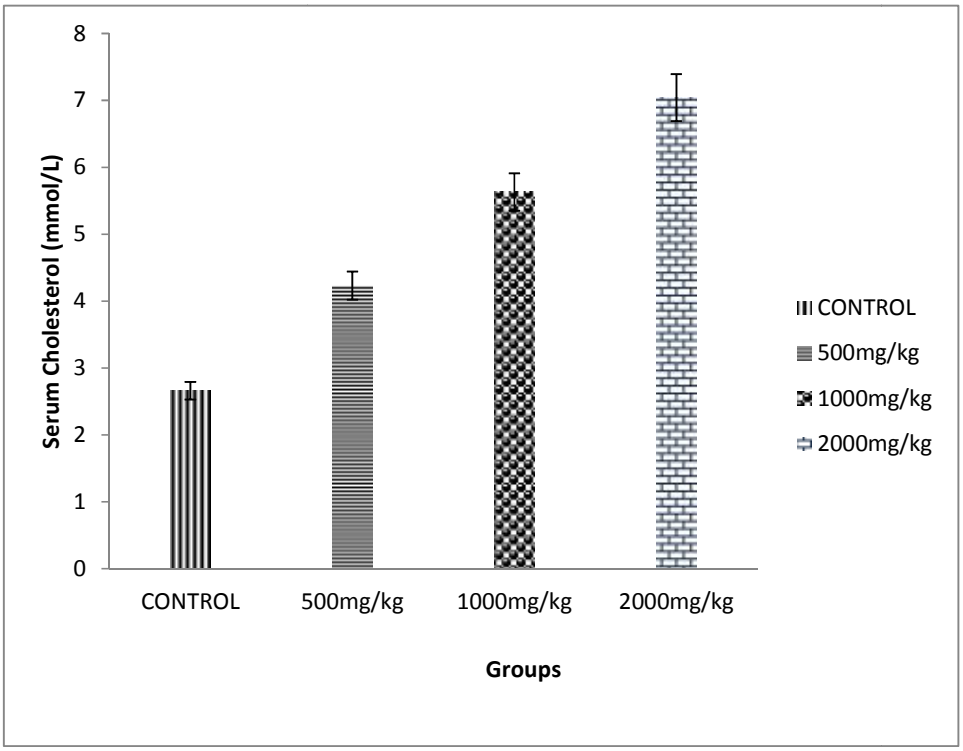

Fig. 7. Effect of *A. melegueta* petroleum ether extract on serum cholesterol in rats

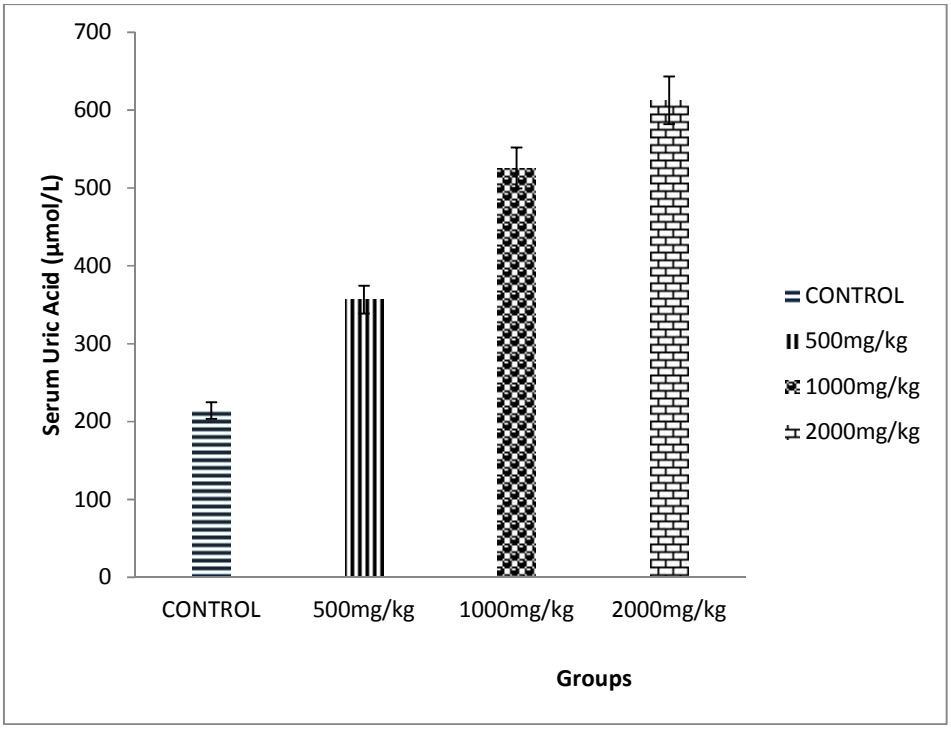

Fig. 8. Effect of *A. melegueta* petroleum ether extract on serum uric acid in rats

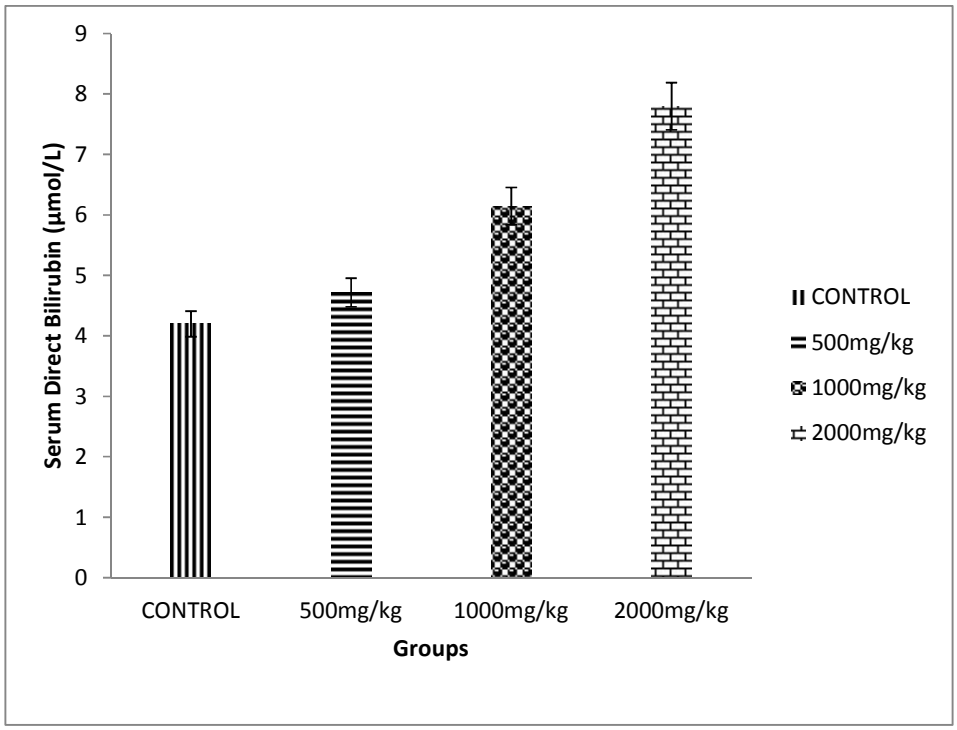

Fig. 9. Effect of *A. melegueta* petroleum ether extract on serum direct bilirubin in albino rats

Normally, levels of ALT in the blood are low, however very high levels of ALT (more than 10 times normal) are usually due to acute hepatitis and sometimes may be due to a viral infection. In most types of liver diseases, the ALT level is higher than AST, and the AST/ALT ratio will be low (less than 1). There are a few exceptions where the AST/ALT ratio is usually greater than 1, this can be found in alcoholic hepatitis, cirrhosis, and with heart or muscle injury and may be greater than 1 for a day or two after onset of acute hepatitis. ALT is often performed together with a test for AST or as part of a liver panel [13].

The liver releases ALT, AST, and creatinne while kidney releases creatinne, urea, uric acid. Increase of all these biomarker into the serum indicates liver and kidney damage. The increase of cholesterol level in the serum of treated animals indicates the lipoprotein metabolism disorder.

Naturally urea, uric acid and creatinine should be excreted from the body system but when the kidney is damage, it is unable to filter the blood in other words these biomarkers will be high in the serum which is as a result of malfunctioning of the kidney system.

5. CONCLUSION

At doses above 500 mg/kg, the petroleum ether extract of *A. melegueta* (AMPE) caused enzymatic leakages from the organs (kidney and liver) into the serum, which reveal that there is damage to the organs. Also high level of bilirubin in the serum which results from malfunctioning of the liver further indicates a damaged effect by the liver.

Furthermore, high level of serum creatinine indicate serious damaged to the renal function, if the filtration process in the kidney is deficient, creatinine blood levels will rise and this reveal a damaged to the kidney, also a lower values total protein may be an indication of liver and kidney damage. This showed that higher doses of *Aframomum melegueta* extract oil may still have some toxicity associated with them because damage of organs were noticed at higher doses, and thus causes damage to cellular organs. Treated stored products at dosages above 500 mg/kg should be used as seeds for planting rather than for consumption.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards.

ACKNOWLEDGEMENTS

Authors are grateful to Prof. G. Oboh for permission to use his laboratory for the biochemical studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Osunwole SA. The Nigerian traditional medicinal. 1999;64:168-173.
2. Oyagade JO, Awotoye OO, Adewunmi JT, Thorpe HT. Antimicrobial activity of some Nigerian medicinal plants, screening for antibacterial activity. Journal of Bioscience research Communication. 1999;11:193-197.
3. Sudha A, Sumathi K, Manikandaselvi S, Prabhu NM, Srinivasan P. Anti-hepatotoxic activity of crude flavonoid fraction of *Lippia nodiflora* L. on Ethanol Induced Liver Injury in Rats. Asian Journal of Animal Sciences. 2013;7:1-13.
4. Okwu DE. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. Journal of Sustain Agricultural environment. 2004;6:30-34.
5. Lale NES. Textbook of stored product entomology and acrarology in tropical Africa. 1st ed. Mole Publication, Maiduguri. 2002;204.
6. Ukeh DA, Umoetok SB, Bowman AS, Mordue AJ, Pickett JA, Birkett MA. Textbook of reduce stored maize infestation by the maize weevil, *Sitophilus zeamais* in traditional African granaries. Crop Protection. 2011;32:99-103.
7. Adeyemo AC, Ashamo MO, Odeyemi, OO. *Aframomum melegueta*: A potential botanical pesticide against *Sitotroga*

cerealella infestation on two paddy varieties. Archives of Phytopathology and Plant Protection. 2014;47:(15):1841-1851.

8. DHHS. Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council. National Academy, Washington, D.C; 1985.
9. OECD. Guidelines for the testing of chemicals, acute oral toxicity – up-anddown- procedure, no. 425, Adopted 3; 2008.
10. Reitman S, Frankel S. Colourimetric method for the determination of serum transaminases. American Journal of Clinical Pathology. 1957;28:56-61.
11. Wallace LA, Ott WR. Personal monitors: A state-of-the-art survey. J Air Pollution Control Associa. 1982;32:601.
12. Shemesh O, Golbetz H, Kriss JP, Myers BD. Kidney Int. 1985;28(5):830–8.
13. Ghouri N, Preiss David, Sattar Naveed. Textbook of Hepatology. 2010;52(3):1156–61.

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