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Toxicity Evaluation of a Commercial Herbal Preparation Commonly Used in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EEO, IEE and ADL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EEO, IEE, MIS and MEG managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the safety of a popular polyherbal decoction, consisting of *Alstonia boonei* stem bark and *Fagara zanthoxyloids* stem bark, used in the management of arthritis and rheumatism in Nigeria.
Place and Duration of Study: Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu, Anambra State, Nigeria between the months of June and November, 2013.
Study Design: In acute toxicity study, 2 -10 ml/kg were administered to rats and mice and obvious toxic signs and mortality were observed for 24 hours post-administration. In sub-chronic toxicity study, 1.29, 2.97 and 5.14 ml/kg, of the herbal preparation were orally administered to three groups (five animals per group) of Wistar rats daily for 90 days. The control group of animals received 10 ml/kg of normal saline. Body weight changes were determined weekly, while hematological

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parameters, biochemical parameters, ulcer index and liver and kidney histologies were evaluated on 31, 61 and 91st days. Post-treatment studies were also carried out.
Results: All the animals survived after twenty four (24) hours of observation in acute toxicity study and the LD_{50} was estimated to be above 10 ml/kg. In sub-chronic toxicity studies, there were insignificant ($P>.05$) changes in body weight and hematological parameters. However, some blood chemistry indices were significant ($P<.05$). The histologies of the liver and kidney showed irreversible toxicities especially at high doses and duration of administration.
Conclusion: These findings indicate that the herbal preparation may be nephrotoxic and hepatotoxic especially at high doses on long term use, suggesting that it may not be safe in the treatment of arthritis and rheumatism and other chronic diseases.

Keywords: Toxicity; anti-arthritic; anti-rheumatic; herbal decoction.

1. INTRODUCTION

Herbal medicines are relied on for the treatment of diseases because they are relatively affordable, available and accessible. In most cases, polyherbal preparations are preferred because of their assumed additive or synergistic effects [1].

Potential adverse effects are associated with herbal decoctions especially in chronic conditions, due to bioaccumulation and altered detoxification processes [2].

Alstonia boonei is a native to tropical and subtropical Africa, Central America, Southeast Asia and Australia. Its stem bark is used in the treatment of rheumatism, muscular pain, insomnia, and hypertension [3-4]. Cold infusion made from the fresh or dried bark of *Alstonia* taken orally two to three times daily is used in the treatment of diabetes [4]. Studies carried out on *Alstonia boonei* had revealed properties which include; antipyretic, analgesic and anti-inflammatory properties of the stem bark [5], reversible anti-infertility effect of the stem bark [6]. The stem bark of the plant has been reported to possess potent neuroleptic and anxiolytic properties in mice [7]. *A. boonei* stem bark extract had been reported to possess hypoglycemic properties via decreased production of regulatory hepatic glucogenic enzymes [8]. The aqueous ethanol stem bark extract of *A. boonei* had been reported to be nephrotoxic especially at higher doses in Guinea pigs [9]. Acute toxicity, lipid peroxidation and ameliorative properties of its ethanol leaf extract on the kidney markers of alloxan induced diabetic rats had also been reported [10].

Fagara zanthoxyloides are distributed in Africa. Throughout West Africa, its aromatic roots, stem, bark and leaves are commonly used in treating elephantiasis, toothache, sexual impotence, gonorrhoea, dysmenorrhoea, abdominal pain, rheumatic pain, arthritic pain and hernia [11]. Root and stem bark decoctions or infusions are widely taken to treat malaria, fever, sickle cell anaemia, tuberculosis, paralysis, oedema and general body weakness, stimulant and to treat pain during childbirth, migraine and neuralgia [12]. Several studies on the various effects of *F. zanthoxyloides* extracts have been carried out. Aqueous extracts from the plant had been reported to have shown activities against bacteria significant for periodontal disease [13]. The anthelmintic activity of the methanol extract of the root-bark of *F. zanthoxyloides* had also been reported [14]. An anti-sickling activity of the root [15], anti-malarial activity of the root extract and anti-inflammatory [16] and amide isolation from *F. zanthoxyloides* [17] had been reported. The LD_{50} of its methanol root bark extract had been shown to be 5.0 g/kg body weight in mice, however, the mice showed signs of cerebral irritation before dying. Histopathological examinations of the viscera showed congestion and focal necrosis of the liver and renal tubules [18]. Phytochemical screening done on *Z. zanthoxyloides* had revealed the presence of cardiac glycosides, alkaloids, saponins, tannins and flavonids [19]. Administration of the *Z. zanthoxyloides* ethanol stem bark extract daily for three weeks to Wistar rats revealed weight gain, significant decrease in serum concentrations of AST, ALT, urea, creatinine levels and hepatic malondialdehyde concentration with high dose (1.5–3 mg/kg.bw). There was no significant effect on serum triglyceride levels in all the treated groups. The administration of 1.5 mg/kg b.w for 21-days resulted in very mild diffuse hydropic degeneration of the hepatocytes, while no visible lesion was found on the kidney [20].

The commercial herbal decoction made from *Alstonia boonei* stem bark and *Fagara zanthoxyloids* stem bark is commonly used in Nigeria for the treatment of arthritis and rheumatism. Despite the popular use of this decoction, no information exists about its safety. Since arthritis and rheumatism are chronic disorders, this study evaluated the acute and sub-chronic toxicities of this herbal decoction in Wistar rats.

2. MATERIALS AND METHODS

2.1 The Herbal Decoction

Freshly prepared products made from *Alstonia boonei* stem bark*,* family Apocynaceae and *Fagara zanthoxyloids* stem bark*,* family Rutaceae were bought from the manufacturer. The dosage specification for adult humans was 60 ml, three times (ie 180 ml) daily.

2.2 Chemicals and Reagents

Sodium chloride (Analar: Product no: 10241) BDH Chemicals Ltd England. Sodium hydroxide pellets (Avondale Lab Ltd, Benbury, Oxon. England UN: 1823) Ethanol, Haematoxylin and Eosin stains, Chloroform, Paraffin wax, Formaldehyde 40%w/v (May and Baker Ltd Dagenham, England: F/65/450-4). Reagent kits include alkaline phosphatase (ALP), aspertate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL-c), all are products of Randox Laboratories Limited, Country Atrium, United kingdom. Other kits include; Hemoglobin, bicarbonate, sodium, chloride and potassium, all are products of Teco diagnostics, California U.S.A.

2.3 Animals

Adult Wistar rats (127.35 ± 5.71 g) and albino mice (20.49 ± 0.64 g) of either sex were employed in the study. All the animals were procured from the animal house of the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu campus. The animals were housed under standard laboratory conditions and fed with rodent feed (Pelletized vital grower feed: Batch no: 4228051). They were acclimatized for two weeks with free access to food and water *ad libitum*. Animals were conducted in compliance with NIH Guide for care and use of Laboratory animals (pub. No. 85-23 Revised 1985), and approved by the Nnamdi Azikiwe University's ethical committee for the use of Laboratory animals.

2.4 Acute Toxicity Study

The acute toxicity (LD_{50}) study was carried out following modified Lorke's method [21]. Animals (rats and mice) of either sex were fasted overnight prior to the study. Dosage selection of the polyherbal remedy was based on the organization of economic corporation and development's (OECD's) guidelines, where 10 ml/kg body weight of aqueous solution of test substance could be considered for acute toxicity testing [22].

2.4.1 Phase 1

The rats and mice were placed in four groups (n = 3). Group 1 served as control and received 10 ml/kg of normal saline while groups 2, 3 and 4 received 2, 4 and 5 ml/kg of polyherbal remedy respectively. The animals were observed for signs of toxicity and death for 24-hours post-administration.

2.4.2 Phase 2

In this phase, four groups (n=3) of mice and rats were used. Group 1 served as control and received 10 ml/kg of normal saline, while groups 2, 3 and 4 received 6, 8 and 10 ml/kg of polyherbal remedy respectively. The animals in each group were also observed for obvious toxic signs and mortality 24 hours post-administration.

2.5 Sub-chronic Toxicity Study

Eighty (80) rats were randomized into four groups of twenty (20) rats each. After pretreatment studies, animals were carefully restrained and dosed orally with the aid of improvised medicut oral canula [23] based on adults human daily dose (60 mls/70kg adult three times daily = 180 ml/70kg adult) of the polyherbal remedy as follows;

$\frac{180\ ml}{70\ kg}$ = 2.57 ml/kg/day. On the basis of this dosage selection, twice of adult dose (i.e. 2 x 2.57 ml/kg = 5.14 ml/kg) and half of adult dose (i.e. $\frac{1}{2}$ × 2.57 ml/kg = 1.29 ml/kg) were selected as high dose (**Group D**) and low dose (**Group B**) respectively. While the actual adult dose

(2.57 ml/kg) was selected as medium dose (Group C) for the sub-chronic toxicity studies. Group A served as control and received normal saline, 10 ml/kg. Animals were observed daily for toxicity manifestation and weekly body weight changes were recorded.

Five animals were randomly selected from each group and sacrificed on 31, 61 and 91st days respectively and blood samples collected by capillary puncture from animals' retro-orbital plexuses into EDTA tubes were used to determine hematological parameters, PCV, RBC, WBC and hemoglobin [24-25]. Blood samples collected into plain tubes were allowed to coagulate for 30 minutes and clear serum obtained after centrifuging at 3000 rpm for 10 minutes were used for the analysis of biochemical parameters, AST and ALT [26], ALP [27], Bicarbonate [28], Chloride [29], Sodium [30], Potassium [31], Creatinine [32], Urea [33], Triglyceride [34], Total cholesterol [35], HDL-cholesterol [36] and LDL-cholesterol [37].

Liver and kidney isolates were fixed in 10% formal saline for histopathological studies [38]. The stomach of each animal was isolated, cut along the greater curvature, washed with tap water and presence or absence of ulcer was scored [39]. At the end of administration period (90-days), the remaining five rats in each group were used for post-treatment recovery studies by placing them only on feed and water *ad libitum*. On the 29th day, they were sacrificed and samples were collected for hematological, biochemical and histopathological studies.

2.6 Method of Data Analyses

Results were presented as mean ± Standard error of mean (SEM) of sample replicates (n=5). Raw data were subjected to statistical analyses by one way analyses of variance (ANOVA), followed by post hoc Turkey's test using statistical package for social Science (SPSS, verson-16). *P* values >.05 were considered statistically non-significant while *P* values <.05 were be considered statistically significant.

3. RESULTS AND DISCUSSION

Acute toxicity results revealed no obvious signs of toxicity in all treatment groups in both specie (rat and mice) and phases (1 and 2) after the administration of the polyherbal remedy to the experimental animals. All the animals survived after twenty four (24) hours post-administration. The LD_{50} of the polyherbal remedy was above 10 ml/kg, suggesting that it has no short term toxicity.

No death was recorded and there was no injury to the gastric mucosa of experimental animals after 90 days of exposure of animals to the polyherbal remedy. Absence of gastric lesion indicates that the polyherbal remedy does not have ulcerogenic potential on long term consumption [39].

There was no significant difference (*P*>.05) in percentage weight gain (Table 1) in the polyherbal remedy treated groups on 31, 61 and 91st days when compared to their respective control groups. This suggests that the polyherbal remedy may not cause weight reduction due to nutrient malabsorption [40]. Also, the withdrawal of the polyherbal remedy in the post-treatment periods for 28-days did not reduce the body weights of the animals. This suggests that the polyherbal remedy did not affect appetite of the animals.

Administration of polyherbal remedy revealed non-significant reduction in total cholesterol (Table 2), triglyceride (Table 2), high density lipoprotein-cholesterol (Table 2) and low density lipoprotein cholesterol (Table 2) when compared to control groups on 31, 61 and 91st days. This suggests the unlikelihood of the polyherbal remedy to cause atherosclerosis and its associated coronary heart diseases [41-42].

Table 1. Effects of polyherbal remedy on percentage body weight gain in Wistar rats

Treatment	Percentage body weight gain (%)			
	31st day	61st day	91st day	Post-treatment
Group A: Control	26.91 ± 2.62	47.20 ± 4.98	51.25 ± 4.49	58.17 ± 6.95
Group B: Low dose	32.69 ± 5.74	33.37 ± 7.39	46.71 ± 8.95	62.88 ± 1.93
Group C: Medium dose	35.59 ± 1.85	45.23 ± 7.73	50.52 ± 7.99	44.26 ± 6.90
Group D: High dose	27.51 ± 7.98	36.64 ± 1.42	55.43 ± 5.13	47.51 ± 2.41

n= 5 for each condition, values represent mean ± S.E.M

Table 2. Effects of polyherbal remedy on serum lipid profile level in Wistar rats

	Pretreatment	31st day	61st day	91st day	Post-treatment
Serum total cholesterol (mg/dl)					
Group A:	145.57 ± 7.08	135.91 ± 20.78	149.21 ± 4.90	159.56 ± 25.68	186.44 ± 30.63
Group B:	146.21 ± 0.15	123.59 ± 5.25	124.21 ± 11.38	133.79 ± 7.74	172.10 ± 24.48
Group C:	138.35 ± 0.64	126.81 ± 7.92	127.84 ± 16.61	137.56 ± 6.99	145.28 ± 31.76
Group D:	141.96 ± 1.27	121.92 ± 10.90	133.08 ± 12.97	126.26 ± 6.89	186.44 ± 24.99
Serum triglyceride (mg/dl)					
Group A:	137.23 ± 2.89	135.58 ± 29.79	184.05 ± 19.16	151.30 ± 7.75	164.49 ± 5.14
Group B:	135.11 ± 1.67	161.68 ± 17.42	168.68 ± 21.08	157.95 ± 18.39	169.81 ± 14.06
Group C:	138.70 ± 3.89	103.26 ± 11.52	151.39 ± 14.02	137.54 ± 9.81	163.03 ± 14.99
Group D:	143.31 ± 3.45	106.56 ± 13.57	144.67 ± 26.43	156.57 ± 20.14	150.57 ± 16.97
Serum HDL-cholesterol (mg/dl)					
Group A:	75.01 ± 1.62	69.13 ± 5.75	55.73 ± 3.52	59.71 ± 10.19	68.51 ± 5.54
Group B:	81.72 ± 2.59	78.62 ± 9.95	55.35 ± 2.01	64.24 ± 5.88	76.71 ± 5.02
Group C:	80.47 ± 1.10	67.75 ± 5.30	50.22 ± 4.12	64.15 ± 6.45	60.22 ± 4.54
Group D:	75.63 ± 2.43	66.37 ± 2.83	53.17 ± 4.07	59.01 ± 5.67	65.14 ± 3.75
Serum LDL-Cholesterol (mg/dl)					
Group A:	43.11 ± 8.89	39.67 ± 22.35	56.67 ± 6.06	69.59 ± 31.83	86.02 ± 31.16
Group B:	37.47 ± 2.10	12.86 ± 4.01	35.12 ± 13.53	37.97 ± 6.61	61.43 ± 24.08
Group C:	30.14 ± 2.51	40.42 ± 8.60	43.34 ± 17.42	45.91 ± 11.56	67.39 ± 26.54
Group D:	37.67 ± 4.39	34.24 ± 10.39	50.98 ± 13.56	36.74 ± 9.31	91.18 ± 27.58

n= 5 for each condition. Values represent mean ± S.E.M. Group A: Control, Group B: Low dose, Group C: Medium dose, Group C: High dose

Table 3. Effects of polyherbal remedy on serum electrolyte levels in Wistar rats

	Pretreatment	31st day	61st day	91st day	Post-treatment
Serum potassium (mEq/L)					
Group A:	5.13 ± 0.27	3.85 ± 0.14	3.57 ± 0.60	5.49 ± 0.80	5.60 ± 0.77
Group B:	4.79 ± 0.34	5.50 ± 0.37*	3.24 ± 0.75	5.97 ± 0.56	5.32 ± 0.61
Group C:	4.91 ± 0.15	6.82 ± 0.39*	3.66 ± 0.71	5.31 ± 0.80	5.80 ± 0.41
Group D:	5.56 ± 0.37	6.52 ± 0.27*	4.88 ± 0.34	6.66 ± 0.45	7.12 ± 0.18
Serum chloride (mEq/L)					
Group A:	88.30 ± 1.42	80.52 ± 2.69	96.77 ± 1.63	119.71 ± 2.36	86.42 ± 10.82
Group B:	88.26 ± 1.29	88.61 ± 2.66	95.19 ± 3.23	123.85 ± 3.47	102.07 ± 4.84
Group C:	92.02 ± 0.71	92.43 ± 6.31	89.77 ± 5.50	127.11 ± 2.36	87.97 ± 4.47
Group D:	87.05 ± 1.50	70.17 ± 5.32	91.05 ± 3.07	141.87 ± 5.90*	97.49 ± 7.89
Serum bicarbonate (mmol/L)					
Group A:	24.64 ± 0.12	28.99 ± 3.46	24.00 ± 2.68	21.38 ± 2.06	30.92 ± 4.33
Group B:	26.80 ± 2.89	35.33 ± 1.10	25.36 ± 3.38	24.32 ± 2.34	27.92 ± 1.72
Group C:	32.81 ± 0.34	25.16 ± 2.53	26.45 ± 2.72	27.27 ± 1.63	24.55 ± 2.87
Group D:	31.87 ± 2.87	25.12 ± 1.45	29.45 ± 3.57	24.75 ± 3.31	25.35 ± 2.36
Serum sodium (mEq/L)					
Group A:	186.21 ± 5.55	195.30 ± 5.46	134.22 ± 7.36	77.86 ± 24.03	148.21 ± 20.23
Group B:	185.12 ± 5.81	210.50 ± 27.40	118.17 ± 6.62	122.14 ± 9.32	136.21 ± 6.27
Group C:	183.45 ± 0.41	223.81 ± 21.24	139.96 ± 5.42	148.10 ± 12.57*	135.67 ± 19.11
Group D:	184.40 ± 0.77	273.48 ± 15.72	143.74 ± 9.35	143.81 ± 9.19*	141.94 ± 22.26

*n= 5 for each condition. Values represent mean ± S.E.M. * denotes significance at P<.05. Group A: Control, Group B: Low dose, Group C: Medium dose, Group C: High dose*

The polyherbal remedy significantly increased (P<.05) potassium level when compared to control group on the 31st day. However, there was non-significant increase (P>.05) in potassium level on days 61 and 91st (when compared to control) (Table 3). The significant increase (P<.05) in serum potassium levels on 31st day, may be due to ineffective elimination of potassium by the kidney [43-44].

There was no significant difference (P>.05) in average chloride level in polyherbal remedy treated groups compared to control groups on days 31 and 61st, except at high dose on 91st day

(Table 3). Increased serum chloride could be associated with dehydration, renal tubular acidosis, and acute renal failure [45]. In this study, significant increase in serum chloride level may be attributed to dehydration associated with the polyherbal remedy. Reduction observed in post-treatment period suggests possible reversibility in serum chloride level.

There was no significant difference (P>.05) in average bicarbonate level of polyherbal remedy group on 31, 61 and 91st day when compared to control (Table 3). This suggests the unlikelihood of the polyherbal remedy to cause metabolic alkalosis and respiratory acidosis or metabolic acidosis and respiratory alkalosis, which are associated with abnormal levels of serum bicarbonate [25,45].

There was non-significant increase (P>.05) in average sodium values on 31 and 61st days, but there was significant increase (P<.05) in sodium level on 91st day when compared to control group (Table 3). Increase in sodium level suggests hypernatremia [46]. However, reduction in sodium values at post-treatment period, suggest that the induced hypernatremia is reversible.

The polyherbal remedy caused dose dependent significant increase (P<.05) in serum urea (Table 4) on 61st and 91st days and creatinine (Table 4) on 91st days and their increased values were not reversible at post-treatment. Determination of urea and creatinine in the serum can be used to assess the functional capacity of the kidney [47]. Significant increase (P<0.05) on 61 and 91st days of serum urea and significant increase (P<.05) on day 91st creatinine level suggests interference of the polyherbal remedy with the renal capacity to excrete these metabolites [42]. Such effect may not be reversible even after the withdrawal of the polyherbal remedy, as evidenced by the post-treatment serum urea and creatinine values. This is corroborated by kidney poisoning as shown in the photomicrograph during treatment and post-treatment periods (Figs. 6 - 8).

In 2007, Oze and co-workers [9], using only the stem bark of *Alstonia boonei* (50 mg/kg and 200 mg/kg) reported significant dose and time dependent increase in chloride, potassium, urea, creatinine levels and decreased sodium levels. Their assessment however was a 4-week study in guinea pig, which are obviously less than 90 days and in different animal specie. Also, three weeks treatment of Wistar rats with *Z. zanthoxyloids* (0.37, 0.75, 1.5 and 3.0 mg/kg bw) showed non-significant body weight gain, significant decrease in serum urea, creatinine levels and hepatic malondialdehyde concentration [20].

There was no obvious change in kidney photomicrograph (in low dose on the 31st day). There was mild reactions (in low dose on the 61st day), glomerulonephritis (in low dose on the 91st day) (Figs. 5-7). However, after the post-treatment period, there was no apparent change in the kidney architecture in low dose indicating that the toxic effects at low doses could be reversible. At the medium dose level and the 61st day, the kidney photomicrograph showed severe nephritis and oedema in the tubular area of the kidney (Fig. 6).

Also at the highest dose used for this study and the 91st day, the kidney photomicrograph showed oedema in the medulla and proteinatious coating and hyperaemia (Fig. 7), indicating time and dose dependent toxicities of the herbal remedy. Post-treatment studies indicate that the medium and high doses effects were irreversible (Fig. 8).

Table 4. Effects of polyherbal remedy on serum renal function parameters level in Wistar rats

	Serum urea (mg/dl)				
	Pretreatment	**31st day**	**61st day**	**91st day**	**Post-treatment**
Group A:	27.69 ± 1.18	29.38 ± 2.37	26.48 ± 0.87	25.24 ± 0.96	31.34 ± 0.80
Group B:	26.17 ± 0.73	29.78 ± 3.76	31.74 ± 0.82	32.18 ± 1.46*	35.42 ± 0.59
Group C:	27.32 ± 0.41	32.83 ± 3.03	34.86 ± 1.72*	33.70 ± 1.75*	36.83 ± 1.48
Group D:	26.27 ± 0.84	31.63 ± 2.66	39.59 ± 2.03*	40.30 ± 1.37*	41.19 ± 0.77
Serum creatinine (mg/dl)					
Group A:	0.93 ± 0.03	0.79 ± 0.04	1.00 ± 0.15	0.69 ± 0.02	0.88 ± 0.01
Group B:	0.88 ± 0.05	0.75 ± 0.04	0.98 ± 0.14	0.86 ± 0.03*	0.94 ± 0.02
Group C:	0.96 ± 0.08	0.77 ± 0.01	1.16 ± 0.12	0.90 ± 0.04*	1.09 ± 0.04
Group D:	0.89 ± 0.08	0.71 ± 0.02	1.36 ± 0.05	1.14 ± 0.06*	1.21 ± 0.06

*n= 5 for each condition. Values represent mean ± S.E.M. * denotes significance at P<.05. Group A: Control, Group B: Low dose, Group C: Medium dose, Group C: High dose*

The study by Oze et al. in 2007 [9] showed infiltration of the glomerular tuft, enlarged lobule, vacuolated cytoplasm, oedema, and glomerular degeneration.

Nwozo and coworkers in 2011 [20] found no lesion in the kidney of Wistar rats. However, his study was for only 3 weeks (with low concentrations of 0.37, 0.75, 1.5 and 3.0mg/kg bw) which corroborate the effects in the short term use of the polyherbal remedy. However, the use of a polyherbal remedy containing the active ingredients of two plants may account for the level of the observed renal impairment in this present study.

The polyherbal remedy caused dose and time dependent significant increase (P<.05) in serum AST level (Table 5), ALT level (Table 5), and in average ALP level (Table 5) on 61 and 91st day when compared to control. Alanine amino transferase (ALT) and Aspartate amino transferase (AST) are largely used in the assessment of liver damage by drugs or other hepatotoxins [48]. Liver cell permeability, congestion or cell rupture are characterized by a rise in serum enzymes like AST, ALT and alkaline phosphatase (ALP) [49]. Dose-time dependent significant (P<.05) increase in AST, ALT and ALP could indicate disruption in the integrity of the plasma membrane of the hepatocytes and loss of cellular components into the blood [50] or bile duct blockade or injury within the liver [51-52]. The irreversibility of these effects is supported by the post-treatment photomicrograph (Fig. 4).

However, in 2011, Nwozo and co-workers [20] reported mild hydropic degeneration of the hepatocytes and significant decrease in serum level of these liver toxicity markers after treatment of Wistar rats for just twenty one days using only *Z. zanthoxyloides*. At present, there are no available *in vivo* toxicity studies on *Alstonia boonei* on the liver of experimental animals that can be used to corroborate or contradict these present findings.

The polyherbal remedy did not cause a significant alteration in average packed cell volume (Table 6), hemoglobin (Table 6), white blood cell count (Table 6) and red blood cell count (Table 6) on 31, 61, and 91st days when compared to their respective control groups. These suggest the unlikelihood of the polyherbal remedy to induce anaemia or disrupt the circulating cells of the immune system on long term use [24].

Photomicrograph of liver (Figs. 1-4) and kidney (Figs. 5-8) revealed dose and time dependent poisoning. There was no reversibility in liver and kidney poisoning at post-treatment period in all groups (except low dose kidney histology).

This suggests that the polyherbal remedy can inflict irreversible damage on hepatic and renal functions if high doses are used over a long period of time.

Table 5. Effects of polyherbal remedy on serum liver enzymes level in Wistar rats

	Pretreatment	**31st day**	**61st day**	**91st day**	**Post-treatment**
Serum aspartate aminotransferase (U/L)					
Group A:	27.80 ± 0.40	36.4 ± 2.93	79.5 ± 4.99	67.60 ± 10.8	45.55 ± 2.22
Group B:	27.33 ± 2.06	25.20 ± 2.67	74.40 ± 5.21	81.60 ± 2.68*	68.04 ± 2.27
Group C:	27.26 ± 1.43	30.8 0 ± 1.36	60.40 ± 8.80	86.40 ± 2.25*	78.67 ± 2.65
Group D:	27.36 ± 0.36	39.80 ± 5.87	72.00 ± 6.00	93.80 ± 1.39*	94.99 ± 1.61
Serum alanine aminotransferase (U/L)					
Group A:	4.76 ± 0.20	11.7 ± 0.64	27.10 ± 1.41	14.80 ± 1.14	18.99 ± 1.39
Group B:	4.15 ± 0.32	10.15 ± 0.61	35.40 ± 2.27*	25.42 ± 1.45*	24.00 ± 1.01
Group C:	4.70 ± 0.09	10.10 ± 0.80	35.10 ± 2.38*	30.38 ± 1.65*	30.59 ± 1.68
Group D:	4.99 ± 0.18	12.35 ± 1.54	40.20 ± 1.59*	36.08 ± 0.91*	38.36 ± 0.77
Serum alkaline phosphatase (U/L)					
Group A:	127.09 ± 0.25	255.21 ± 39.41	226.83 ± 2.38	241.24 ± 5.91	219.45 ± 4.57
Group B:	111.11 ± 2.89	330.10 ± 36.05	264.68 ± 8.16*	269.81 ± 5.45*	264.03 ± 5.95
Group C:	123.25 ± 3.18	343.71 ± 47.76	312.18 ± 4.92*	316.13 ± 7.44*	324.82 ± 6.22
Group D:	120.52 ± 1.48	361.74 ± 32.51	364.70 ± 4.56*	371.80 ± 6.80*	363.51 ± 17.23

*n= 5 for each condition. Values represent mean ± S.E.M. * denotes significance at P<.05. Group A: Control, Group B: Low dose, Group C: Medium dose, Group C: High dose*

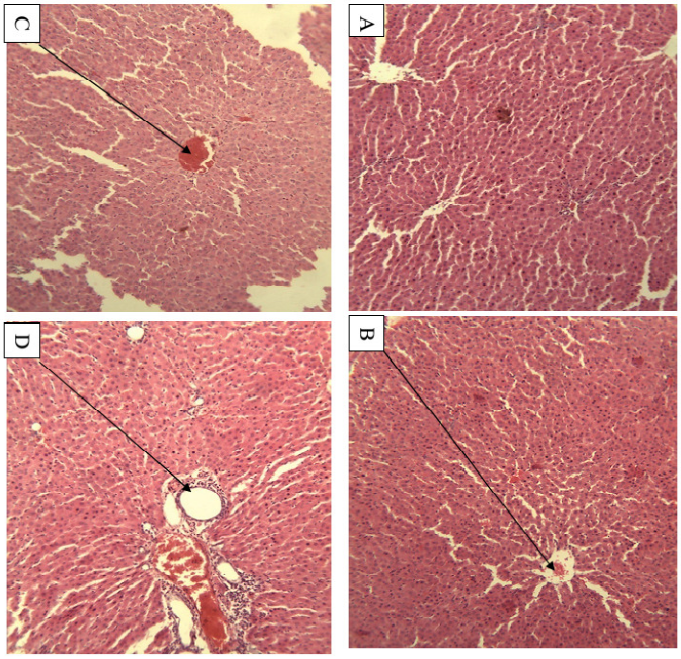

Fig.1. 31st day liver photomicrograph, Plate A: Control (Normal liver architecture), Plate B: Low dose (Congestion around hepatic triad), Plate C: Medium dose (Central vein plugged by proteinaceous material) Plate D: High dose (Severe reaction around hepatic triad)

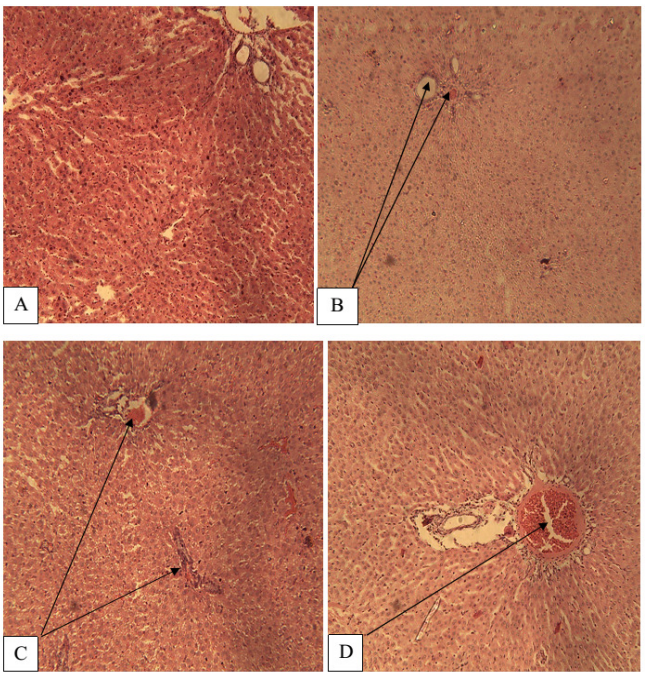

Fig. 2. 61st day liver photomicrograph, Plate A: Control (Normal liver architecture), Plate B: Low dose (Necrosis around hepatic triad and hepatocytes, pan-lobular), Plate C: Medium dose (Necrosis around hepatic triad), Plate D: High dose (Necrosis around hepatic triad and inflammatory reactions)

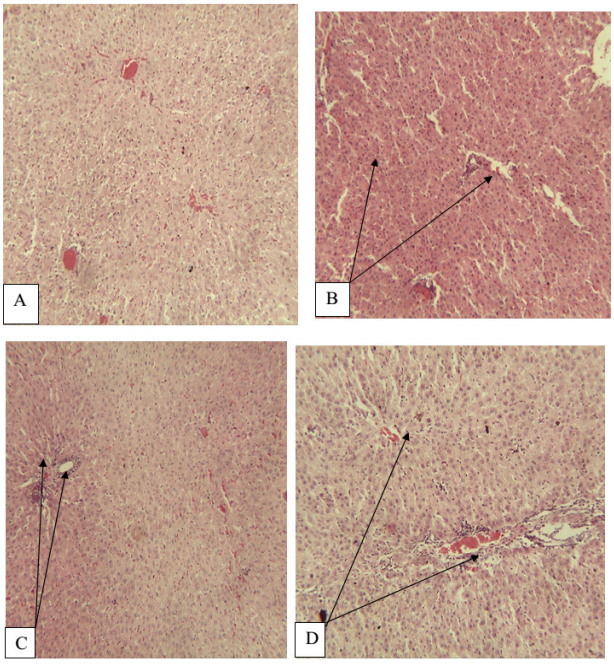

Fig. 3. 91st day liver photomicrograph. Plate A: Control (Normal liver architecture), Plate B: Low dose (Necrosis of hepatocytes), Plate C: Medium dose (Congestion Observed at the hepatic triad), Plate D: High dose (Severe necrosis around the hepatic triad)

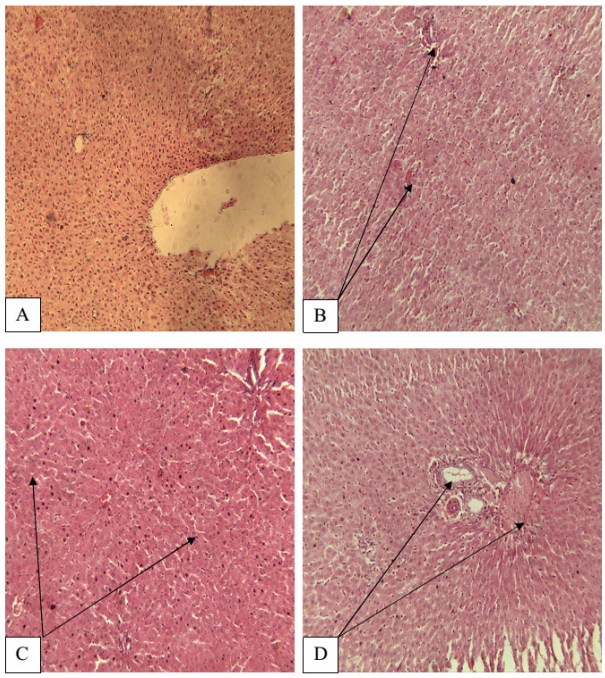

Fig. 4. Post-treatment liver photomicrograph. Plate A: Control (Normal liver architecture. Ignore the artifact), Plate B: Low dose (Necrosis of hepatic triad, lymphocytic infiltration in the hepatic vein observed), Plate C: Medium dose (necrosis of the hepatocyte) Plate D: High dose (Congestion and severe necrosis around the hepatic triad)

Table 6. Effects of polyherbal remedy on hematological parameters in Wistar rats

	Pretreatment	31st day	61st day	91st day	Post-treatment
Packed cell volume (%)					
Group A:	38.50 ± 0.30	47.40 ± 2.2	39.80 ± 2.62	40.00 ± 1.97	45.20 ± 2.80
Group B:	41.65 ± 1.09	44.80 ± 1.93	40.20 ± 1.77	40.60 ± 0.51	40.60 ± 2.29
Group C:	40.66 ± 0.33	46.8 ± 0.58	37.40 ± 1.03	41.00 ± 1.00	40.80 ± 0.86
Group D:	41.39 ± 1.61	47.40 ± 2.38	39.60 ± 1.21	41.60 ± 0.68	43.00 ± 1.18
Hemoglobin concentration (g/dl)					
Group A:	10.51 ± 1.94	15.68 ± 1.45	14.24 ± 0.47	17.55 ± 0.83	19.26 ± 0.43
Group B:	11.32 ± 1.75	15.77 ± 1.49	13.88 ± 0.85	18.46 ± 1.19	17.87 ± 1.03
Group C:	10.60 ± 1.87	16.82 ± 1.30	12.62 ± 0.55	17.15 ± 1.62	19.23 ± 0.81
Group D:	11.86 ± 1.86	15.89 ± 1.41	14.00 ± 0.26	20.00 ± 1.06	19.89 ± 0.80
White blood cell (Cell count × 10^9/L)					
Group A:	5.70 ± 0.28	5.26 ± 0.53	4.86 ± 0.30	4.25 ± 0.11	7.30 ± 1.11
Group B:	5.62 ± 0.06	5.66 ± 0.51	3.90 ± 0.62	4.06 ± 0.17	4.94 ± 0.29
Group C:	6.17 ± 0.20	4.76 ± 0.25	5.21 ± 0.29	4.26 ± 0.34	5.42 ± 0.43
Group D:	5.58 ± 0.05	4.90 ± 0.19	4.75 ± 0.58	4.57 ± 0.52	5.48 ± 0.15
Red blood cell (Cell count × 10^{12}/L)					
Group A:	7.99 ± 0.20	3.15 ± 0.92	7.41 ± 1.46	6.40 ± 1.12	9.28 ± 1.72
Group B:	7.57 ± 0.03	3.66 ± 0.67	7.34 ± 1.16	4.00 ± 0.65	7.74 ± 2.31
Group C:	7.88 ± 0.27	7.00 ± 1.34	9.60 ± 2.73	5.40 ± 1.40	6.78 ± 0.68
Group D:	7.05 ± 1.51	6.58 ± 1.3	6.97 ± 1.07	7.00 ± 2.07	7.06 ± 0.95

n= 5 for each condition. Values represent mean ± S.E.M. Group A: Control, Group B: Low dose, Group C: Medium dose, Group C: High dose

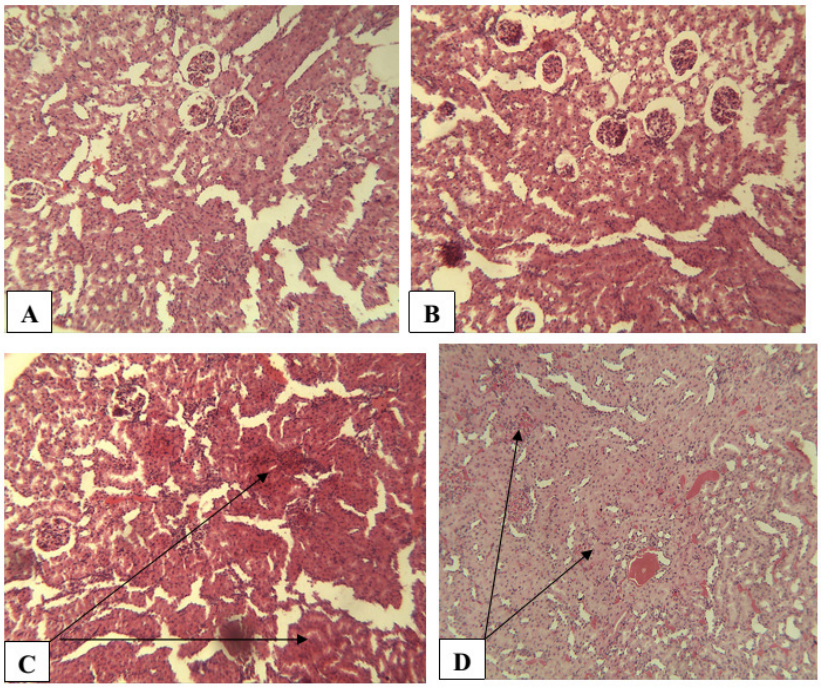

Fig. 5. 31st day kidney photomicrograph. Plate A: Control (Normal kidney architecture), Plate B: Low dose (No obvious change in kidney architecture), Plate C: Medium dose (Nephritis observed), Plate D: High dose (Necrosis of tubules)

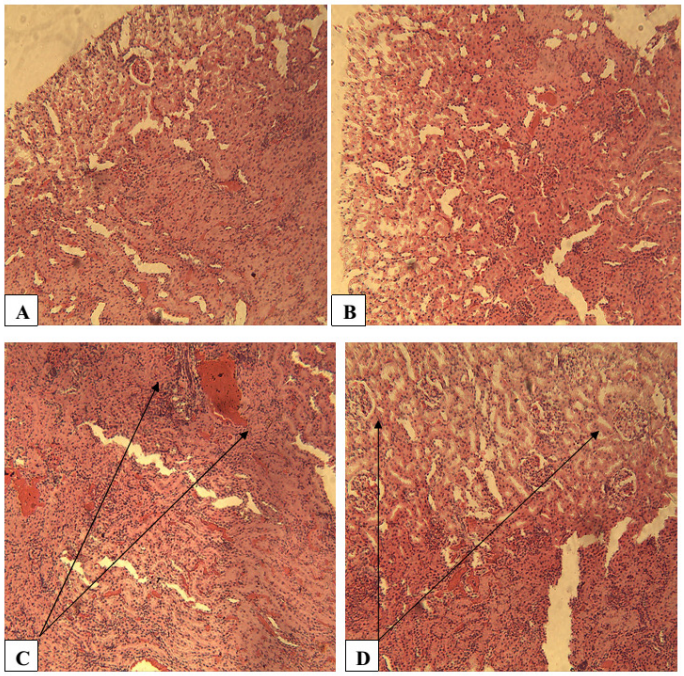

Fig. 6. 61st day kidney photomicrograph. Plate A: Control (Normal kidney architecture), Plate B: Low dose (Mild reactions), Plate C: Medium dose (Severe nephritis), Plate D: High dose (Oedema in the medulla)

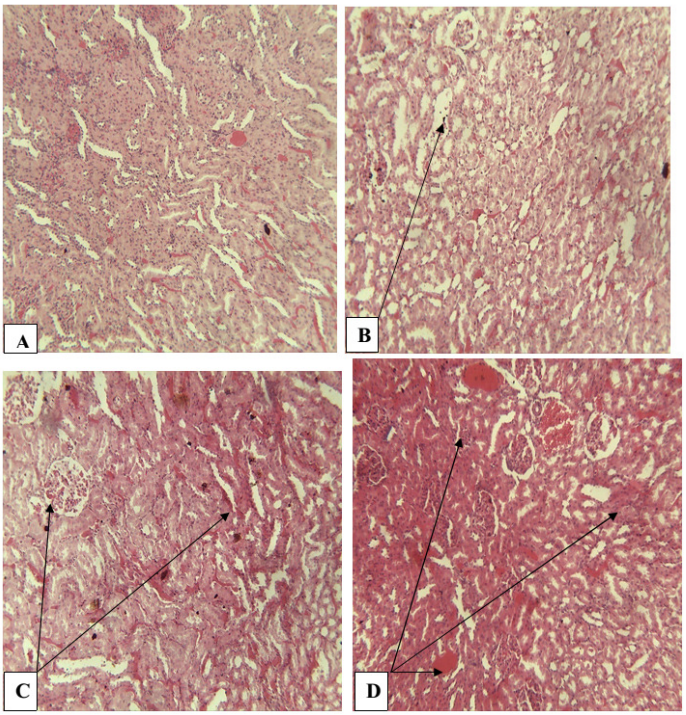

Fig. 7. 91st day kidney photomicrograph. Plate A: Control (Normal kidney architecture), Plate B: Low dose (Glomerulonephritis), Plate C: Medium dose (Edema in the tubular area of the kidney). Plate D: High dose (Proteinaceous coating- short arrow and hyperemia-long arrows observed

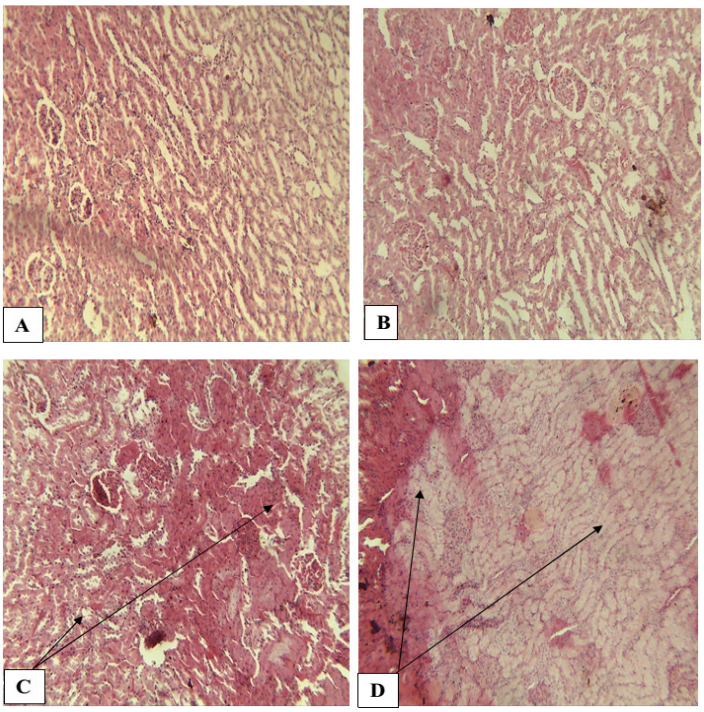

Fig. 8. Post-treatment kidney photomicrograph. Plate A: Control (Normal kidney architecture), Plate B: Low dose (Tubular degeneration-short arrow, Congestion in the glomeruli showing oedema in the medulla-long arrow), Plate D: High dose (Hyperemia affecting the nephrons-nephritis)

Although, we are much encouraged with the apparent effects of the polyherbal remedy on Wistar rats, further studies of the mutagenic and teratogenic effects on Wistar rats is needed to fully characterize the toxicity of this polyherbal herbal remedy.

4. CONCLUSION

Single dose administration of this polyherbal remedy appears to be relatively non-toxic. Sub-chronic toxicity results suggest that this polyherbal remedy has no ulcerogenic potential in the stomach, but nephrotoxicity and hepatotoxicity may occur especially at high doses. In view of these findings, patients receiving larger doses, or under-going prolonged medication with this polyherbal remedy should have their renal as well hepatic functions evaluated regularly. The enlightment of the traditional medicine practitioners and the general public on the dangers of chronic and high dose administration of this polyherbal remedy is imperative.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by the Nnamdi Azikiwe University's ethical committee for the use of Laboratory animals.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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