

EVALUATION OF TOXICOLOGICAL PROFILE OF KOJU[®] - A NIGERIAN POLYHERBAL FORMULATION, IN WISTAR RATS

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ABSTRACT

Increased morbidity and mortality associated with the use of herbs/herbal products has raised universal attention in the last few years, making the safety and toxicity evaluations of these preparations imperative. This study evaluated the toxicological profile of Koju[®] (KPF) - a common Nigerian polyherbal formulation developed from *Xylopiya aethiopica* (root bark), *Securidaca longepedunculata* (stem bark), *Magnifera indica* (stem bark), *Allium sativum* (seed), *Citrus aurantifolia* (root bark), *Morinda lucida* (seed) and *Saccharum officinarum*. Acute oral toxicity (LD₅₀) was carried out in albino rats according to Lorke's method while sub-chronic toxicity study was carried out with 24 adult albino rats which were divided into 4 groups of 6 animals each. Group one served as control and received normal

saline while Groups 2 to 4 received 500, 1000 and 2000 mg/kg KPF respectively for 30 days. Food/ water intake and body weight of the rats were monitored while on day 31, the rats were sacrificed and blood samples and organs were collected for biochemical /hematological analysis and histopathological examination respectively. Results showed that KPF is safe up to 5000 mg/kg following acute oral toxicity test. The sub-chronic toxicity test revealed that KPF had no significant effects on the biochemical, hematological and histopathological parameters. However, the body weight of the animals significantly increased. It was concluded that KPF is safe but despite its safety in few animals, clinical trials and more investigations on a large number of animals are essentially needed to establish safety and efficacy of the herbal formulation.

KEYWORDS: Toxicological, Koju[®], Polyherbal, Formulation, Sub-chronic.

INTRODUCTION

Herbal medicine or phytomedicine is acknowledged as the most common form of alternative medicine.^[1] Long in the creation of mankind, plants have been used medicinally.^[2] The World Health Organization (WHO) estimates that about 80% of the world's population relies on these unconventional plant-based medicines as their primary medical intervention especially in the developing as well as in the developed countries where modern medicines are largely used.^[3] This global upsurge in the use of herbs and herbal products is largely due to the wide acceptability; accessibility and affordability of these herbs/ herbal products.

Herbal medicines are also usually thought to be safe due to their 'natural origin'. However, this assumption is wrong as herbal preparations could be inherently toxic and could also be contaminated with microbial and foreign materials such as heavy metals, pesticide residues or even aflatoxins. The presence of any of the possible contaminants is a potential health risk to a vast population that depends on herbal medicine for their health care need. Increased morbidity and mortality associated with the use of herbs has raised universal attention in the last few years.^[4] Upon exposure, the clinical toxicity may vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations imperative.

Polyherbal formulations are combinations of many plant parts obtained from various plant species and families and may contain multiple bioactive constituents. They may have multiple physiological activities and could be used in the treatment of a variety of disease conditions.^[5] Many people believe that polyherbal formulations are just effective as the conventional drugs. Herbalists suggest that nature provide other ingredients that may act as buffers, synergists or counterbalances, working in harmony with the more powerful ingredients. Therefore, by using herbal combination in their complete form, the body's healing process utilizes a balance of ingredients provided by nature.^[6] Some polyherbal extracts have been scientifically proven for efficacy in the treatment of diseases while many others are yet to be investigated.^[7]

Since polyherbal formulations contain multiple bioactive constituents which are usually difficult to characterize, the chance of possible interactions with each other in the solution is usually high. The quality as well as the safety criteria for polyherbal formulations may be based, therefore, on a clear scientific definition of the raw materials used for such preparations. Also polyherbal formulations may be administered in most disease conditions over a long period of time without proper dosage monitoring and consideration of toxic

effects that might result from such prolonged usage. The danger associated with the potential toxicity of such therapy and other herbal therapies used over a long period of time could be averted if proper toxicological investigations are carried out on such preparations and reported accordingly.^[8]

Koju[®] is a polyherbal formulation specially prepared for pile, dysentery, menstrual, waist pain and as an aphrodisiac. It is prepared from the following plant materials- *Xylopi aethiopica* (root bark), *Securidaca longepedunculata* (stem bark), *Magnifera indica* (stem bark), *Allium sativum* (seed), *Citrus aurantifolia* (root bark), *Morinda lucida* (seed) and *Saccharum officinarum* (Bark). It is a popular and widely consumed polyherbal formulation. To the best of our knowledge, its toxicological profile has not been evaluated as no such information is included on the 'product information leaflet'. Thus, the aim of this study therefore was to evaluate the toxicological profile of this polyherbal preparation, by carrying out acute and sub-chronic toxicity studies using Albino Wistar rats.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Test Substance

Koju[®] is a polyherbal formulation developed by Kojumaribi Trado Medical Nig. Ltd. It contains *Xylopi aethiopica* (root bark) (3.5%), *Securidaca longepedunculata* (stem bark) (5.5%), *Magnifera indica* (stem bark) (7.5%), *Allium sativum* (seed) (8.5%), *Citrus aurantifolia* (root bark) (9.5%), *Morinda lucida* (seed) (10.5%), *Saccharum officinarum* (Bark) (25.0%) and water (q. s).

2.1.2 Drugs and Chemicals

All the chemicals used were of analytical grade and either Sigma or Merk chemicals.

2.1.3 Equipments

UV-visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom), animal cages.

2.1.4 Experimental animals

Wistar rats weighing 180–250g were procured from the animal house facility of the Department of Biochemistry, Salem University, Lokoja and handled according to the guide for the Care and Use of Laboratory Animals, published by the National Institute of Health

(NIH), USA. The rats were maintained at 25.0 ± 2 °C on a 12 h light/dark cycle with access to standard animal feed and water ad libitum for 7 d before the commencement of the experiment.

2.2 Methods

2.1.2 Preparation of Koju[®]

The plant materials- *Xylopi aethiopia* (root bark), *Securidaca longepedunculata* (stem bark), *Magnifera indica* (stem bark), *Allium sativum* (seed), *Citrus aurantifolia* (root bark), *Morinda lucida* (seed) and *Saccharum officinarum* (Bark) were bought from 'Old Market' in Lokoja, Kogi State, Nigeria and authenticated by an Ethno-botanist at the Herbarium unit of the Department of Biological Sciences, Federal University Lokoja. The samples were thoroughly washed, thereafter dried and pulverized, using electric blender. The crude powders obtained from the plants materials were mixed in appropriate proportions [(according to the product label), *Xylopi aethiopia* root bark (175g), *Securidaca longepedunculata* stem bark (275g), *Magnifera indica* stem bark (375g), *Allium sativum* seed (425g), *Citrus aurantifolia* root bark (475g), *Morinda lucida* seed (525g), and *Saccharum officinarum* Stem bark (1250g)] was weighed and extracted with 10000 ml (10L) of distilled water. After 48 hours, the mixture was filtered using muslin sieve followed by Whatmann filter paper (No 1). The filtrate was then dried and the extract was stored in the refrigerator for subsequent analysis. The extract will henceforth be referred to as KPF (Koju Polyherbal Formulation).

2.3 Acute Toxicity Study of KPF

The oral median lethal dose (LD₅₀) of KPF was determined in rats according to the method of Lorke^[9] with slight modifications. The study was carried out in two phases. In the first phase, 9 rats were divided into 3 groups of 3 rats each and were treated with KPF at doses of 10, 100 and 1000mg/kg body weight respectively after which they were observed for 24 hours for signs of toxicity and/ or mortality. Based on the results of the first phase, 9 rats were again divided into 3 groups of 3 rats each and were also treated with KPF at doses of 1600, 2900 and 5000 mg/kg body weight respectively in the second phase. The rats were then monitored 24 h after treatment and for signs of toxicity and/or mortality. The median lethal dose (LD₅₀) of KPF was estimated based on the observations in the second phase.

2.4 Sub- chronic Toxicity Study of KPF

This was performed according to the modified method of Maphosa^[10] and Organization of Economic Co- operation and Development (OECD) guidelines. Adult male albino rats were randomly divided into 4 groups of 6 rats each. Rats in Groups 1, 2 and 3 were administered 500, 1000 and 2000 mg/kg of KHF while Group 4 served as control and administered 1ml Normal saline for 30 days. The rats were maintained on diet and tap water ad libitum throughout the study. All groups were observed for signs of toxicity and mortality for the 1st, 2nd, 3rd and 4th hours and thereafter, daily for 30 days. On day 31, all rats were fasted overnight and then euthanized using chloroform, organs were harvested for histopathological examination and blood samples were collected via cardiac puncture into EDTA/ plain bottles for the following analyses.

2.4.1 Parameters Monitored/ Determined

2.4.1.1 Weekly Food and water intake

The quantity of food and water consumed by rats in each group was measured daily as the difference between the quantity of feed and water supplied and the quantity remaining after 24 hours respectively and a weekly average determined.

2.4.1.2 Weekly body weight changes

Rats in all groups were weighed weekly during the period of treatment and on the day of sacrifice for changes in body weight.

2.4.1.3 Liver function biomarkers

Aspartate aminotransferase (AST) was evaluated using the method of Reitman and Frankel^[11] as described by Randox laboratories, United Kingdom using Randox kits; Alanine aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine using the method of Reitman and Frankel^[11] as described in Randox kits; alkaline phosphatase (ALP) was assayed based on the methods of Kind and King^[12]; total protein in serum was assayed using direct Biuret method^[13] while total and conjugated bilirubin were determined according to the method of Jendrasik and Grof.^[14]

2.4.1.4 Kidney function biomarkers

Serum urea: This was done following the method of Bauer.^[15] Urea in serum was hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically by Berthelot's reaction.

Serum creatinine: The method of Cockcroft and Gault^[16] was employed for the estimation of serum creatinine in which at alkaline pH values; creatinine reacts with picric acid to produce a colored compound creatinine alkaline picrate which is photometrically read at 546nm.

2.4.1.5 Lipid profile assay

Total cholesterol was evaluated using enzymatic colorimetric CHOD- POD test method described by Allain^[17] with Quimica Applicada test kit; Triglycerides was also determined spectrophotometrically using the method of Tietz^[18]; High density lipoproteins (HDL) was evaluated by the method of Grove^[19] as described in Quimica Clinica Applicada test kit; low density lipoprotein (LDL) was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulfate (PVS) in the presence of polyethylene- glycol monomethyl ether.^[20]

2.4.1.6 Oxidative stress biomarkers

Malondialdehyde (MDA): Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, Malondialdehyde (MDA) as described by Draper and Hadley^[21] where MDA reacts with thiobarbituric acid (TBA) to form a red or pink colored complex which absorbs maximally in acid solution at 532nm.

Superoxide dismutase (SOD): Adrenaline (10mg) was dissolved in 17ml of distilled water to make adrenaline solution. Serum (0.1ml) was added to 0.9 ml of phosphate buffer (pH7.8). 0.2 ml of the extract was taken in triplicate and 2.5 ml of buffer added inside a cuvette and 0.3 ml of adrenaline solution added, mixed well and absorbance was read at 450 nm at 30 s interval for 5 times.^[22]

Catalase: This assay was done according to the method of Aebi.^[23] This was done using the principle that the ultra violet absorption of hydrogen peroxide can be easily measured at 240 nm and that as hydrogen peroxide decomposes with catalase, the absorption decreases with time, hence, catalase activity can be measured.

2.4.1.7 Hematological parameters

The haematological parameters was determines using hematological auto-analyzer (Mindray BC-2800 Auto Hematological Analyzer, England).The hematological parameters analyzed included: Packed cell volume (PCV), Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Hemoglobin (Hb) concentration. Others were; mean corpuscular volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

2.4.1.8 Histopathology

The organs (liver, kidney, heart, brain and lungs) were collected for histopathological studies. Tissue samples collected were fixed in 10% formal saline for 24h. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome and stained with haematoxylin and eosin (H and E) and mounted on Canada balsam.^[24] All the sections were examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

2.5 Data and statistical analysis

All data were expressed as Mean \pm SEM and statistical differences between means were determined by one- way ANOVA followed by Duncan's *post -hoc* test for multiple comparison tests using GraphPad version 5. 0. Values were considered significant at $P \leq 0.05$.

3.0 RESULTS

3.1 Acute toxicity test

In both phases of the acute toxicity study, no death or signs of toxicity was observed in the rats treated with different doses (10, 100, 1000, 1600, 2900 and 5000mg/ kg) of KPF. Thus, the oral LD₅₀ of KPF was taken to be > 5000mg/ kg.

3.2 Effect of Koju Polyherbal Formulation (KPF) on Food Intake of Wistar Rats

As shown in Table 1, KPF at all doses administered did not produce statistically significant changes in the food intake of rats compared to the control group.

Table 1: Food Intake (g) of Wistar Rats Administered Graded Doses of Koju Polyherbal Formulation (KPF).

Treatment group				
WEEK	Control	500mg /kg KPF	1000 mg/kg KPF	2000 mg/ kg KPF
1	132.0±2.68	125.2±4.34	132.1±4.11	139.9±2.10
2	124.1±3.71	118.1±2.93	128.7±4.15	142.1±4.25
3	128.3±3.23	120.3±4.65	131.2±5.61	137.2±4.21
4	136.6±5.21	122.4±4.19	135.1±2.81	141.3±1.20

Data represented as mean ± S.E.M (n=6)

3.3 Effect of Koju Polyherbal Formulation (KPF) on water Intake of Wistar Rats

KPF at all doses administered did not produce statistically significant changes in the water intake of rats compared to the control group (Table 2).

Table 2: Water Intake (ml) of Wistar Rats Administered Graded Doses of Koju Polyherbal Formulation (KPF).

Treatment group				
WEEK 1	Control	500 mg/ kg KPF	1000 mg/ kg KPF	500 mg/ kg KPF
1	162.1±2.51	165.4±3.14	168.1±4.31	161.2±1.10
2	159.3±3.28	154.1±4.36	165.6±3.15	163.4±3.21
3	148.5±4.11	164.4±3.62	161.3±5.16	166.6±4.21
4	168.2±4.13	163.6±4.19	162.5±3.17	164.7±2.58

Data represented as mean ± S.E.M (n=6)

3.4 Effect of Koju Polyherbal Formulation (KPF) on Body Weight of Wistar Rats

Table 3 shows the changes in the body weights of rats treated with different doses of KPF. KPF produced dose- and time- dependent increases in the body weights within the period of treatment. At 500 mg/ kg, KPF produced a significant ($p < 0.05$) increase in body weight on day 28 compared to the control group. KPF at 1000 mg/kg produced significant ($p < 0.05$), ($p < 0.01$) increase in the body weights of the rats on days 21 and 28 respectively while at 2000mg /kg, KPF produced highly significant ($p < 0.01$), ($p < 0.001$) increase in the body weights of the rats on days 21 and 28 respectively when compared to the control group.

Table 3: Body Weight Changes in Wistar Rats Administered Graded Doses of Koju Polyherbal Formulation (KPF).

Treatment group				
Day	Control	500 mg/ kg KPF	1000 mg/ kg KPF	2000 mg/ kg KPF
0	132.5±4.15	135.8±3.31	135.1±4.11	136.1±5.19
7	134.7±5.63	136.2±5.42	137.3±3.32	138.9±4.31
14	139.2±7.13	138.1±4.23	140.5±4.37	141.1±6.13
21	140.2±5.61	141.6±3.31	145.1±4.22*	148.2±4.12**
28	141.1±6.11	147.4±4.10*	149.7±5.13**	152.1±5.53***

Data represented as mean ± S.E.M (n=6), analysed by one- way ANOVA followed by Dunnett's *post – hoc* test for multiple comparisons. *p < 0.05, ** p < 0.01, ***p< 0.001, statistically significant increase in body weight of rats compared to the control

3.5 Effect of Koju Polyherbal Formulation (KPF) on Liver Function Biomarkers in Wistar Rats

Table 4 shows the values of the liver function biomarkers of rats treated with different doses (500, 1000 and 2000 mg/kg) of KPF. KPF showed no significant changes in most of the parameters (ALT, AST, ALP, GGT, T. Bil and D. Bil). However, KPF at 2000 mg/kg produced a significant (p< 0.05) increase in Total protein and Albumin compared to the control.

Table 4: Effect of Koju Polyherbal Formulation (KPF) on Liver Function Biomarkers in Wistar Rats.

Treatment group				
Parameter	Control	500 mg/ kg KPF	1000 mg/ kg KPF	2000 mg/ kg KPF
ALT (U/L)	32.5±2.35	31.8±1.31	32.10±1.13	31.91±1.09
AST (U/L)	119.2±3.72	120.9±3.82	120.5±2.25	118.3±6.23
ALP (U/L)	32.9±4.18	31.5±3.63	31.8±4.65	31.4±3.26
GGT (U/L)	3.1±0.31	3.2±0.59	2.8±0.87	2.9±0.50
TP (g/dl)	72.5±2.18	74.8±1.32	73.0±2.19	78.4±4.08*
ALB (g/dl)	42.63±3.16	43.38±4.53	46.08±3.14	49.6±5.30*
T. Bil (µmol/L)	1.57±0.03	1.94±0.29	1.73±0.55	1.61±0.95
D. Bil (µmol/L)	0.78±0.11	0.83±0.12	0.84±0.13	0.75±0.08

Values are Mean±SEM (n= 6) *p < 0.05 statistically significant increase compared to the control

3.6 Effect of Koju Polyherbal Formulation (KPF) on Kidney Function Biomarkers in Wistar Rats

KPF showed no significant changes in serum creatinine and urea levels of the treated rats compared to the control.

Table 5: Effect of Koju Polyherbal Formulation (KPF) on Kidney Function in Wistar Rats.

Parameter	Treatment group			
	Control	500 mg/ kg KPF	1000 mg/ kg KPF	2000mg/ kg KPF
Creat ($\mu\text{mol/L}$)	62.31 \pm 2.05	61.25 \pm 3.41	61.09 \pm 4.50	59.18 \pm 4.25
Urea (mmol/L)	7.58 \pm 1.01	6.99 \pm 0.98	7.87 \pm 1.23	7.51 \pm 1.91

Values are Mean \pm SEM (n= 6)

3.7 Effect of Koju Polyherbal Formulation (KPF) on Serum Lipid Profile of Wistar Rats

The result of the effect of sub- chronic exposure of rats to KPF on the Lipid profile of rats is presented in Table 6. KPF did not cause significant changes in the levels of TAG, T. cholesterol and LDL compared to the control. However, at 2000 mg/ kg, KPF produced a significant ($p < 0.05$) increase and decrease in the levels of HDL and LDL respectively when compared to the control.

Table 6: Effect of Koju Polyherbal Formulation (KPF) on serum Lipid Profile of Wistar Rats.

Parameter	Treatment group			
	Control	500 mg/ kg KPF	1000 mg/ kg KPF	2000 mg/ kg KPF
TAG (mg/dl)	35.4 \pm 2.93	33.8 \pm 2.11	36.5 \pm 1.97	38.11 \pm 1.45
T. Chol (mg/dl)	84.6 \pm 1.73	86.9 \pm 1.89	85.7 \pm 1.55	84.3 \pm 1.55
HDL (mg/dl)	43.2 \pm 1.12	45.3 \pm 1.63	44.1 \pm 1.23	49.1 \pm 2.13*
LDL(mg/dl)	14.0 \pm 0.51	13.1 \pm 0.39	12.2 \pm 0.02	9.9 \pm 0.31*

Values are Mean \pm SEM (n= 6) * $p < 0.05$ statistically significant increase compared to the control

3.8 Effect of Koju Polyherbal Formulation (KPF) on Oxidative Stress Biomarkers in Wistar Rats

The result of superoxide dismutase activities of both KPF treated and control rats are presented in Fig. 1. The results showed that there was no significant change in the SOD activities of the 500, 1000 and 2000 mg/kg KPF- treated rats compared to the control. Fig. 2 shows the mean level of MDA activities in KPF- treated rats. The result showed that there

was no significant changes in the MDA levels of KPF- treated rats compared to the control. The catalase activity of KPF- treated rats is shown in Fig. 3. There was no significant difference between the catalase levels of the treated and control rats.

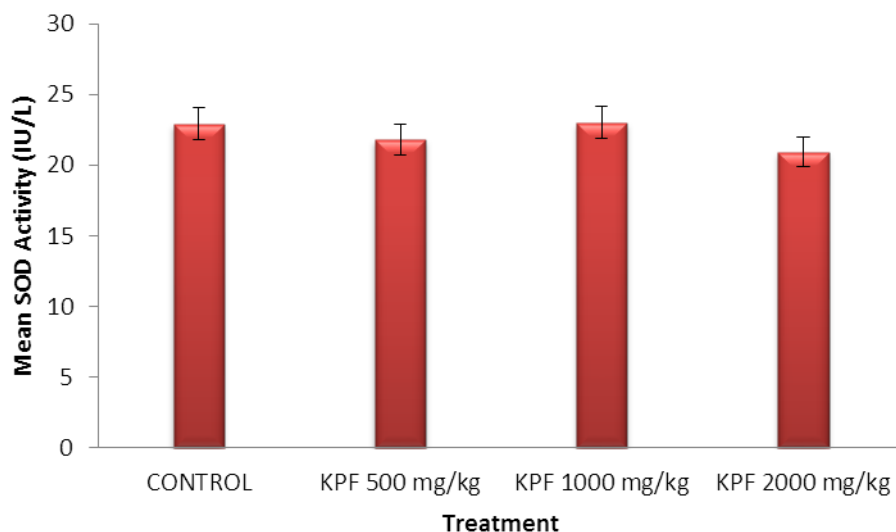


Fig. 1: Effect of Koju Polyherbal Formulation (KPF) on superoxide dismutase activities in wistar rats.

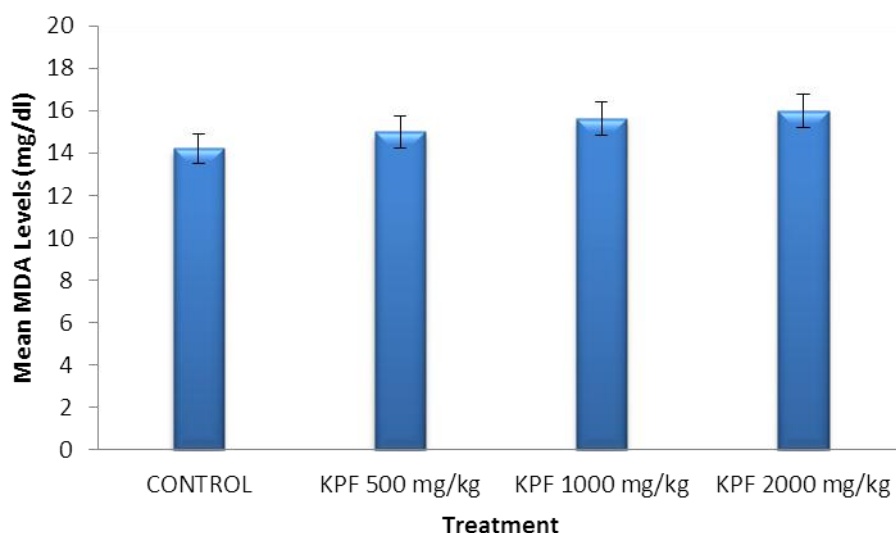


Fig. 2: Effect of Koju Polyherbal Formulation (KPF) on MDA levels in wistar rats.

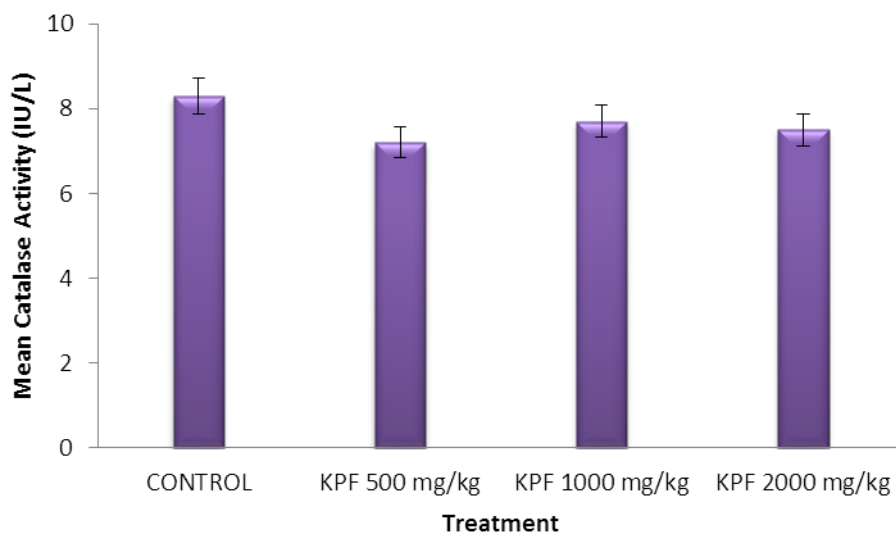


Fig. 3: Effect of Koju Polyherbal Formulation (KPF) on Catalase activities of wistar rats.

3.9 Effect of Koju Polyherbal Formulation (KPF) on Serum Haematological Parameters of Rats

Table 7 shows the result of the hematology of rats exposed to sub-chronic treatment with different doses (500, 1000 and 2000 mg/kg) of KPF. KPF at all the dose levels produced no significant changes in the hematological parameters (RBC, MCV, MCH, MCHC, RDE, WBC, hemoglobin concentration and MPV) when compared to control.

Table 7: Effect of Koju Polyherbal Formulation (KPF) on serum Haematological Parameters.

Parameter	Treatment group			
	Control	500 mg/ kg KPF	1000 mg/ kg KPF	2000 mg/ kg KPF
RBC (x 10 ⁶ /μL)	8.52±0.38	9.2±0.45	8.35±0.28	8.93±0.15
MCV (μ ³)	61.3±3.13	65.1±4.73	63.7±3.15	60.4±3.29
HCT (%)	52.9±1.02	51.5±0.99	531.4±1.15	54.3±1.02
MCH (pg)	18.2±0.11	18.2±0.53	18.4±0.84	17.3±0.38
MCHC (g/L)	32.3±1.12	33.7±1.30	29.9±1.14	33.6±1.02
RDW (%)	35.5±1.02	37.4±1.21	38.5±1.53	37.1±1.18
WBC (x 10 ³ /μL)	8.7±0.19	8.9±0.77	9.8±0.91	10.7±0.76
HGB (g/L)	14.9±0.95	15.3±0.91	15.3±0.59	16.9±0.88
PLT (x 10 ³ /μL)	519.3±10.12	523.5±11.13	530.8±12.63	518.6±10.89
MPV (μ ³)	6.3±0.15	6.9±0.21	7.3±0.61	7.9±0.95
LYMPH (#)	6.98±0.09	7.21±0.26	7.57±0.29	7.21±0.16

Values are Mean±SEM (n= 6)

3.8 Histopathology of Organs of Wistar Rats after 28 days of KPF oral Administration

Following 28 days of administration, the effect of KPF at 500, 1000 and 2000 mg/kg on the histological appearance of the selected organs- liver, kidney, heart, brain and lungs was microscopically evaluated after Haematoxylin and Eosin stain. Plates I- V show the photomicrographs of the liver, kidney, heart, brain and lung sections of the control and treated rats respectively. No remarkable changes occurred in the structural architecture of all the organs.

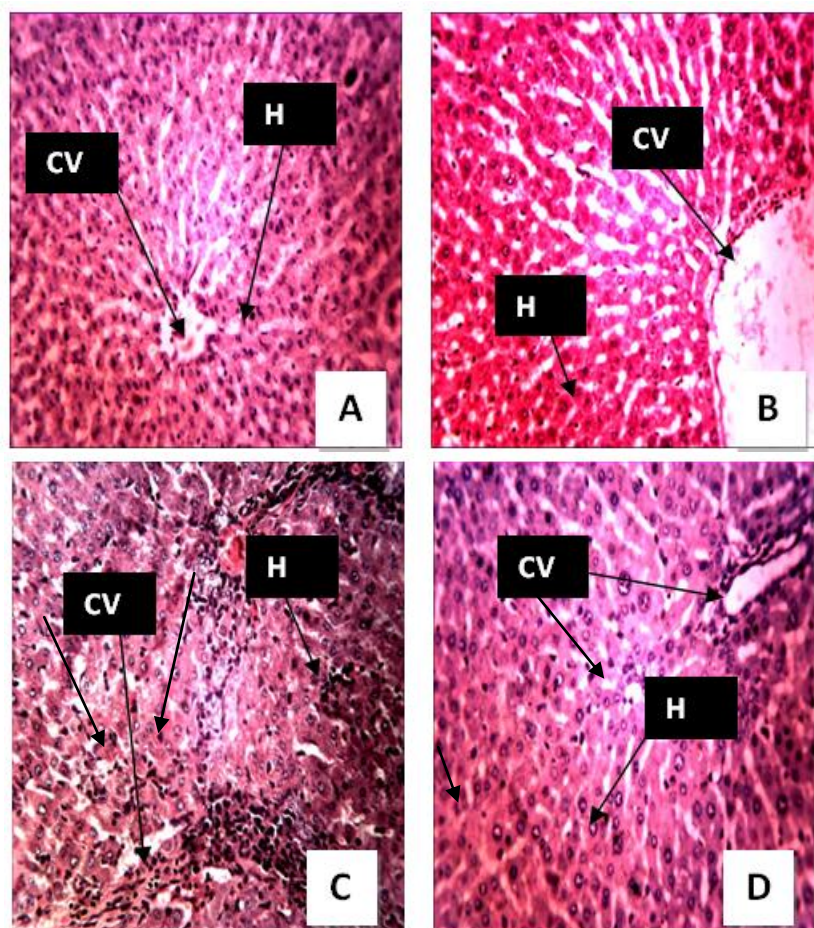


Plate 1: Photomicrograph of a section of the liver of a (A) control rat (B), (C) and (D) 500, 1000 and 2000 mg/kg KPF- treated rat respectively. All showing normal lobular architecture at 30 day post treatment (HE x250), CV= Central vein, H= Hepatocytes.

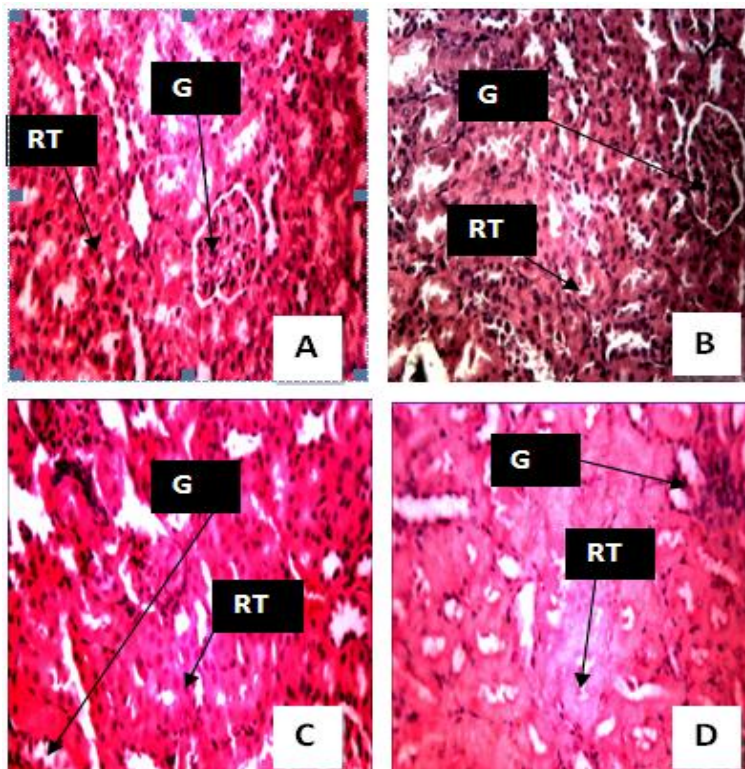


Plate II: Photomicrograph of a section of the kidney of a (A) control rat (B), (C) and (D) 500, 1000 and 2000 mg/kg KPF- treated rat. All showing normal architecture at 30 day post treatment (HE x250), RT= Renal tubule, G= Glomerulus.

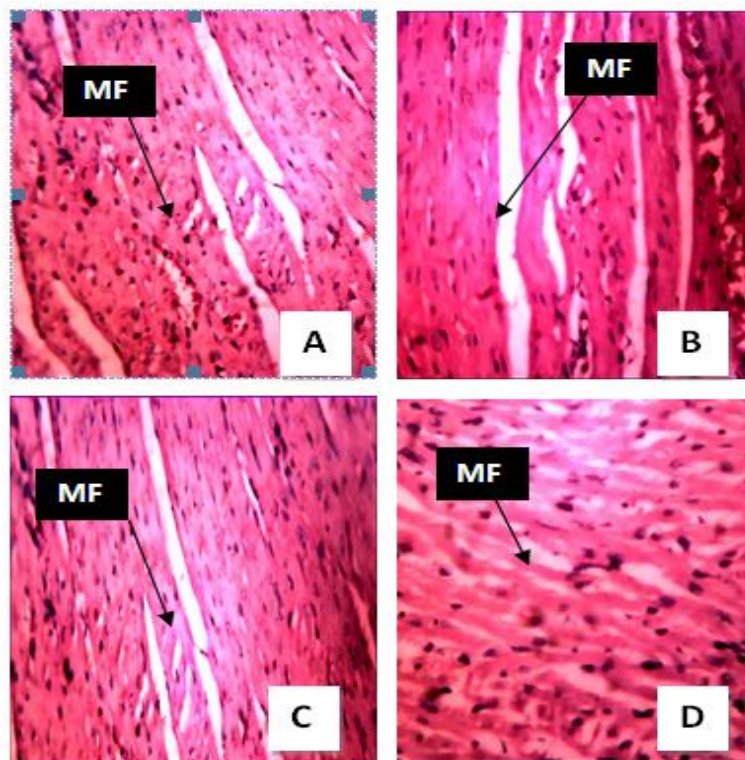


Plate III: Photomicrograph of a section of the heart of a (A) control rat (B), (C) and (D) 500, 1000 and 2000 mg/kg KPF- treated rat respectively. All showing normal architecture at 30 day post treatment (HE x250), MF= Muscle fibres.

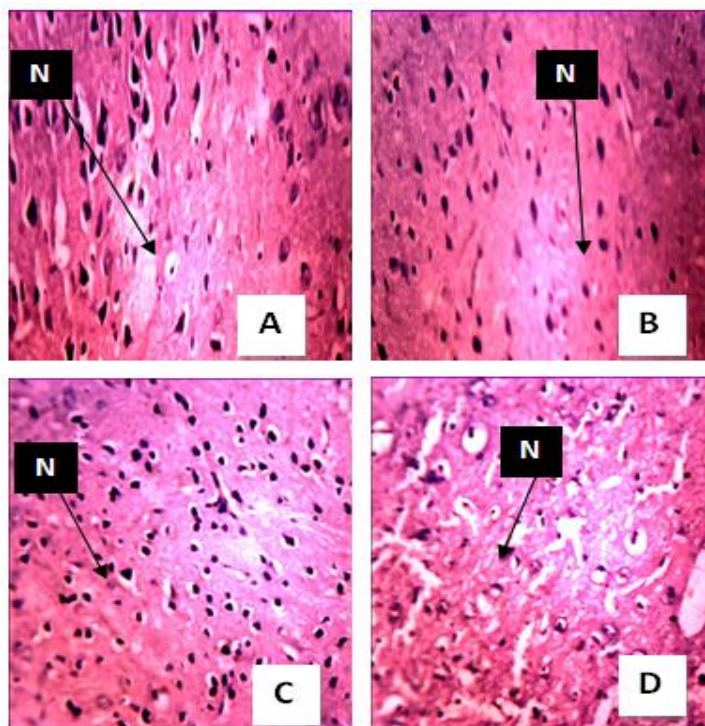


Plate IV: Photomicrograph of a section of the brain of a (A) control rat (B), (C) and (D) 500, 1000 and 2000 mg/kg KPF- treated rat respectively. All showing normal architecture at 28 day post treatment (HE x250), N= Neurones.

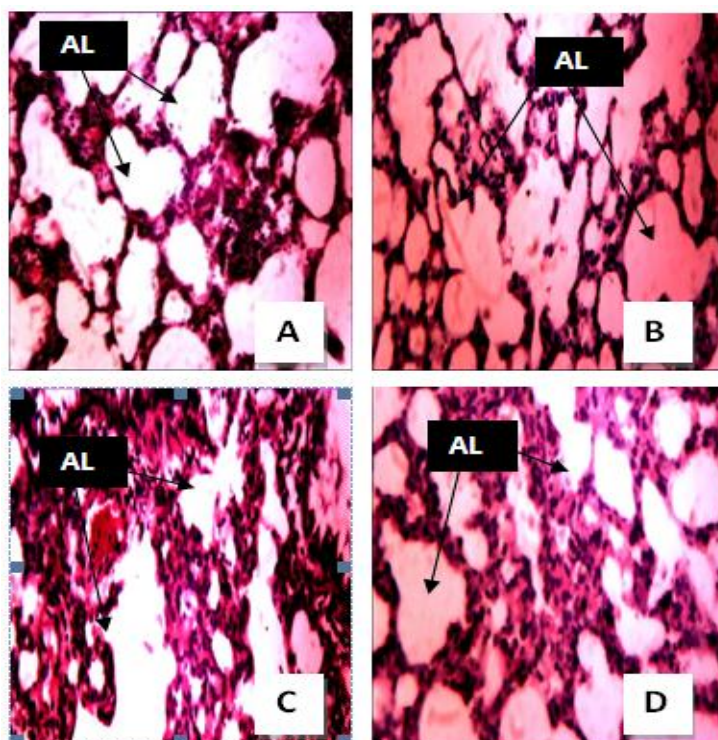


Plate V: Photomicrograph of a section of the lung of a (A) control rat (B), (C) and (D) 500, 1000 and 2000 mg/kg KPF- treated rat respectively. All showing normal architecture at 28 day post treatment (HE x250), AL= Alveoli.

DISCUSSION

The chronic nature of some diseases warrants life- long treatment. Some patients consume herbs and herbal products to treat such disease conditions paying little or no attention to the possible deleterious effects it might have on them in the long run. Safety profile on medicinal plants is important as safety of herbal medicine use has recently been questioned due to reports of illnesses and fatalities.^[25] Consequently, it has become pertinent to scientifically evaluate the toxicological profiles of herbs and herbal products and this is usually done via acute toxicity test, sub-chronic and chronic toxicity studies. This study evaluated the acute and sub- chronic toxicity of Koju- a polyherbal formulation (KPF) that is common and widely consumed in Nigeria, using Wistar rats.

In acute study, there were no observable signs of morbidity or mortality throughout the duration of the experiment at all the doses used, which is an indication that KPF was well tolerated by the rats. In the sub- chronic toxicity evaluation, administration of KPF at graded doses of 500, 1000 and 2000 mg/kg did not affect the eating and drinking patterns of the rats significantly. However, body weight which is one of the critical parameters for the evaluation of first signs of toxicity^[26] increased in all the groups but was more prominent in the 2000 mg/kg extract treated group of rats. This may be attributed to normal growth of the rats with age and also may be due to improved feed consumption by the rats.

The liver and kidneys have demonstrated to play crucial roles in various metabolic processes and are, therefore, particularly exposed to the toxic effects of exogenous compounds.^[27] Levels of serum liver biomarker enzymes are biochemical parameters usually performed in order to evaluate any toxic effects on the liver.^[28] AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function.^[29] Therefore an elevation in plasma concentration of ALT is an indication of liver damage.^[30] AST is mostly present in the myocardium, skeletal muscle, brain and kidneys.^{[31], [32]} Thus, the liver and heart release AST and ALT and an elevation in plasma concentration are an indicator of liver and heart damage.^{[33], [29]} ALP is present mostly in cells lining the biliary duct of the liver and is used to diagnose obstruction to the biliary system. Therefore, its elevation in the blood indicates cholestatic diseases such as gall stone or tumor blocking the bile duct.^[34] In this study, chronic exposure of rats to KPF at different doses (500, 1000 and 2000mg/kg) did not cause significant increase in AST, ALT and ALP values when compared to the control group. This might be an indication that KPF is not toxic to the

liver when consumed sub-chronically. Aside the liver, this demonstrates that KPF also had neither nephrotoxic nor deleterious effects on the heart when consumed sub-chronically. This is further confirmed in the histopathological observations.

Bilirubin is a break down product of hemoglobin. Serum bilirubin levels could be expressed as total bilirubin comprising of conjugated and non conjugated or as direct bilirubin comprising only of the conjugated bilirubin. An elevation in serum bilirubin level could be attributed to three major causes such as hemolysis, biliary obstruction and liver cell necrosis.^[35] Increased bilirubin levels also reflect the depth of jaundice.^[36] In this study there was no significant change in the levels of serum bilirubin and albumin of both the treated and control rats. This suggests that KPF may have no toxic effect on the erythropoietic system as was confirmed by the hematological parameters.

There was a significant increase in the mean total protein level of the treated rats at the dose of 2000 mg/kg (Table 4). It can be suggested that the observed increases in total protein at that dose level may be due to increased synthesis of globulin in the lymphoid organ and albumin with possible involvement of the liver.^[37] The increase in total protein level could also be linked to the protective effect of KPF against oxidative damage to the liver as Oyagbemi^[38] linked an increase in total protein level to the hepato-protective ability of medicinal plants.

Creatinine is excreted by glomerular filtration and the clearance is dependent on the rate at which it is removed from the blood by the kidneys, therefore, an increase in the plasma creatinine level suggests kidney damage specifically renal filtration mechanism.^[33] In this study, KPF at all the doses had no significant effect on the serum and urea levels. This observation is a further confirmation of the safety of the polyherbal formula at least when consumed sub-chronically.

After 30 days of HPF administration, all the doses used did not produce significant changes in the serum total cholesterol and triglyceride. KPF at 2000 mg/kg however produced a significant increase in serum HDL and a corresponding decrease in LDL. An elevation of serum LDL levels is usually an indication of coronary events such as atherosclerosis and coronary heart disease.^[39] In this case, however, where the LDL level decreased and HDL increased considerably, HPF might be said not to be harmful to the cardiovascular system.

More so, the photomicrograph of cardiac tissue of the treated animals showed normal appearance.

The effect of HPF on the oxidant- antioxidant balance of the rats was evaluated via estimation of the level of activity of catalase and superoxide dismutase (SOD) enzymes and malondialdehyde (MDA) level. Decreases in the levels of catalase and SOD activities and increases in the level of MDA signifies increased oxidative stress and reduced antioxidant activities in the system which reduces the capability of the body to get rid of free radicals.^[40] In this study, HPF at all the doses used did not cause significant changes in the activities of catalase and SOD, also MDA Levels when compared to the control. This suggests that HPF when consumed sub- chronically might be free of inducing oxidative stress.

Study of the hematological parameters like hemoglobin concentration, haematocrit, RBC, WBC, and platelet count gives valuable information about the drug-induced hematological toxicities. KPF at all the dose levels produced no significant changes in the hematological parameters. KPF at all the doses administered also did not reveal any macroscopic and microscopic changes in all the organs examined. This observation from the histopathological study further validates the observations made in the serum biochemistry.

5.0 CONCLUSION

Acute and sub-chronic investigation of the biochemical, hematological and histopathological parameters of rats administered KPF did not reveal any significant toxicity; thus it may be concluded that Koju[®] is a safe polyherbal formulation. However despite the safety of the drug in few animals, clinical trials and more investigations on a large number of animals are essentially needed to establish safety and efficacy of the herbal formulation.

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