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Evaluation of toxicity profile and pharmacological potentials of *Aju Mbaise* polyherbal extract in rats

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ABSTRACT

Aju Mbaise polyherbal extract (APE) is used in Southeast Nigeria by many women to enhance labour, remove retained placenta after delivery, and for managing pains from postnatal and menstrual cramps. This study investigated the toxicity and the pharmacological activities of APE in Wistar rats. A single dose of 5000 mg/kg APE was orally administered to the mice for acute toxicity study. In the subacute toxicity, forty female rats (10/group) received 0.25 mL distilled water, 200, 400 and 800 mg/kg of APE respectively for 28 days. For analgesic (50 rats) and anti-inflammatory investigations (25 rats), were randomly grouped into 5 respectively. Group 1 rats received 0.2 mL normal saline. Group 2 received 100 mg/kg of aspirin while groups 3, 4 and 5 received 200, 400 and 800 mg/kg body weight of APE respectively. The biochemical parameters, *in vitro* osmotic fragility, analgesic and anti-inflammatory activities were evaluated. The LD_{50} of APE was $>$ 5000 mg/kg. Oral intake of APE did not cause body weight retardation. Various doses of APE significantly increased ($p<0.05$) high density lipoprotein cholesterol (HDL-C). Again, there were slight elevations within the reference limits in creatinine, urea, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in test groups. APE had a lower percentage hemolysis value compared to aspirin and significantly lowered onset time and number of writhes in the pretreated groups. The results of this study establish the acclaimed usage of APE in the management of post-delivery and menstrual cramps. However, prolonged treatment with APE above 400 mg/kg may lead to hepatotoxic and nephrotoxic effects. Therefore, lower doses of APE should only be used for therapeutic purpose.

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Introduction

The substantial worldwide interests of herbal and polyherbal extracts in the treatment of diseases have been reported in Ayurveda literature. Several reports from Ayurveda have revealed that the use of single herbs may have clinical insufficiency

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to the desired therapeutic roles. However, the use of optimized concentrations and ratios of different herbs, offers more remedy with lower toxicity [7].

These growing interests in herbal medicines have been underscored by their efficacies and roles in alleviating extreme cases of ailments. Again, its exploration and utilization by inhabitants of developing and developed countries are factored on their affordability, accessibility, acclaimed non-toxicity and as a natural healing agent [7,19]. However, the cost of procuring individual herbs against various microbial agents including antimicrobial, antifungal and anti-inflammatory confirms the cost advantage of polyherbal remedy which may contain all in one of the aforementioned biological activities [12]. Furthermore, herbs have shown promising therapeutic attributes in the treatment of plethora of ailments including arthritis, diabetes, liver, renal, obesity and cardiovascular diseases [32]. For example; *Allium sativum* as a hypocholesterolemic agent, Commiphora (Myrrh) as cough remedy among other herbal extracts [6,16].

In Southeast part of Nigeria, various herbs have been utilized in the treatment of different diseases. One of the utilized polyherbal formulations is "*Aju Mbaise*". *Aju Mbaise* is a polyherbal formulation used by many women to enhance labor, remove retained placenta after delivery, and for managing pains from postnatal and menstrual cramps [18]. *Aju Mbaise* got its name from Mbaise, a large community in Imo State, southeast Nigeria [24]. This herbal remedy is made up of *Euphorbia convolvuloids* (3.72%), *Uvaria chamae* (10.09%), *Spondias mombine* (11.45%), *Ceiba petandra* (16.60%), *Napoleona vogelli* (23.72%) and *Barteria fistulosa* (34.97%) plants [17,18]. Ethnopharmacologically, the individual plants that make-up APE has been reportedly used in the treatment of different diseases. For example; *E. convolvuloids* is used for treatment of diarrhea, dysentery and respiratory tract infections [18], *U. chamae* has antimalarial, anti-inflammatory and anti-anemic effects [13]. *S. mombin* has uterotonic [22], wound healing, and anti-inflammatory effects. It is also used in treatment of hemorrhoids, stomach pains, diarrhea, gonorrhea and dysentery [21]. *C. petandra* is used in the management of hypertension, diabetes mellitus and fever [10]. *N. vogelli* is used for wound healing, management of hypertension and treatment of bacterial infections [5] while *B. fistulosa* is used for treating wounds, toothache, stomach pains, fever and anemia [8]. Experimental investigation has shown that the decoction of APE caused inhibition of *Escherichia coli*, however, *Staphylococcus aureus* was insusceptible to the decoction [24].

The GC–MS analysis of APE as previously reported in our study revealed that APE is enriched with the following bioactive components; hexadecanoic acid, n-octadecanoic acid, N-(furan-3-yl) acetamide, 17-carboxyheptadec-9-en-1-ylium, nicotinic acid, ethyl palmitate, methyl 2-(4-chlorophenoxy)-2 methylpropanoate, 20-carboxydodec-8-en-1-ylium, S-methyl-L-cysteine, benzyl benzoate, 1,3-oxazolidine-2-thione, pyridine-4-carboxylic acid, ρ-nitrocinnamic acid, methyl (2)-3-cyanoprop-2-enoate, pyroglutamic acid and 2-ethyl-2-hexen-1-ol [18]. It was observed that some of these identified bioactive components have medicinal properties, for instance, hexadecanoic acid with the peak area of 32.65% has been reported by Aparna et al. [3] as a known anti-inflammatory compound that inhibits phospholipase.

Many researchers have demonstrated that polyherbal formulation elicit various pharmacological properties like anti-inflammatory, anti-anemic, antioxidant, anti-malarial, anti-diarrhea, anti-cancer, wound healing and hepato/renoprotective effects [12,18,[27]. In spite of these acclaimed pharmacological relevance of polyherbal medicines, there are insufficient and dearth data on the toxicological indices as well as their safety. Again, the required doses, time and side effects of these herbal remedies may not be ascribed on their label. Today, researchers all over the world have been propelled into the investigation of spectral doses that may be recommended as effective concentrations of these phytomedicines. Therefore, there is need to investigate for documentation the toxicity and pharmacological potentials of *Aju Mbaise* polyherbal formulation; a utilized and popular herbal remedy used by indigenous people of Southeast Nigeria which the present study investigated.

Materials and methods

Sample collection, identification and preparation of extract

Aju Mbaise polyherbal heads used in this study were procured at Onuimo market in Obowo, Imo State. It was air-dried for 21 days at room temperature and thereafter milled into powder using multipurpose milling machine (Honda-G-TECH, Japan). Exactly 50 g of the milled samples were weighed into thimble, the thimble containing the sample was then inserted into Soxhlet extraction chamber and then the sample extracted using 96% ethanol for 4 h at 60 °C. After the extraction, ethanol was removed by placing the extracted sample on a hot air oven at low temperature. The crude extract obtained was stored at 4 °C until they are required for the experiment.

Experimental animal

Healthy and unused rats and mice used in this study were procured from the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike. The rats were allowed to acclimatize for 14 days and were maintained in standard laboratory conditions (temperature 22–25 °C, humidity 40–60% and 12 h light/12 h dark cycle). The animals had free access to feed (Vital feed, Nigeria) and water *ad libitum*. The animal experiments were carried out in strict compliance with the University ethics committee guidelines and regulations on the welfare and use of laboratory animals. Also ethical approval was obtained for this study (VPP/EC/2016/002).

Acute toxicity study (LD_{50}) of APE

A total of 30 female mice (30–35 g) were randomly divided into six (6) experimental groups. Then, different doses of 500, 1000, 2000, 3000, 4000 and 5000 mg/kg APE were administered respectively to the mice. The mice were monitored for 24 h and thereafter for 14 days for any sign of toxicity and death.

Subacute toxicity study

Forty (40) female rats were divided into four (4) experimental groups. Group 1 served as control and received a placebo of 0.25 ml distilled water. Groups 2, 3 and 4 received 200, 400, and 800 mg/kg APE respectively. The rats were treated with the APE orally for 28 days. After the treatment period, the rats were humanely sacrificed and the blood collected via cardiac puncture into EDTA bottles for hematological analysis and plain bottles for serum chemistry tests.

Determination of biochemical parameters

Lipid profile tests; total cholesterol (TC), triglyceride, low density lipoprotein cholesterol, and high-density lipoprotein cholesterol were determined by enzymatic spectrophotometric method using commercial laboratory kits (Randox Laboratory Ltd., Co. Antrim, UK), and were measured at 546 nm. Also, AST and ALT were measured at 405 nm while ALP was measured at 405 nm. Urea and creatinine were measured at 560 and 492 nm respectively. The absorbance was read using nano-UV/vis spectrophotometer (Optima, USA).

In vitro *osmotic fragility effect of APE*

This was carried out according to the method used by Dependra and Bisu [11]. Red blood cell suspension was prepared by collecting 5 mL of whole blood from a matured rat into a test tube. The collected blood was mixed with equal volume of sterilized Alsever's solution before centrifuging for 10 min at 3000 rpm, and then the packed cells were rinsed 4 times with isosaline. Thereafter, the blood volume was measured and reconstituted with 10% v/v isosaline.

For *in vitro* osmotic fragility, various concentrations of APE were prepared in different test tubes (500, 1000, 2000, 3000, 4000 and 5000 µg/mL), thereafter 1 mL phosphate buffer, 2 mL hyposaline and 0.5 mL RBC suspension were added to each of test tubes. Aspirin was used as a standard drug and prepared at the same concentrations (500–5000 µg/ml) with that of the APE. The control and the standard did not receive any APE. The test tubes were incubated for 30 min at 37 °C. Immediately after the incubation period, the tubes were centrifuged for 20 min at 3000 rpm. The supernatants in each tube were used to estimate the hemoglobin content by reading their respective absorbance at 560 nm using spectrophotometer (722N, Mindray Co., China). Percentage hemolysis was ascertained using the expression below;

$$\text{Percentage Haemolysis} = \frac{\text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

Analgesic effect of APE

The method described by Adeyemi et al. [2] with little modifications was used. Fifty female rats were randomly divided into five groups. Group 1 was used as the control group and received 0.2 mL/kg normal saline. Group 2 was administered 100 mg/kg aspirin, whereas groups 3, 4 and 5 received 200, 400 and 800 mg/kg APE respectively. After 30 min of oral treatment, the rats in each of the groups received intraperitoneally 10 mL/kg (0.6%) body weight of acetic acid. The number of writhes made by each rat within 30 min was counted.

Percentage inhibition of pain was evaluated for each rat using the expression below:

$$\text{Percentage Inhibition} = \frac{\text{Writhes in control} - -\text{Writhes in test}}{\text{Writhes in control}} \times 100$$

Anti-inflammatory effect of APE

In this experiment, 25 female rats were divided into 5 groups. Group 1 was used as the control group and received 0.2 mL/kg normal saline. Group 2 was administered 100 mg/kg aspirin, whereas groups 3, 4 and 5 received 200, 400 and 800 mg/kg APE respectively. After 30 min of oral treatment, 0.1 mL egg albumin was injected to each rat via the right hind paw to induce paw edema. After the induction, paw circumferences of the rats were measured and recorded at 0 min, 30 min, 1 h and 2 h. The degree of edema was estimated by subtracting initial paw circumference from the final paw circumference.

Data analysis

Data obtained from this study were presented as mean ± SD. Data were tested for significance difference at 95% level of confidence using one-way ANOVA-analysis of variance with Tukey test post-hoc using R^{TM} Statistical software; version 3.0.3.

Table 1
Influence of APE on mean body weight changes of female Wistar rats.

Dose of APE mg/kg	Body weight (g) Day 1	Day 28	Weight gain	Percentage weight gain (%)
Control (0)	128.38±0.98	173.50±4.24	45.12±2.78	35.14
200	130.07±1.09	168.84±6.22*	38.77±4.06*	29.80
400	137.32±2.06*	169.83±8.88*	32.51±0.95*	23.67
800	134.92±6.90*	165.66±8.01*	30.74±2.88*	22.78

Values are presented as mean ± SD, $n = 6$. Superscript asterisk (*) in the same column represents significant difference at ($P < 0.05$).

Table 2
Effect of APE on lipid profile of female Wistar rats.

Doses of APE (mg/kg)	TC (mg/dl)	HDL-C (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)
Control (0)	110.62±2.02	56.39±0.34	62.12±0.43	41.81±2.03
200	106.29±1.22	57.32±0.46	64.14±0.50	36.14±0.83
400	104.94±4.41	59.46±0.42*	63.05±0.84	32.87±1.64*
800	101.02±1.68*	60.58±0.25*	69.87±0.36*	26.47±1.70*

Values are presented as mean ± SD, $n = 6$. Superscript asterisk (*) in the same column represents significant difference at ($P < 0.05$). TC = Total Cholesterol, HDL= High Density Lipoprotein, LDL= Low Density Lipoprotein.

Table 3
Influence of APE on liver and kidney parameters of female Wistar rats.

Dose of APE (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	Bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Control (0)	32.20±0.47	40.90±0.23	83.00±1.10	0.66±0.01	22.50±0.19	1.35±0.05
200	32.60±0.62	41.30±0.34	82.40±2.24	0.66±0.01	23.40±0.21	1.55±0.02*
400	34.60±0.40*	42.50±0.73	87.30±1.42	0.75±0.02*	24.34±0.34*	1.63±0.02*
800	36.20±0.42*	45.20±0.36*	85.30±1.66	0.70±0.01*	24.18±0.19*	1.65±0.03*

Values are presented as mean ± SD, $n = 6$. Superscript asterisk (*) in the same column represents significant difference at ($P < 0.05$) when compared to the control group. AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase.

Results

In the acute toxicity test, the highest single dose of APE-5000 mg/kg administered to the mice did not cause any toxicity signs or death within 24 h and 14 days indicating that LD_{50} of APE is greater than 5000 mg/kg (see supplementary data Table 1).

Table 1 shows the effect of APE on mean body weight changes of female Wistar rats. All the Wistar rats used in this study gained weight. However, observable dose-dependent reduction in % body weight was noted as the doses of APE increases.

From the result of the lipid profile (Table 2), dose-dependent decrease ($p< 0.05$) were recorded in TC and LDL-C as the doses of the extract increases, whereas dose-dependent increase ($p< 0.05$) in HDL-C in the 400 and 800 mg/kg APE treatment groups were observed. The group administered 800 mg/kg APE had a significant increase ($p< 0.05$) in triglyceride when compared to the other groups.

Table 3 showed that 400 and 800 mg/kg APE doses significantly elevated ($p<0.05$) AST, ALT, bilirubin, urea and creatinine levels, whereas ALP was not affected.

The protection offered by APE on the osmotic fragility of red blood cells did not produce any significant effect from that of aspirin ($P>0.05$). However, red blood cells placed in distilled water had 100% hemolysis, while those placed in 500 µg/mL APE and aspirin had 84.18 ± 0.52% and 80.20 ± 0.24% respectively. At a higher concentration (3000 µg/mL), percentage hemolysis for APE and aspirin were 67.36 ± 0.27% and 77.79 ± 0.28% respectively, and when the concentrations were increased to 5000 µg/ml, percentage RBC hemolysis for APE and aspirin were 48.72 ± 0.33% and 44.68 ± 0.22% respectively (Fig. 1).

Dose-dependent percentage inhibition of writhing reflexes was noted in the APE pretreated rats and the standard drug (aspirin). Percentage inhibitions of pain were also higher in the 400 and 800 mg/kg APE groups than the group treated with aspirin (Table 4). Again, the time before onset of writhing reflex was also higher in test groups treated with APE and the standard drug (aspirin).

Table 5 shows anti-inflammatory effects of APE. The result revealed that treatment with APE caused a significant ($P<0.05$) inhibition of egg albumin induced paw edema in the rats. However, the anti-inflammatory effect of APE was slightly lower than that of aspirin.

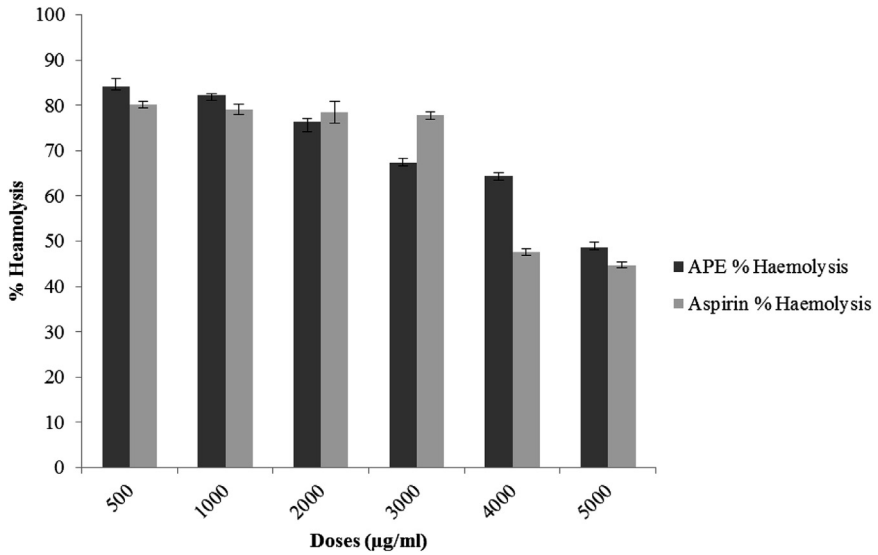

Fig. 1. *In vitro* comparative investigation of APE and aspirin on red blood cell osmotic fragility. Values are mean ± SD ($n = 6$).

Table 4
Effect of APE on pain onset and sensations.

Group	Time before 1st writhe (s)	No. of writhing reflexes in 30 min	% Inhibition of pain at 30 min	% Inhibition of pain at onset
Normal Control	15.70±2.75	116.10±3.48	–	–
100 mg/kg Aspirin	89.30±3.23*	50.40±4.79*	56.49±5.10*	486.55±116.73*
200 mg/kg APE	69.00±1.70*	93.30±14.49*	19.64±12.20*	354.89±103.28*
400 mg/kg APE	138.40±2.91*	53.20±4.05*	54.18±3.15*	811.33±195.70*
800 mg/kg APE	363.70±24.29*	43.00±6.31*	62.39±6.36*	2281.82±439.87*

Values are expressed as mean ± SD, $n = 6$. Superscript asterisk (*) in the same column represents significant difference at ($P < 0.05$) when compared to the control group.

Table 5
Anti-inflammatory effects of graded doses of APE in rats.

Group	PC in mm at 0 min	PC in mm at 30 min	PC in mm at 60 min	PC in mm at 120 min
Control	21.40±0.27	35.10±0.85	33.30±0.74	32.50±1.08
100 mg/kg Aspirin	21.60±0.39	29.50±0.69*	27.60±0.63*	23.30±0.76*
200 mg/kg APE	20.90±0.61	32.20±0.64*	30.40±0.47*	26.50±0.55*
400 mg/kg APE	20.70±0.55	32.50±0.60*	30.60±0.4*	27.20±0.71*
800 mg/kg APE	21.20±0.71	31.40±0.55*	29.90±0.65*	26.50±0.45*

Values are presented as mean ± SD, $n = 6$. Superscript asterisk (*) in the same column represents significant difference at ($P < 0.05$). PC = paw circumference while mm = millimeters.

Discussion

Worldwide, various parts of medicinal plants have been widely utilized as primary supplements for therapy [26]. These herbal remedies are often abused by the users. Based on this premise, there is a great need to evaluate the toxicity as well as their acclaimed analgesic effects. This study investigated the toxicity profile and pharmacological potentials of *Aju Mbaise* polyherbal extract (APE). The report of Organization for Economic Co-operation has shown that toxicity evaluation is used to ascertain the safety of plant-based therapy or food samples. This could be ascertained by carrying out experimental analysis with laboratory animals [23]. APE did not cause mortality in mice at a single dose of 5000 mg/kg and therefore LD_{50} of APE is greater than 5 g/kg. Hence, APE can be classified as a nontoxic substance. This agrees with the reports of Ugbogu et al. [31] who made an assertion that if up to 5 g/kg of substance did not produce lethality, that the said substance is nontoxic.

Body weight assessment post biotransformation of xeno-compounds could be used to ascertain the *in vivo* effect of the ingested compounds. This is because decrease in body weight could be a sign of adverse effect [29]. The administration of various doses of APE did not induce weight retardation.

Lipid profile estimation of Wistar rats administered with APE revealed an increased serum HDL-C. Again, APE significantly decreased LDL-C and TC. High concentrations of LDL have been reported to elevate the risk of cardiovascular diseases

[4]. Dose-dependent decrease in TC and LDL-C and dose-dependent increase in HDL-C suggest that APE could have cardio-protective potential due to its hypolipidemic effects [20].

In liver enzymes, there were observable dose-dependent elevations in AST and ALT values in the APE groups. However, the observed increase still falls within safety range. Thapa and Anuj [30] had reported that standard range of accepted values for liver function tests, beyond which liver damage may be suspected is ALT (10–55 U/L), AST (10–40 U/L), and ALP (45–115 U/L). These enzymes are raised in the serum when the integrity and functionality of the liver is compromised. Therefore, they are used as biomarker in monitoring the integrity of the liver [14]. Hence, they serve as the major biomarker in the diagnosis of liver problem [1,15]. This study shows that prolonged treatment of APE above 400 mg/kg body weight could lead to hepatotoxic effect as shown by AST and ALT results. ALP is linked to biliary duct [14]. The levels of these enzymes in all pretreated rats remained within safety range giving credence to the use of APE for medicinal purposes. Again, there were significant elevations in bilirubin, urea and creatinine following the APE treatment, although these biomarkers still fall within safety range. The increase in bilirubin concentration may be associated with mild hemolysis associated with high dose APE. This agrees with Shivaraj et al. [28] who asserted that an elevation of bilirubin in small animals is often resulted from acute and severe hemolysis. The elevations of urea and creatinine beyond acceptable limits are secondary to kidney insult [9]. The mild elevation in urea value following treatment with high doses of APE may be related to high protein diet (Vita growers' mash). High protein diets may cause some degree of rise in serum urea concentration. Creatinine concentration was significantly affected ($p<0.05$) by all doses of APE. This shows that APE concentration greater than 800 mg/kg body weight may cause nephrotoxic effects.

The steady performance of a normal red blood cell (RBC) depends significantly on the stability, integrity and ability of the RBC membrane to resist lysing attacks [25]. However, in human body activities like lipid peroxidation and free radicals may increase percentage hemolysis. *In vitro* osmotic fragility activity of APE extract revealed a dose dependent decrease in percentage hemolysis as concentration of the extract increases from 500 to 5000 µg/mL (Fig. 1). At higher concentrations of 4000 and 5000 µg/mL, APE had a lower percentage hemolysis value compared to the standard drug (aspirin). Traditionally, APE is used to manage pains and cramps post child's delivery and also in painful menstruation [18]. Considering this acclaimed analgesic activity, acetic acid model was used to generate a typical visceral pain similar to those experienced by women in reproductive and menstrual matters. Results obtained show that APE significantly inhibited pain in all pretreated rats, lowering both the onset time and number of writhes reflexes (Table 4). Again, the time before onset of writhing reflex was also higher in the test groups treated with APE and the standard drug (aspirin) when compared to control. Furthermore, the antinociceptive or analgesic effect of APE was found to be greater than that of aspirin. The APE may have achieved this analgesic effect through the inhibition of the cyclooxygenase (COX) activity which subsequently inhibited the biosynthesis of prostaglandins. This may be the possible mechanism by which APE reduces pains. The results obtained from the anti-inflammatory effects of APE revealed that treatment with the extract significantly inhibited egg albumin induced paw edema in the rats. Although, the anti-inflammatory effect of APE was slightly lower than that of aspirin, however, it inhibited the paw circumference which justified its anti-inflammatory activities.

Conclusion

In this study, the acute toxicity test (LD_{50}) suggests that APE is practically non-toxic at the dose $\leq$5000 mg/kg. The elevations observed in HDL value following the treatment with APE suggest that APE could have positive effects in managing cardiovascular problems associated with lipid abnormalities. This study also justifies the use of APE as a hypolipidemic agent. Also, the results obtained from the osmotic fragility, analgesic and anti-inflammatory effects may be the reasons APE is used traditionally used in the management of pains resulting from child's delivery and menstrual cramps. However, biochemical activity of the liver and renal functions post administration of APE suggests that APE is relatively toxic at higher doses $\geq$400 mg/kg body weight. This is an indication that prolonged treatment with APE above 400 mg/kg body weight could lead to hepatotoxic and nephrotoxic effects. Therefore, lower doses of APE should be used for therapeutic purpose.

Declaration of Competing Interest

None.

Author contributions

Ijiomah, S.N, Emmanuel O., Nosiri, C.I. and Ugbogu, E.A contributed equally in designing and conducting the experiment; analyzing the data and writing the manuscript.

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Supplementary materials

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