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Effect of Ethanolic Extract of Traditional Herbal Tea (Aju-Mbaise) on Lipid Profile and Some Liver Enzymes in Dyslipidaemic Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Considering the recent popular use of a herbal tea— *Aju Mbaise,* in Southern parts of Nigeria, for weight shedding, the present study evaluated the effect of Ethanolic Extract of Aju Mbaise (EEAM) herbs consumption on lipid profile, some liver enzymes and body weight in high fat-diet induced dyslipidemic female Wistar rats were evaluated. Thirty (30) female Wistar rats weighing between 120-180g were used for this study and were divided into six (6) groups of 5 rats each: Group 1 rats (Negative Control) were allowed access to normal rat feed and water *ad libitum*. Groups 2, 3, 4, 5, and 6 rats were allowed access to only high-fat diet (HFD) and water *ad libitum* from the three weeks of acclimatization and throughout the period of administration. Group 2 served as positive

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control (received no treatment) while Group 3, was administered 1ml of 0.04mg simvastatin (a standard antidyslipidemic drug), and groups 4, 5 and 6 were administered 150mg/kg, 300mg/kg and 600mg/kg EEAM respectively. The body weights of the rats were recorded weekly. After the 21 days of administration, blood samples were collected from the rats via cardiac puncture after properly sedating them with 80% trichloromethane. The blood samples were then subjected to lipid profile and liver enzyme screening. Quantitative data obtained were then statistically analysed using IBM Statistical Package for Social Sciences SPSS. The HFD elevated serum levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) were significantly ($P<0.05$) reduced in groups 3 to 6 (treated rats) when compared to mainly group 2 and occasionally group (untreated rats). The serum levels of some liver enzymes particularly alanine transaminase (ALT) and alkaline phosphatase (ALP) were found to be mostly significantly ($p<0.05$) elevated in the treated rats when compared to those of Groups 1 and 2. The percentage change in body weight of the rats in the treated groups (3, 4, 5 and 6) were also found to be significantly ($P< 0.05$) reduced when compared to the positive control (Group 2) which was not treated. Thus, while EEAM may be beneficial in regulating lipid profile and weight gain check, it may possibly result in significant hepatotoxicity in increasing EEAM doses and its continuous consumption.

Keywords: Aju mbaise herbs; body weight; lipid profile; liver enzymes.

1. INTRODUCTION

The quest to lose weight, especially, amongst obese people in recent times has led many to resort to wrong practices such as skipping meals, taking unhealthy diet-modulating pills, reliance on unsafe slimming agents, amongst others [1,2]. Often, these are done with complete disregard to the possible associated adverse effects, such as cardiovascular disorders, hepato-renal toxicities, etc., [3,4]. On the other hand, while the idea of taking slimming and detox teas is not very new, it is however gaining more popularity recently, as more people in the weight loss community are taking on it [5,6]. These teas, which come in various brands, often carry the same message of quick weight loss with no exercise [7]. The thought that different brands of slimming teas act by stimulating digestion, aiding metabolism, and, in other cases, rids the body of toxins [8] often lack scientific backing [3].

Like the foreign brands of slimming teas, in our local, South-south and South-east regions of Nigeria, a typical herbal tea called “Aju Mbaise” with similar use is now frequently used for attaining flat tummy and weight loss. It is believed to possess a fat-burning effect [9]. Aju Mbaise means “a wrap from Mbaise”. This name is derived from its producers, people of *Mbaise* sub-ethnicity in Imo State of Nigeria. The Aju Mbaise traditional tea is known to be made up of nine (9) different plants parts which include leaves, roots and barks of medicinal plants wrapped together in a circular shape. Some folkloric applications of this traditional tea include trimming down accumulated post-pregnancy fat, flat tummy and weight loss, fertility boosting, anti-microbial activities, correction of menstrual irregularities and fibroid treatment [9,10].

Considering the recent popular and numerous folkloric uses of the *Aju Mbaise* and the need to validate its effects and safety especially on the cardiovascular system and the liver [7] in the biological system, the present study is aimed at investigating the effect of ethanolic extract of a traditional herbal tea (*Aju-Mbaise*) on lipid profile and some liver enzymes in hyperlipidaemic female Wistar rats.

2. MATERIALS AND METHODS

All reagents used are of analytical grade and the method of plants/tea extraction was adopted from Nnadiukwu et al., (2019).

2.1 Collection of Plant Materials and Preparation of Extract

Fresh wraps of Aju Mbaise were obtained from Umunakukwu Umuchoko, Chokoneze of Ezinite Mbaise Local Government Area of Imo State, Nigeria. Each wrap with the various components (including *Cnestis feruginrea* (Oko-Aja), *Xylopia aethiopica* (Guinea pepper, Uda); *Uvaria chamae* (Bush banana, Ehuru); *Palisota hirsute* (Ikpeleatulu); *Scleria sp* (Nutrushes); *Napoleona imperialis* (Napoleon’s hat); *Dialium guineense* (Velvet tamarid); *Combretum racemosun* (Christmas rose); *Heterotis rotundifolia* (Spanish shawl) [11,12] were air-dried for 14 days and thereafter, pulverized into powder using a local

milling machine powered by a petrol motor (Honda). The powdered sample was then soaked in 95% ethanol in covered jars. The jars were macerated continually to enable proper mixing and each allowed to stand for about 72 hours. Subsequently, each sample was filtered and concentrated into semi-liquid extracts using a rotary evaporator and then water bath at 45^{o}C. The semi-solid extract of the *Aju Mbaise* herbs was then obtained, properly labeled and stored at about 4°C until ready for experiments. The rationale for the ethanolic crude extraction of the plants/tea constituents are that the study targets to extract ethanol soluble ingredients that may not be water soluble and that they are also traditionally soaked and used alongside ethanol. It, thus, validates the need for multiple solvent extractions for active ingredients in plants which has been emphasized by some earlier reports [9,10].

2.2 Handling of Experimental Animals

Thirty (30) female Wistar rats weighing between 120 and 180g were obtained from the Animal House of the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were housed in standard wire-gauzed plastic cages under 12 hours light/dark cycles at about 25^{o}C. The rats were selected into six (6) groups of 5 rats each. The rats under group 1 served as negative control and were allowed access to normal rat chow and tap water *ad libitum* throughout the study period but experimental hyperlipidaemia was induced by allowing the rats in groups 2, 3, 4, 5 and 6 to freely access high-fat diet (a specially formulated grower mesh animal feed supplemented with high sugar—15% weight of meal served per day and baker's butter—15% weight of meal served per day respectively) and water from the three weeks of acclimatization and throughout the period of administration. The above formulated high fat diet (HFD) used to induce dyslipidaemia in the current study was according to the methods of Maioli et al. [13].

It is noteworthy that, the lipid profile of rats in the dyslipidaemic groups were confirmed by initially harvesting blood samples from the study animals (i.e. added number) and preliminary laboratory/numerical data of high fat diet fed animals were found to be significantly ($p<0.05$) higher compared to those of non-high-fat-diet-fed group. It was after the foregoing that the treatments with different doses of EEAM and standard drug commenced.

2.3 Experimental Protocol

The rats were randomly selected into 6 groups of 5 rats each and they were namely:

Group 1: Negative control, received normal feed + 1ml of distilled water.
Group 2: Positive control, received high-fat diet (HFD) + 1ml of distilled water.
Group 3: Standard control, received HFD + 20mg simvastatin.
Group 4: 150mg/kg Ethanolic Extract of Aju Mbaise (EEAM) + HFD
Group 5: 300mg/kg EEAM + HFD
Group 6: 600mg/kg EEAM + HFD

It is noteworthy that the route of administration was per oral using oral gavage for rats and the length of administration was 21 consecutive days. On the 22nd day, blood samples were obtained from the study rats via cardiac puncture after sedating them with 80% trichoromethane (chloroform). The blood sample was immediately transferred into properly labeled lithium heparin bottles. Afterwards, the lipid profiles of the rats were screened in the laboratory following the specified procedure by the different kit manufacturers.

2.4 Statistical Analysis

Numeric data obtained from the study were subjected to statistical analysis using the IBM statistical package for social sciences (SPSS) version 20.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Post-Hoc multiple comparison test. A p-value less than 0.05 were considered statistically significant.

3. RESULTS

The result in Table 1 showed the effects of ethanolic extract of *Aju-Mbaise* herbs (EEAM) administration on some lipid profile parameters in female Wistar rats. The total cholesterol (TC) level of group 2 (Positive Control-l HFD rats with no treatments) was found to be significantly ($p<0.05$) higher than that of group 1 (normal control: Non-HFD rats with no treatments). Only group 5 (HFD + 300 mg/kg EEAM treated) rats had a significantly ($p<0.05$) decreased level of TC when compared to group 1. Interestingly, the TC levels of all treated groups (groups 3, 4, 5 and 6) indicated significantly ($p<0.05$) reduced TC levels when compared to that of group 2. The TC levels of groups 4, 5 and 6 (HFD rats treated

with 150, 300 and 600 mg/kg EEAM respectively) showed significantly ($p<0.05$) reduced levels compared to group 3 (20mg simvastatin treated rats).

Considering the changes in triglyceride (TG) levels of the experimental rats, groups 2, 3, 4 and 6 indicated significantly ($p<0.05$) higher levels when compared to that of group 1. Only groups 3 and 5 had a significantly ($p<0.05$) lower levels of TG compared to that of group 2. Group 5's (300mg/kg EEAM treated rats) TG level was significantly ($p<0.05$) lower than that of groups 4 and 6 (150 and 600mg/kg EEAM treated rats).

The changes in the level of HDL-C, only showed significantly ($p<0.05$) elevated levels in groups 3, 5 and 6 when compared to group1. These elevations were highest in group 2 which was followed by those of groups 5 and then 4. Other groups did not indicate any significant ($p>0.05$) change in their HDL-C).

Looking at the effects of the extract on LDL-C levels in the study rats, only group 2 (HFD untreated) rats had significantly ($p<0.05$) elevated LDL-C but group 5 indicated a significantly ($p<0.05$) reduced level of LDL-C. Notably, both the simvastatin and extract treated animals showed significantly ($p<0.05$) lower levels of LDL-C compared to that of group 2. The LDL-C level of group 5 (i.e. the 300mg/kg EEAM treated rats) had the significantly ($p<0.05$) lowest level.

For the changes in VLDL levels, all groups except group 5 indicated significantly ($p<0.05$) raised levels when compared to that of group 1. Groups 3 and 5 indicated a significantly ($p<0.05$) reduced level of VLDL when compared to that of group 2. On the other hand, the VLDL level of group 6 was found to be significantly ($p0<0.05$) higher when compared to those of groups 3 and 5. Finally on Table 1, the atherogenic index of plasma (AIP) level was seen to be significantly ($p<0.05$) lower in groups 3 (HFD + simvastatin treated rats) and 5 (HFD + 300mg/kg EEAM treated rats) when compared to that of group 2 (HFD untreated rats).

Table 2 displays the result on the effects of ethanolic extract of Aju-Mbaise herbs (EEAM) administration on some liver enzymes in female Wistar rats. Considering the changes in AST levels, group 5 indicated a significantly ($P<0.05$) reduced level when compared to groups 1 and 4.

Considering the ALT levels, groups 3 and 4 indicated significantly ($P<0.05$) elevated levels compared to group 2. The ALT levels of groups 5 and 6 were seen to be significantly ($P<0.05$) lower than those of groups 3 and 4.

The ALP levels of all test groups were found to be significantly ($P<0.05$) raised when compared to group 1. Again, groups 3, 4 and 5 were also observed to be significantly ($P<0.05$) elevated with respect to group 2. Group 5 ALP mean value was also significantly ($P<0.05$) higher than that of group 3 and group 6 significantly ($P<0.05$) lower than group 5.

The result in Fig. 1 indicates the effects of ethanolic extract of Aju-Mbaise herbs (EEAM) administration on percentage change in body weights of female Wistar rats over the treatment interval. After week one week (first week of acclimatization), the percentage change in body weights (PCBW) of the rats were all in the positive values, meaning increases in the body weights and these values were significantly ($P<0.05$) higher in groups 2, 3, 4, 5 and 6 (which were the high fat diet induced dyslipidaemic rats) and PCBW value of group 6 was found to be significantly ($P<0.05$) lower compared to those of groups 3 and 5 respectively.

Similarly, after week 2 (second week of acclimatization), the PCBW values of groups 2 to 6 were found to be significantly ($P<0.05$) higher compared to that of group 1. Only groups 4 and 6 (i.e. 150 & 600mg/kg EEAM treated rats) indicated a lower PCBW when compared to that of positive control (group 2). Interestingly, after week 3 (first week of EEAM administration), only group 2 had a significantly ($P<0.05$) higher level of PCBW compared to group 1 but all treated groups (3, 4, 5 and 6) had significantly ($P<0.05$) reduced levels of PCBW when compared to that of group 2.

Furthermore, after weeks 4 and 5 (second and third week of EEAM administration), while only group 2's PCBW level were significantly ($P<0.05$) higher when compared to group 1, all treated groups (3, 4, 5 and 6) had PCBW values that significantly ($P<0.05$) reduced when compared to those of groups 1 and 2.

Table 1. Effects of ethanolic extract of Aju-Mbaise herbs (EEAM) administration on some lipid profile parameters in female Wistar Rats

Group	Lipid profile parameters					
	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	VLDL (mmol/L)	AIP
Group 1: Negative Control	3.14±0.10	1.31±0.02	1.52±0.15	2.20±0.22	0.59±0.01	-0.06±0.03
Group 2: Positive Control	3.94±0.15 [a]	1.820.06 [a]	1.81±0.06	2.96±0.19 [a]	0.83±0.03 [a]	0.00±0.01
Group 3: (20mg Simvastatin)	3.46±0.09 [b]	1.59±0.09 [a, b]	2.02±0.09 [a]	2.16±0.12 [b]	0.72±0.04 [a, b]	-0.10±0.02 [b]
Group 4: 150 mg/kg EEAM	3.34±0.22 [b, c]	1.78±0.05 [a]	1.79±0.12	2.36±0.30 [b]	0.81±0.02 [a]	0.00±0.02 [c]
Group 5: 300 mg/kg EEAM	2.66±0.12 [a, b, c, d]	1.37±0.12 [b, c, d]	1.89±0.08 [a]	1.39±0.12 [a, b, c, d]	0.62±0.05 [b, d]	-0.14±0.02 [a, b, d]
Group 6: 600 mg/kg EEAM	3.12±0.08 [b, c, e]	1.84±0.05 [a, c, e]	1.85±0.14 [a]	2.02±0.13 [b, e]	0.84±0.02 [a, c, e]	0.00±0.04 [c, e]

Values represent mean ± standard error of mean (SEM), n=5; [a] Significant at P<0.05 when compared to group 1 (Negative control); [b] Significant at P<0.05 when compared to group 2 (Positive Control: High fat diet induced dyslipidaemic—HFD rats without any treatment); [c] Significant at P<0.05 when compared to group 3 (HFD + Simvastatin treated rats); [d]Significant at P<0.05 when compared to group 4 (HFD + 150 mg/kg EEAM treated); [e]Significant at P<0.05 when compared to group 5 (HFD + 300 mg/kg EEAM treated). Note: EEAM= Ethanolic Extract of Aju-Mbaise

Table 2. Effects of ethanolic extract of Aju-Mbaise herbs (EEAM) administration on some liver enzymes in female Wistar Rats

Group	Some liver enzymes levels		
	AST (U/L)	ALT (U/L)	ALP (U/L)
Group1: Negative Control	44.60±1.78	11.28±0.68	23.00±1.00
Group2: Positive Control	43.00±1.82	12.94±0.47	40.80±1.39 [a]
Group3: (20mg Simvastatin)	44.20±2.33	16.66±0.60 [a, b]	48.00±2.36 [a, b]
Group 4: 150 mg/kg EEAM	48.60±2.48	15.46±0.64 [a, b]	49.20±1.88 [a, b]
Group 5: 300 mg/kg EEAM	37.60±2.67 [a, d]	12.18±0.68 [c, d]	53.40±1.66 [a, b, c]
Group 6: 600 mg/kg EEAM	42.40±2.38	12.94±0.59 [c, d]	44.80±1.93 [a, e]

Values represent mean ± standard error of mean (SEM), n=5; [a] Significant at P<0.05 when compared to group 1 (Negative control); [b] Significant at P<0.05 when compared to group 2 (Positive Control: High fat diet induced dyslipidaemic—HFD rats without any treatment); [c] Significant at P<0.05 when compared to group 3 (HFD + Simvastatin treated rats); [d]Significant at P<0.05 when compared to group 4 (HFD + 150 mg/kg EEAM treated); [e]Significant at P<0.05 when compared to group 5 (HFD + 300 mg/kg EEAM treated). Note: EEAM= Ethanolic Extract of Aju-Mbaise

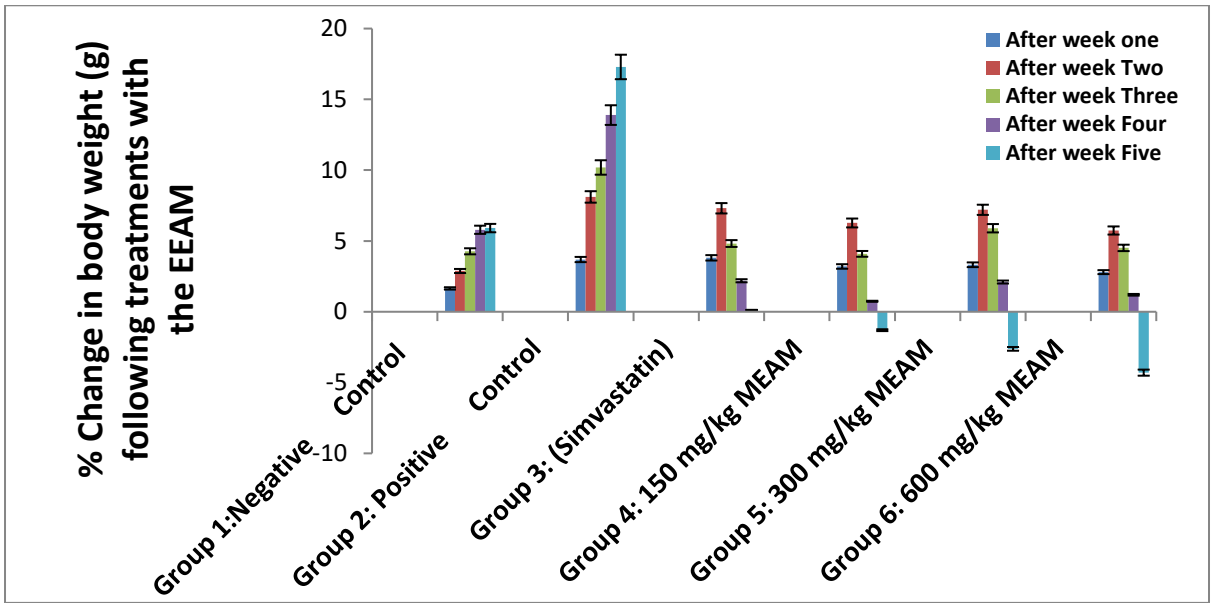

Fig. 1. Effects of Ethanolic Extract of Aju Mbaise Herbs (EEAM) administration on percentage change in body weights of female albino Wistar Rats

Key: EEAM= Ethanolic Extract of Aju-Mbaise

4. DISCUSSION

In recent times, studies on the anti-hypercholesterolemic, anti-diabetic, anti-inflammatory effects of herbs and spices are leading amongst others, especially considering the increasing morbidities associated with diabetes, cardio-vascular diseases, arthritis and cancer in different populations [14]. Hypercholesterolemia is an important key contributory factor for ischemic heart disease and is associated with aging, high blood pressure, a family history of hypercholesterolemia, and diabetes [15,16]. *Aju Mbaise* herb is one of such herbs that are now popularly and frequently used in our locality by especially traditional birth attendants and weight-control seeking people, amongst others [9].

In the present study, the effect of ethanolic extract of *Aju Mbaise* on some lipid profile parameters, some liver enzymes and body weight in high fat diet induced dyslipidaemic female Wistar rats were investigated.

The result of the present study on the effects of ethanolic extract of *Aju Mbaise* herbs treatment on lipid profile in female Wistar rats indicated significant reductions of the elevated levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in the EEAM extract treated group of rats compared to those of dyslipidaemic rats without treatments and dyslipidaemic rats treated with 20mg simvastatin. Further, the high-density lipoprotein cholesterol (HDL-C) levels of the extract treated rats were significant elevated compared to those of the normal rats (group 1). The outcome of the present study validates earlier reports that bioactive compounds from herbal plants possess lipid profile regulatory abilities via one or more pathways of lipid metabolism, including inhibition of cholesterol absorption in intestinal enterocytes, reduction of majorly hepatic cholesterol synthesis, elevation of reverse cholesterol transport, and promotion of cholesterol excretion in the liver [17,18]. These possible anti-hyperlipidaemic effects exerted by EEAM herbs may not be clearly understood for now, but could possibly be due to the modulation of various molecular targets and related pathways [18]. Similarly, the possible elevation of HDL-C by EAAM is indicative of a potential beneficial regulatory effect on lipid profile. This finding is similar to the report of [19]. Of course, HDL-C is known to promote the storage of plasma cholesterol in the liver.

Thus, the earlier reported abundant phytochemical composition of the EEAM [12,20] and its effects on both TC and LDL-C levels in this study is suggestive of the fact that, this herbal tea may possess anti-hyperdyslipidaemic agent. And this could be a good scientific justification of the varied folkloric application of the Aju-Mbaise herbal tea in our local.

The result of the present study on the effects of EEAM administration on some liver enzymes in female Wistar rats indicated significant elevations in the liver specific enzyme—ALT and then ALP levels in all treated rats. This was even more in the simvastatin treated group followed by those treated with EEAM when compared to both the normal rats and dyslipidaemic rats without treatments. Specifically, for the EEAM, it implies that indiscriminate and chronic consumption of EEAM, being a crude/unpurified extract, may elicit an appreciable level of hepatotoxicity. And generally, this reveals the need for proper screening of both synthetic and natural antihyperlipidaemic drugs and agents in order to check their cytotoxic effects particularly on the liver. This finding of the current study is consistent with the report of Ijioma et al. [19], which also recorded significant increases in the same parameters and others following treatments with *Aju Mbaise* polyherbal extract. Considering this characteristic of EEAM, it is worthy of note to state that cautions should be exercised in the dose and duration of consumption of it.

The result of the effect of EEAM on body weights of the study animals in the present study indicates a weight reducing effect of the different doses of the extract in similar fashion as simvastatin. The mechanisms of action of most tea brands with widely acclaimed weight reduction effects are yet to be defined, however, Bei et al. [21] and Yang et al. [4] linked this attribute of teas/herbs to one, a possible decrease in intestinal absorption of lipids and proteins by antinutrient-like constituents of the teas, thus reducing calorie intake. And two, by possible stimulation of AMP-activated protein kinase (AMPK—a known central regulator of energy homeostasis) by the teas' polyphenols in the liver, skeletal muscle, and adipose tissues. Thus, the possible weight reducing effect of the different doses of the Aju-Mbaise herbal tea in the present study is indicative that intestinal absorption of lipid may have been impeded (even if the mechanism is not yet understood. This may hold true as the high fat induced weight gained in the study rats were seen to significantly reduce within fourteen days of treatments with the different doses of the extract. Consequently, this outcome justifies a major rationale for the ethnomedicinal application of the Aju-Mbaise herbal tea amongst weight reduction seeking individuals in our locality. It is therefore suggestive to characterize the different constituents of the EEAM in further studies as to better understand the possible mechanism (s) of action of the EEAM herbal extract on weight reduction.

5. CONCLUSION

The present study has thus shown scientific and beneficial anti-hyperlipidaemic and weight-gain control properties of the ethanolic extract of Aju-Mbaise herbs in experimentally induced hyperlipidaemic female Wistar rats but with a possible significant hepatotoxic potential in increasing doses and continuous consumption. Thus, extrapolating from the present experimental study, caution should be exercised in the indiscriminate and chronic consumption of the Aju-Mbaise herbs. The conduction of in debt safety evaluations of the EEAM in human volunteer subjects would help validate this preliminary finding in an animal model.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Approval for this study was obtained from the Ethics Committee of Centre for Research Management and Development, University of Port Harcourt, Nigeria (with reference number: UPH/CEREMAD/REC/04, dated August 1, 2017).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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