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Relative Organ Weights and Histological Changes in Wistar Rats Treated with a South East Nigerian Polyherbal Formulation (*Ajumbise*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SNI designed the study, wrote the first draft of the manuscript, wrote the protocol and author NIN performed the statistical analysis. Authors EEO and CBU managed the analyses of the study. Author OCN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and Aim: *Ajumbise* is a polyherbal formulation used in Southeast Nigeria for enhancing labour, facilitating the expulsion of retained placenta, relieving menstrual and post-delivery pains and promoting involution of the uterus. In this study, the effect of the Polyherbal formulation on body weights, relative organ weights and liver and kidney histology was evaluated.
Methods: Forty rats were divided into four groups of ten rats each and were assigned daily oral administration of the extract for 28 days. While group 1 served as the control, groups 2, 3 and 4 were administered increasing doses of the extract. At the end of treatment organs were collected for

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histological analysis respectively. Students's t-test at 95% level of significance was used for statistical analysis.

Results: Acute toxicity study result indicated zero mortality in all groups within the 24 hours of thestudy, even at a dose of 6000 mg/kg body weight. Body weight gain was significantly lowered in all treatment groups when compared with the control group ($P<0.05$). Relative liver weight did not significantly differ from that of the control except for the 800 mg/kg treated group where significant elevation was observed ($P< 0.05$). Relative kidney weights was significantly elevated in groups treated with 200 and 400 mg/kg ($P<0.05$). No significant histological changes were observed between treatment groups and control except for 800 mg/kg treated group where some inflammatory cells were observed masking the features of the portal triad. The arrangement of the hepatocytes, architecture of the portal triad comprising of the bile duct, hepatic portal vein and hepatic artery and central vein were essentially normal and had neither congestions nor necrosis. Histological presentations of the kidneys in all groups were normal and did not significantly differ from control.

Conclusion: We therefore conclude that *Ajumbise* polyherbal may be safe at low to moderate doses and at such doses does not pose any threat to the liver and kidney cells.

Keywords: Liver; kidney; Ajumbise polyherbal; toxicity.

1. INTRODUCTION

The role of the liver and kidney in the maintenance of life cannot be overemphasized. As the largest internal and major organ in the body the liver metabolizes and detoxifies substances and also helps in the regeneration of body cells. The kidney on the other hand maintains health via its role in the elimination of waste materials such as urea, creatinine, water etc and maintenance of body electrolytes. Failure by these organs in part or full to perform these life functions impairs metabolic activities and causes accumulation of waste materials in the body causing toxicity effects including and death [1,2,3]. The current high prevalence rate of diseases like diabetes mellitus [4] hypertension [5], glomerulonephritis [6], malaria [7] system lupus erythematosus [8], polycystic diseases [9], pyelonephritis [10,11] Jaundice [12] etc and environmental conditions such as exposure to harmful chemicals [13] and over consumption of some medications [14] with other conditions which favor the generation of free radicals in the body have continued to negatively affect the activities of the liver and kidney [15].

Amongst these popularly consumed medications are herbal medicines. It has indeed been reported that over 80% of the world's population is currently relying on herbal medicines [16]. In southeast, Nigeria, herbal medications are prepared as infusions, concoctions or decoctions of a part or parts of single or more plants. These preparations are most times taken without knowledge of their likely toxicity effects on the liver and kidney, despite several reports which implicated plants as sources of toxic substances [17,18]. The polyherbal formulation (*Ajumbise*) used in this study is a combination of the leaves, stems, bark and roots of different species of plants put together in various proportions. We had reported in our other publication that this herbal formulation is composed of parts of six different plants including *Barteria fistulosa* (34.97%), *Napoleona vogelli* (23.72%), *Euphorbia convolvuloids* (3.72), *Spondias mombine* (11.45%), *Uvaria chamae* (10.09%) and *Ceiba petandra* (16.60%) with phytocomponents such as flavonoids, steroids, terpenes, phenolic compounds, alkaloids, saponins and tannins [16]. Identified compounds by Gas Chromatography- Mass Spectrometry (GC-MS) included Methyl (2)-3-cyanoprop-2-enoate (1.22%), 2-Ethyl-2-hexen-1-al (0.80%), 1,3-oxazolidine-2-thione (2.54%), Benzyl benzoate (2.71%), Methyl 2-(4-chlorophenoxy)-2 methylpropanoate (5.00%), Hexadecanoic acid also known as Palmitic acid (32.65%), Ethyl palmitate (6.74%), S-methyl-L-cysteine (3.49%), Niacin or nicotinic acid (6.96%), *N*-(furan-3-yl)acetamide (10.60%), Stearic acid or n-Octadecanoic acid (12.34%), and Pyridine-4-carboxylic acid (1.94%). Others are Pyroglutamic acid (0.91%), Pyroglutamic acid (0.91%), ρ-nitrocinnamic acid, methyl ester (1.42%), 17-carboxyheptadec-9-en-1-ylium (7.10%) and 3.58% of 20-carboxydodec-8-en-1-ylium [16]. The formulation which is native to the Mbise Community in Mbise Local Government area of Imo State, Nigeria is used for various purposes including, enhancing labour, facilitating the expulsion of retained placenta, relieving menstrual and post-delivery pains and promoting involution of the uterus. In this publication, we present the effects of the polyherbal extract on

relative organ weights and histology of liver and kidney in rats.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Heads of *Ajumbise* polyherbal were purchased from Onu-imo herbal market in Obowo Local Government Area of Imo State, Nigeria. Some were separated into its component plants and were identified at the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture. Umudike. The identified plants were assigned voucher numbers and specimens were preserved in the herbarium of the Department of Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike.

Plate A. Freshly prepared *Ajumbise* polyherbal formulation purchased from Onu-imo herbal market, Obowo local government area, Imo state, Nigeria

Plate B. *Barteria fistulosa* leaves

Plate C. *Napoleona vogelli* leaves

Plate D. *Euphorbia convolvuloids* whole plant

Plate E. *Spondias mombin* leaves

Plate F. *Ceiba pentandra* bark

Plate G. *Uvaria chamae* stems

2.2 Preparation of Plant Extract

Extracts were prepared for the Polyherbal as a unit and also for the individual plant components. To achieve this, the plant materials were air dried at room temperature for 21 days and then ground to coarse powder using a locally fabricated milling machine powered by a petrol motor (Honda Company, Japan). For each round of extraction, fifty (50) grams of the powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 60ºC for 48 hours. At the end of the period, the ethanol was evaporated at low temperature in a hot air oven to obtain crude extract which weighed 8.69 g and represented a percentage yield of 17.38% and is hereafter referred to as *Ajumbise* Polyherbal Extract (APE).

2.3 Animals

Thirty mice (30-35 g) and forty (40) rats (120-160 g), 8 to 10 weeks old andof both sexesobtained from the Animal production unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike were used. They animals were housed under specific pathogen free (SPF) conditions and were provided standard feed (Vital feed, Nigeria) and water *ad libitum*. The experiment was carried out in compliance with NIH guidelines for Care and Use of Laboratory Animals [19] and was carried out in the Physiology Laboratory of the Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria where ethical approval was obtained.

2.4 Acute Toxicity Test (LD_{50})

Thirty mice were divided into 6 groups of five mice each and each group was assigned a particular oral dose level of the crude extract in the order: 1000, 2000, 3000, 4000, 5000, and 6000 mg/kg body weight. The mice were thereafter monitored for toxicity signs and deaths within 24 hours. The number of deaths recorded in each group by the end of 24 hours was used to estimate the LD_{50} value for the extract according to Karber's method, as was used by Akomas et al. [20] in accordance with OECD guideline 423.

2.5 Grouping of Animals and Treatment for Histological Study

Forty rats were divided into four groups of ten rats each. Each group was housed in an aluminum cages and were assigned treatment in the order below:

Group 1: Normal control
Group 2: Treated with 200 mg/kg APE
Group 3: Treated with 400 mg/kg APE
Group 4: Treated with 800 mg/kg APE

Treatment was done orally by gavage and lasted for twenty eight (28) days during which body weights of the animals were taken at the beginning and end of treatment and was followed by sacrificing the animals for collection of organs (liver and kidneys). The organs were weighed on an electronic balance and expressed as percentages of the body weight before being transferred into separate organ bottles containing 10% formalin for histological studies.

2.6 Preparation of Liver and Kidney Slide for Histological Studies

Slides of the harvested livers and kidneyswere prepared according to the method used by Ogwo et al. [21] and were observed under the microscope with magnifications x400. Selected images were captured using a moticam 2.0 digital camera attached to a computer.

2.7 Statistical Analysis

Results were expressed as means ± standard error of mean (SEM). Statistical analysis was done using one way analysis of variance (ANOVA). Students's t-test at 95% level of significance was used to access significant difference between control and treated groups. P values less than 0.05 were considered as significant. Computer software package, SPSS version 21 was employed.

3. RESULTS

3.1 Acute Toxicity Study

No death was recorded within the 24 hours of acute toxicity study, even at the highest dose of 6000 mg/kg body weight. The mice instead had normal disposition both physically and mechanically and were emotionally stable and all survived the 24 hours period of acute toxicity study and suggest that LD_{50} value of the extract

must be above 6000 mg/kg body weight. Observation of the animals for a further 7 days showed no form of delayed toxicity and no mortality was observed.

3.2 Effect on Body Weight Changes

Body weight gain was significantly higher in the control group when compared with the treated groups (P<0.05). Group 2 rats treated with 200 mg/kg had a mean body weight gain of 39.77±1.91, while in groups 3 and 4 body weight changes were 49.51±3.46 and 32.74±1.86 respectively. The values so obtained were significantly lowered when compared with the control group in which mean body weight gain was 62.12 ± 2.14 g (P< 0.05) (Table 1). Percentage gain in body weight in groups 2, 3 and 4 were 51.04 ± 3.49%, 68.64 ± 7.29% and 37.96 ± 2.46 respectively and significantly differed from that of the control group which was 69.44±4.29% (P<0.05).

3.3 Effect APE on Relative Organ Weights (ROW) in Percentages

Liver to body weight ratios was not significantly affected in groups 2 and 3 treated with 200 and 400 mg/kg of APE respectively when compared with the control (P< 0.05). In the control group the relative liver weight was 4.15±0.02% while in groups 2 and 3 the values were both 4.15±0.01%. However, in group 3 treated with 800 mg/kg of the extract, relative liver weight was significantly higher when compared with control (P< 0.05). The value in the control group was 4.15±0.02% (Table 3), the relative kidney weight in group 4 (0.71±0.01 %) did not significantly differ from that of the control group (P< 0.05) in which ROW value was 0.71 ± 0.01%. However relative kidney weight for groups 2 and 3 treated with 200 and 400 mg/kg of the extract were significantly higher when compared with control (P < 0.05). The values in these groups were 0.74±0.01% and 0.73±0.02% respectively (Table 2).

3.4 Effect of APE on Liver Histology

A section of control liver showed normal arrangement of the hepatocytes (liver cells) in cords. The architecture of the portal triad comprising of the bile duct, hepatic portal vein and hepatic artery were all normal (Plate L1A). The central vein was also found to be normal (Plate L1B). Treatment with 200 mg/kg of APE produced no significant histologic changes when compared with control as the hepatocytes appeared normal. No inflammatory cells were seen in the connective tissue of the portal triad. No necrosis was also observed and the central vein showed no form of congestion and no infiltration with inflammatory cells (Plates L2A & L2B).

3.4.1 Liver architecture of group 3 (400 mg/kg, APE) compared with control

Treatment with 400 mg/kg of APE also produced no significant histologic changes when compared with control as the hepatocytes appeared normal. No inflammatory cells were seen in the connective tissue of the portal triad. No form of necrosis was also observed and the central vein showed no form of congestion and no infiltration with inflammatory cells (Plates L3A & L3B).

3.4.2 Liver architecture of group 4 (800 mg/kg, APE) compared with control

For animals treated with 800 mg/kg of APE, infiltration of inflammatory cells into the connective tissues of the portal triad was observed. These inflammatory cells were seen masking the features of the portal triad, even though the nucleus, cytoplasm and central vein still had normal presentations as in the control group. No necrosis and fatty deposits were seen (Plates L4A & L4B).

Table 1. Effect of APE on body weight changes

	Day 1 body weight (g)	Day 28 body weight (g)	Body weight gain (g)	% Rise in body weight
Normal control	91.38±12.98	153.50±13.24	62.12±6.78	69.44±13.56
200 mg/kg APE	79.07±7.09*	118.84±6.22*	39.77±6.06*	51.04±11.03*
400 mg/kg APE	74.32±9.06*	123.83±8.88*	49.51±10.95*	68.64±23.05
800 mg/kg APE	86.92±6.90	119.66±8.01*	32.74±5.88*	37.96±7.79*

*Values are mean ± SEM. Means marked * is significantly different from normal control at 0.05 sig. level*

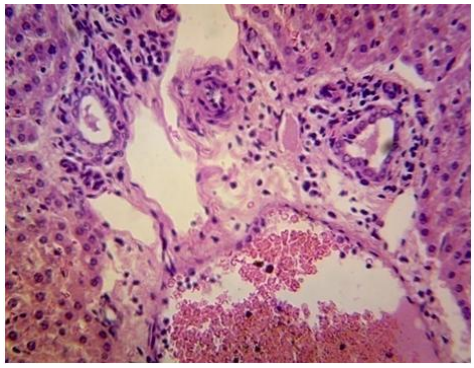

Plate L1A: Group 1 H&E X400

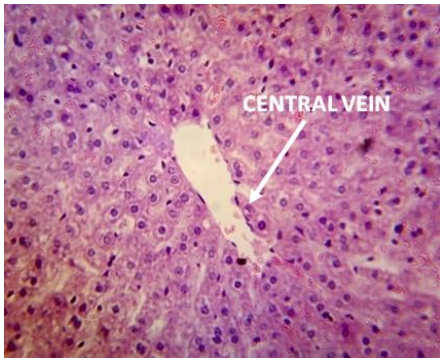

Plate L1B: Group 1 H&E X400

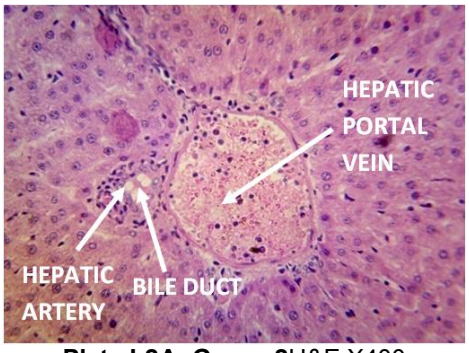

Plate L2A: Group 2H&E X400

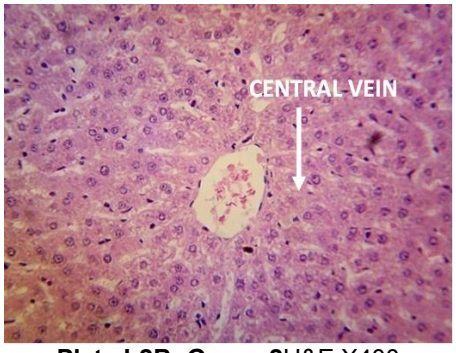

Plate L2B: Group 2H&E X400

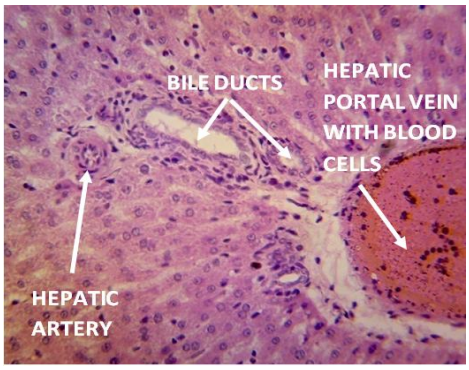

Plate L3A: Group 3 H&E X400

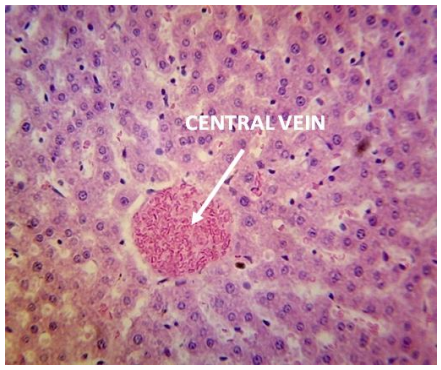

Plate L3B: Group 3 H&E X400

Table 2. Effect of APE on relative organ weights

	Liver (%)	Kidney (%)
Normal control	4.15±0.02	0.71±0.00
200 mg/kg APE	4.15±0.01	0.74±0.01*
400 mg/kg APE	4.15±0.01	0.73±0.02*
800 mg/kg APE	4.45±0.05*	0.71±0.01

*Values are mean ± SEM. Means marked * is significantly different from normal control at 0.05 sig. level*

3.4.3 Kidney architecture of group 2 (200 mg/kg, APE) compared with control

No pathological changes were observed in the section of the kidneys after treatment with APE. The renal tubules, glomerulus and renal vein and artery all appeared normal (Plate K1) and did not significantly differ from that of the control group (Plate K2).

3.4.4 Kidney architecture of groups 3 and 4 (400 and 800 mg/kg, APE) compared with control

No pathological changes were observed in the section of the kidneys after treatment with APE. The renal tubules, glomerulus and renal vein and artery all appeared normal (K3 and K4) and also did not significantly differ from that of the control group (K1).

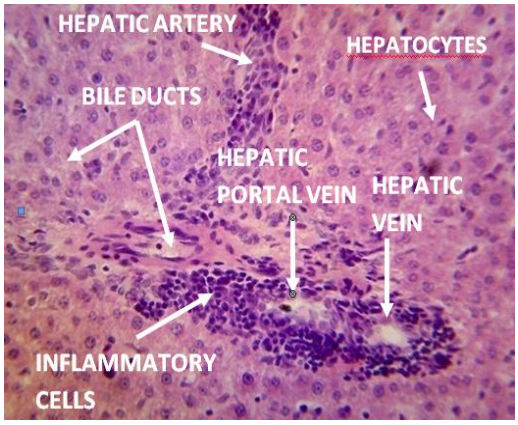

Plate L4A: Group 4 H&E X400

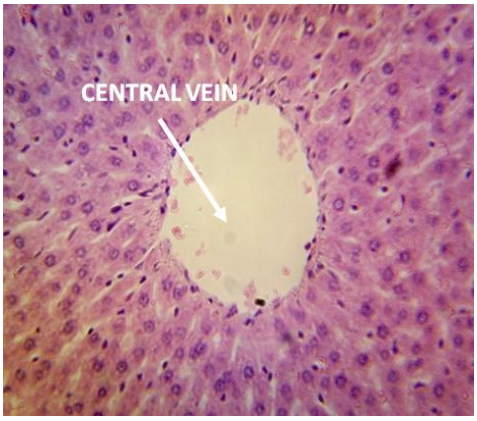

Plate L4B: Group 4 H&E X400

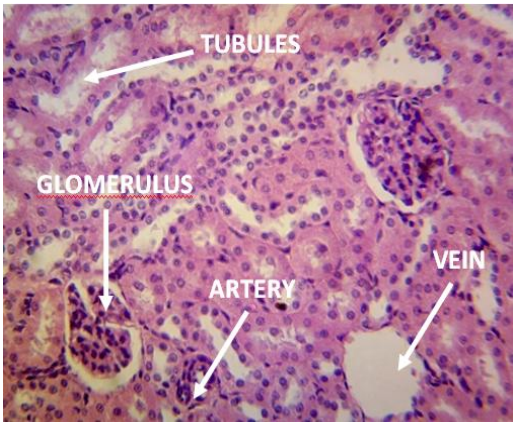

Plate K1: Group 1 H&E X400

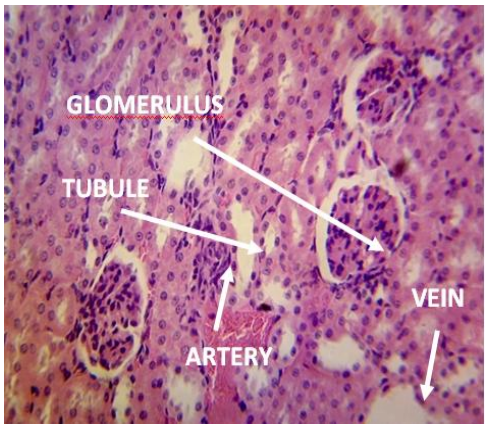

Plate K2: Group 2 H&E X400

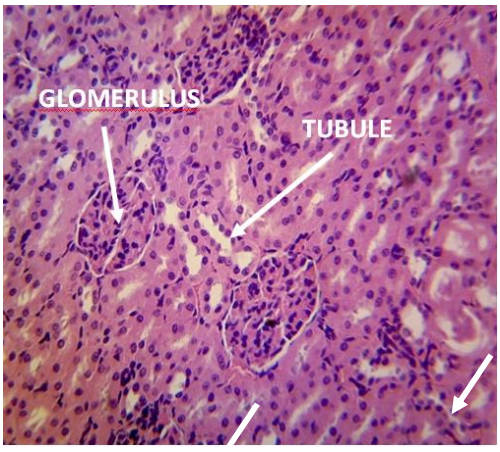

Plate K3: Group 3 H&E X400

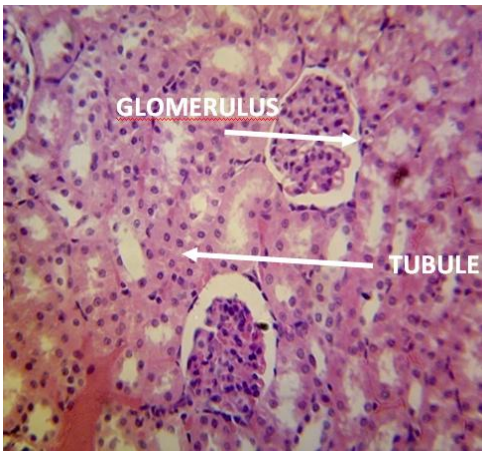

Plate K4: Group 4 H&E X400

4. DISCUSSION

The LD_{50} results suggest that APE is completely free from any form of acute toxicity as all animals treated with the extract during the acute toxicity study period showed no sign of toxicity but instead looked physically healthy and emotionally stable all through the period, including those which were given 6000 mg/kg body weights of APE. This conclusion is in line with the OECD guideline for acute toxicity studies. It has been reported that mortality is the expected end point of acute toxicity and non observation of mortality within a population treated with a dose range at

which mortality is expected indicates tolerance or lack of acute toxicity [19]. Similar conclusions were drawn in other acute toxicity investigations involving plant materials [20].

In this study, body weight gain was significantly lowered in the groups treated APE when compared with the control group and may be due to the inhibitory effect of the extract on the gastrointestinal tract. Although the higher brain centers maintains body weight via the process of energy homeostasis [22] and modulation of appetite, signals from the GIT are important regulators of satiety and have implications for the control of body weights [23]. Inhibition of GIT motility suggest possible decline in the quantity of food consumed and lowering of caloric value and may have been responsible for the observed change in body weight when compared the normal rats. More over the weight maintenance effect of APE may also be related to its hypolipidaemic activity and lowering of fat deposits in the body. APE may have increased the catabolism of lipids accumulated in adipose tissue resulting in decreased body weight gain when compared to control. This mechanism has been implicated in weight loss following administration of plant extract [24]. This agrees with a local claim on the *Ajumbise* polyherbal formulation. Local users of APE had explained that APE maintains body weight by facilitating the burning off of excess fat, particularly those lining the stomach wall (Personal conversation). Further evaluation is however required here because change in body weight can occur through other mechanisms such as alterations in growth (e.g agents that modify secretion of growth hormone or somatostatin), alterations of hormonal status (e.g agents that modify secretions of sex steroids and thereby alter maturation patterns), changes in concentration of neurotransmitters that affect food consumption, reduced palatability of diets containing the test compound and through non-specific toxicity [25].

It is a common practice to present organ weight data relative to the animal's body weight since this will help to remove bias due to differences in body weights; hence relative organ weights (ROW) was also evaluated in this work. Comparing relative organ weight between treated and untreated groups of animals have conventionally been used to evaluate the toxic effect of a test substance and is an important end point in many toxicity studies [26]. Indeed organ weight is one of the most sensitive indicators of an effect of a test substance as significant differences in organ weights between treated and untreated (control) animals may occur in the absence of any morphological changes [27]. The slight changes observed in relative organ weights (ROW) in few of the APE treated groups may be due to the observed body weight changes. ROW is usually evaluated by dividing the organ weight (liver or kidney) by the body weight of the animal and expressed as a percentage of the whole body. Hence any slight decline in body weight rise without corresponding effect on the organ weight will lower ROW values. The slight changes observed in few treatment groups may be due to mild enzyme induction in the organs. It has been reported that minimal increase in liver weights without any microscopic lesion can be correlated with enzyme induction [27].

A section of control liver showed normal arrangement of the hepatocytes (liver cells) in cords. The architecture of the portal triad comprising of the bile duct, hepatic portal vein and hepatic arteries were all normal. The central veins were also found to be normal. No inflammatory cells were seen in the connective tissue of the portal triad. No necrosis was observed and the central vein showed no form of congestion and no infiltration with inflammatory cells. However for animals treated with 800mg/kg of APE, infiltration of inflammatory cells into the connective tissues of the portal triad was observed. These inflammatory cells were seen masking the features of the portal triad, even though the nucleus, cytoplasm and central vein still had normal presentations as in the control group. The hepatocytes make up 70 -85% of the liver mass and are sites for protein synthesis, storage and transformation of carbohydrates. The hepatocytes also synthesis cholesterol, bile salts and phospholipids and initiate the formation and secretion of bile and play major role in detoxification and excretion of exogenous and endogenous substances [2]. Negative histological changes in the structure and architecture of these liver cells invariably will affect the entire functioning of the liver [28]. The fact that these cells were found intact following treatment with low to moderate doses of APE suggests that the consumption of the extract may not be toxic to liver cells.

The portal triad also known as the portal field, portal area or portal tract is a distinctive arrangement in the liver and consist of three major tubes including hepatic artery, hepatic

portal vein and common bile duct, all surrounded by the hepatoctes. Lymphatic vessels and a branch of the vagus nerve have also of late been associated with the portal triad [28]. The portal artery carries oxygenated blood to the hepatocytes while branches of the portal vein carry blood with nutrients from the small intestine. The bile duct carries bile products away from the hepatocytes, to the larger ducts and gall bladder. All these liver structures remained intact with APE treatment and suggest a degree of safety. It has been reported that disturbances in the architecture of both the hepatocyes and portal triad with elevated enzyme levels are clear indicators of liver toxicity [29]. In fact, severe liver injuries including acute and chronic abnormalities and even cirrhotic transformation and liver failure have been reported after the ingestion of a wide range of herbal products [30]. The inflammatory cells observed in the group treated with 800mg/kg may be due to the consumption of high amounts of phytochemical components in APE. Ingestion of high amounts of phytochemical substances has been implicated in the formation of inflammatory cells in the liver [31]. The presence of inflammatory cells in the liver of the rats in this group may also be the reason for their higher relative liver weights.

The kidneys in both the control and treated groups were normal showing numerous renal tubules interstitials tissues and no signs of inflammations. The glomerulus and renal vein and artery also appeared normal. The kidney histology also suggests that APE did not induce any form of renal toxicity. This result agrees with that of the relative kidney weights which clearly did not show any difference between control and treatment groups and suggest that kidney functions in all groups were essentially normal. This has also been reported to be a mark of safety [31].

5. CONCLUSION

From results obtained, we conclude that *Ajumbise* polyherbal may be safe and does not pose any threat to the liver and kidney cells when used at low to moderate doses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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