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Toxicological effects of polyherbal formulations for malaria, yellow fever and haemorrhoids in Ilorin metropolis, Nigeria, on male Wistar rats: A comparative biochemical and histological study

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ABSTRACT**:** This study evaluated the toxicological effects of polyherbal formulations used for the treatment of malaria (*Agbo iba*, AI), yellow fever (*Agbo kojupon*, AK) and haemorrhoids (*Agbo jedi*, AJ) on the function indices and histoarchitecture of liver and kidney of male Wistar rats. Forty rats were randomised into two groups (A and B) of twenty animals each such that the 20 rats in group A were further reassigned into A_1, A_2, A_3 and A_4 and administered distilled water (0.5 ml), and AI, AK and AJ at 21 mg/kg body weight respectively for 4 days. Animals in group B were correspondingly assigned and treated like those of group A except that the administration lasted for 10 days. The results revealed that AI, AK and AJ contained alkaloids, saponins, phenolics and flavonoids whilst anthraquinones were not detected. The AI and AK contained tannins whereas AK and AJ contained cardiac glycosides. The AI, AK and AJ reduced the liver- and kidney-body weight ratios, activities of both the liver alkaline phosphatase and aspartate aminotransferase, levels of serum total protein, albumin, globulin, total bilirubin and conjugated bilirubin, and increased the activities of liver alanine aminotransferase, serum alkaline phosphatase, serum aspartate aminotransferase, Na^+ and Cl^- on days 4 and 10. The AK and AJ significantly ($p<0.05$) reduced the activity of serum alanine aminotransferase and increased K^+. The AK, AI and AJ increased serum creatinine content on day 4 whereas only AK increased it by day 10. Serum uric acid was not altered by all the treatment on days 4 and 10. The administration of AK and AJ induced moderately swollen hepatocytes, congested and dilated blood vessels in the liver as well as imposed glomeruli and renal tubules in the kidney whereas the lobules, glomeruli and tubules were within normal liver and kidney histology after the administration of AI. Overall, the AK and AJ exhibited both functional and structural toxicities whilst the AI displayed only functional toxicity at 21 mg/kg body weight and during the 10 days of administration. The *Agbo jedi* exhibited the highest degree of toxicity whilst the *Agbo iba* was the least toxic with respect to the dose and duration of the study.

Keywords: polyherbal formulation, functional toxicity, structural toxicity, toxicity.

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Introduction

For centuries, medicinal herbs either singly or as mixtures of herbs (polyherbal formulations or mixtures) have been used as ethnopharmacological therapies for the management of several diseases and their complications. According to traditional medicine practitioners, treatment of diseases like malaria, yellow fever and haemorrhoids with polyherbal mixtures may be far more beneficial than monoherbal therapies [1]. The synergistic effect of the medicinal herbs used in polyherbal preparations leads to a better therapeutic outcome by increasing their efficacies and reducing their toxicity [2]. However, some of these polyherbal formulations may exhibit functional toxicity, structural toxicity, genotoxicity and cytotoxicity, while others may possess antioxidant, antimalarial, antidiabetic, antipyretic, aphrodisiac and anti-genotoxic activities with low toxicity even when used in high concentrations [2].

In Nigeria, some of the commonly consumed polyherbal preparations include those used to treat malaria (*Agbo iba*, AI), yellow fever (*Agbo kojupon*, AK) and haemorrhoids (*Agbo jedi*, AJ). These self-medication polyherbal formulations in which the components are largely unknown to the buyers/consumers are sold in motor parks, markets and workshops of artisans among others. Although, the efficacy of AI, AK and AJ may not be in doubt, information on the comparative toxicological implications of the consumption of these polyherbal formulations on key metabolic organs and the general well-being of the consumers are still lacking in open scientific literatures. Herein, the current study provides information on the comparative toxicological implications of AI, AK and AJ on the function indices and histoarchitecture of the liver and kidney of male Wistar rats.

Materials and Methods

Polyherbal formulations

Polyherbal formulations for AI, AK and AJ were obtained from herb sellers at Maraba Central Motorpark, Maraba, and Ipata Market, in Ilorin, Kwara State, Nigeria.

Assay kits and reagents

The assay kits for alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, albumin and creatinine were products of Randox Laboratories Limited, United Kingdom whilst those of urea and uric acid were from Fortress Diagnostics Limited, United Kingdom. Other reagents used were of analytical grade obtained from Sigma-Aldrich, Steinheim, Germany.

Animals

Forty, male, Wistar rats (137.50 ± 1.35 g) obtained from the Animal House Facility of the Department of Biochemistry, University of Ilorin, Nigeria, were used for this study. The animals were housed in clean cages placed in a well-ventilated house with standard conditions (temperature: 23 ± 2°C; photoperiod: 12 h natural light and 12 h dark; humidity: 45-50%). The animals were allowed free access to rat feeds (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and clean tap water throughout the experimental period. The cages were also cleaned of wastes on daily basis throughout the period of the experiment.

Handling of the polyherbal formulations

A known volume (150 ml) each of the AI, AK and AJ were lyophilised (Zirbus, VaCo 5-11, Zirbus Technology, Stephensonstraat, Germany) to give 1.78 g, 1.31 g and 1.26 g respectively. The lyophilisation was repeated five more time to give sufficient quantity for reconstitution of the required dose. Calculated amount of the yields of each of the AI, AK and AJ were reconstituted in distilled to give the required dose of 21 mg/kg body weight. The dose (21 mg/kg body weight) represents what was contained in a cup of 150 ml taken by an adult of 70 kg for the management of malaria, yellow fever and

haemorrhoids within Ilorin metropolis of Kwara State, Nigeria. The AI contained *Mangifera indica* bark (*Eepo mangoro*), *Alstonia boonei* (*Ahun*), *Enantia chlorantia* (*Awopa*), *Lophira alata* (*Panhan*) and *Morinda lucida* (*Oruwo*) whilst the AK was made of *Enantia chlorantia* bark (Eepo *Awopa*) and fermented cereal water (*Omi ogi, Omikan* or *Omidun*) that contained smooth cereal sediment, in this instance, guinea corn. The AJ consisted of *Senna sieberiana* (*Aidan toro*), *Gongronena latifolium* (*Madunmaro*), *Picralima nitida* (*Abere*), *Eugenia aromatica* (*Kanafuru*), *Aristolochia ringens* (*Akogun*) and *Rauvolfia vomitoria* (*Orira*),

Secondary metabolite screening

Preliminary screening to detect the presence of alkaloids, tannins, saponins, cardiac glycosides, steroids, anthraquinones and phenolics in AI, AK and AJ were carried out as outlined by Sofowora [3].

Animal grouping and administration of AI, AK and AJ

Forty Wistar rats were randomly assigned into 2 groups (A and B) of 20 rats each. The 20 rats in group A were assigned into four sub-groups (A_1, A_2, A_3 and A_4) of 5 rats each while those in group B were equally randomised as B_1, B_2, B_3 and B_4 as follows:

Group A:

A_1 (naïve control): administered 0.5 ml of distilled water
A_2: administered 21 mg/kg body weight of AI
A_3: administered 21 mg/kg body weight of AK
A_4: administered 21 mg/kg body weight of AJ

The animals in group A were orally administered once daily the 0.5 ml of distilled water and same volume that corresponded to 21 mg/kg body weight of AI, AK and AJ for 4 days.

Group B:

B_1 (Control): administered 0.5ml of distilled water
B_2: administered 21 mg/kg body weight of AI
B_3: administered 21 mg/kg body weight of AJ
B_4: administered 21 mg/kg body weight of AK

The animals in group B were orally administered once daily the 0.5 ml of distilled water and same volume that corresponded to 21 mg/kg body weight of AI, AK and AJ for 10 days.
The animals were humanely handled in accordance with the guidelines of European Convention for the Protection of Vertebrate Animals and Other Scientific Purposes- ETS-123 [4].

Preparation of serum and tissue supernatant

Twenty-four hours after the completion of 4 and 10 daily doses of the distilled water and the AI, AK and AJ, the rats were anaesthetised in a glass container containing cotton wool soaked with diethyl ether. The neck area was quickly cleared of fur after which the jugular veins were cut. A known volume (5 ml) of the blood was collected from each animal into clean and dry test tubes. Serum was collected with the aid of Pasteur pipette after centrifugation at 685 x *g* for 10 minutes. The rats were quickly dissected and their liver and kidney excised, blotted in clean blotting paper, cleaned of fat and weighed separately. The organs were homogenised (1:5w/v) in ice-cold 0.25M sucrose solution. The homogenates were further centrifuged at 1398 x *g* for 15 minutes to obtain supernatant, which were then stored frozen in specimen bottles for analyses [5].

Determination of biochemical parameters

The biochemical parameters were determined as described for alkaline phosphatase [6], aspartate aminotransferase and alanine aminotransferase [7], total protein [8], albumin [9], globulin, Na^+, K^+ and

Cl^- [10], total bilirubin and conjugated bilirubin [11]. Other parameters included urea [12], uric acid [13] and creatinine [14]. The liver- and kidney-body weight ratios were computed according to the expression of Yakubu *et al* [15].

Histopathological examination

The procedure described by Talukdar *et al* [16] was adopted in the preparation of liver and kidney photomicrographs. Briefly, fixed tissues (liver and kidney) in 10% formalin were dehydrated through ascending grades of ethanol (70%, 90% and 95%). The tissues were then cleaned in xylene, impregnated and embedded in paraffin wax (melting point 56ºC). Tissue sections (5 µm) were floated out on clean microscope slides (which had previously been lightly coated with egg albumin preparation to avoid detachment from slides during the staining procedure) and dried for 2 hours at 37^0C. After staining in aqueous dyes, the slides together with the mounted stained section were passed through ascending concentration of ethanol (20% - 100%) for dehydration and then cleaned with xylene. A permanent mounting medium (balsam) was put on the tissue section. A thin glass cover slip was placed on the covering mounting medium and underlying tissue sections were allowed to dry. The slides were viewed with a light microscope (OLYMPUS, Model: CX21, New York Microscope Company Inc., New York). Cross section of the liver and kidney were captured at x400 with Canon! Image Folio package software (Model: Powershot A2500, Japan).

Statistical analysis

Data were presented as mean ± standard error of mean (SEM) of 5 replicates. Statistical analysis was done with computer assisted software, statistical package program version 20.0 (SPSS Corporation Chicago, Illinois, USA). One-way analysis of variance was used to compare the variables among the different treatments whilst the of significance (*Post hoc* comparisons) among the various treatments was done with Duncan‘s Multiple Range Test. The statistical significance was set at $p < 0.05$.

Results

The three polyherbal formulations of AI, AK and AJ contained alkaloids, saponins, phenolics and flavonoids whilst anthraquinones were not detected (Table 1). The AI and AK contained tannins which were absent in AJ. Furthermore, AK and AJ contained cardiac glycosides whereas cardiac glycosides were not detected in AI. Of the three polyherbal formulations investigated in this study, only AJ contained steroids (Table 1).

Table 1: Secondary metabolite profile of the polyherbal formulations

	Herbal formulations		
Secondary metabolites	*Agbo iba*	*Agbo kojupon*	*Agbo jedi*
Alkaloids	+	+	+
Tannins	+	+	-
Saponins	+	+	+
Cardiac glycosides	-	+	+
Steroids	-	-	+
Anthraquinones	-	-	-
Phenolics	+	+	+
Flavonoids	+	+	+

+ = Present, - = Absent

Table 2: Liver function indices of male Wistar rats after oral administration of the polyherbal formulations

Days of intervention/Polyherbal formulation								
	Day 4				Day 10			
Liver function indices	**Control**	***Agbo iba***	***Agbo kojupon***	***Agbo jedi***	**Control**	***Agbo iba***	***Agbo kojupon***	***Agbo jedi***
Liver-body weight ratio (%)	3.37 ± 0.17	3.25 ± 0.09[a]	3.13 ± 0.01[b]	3.02 ± 0.02[b]	3.43 ± 0.01	3.09 ± 0.03[a]	3.05 ± 0.03[a]	3.01 ± 0.05[a]
Liver ALP (nm/min/mg protein)	3.62 ± 0.02	2.21 ± 0.03[a]	1.25 ± 0.01[b]	0.76 ± 0.01[c]	3.58 ± 0.01	1.19 ± 0.02[a]	2.87 ± 0.02[b]	0.83 ± 0.02[c]
Serum ALP (nm/min/mg protein)	0.51 ± 0.04	0.89 ± 0.02[a]	1.01 ± 0.09[b]	1.24 ± 0.06[c]	0.44 ± 0.02	1.20 ± 0.05[a]	1.19 ± 0.04[a]	1.32 ± 0.02[b]
Liver AST (U/L)	152.00 ± 5.42	136.65 ± 4.54[a]	138.47 ± 3.35[a]	108.00 ± 3.06[b]	149.00 ± 7.24	119.50 ± 5.57[a]	119.65 ± 7.36[a]	73.24 ± 4.45[b]
Serum AST (U/L)	67.50 ± 4.50	133.50 ± 6.97[a]	136.80 ± 5.45[a]	161.20 ± 5.19[b]	70.21 ± 2.20	137.60 ± 3.57[a]	150.55 ± 6.36[b]	167.00 ± 2.45[c]
Liver ALT (U/L)	107.20 ± 5.35	139.08 ± 9.02[a]	152.13 ± 7.14[b]	178.02 ± 8.00[c]	109.80 ± 7.21	138.86 ± 6.19[a]	162.50 ± 5.26[b]	190.69 ± 10.24[c]
Serum ALT (U/L)	57.50 ± 3.19	58.60 ± 3.50	35.00 ± 4.14[a]	31.65 ± 6.00[a]	58.40 ± 3.55	56.20 ± 4.19	29.50 ± 2.26[a]	19.69 ± 2.24[b]
Serum total protein (g/L)	73.17 ± 4.07	58.00 ± 2.11[a]	56.38 ± 1.73[a]	37.50 ± 3.21[b]	69.94 ± 3.41	45.06 ± 1.15[a]	25.87 ± 1.30[b]	15.33 ± 2.02[c]
Serum albumin (g/L)	43.50 ± 2.48	33.21 ± 3.02[a]	31.67 ± 5.11[a]	20.55 ± 2.83[b]	41.10 ± 2.05	28.12 ± 0.07[a]	14.55 ± 1.41[b]	8.13 ± 2.04[c]
Serum globulin (g/L)	29.67 ± 0.19	23.79 ± 1.00[a]	23.71 ± 0.14[a]	15.95 ± 0.04[b]	27.84 ± 1.50	15.94 ± 0.19[a]	10.32 ± 0.26[b]	5.20 ± 0.24[c]
Total bilirubin (g/L)	1.70 ± 0.07	1.10 ± 0.02[a]	1.42 ± 0.01[b]	1.08 ± 0.11[c]	1.74 ± 0.01	1.06 ± 0.01[a]	1.17 ± 0.00[b]	1.33 ± 0.02[c]
Conjugated bilirubin (g/L)	0.32 ± 0.03	0.24 ± 0.02[a]	0.16 ± 0.01[b]	0.10 ± 0.02[c]	0.30 ± 0.04	0.19 ± 0.02[a]	0.10 ± 0.01[b]	0.05 ± 0.01[c]

Values are means of 5 determinations ± SEM. Values with different superscripts from the control across the row for each day of intervention are significantly different ($p < 0.05$)

Administration of AI, AK and AJ significantly ($p < 0.05$) reduced the computed liver-body weight ratio of the animals on days 4 and 10 of the experimental period (Table 2). The AI, AK and AJ

significantly ($p < 0.05$) decreased the activities of both the liver alkaline phosphatase and aspartate aminotransferase of the male rats whereas AI, AK and AJ significantly ($p<0.05$) increased the activities of liver alanine aminotransferase on days 4 and 10 of the experimental period (Table 2). The decreases in the activities of alkaline phosphatase and aspartate aminotransferase in the liver of the male rats were accompanied by corresponding significant ($p < 0.05$) increases in the serum enzymes. In addition, the AK and AJ significantly ($p < 0.05$) reduced the activity of alanine aminotransferase in the serum of the male rats whereas the administration of AI did not significantly ($p > 0.05$) alter the alanine aminotransferase activity in the serum of the animals throughout the 4 and 10 days of experimental period (Table 2). The increases in the liver alkaline phosphatase and aspartate aminotransferase as well as the reduction in both the serum alkaline phosphatase and aspartate aminotransferase were in the order AI > AK > AJ (Table 2). The administration of AI, AK and AJ significantly reduced the levels of serum total protein, albumin, globulin, total bilirubin and conjugated bilirubin on both days 4 and 10 of the experiment (Table 2). In all of this, the AJ exhibited the most pronounced alterations in the level of the proteins.

The administration of AI, AK and AJ significantly ($p < 0.05$) reduced the computed kidney-body weight ratio with the AJ reducing the kidney-body weight ratio the most (51%). Furthermore, the administration of AI and AJ significantly ($p < 0.05$) elevated the levels of serum urea on both days 4 and 10 of the experimental period whilst the AK did not significantly ($p > 0.05$) alter the serum urea content of the animals (Table 3). Although, the creatinine levels were increased by the AI, AK and AJ after 4 daily doses, the increase in creatinine was only sustained after the 10 days of administration of AK. The AI and AJ by the end of the 10 daily doses produced creatinine levels that compared favourably ($p > 0.05$) with that of the distilled water treated control male rats (Table 3). The serum uric acid was not significantly altered by the administration of AI, AK and AJ throughout the experimental period (Table 3). In addition, the 4 and 10 days of administration of AI, AK and AJ significantly ($p < 0.05$) elevated the levels of Na^+ and Cl^- in the serum of the male rats. The AJ produced the most pronounced increase in the serum Na^+ and Cl^- contents of the animals. Although AI did not significantly ($p > 0.05$) alter the levels of K^+ in the serum of the male rats, the administration of AK and AJ increased the levels of serum K^+ content of the animals. The AK produced the most pronounced increase in serum K^+ by day 10 of the experimental period (Table 3).

Compared with the hepatic tissue, lobular architecture and Kupffer cells that were within normal histology in the liver of male rats that received distilled water for 10 days (Plate 1a), administration of AI showed normal lobular architecture with ruptured veins (Plate 1b). Furthermore, AK administration induced moderately swollen cells with empty central veins and infiltration of the neutrophils (Plate 1c) whereas AJ administration induced moderately swollen hepatocytes, congested and dilated blood vessels and activated Kupffer cells (Plate 1d). In comparison with the kidney histoarchitecture of the distilled water treated control rat (Plate 2a) where the glomeruli, tubules and juxtaglomerular apparatus were within normal kidney histology, the 10 daily administration of AI also produced normal kidney histoarchitecture with no evidence of lesion (Plate 2b) in a manner similar to that of Plate 2a. In contrast, administration of AK for 10 days produced glomeruli and renal tubules that were imposed as well as hyper-cellular glomeruli whereas administration of AJ induced severe swollen cells with moderate distortion of the renal architecture and bloody glomeruli (Plate 2d).

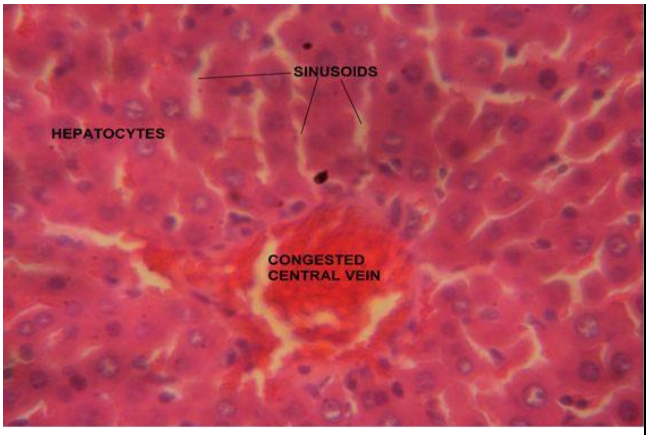

Plate 1a: Cross section of liver of rat orally administered. 0.5ml distilled water for 10 days (x400; H & E).

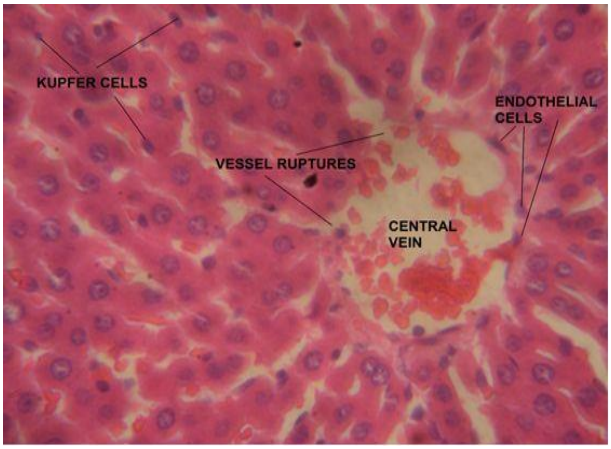

Plate 1b: Cross section of liver of rat orally administered. 21 mg/kg body weight of *Agbo iba* for 10 days (x400; H & E).

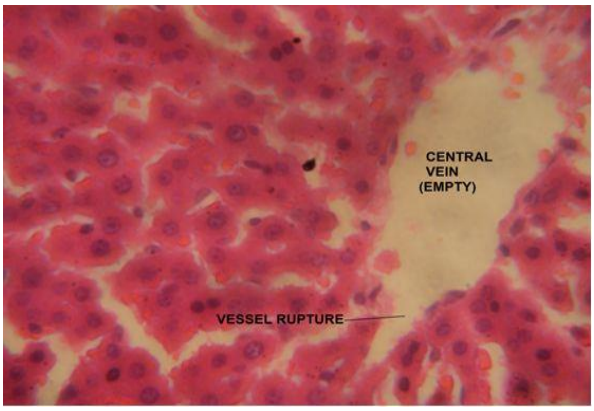

Plate 1c: Cross section of liver of rats orally administered 21 mg/kg body weight of *Agbo kojupon* formulation for 10 days (x400; H & E)

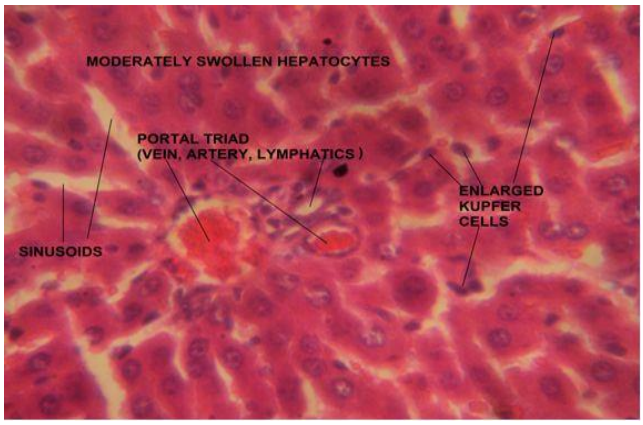

Plate 1d: Cross section of liver of rat orally administered 21 mg/kg body weight of *Agbo jedi* formulation for 10 days (x400; H & E).

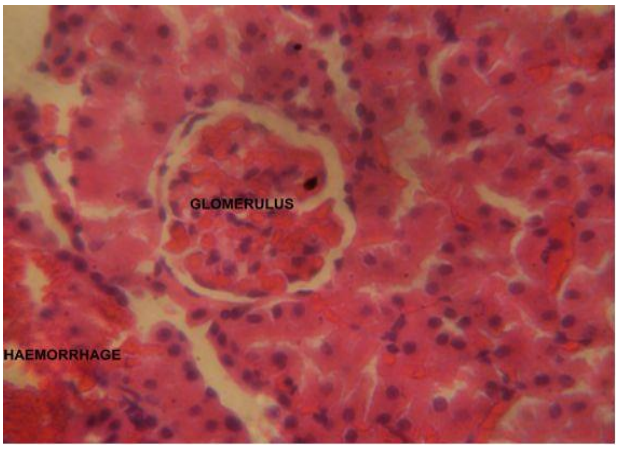

Plate 2a: Cross section of kidney of rat orally administered distilled water for 10 days (x400; H & E)

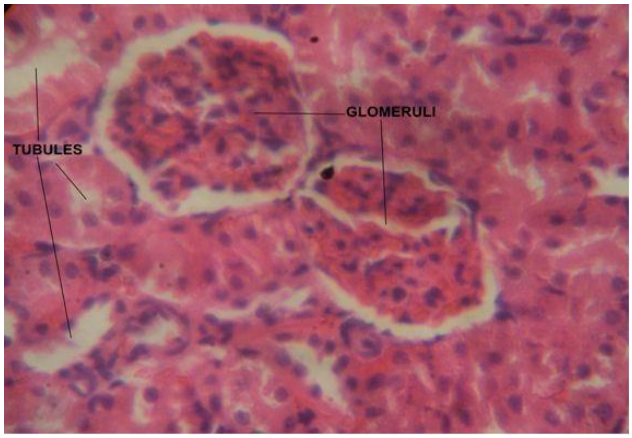

Plate 2b: Cross section of kidney of rat orally administered 21 mg/kg body weight of *Agbo iba* formulation for 10 days (x400; H & E)

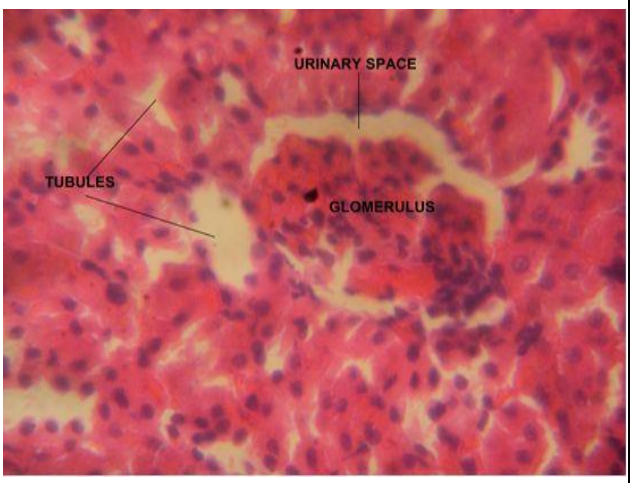

Plate 2c: Cross section of kidney of rat orally administered 21 mg/kg body weight of *Agbo kojupon* formulation for 10 days (x400; H & E)

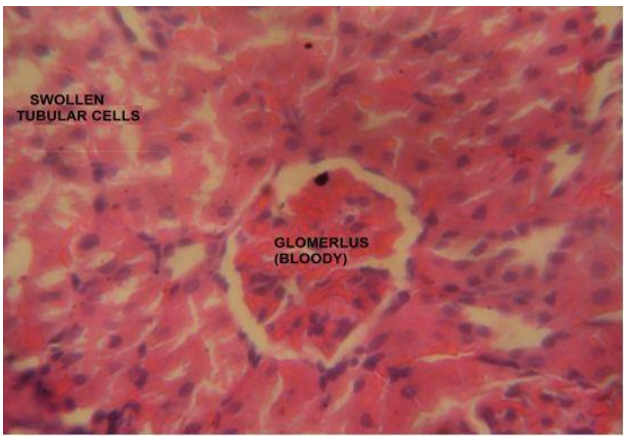

Plate 2d: Cross section of kidney of rat orally administered 21 mg/kg body weight of *Agbo jedi* formulation for 10 days (x400; H & E)

Table 3: Kidney function indices of male Wistar rats after oral administration of the polyherbal formulations

Days of intervention/Polyherbal formulation								
	Day 4				Day 10			
Kidney function index	**Control**	***Agbo iba***	***Agbo kojupon***	***Agbo jedi***	**Control**	***Agbo iba***	***Agbo kojupon***	***Agbo jedi***
Kidney-body weight ratio (%)	0.91 ± 0.09[a]	0.78 ± 0.04[b]	0.74 ± 0.01[a]	0.65 ± 0.03[a]	0.98 ± 0.02[a]	0.93 ± 0.07[a]	0.67 ± 0.04[b]	0.48 ± 0.01[c]
Creatinine (µmol/L)	30.27 ± 0.19[b]	33.08 ± 1.00[d]	34.13 ± 0.14[a]	35.65 ± 0.00[c]	29.80 ± 0.00[a]	29.86 ± 0.19[a]	34.50 ± 0.26[b]	29.69 ± 0.24[a]
Urea (mmol/L)	4.68 ± 0.07[c]	5.09 ± 0.01[d]	4.33 ± 0.01[b]	5.21 ± 0.01[a]	4.64 ± 0.08[c]	5.16 ± 0.01[b]	4.17 ± 0.03[a]	5.33 ± 0.02[d]
Uric acid (µmol/L)	0.11 ± 0.02[a]	0.12 ± 0.01[b]	0.11 ± 0.01[a]	0.11 ± 0.01[a]	0.10 ± 0.02[a]	0.12 ± 0.01[a]	0.12 ± 0.01[a]	0.13 ± 0.01[a]
Na^+ (mmol/L)	101.20 ± 0.73[a]	122.60 ± 0.25[b]	132.40 ± 2.20[c]	125.80 ± 0.49[b]	114.00 ± 0.00[a]	123.00 ± 0.00[a]	144.80 ± 4.16[b]	181.00 ± 0.00[c]
K^+ (mmol/L)	4.12 ± 0.01[a]	4.59 ± 0.01[b]	5.39 ± 0.03[c]	5.38 ± 0.03[c]	4.19 ± 0.02[a]	4.20 ± 0.05[a]	6.37 ± 0.14[b]	8.30 ± 0.00[c]
Cl^- (mmol/L)	69.00 ± 0.00[a]	80.80 ± 0.49[c]	79.40 ± 0.24[b]	83.40 ± 0.24[d]	73.00 ± 0.00[a]	73.00 ± 0.00[a]	92.20 ± 0.73[c]	94.00 ± 0.00[d]

Values are means of 5 determinations ± SEM. Values with different superscripts from the control across the row for each day of intervention are significantly different ($p < 0.05$)

Discussion

Repeated dose oral toxicity studies are carried out to assess the adverse effects of a substance used for short and prolonged periods of time and to obtain information about the potential health hazards that may likely to occur from continuous exposure including information about target organ toxicity, possibilities of cumulative effects, and an estimate of the dose at which there is no observed adverse effect [17]. These toxicities are normally expressed as alterations in the levels of biochemical functional indices of the target organs and histoarchitectural changes leading to functional toxicity and structural toxicity. Furthermore, indiscriminate consumption of herbs/polyherbal formulations may manifest changes in physical performance and/or biochemical changes of various organs in the body.

Computed organ-body weight ratios are valuable tools in toxicity studies because changes in such parameter normally reflect atrophy and/or hypertrophy. Therefore, the reduction in both the computed liver- and kidney-body weight ratios after the administration of AI, AK and AJ suggests atrophic changes to the cells of the liver and kidney of the animals. These atrophic changes were evident from the loss of and/or reduction in metabolic constituents of the cells as evident in this study by the reduction in some liver enzymes, total protein and globulin for the liver and creatinine in the kidney.

The liver contains a myriad of enzymes such as alkaline phosphatase, aminotransferases and gamma glutamyl transferase which can be used to assess the cellular integrity of the organ, functional status of the organ and as indices of damage after the administration of chemical compounds including polyherbal formulations. Hepatotoxic compounds cause damage to the liver cell membrane and these enzymes are leaked out into serum and shows increased activities [18]. The reduction in the activity of the liver

alkaline phosphatase and the corresponding increase in the serum in the present study is an indication of permeability changes due damage to the cell membrane resulting in leakage through the altered cell membrane into the serum and consequently the increase in the serum enzyme. The reduction in the activity of aspartate aminotransferase from the liver is quite understandable since alteration in the composition of cell membrane as evident from the changes in alkaline phosphatase activity in both the liver and serum in this study will also lead to leakage of the cytosolic enzyme by the AI, AK and AJ. Furthermore, the increase in the activity of liver alanine aminotransferase by the AI, AK and AJ and the reduction in the serum enzyme by the AK and AJ is not immediately known but may not be unconnected with the selective cytosolic toxicity of the components of the polyherbal formulations on the enzyme leading to enhanced synthesis of the enzyme in the liver and inactivation of the enzyme molecule in the serum that may be attributed to the presence of cardiac glycosides in the AK and AJ formulations.

Serum total protein, albumin and globulin assay are also useful, reliable and sensitive indicators of the functional status of the liver since they are synthesized and metabolized in the liver and also secreted by the organ whilst bilirubin assay is a pointer to the excretory function of the liver. The reductions in the levels of serum total protein, albumin and globulin after the administration of AI, AK and AJ to the male rats suggest diminished synthetic function of the liver arising from liver disorder or hepatocellular damage. Such reduction in the levels of serum total protein, albumin and globulin may also be attributed to increased catabolism of the proteins, abnormal distribution and/or abnormal or excessive loss of the proteins. Bilirubin is an important catabolic product of blood with biological and diagnostic values. Hepatocytes convert bilirubin to its polar form via conjugation. Conjugation increases solubility in water and thus enhances the ease with which bilirubin becomes excreted in the bile. The hepatotoxicity of the AI, AK and AJ was further substantiated with the diminished hepatic excretory function as evidenced from the reduction in both the level of total and conjugated bilirubin content of the male rats. Overall, the AJ produced the most adverse effects on the synthetic, secretory and excretory function of the liver.

Determination of the levels of urea, uric acid, creatinine and electrolytes in the serum of animals after the administration of medicinal plants and natural products are useful indicators in assessing damage to the kidney at the glomerular and tubular levels. Creatinine and urea are the standard index of renal function and the most reliable clinical estimates of glomerular filtration rates [19]. Musso and Oreopoulos [20] have reported that changes in the serum concentrations of creatinine and urea are the consequences of alterations in the renal blood flow, renal function or urine outflow. Therefore, the increase in the concentrations of creatinine and urea after the administration of AI, AK and AJ for 4 days was an indication of kidney dysfunction due to impaired glomerular filtration leading to poor creatinine clearance by the kidney. Furthermore, since renal tubules play a vital role in the reabsorption of electrolytes by the kidney, the increase in the levels of serum Na^+, K^+ and Cl^- after the 4 days administration of AI, AK and AJ may be an indication of dysfunction at the levels of the tubules. However, the fact that the serum levels of creatinine, urea, uric acid, Na^+, K^+ and Cl^- returned to their respective control levels after 10 days of administration of AI suggest more of physiological adaptation rather than of being of toxicological relevance as is the case with AK and AJ in the present study. This cumulatively suggests that toxicological relevance of the polyherbal formulation is more with the AK and AJ, causing dysfunction at both the tubular and glomerular levels throughout the experimental period of the 10 days.

Another important consideration in toxicological studies is the effect of agents including plant extracts on the histoarchitecture of xenobiotic metabolising organs such as the liver and kidney. This is because histopathological investigation will corroborate or otherwise the biochemical findings and might also reveal the nature and pattern of structural toxicity if any, elicited by such agent. The imposed glomeruli and renal tubules as well as hyper-cellular glomueruli after the administration of AK for 10 days and the severe swollen cells with moderate distortion of the renal architecture and bloody glomeruli after the 10 days administration of AJ further corroborates the earlier position on the biochemical findings that AK and AJ caused tubular and glomerular dysfunction. This further indicated that consumption of AK and AJ for 10 days elicited structural toxicity which did not manifest in the animals exposed to AI. The liver and kidney histoarchitectural damages in the present study are similar to those reported by Saleem et al [21] after the oral administration of two anti-ashmatic polyherbal formulations to Wistar rats.

From the foregoing, the AK and AJ exhibited both functional and structural toxicities whilst the AI displayed only functional toxicity at 21 mg/kg body weight and during the 10 days of administration. The study therefore concluded that *Agbo jedi* exhibited the highest degree of toxicity whilst the *Agbo iba* was the least toxic with respect to the dose (21 mg/kg body weight) and duration (10 days of daily administration) of the study.

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