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Toxicological Studies of a Nigerian Commercial Polyherbal Product in Albino Rats

¹E.U. Etuk, ²V. Igbokwe, ³O.P. Ajagbonna and ¹M.O. Egua

¹Department of Pharmacology,

²Department of Physiology,

³Department of Veterinary Pharmacology and Physiology,

Usmanu Danfodiyo University, Sokoto, Nigeria

Abstract: There have been earlier reports of herbal medicine toxicity elsewhere in Nigeria, China and India. The present study examined the possible acute and subchronic toxic effects of Nasara Pile Syrup (NPS), a Nigerian commercial polyherbal medicine in albino rats. Graded doses (0.5, 1.0, 1.5 and 1.75 mL/100 g) of the herbal medicine were administered to 4 groups of albino rats and their responses observed for 72 h to study the acute toxic effect of the herbal medicine. In the subchronic toxicity study, the rats were treated orally with repeated doses of the extract for 28 days after which the animals were slaughtered and samples from the liver, kidney and heart obtained for histopathological examination. The results showed that, administration of a single dose of the herbal medicine did not produce any harmful effect or death in the animals. But in the repeated dose treatment, the herbal medicine produced a number of deaths and damages on the kidney, liver and heart of the rats that were evidenced by histopathological lesions in a dose dependent manner. Based on the results, it was concluded that, prolong administration of NPS may cause harmful effect in the consumers, therefore, the general public should exercise caution in taking this herbal remedy and they should be aware of the impending health risk that may be associated with it.

Key words: Herbal remedy, albino rats, liver damage, consumers

INTRODUCTION

The contribution of herbal product to modern medicine is well known. Life in most parts of Africa begins and ends with herbal medicine. About 80% of Africans rely on traditional medicine for their health care needs (Calixto, 2000). According to the United Nations Conference on Trade and Development, 33% of total modern drugs produced by industrialized countries are plant based (Raskin *et al.*, 2002). Despite all these antecedent benefits from plants, plant materials and herbal medicine, a significant percentage of human and animal health problems can be traced to the consumption of plant materials either as food or medicine. The injurious elements in plants materials may come from natural components of the plant or may arise from contaminants acquired during the process of preparation (Keen *et al.*, 1994). This calls for a better screening of herbal medicine before it is put to public use.

Over the years, the use of herbal products globally either as food supplement or curative medicine has witnessed a phenomenal growth (Raskin *et al.*, 2002). This is partly related to the fact that, herbal products are cheaper than the orthodox medicine and the erroneous impression by the people that,

Corresponding Author: Dr. E. Etuk, Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria Tel: 2348054693770

herbal product is natural and thus less harmful to the body. The increase demand for herbal products has brought concerns and fears over the quality, efficacy and safety of such products available in the open market. The toxicities of some of the most commonly used herbal medicines have been reported. Ginseng, a Chinese herbal medicine was reported to be associated with apparent aggressive behavioral changes/hypertension in chronic users (Kennedy and Scholey, 2003). There have been confirmed cases of renal failure and liver diseases associated with herbal medicine consumption in South Africa (Calixto, 2000).

The present study examined Nasara Pile Syrup (NPS), a commercial herbal medicine that enjoys wide patronage among the populace in Sokoto state, Nigeria because of its acclaimed sexual stimulation and provision of a general sense of well being after consumption. The phytochemical compositions as well as the effects of the herbal medicine on the liver, heart and kidney following repeated oral administration for 28 days in albino rats will be investigated. The study protocol was in line with OECD guidelines and approved by the Institutional Ethical Research Committee.

MATERIALS AND METHODS

This study was carried out in the Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria between February and June 2007.

Herbal Medicine

The herbal medicine under study; Nasara Pile Syrup (NPS) was purchased from a retail shop in the Central motor park area in Sokoto State, Nigeria. NPS is a red wine colour homogenous liquid, sealed in a 1 L bottle with a clearly identifiable label on the bottle carrying a batch number of the product as 04. There is no indication of official registration, product composition or side effect. Several illnesses including infertility, asthma, stomach ulcer, hypertension, etc. are listed as indications for the herbal medicine. A sample of NPS was deposited in the Research Laboratory of the Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto (UDUS) for possible future reference.

Yield Value of Extract

Five milliliters of the NPS was placed in a 25 mL beaker and evaporated slowly in a water bath maintained at 50°C. A dried powder was obtained and the percentage yield was calculated (Hazel and Robert, 1997).

Tests for Acidity/Alkalinity of NPS

Five milliliters of NPS was warmed in a hot water bath maintained at 38±2°C for about 30 min and then allowed to cool. A piece of water wet litmus paper was dipped into the extract and the colour change on the litmus paper noted (Hazel and Robert, 1997).

Animals

Albino rats of both sexes weighing between 180-220 g and age about 8-12 weeks were obtained from Faculty of Agricultural Sciences, Usmanu Danfodiyo University, Sokoto (UDUS). Animals were kept in a constant 12 h light/dark cycle and maintained at 35±2°C for 2 weeks for acclimatization in the Pharmacology Laboratory, UDUS. The rats were fed with rat normal pellets (growers mash) and had free access to tap water. NPS was administered in its original liquid form to the animals and the dosage measured in mL/100 g body weight of the animals.

Phytochemicals Analysis of NPS

The phytochemical analysis of NPS was conducted using the method outlined by Odebiyi and Sofowora (1979). The extract was analyzed for alkaloids, flavonoids, glycosides, tannins, saponins, steroids, reducing sugar, cardiac glycosides, anthraquinones and vitamin C.

Acute Toxicity Test

The animals were randomly divided into four groups of ten rats in each group and labeled A to D. The rats in group A were treated orally with 0.5 mL/100 g (b.wt.) of NPS. They were maintained and observed for 72 h for signs of acute toxicity or death. Thereafter, the rats in groups B, C and D were given 1.0, 1.5 and 1.75 mL/100 g (b.wt.) of NPS, respectively and also observed for the same period for signs of acute toxicity or death (Schlede *et al.*, 1995).

Subchronic Toxicity Study

Forty rats of both sexes were randomly selected and divided into four groups labeled A to D. The initial weights of all the animals were recorded before the extract administration. The rats in group A, B and C were given 0.5, 1.0 and 1.5 mL/100 g (b.wt.) of the herbal syrup, respectively daily for 28 days. While those in group D were given equivalent volume of distilled water. Food and water consumption of the animals were monitored daily during the period. The body weights of the animals were recorded weekly. On the 29th day, the animals were anaesthetized with chloroform and sacrificed. Blood samples were collected by cardiac puncture for haematological and biochemical analysis. Tissue samples were collected from the liver, heart and kidney of the rats for histopathological examinations.

Statistical Analysis

The data obtained from this study was analyzed using Analysis of Variance (ANOVA). Further comparison between groups was carried out using Duncan multiple range test. The values were expressed as Mean \pm SEM. All differences were considered statistically significant at probability value of less than 5%.

RESULTS

Yield Value

Five milliliters of Nasara pile syrup yielded 0.04 g of solid extract powder after complete evaporation.

Phytochemical Composition

The extract residue retained the original red wine colour with no distinctive smell after complete evaporation. Alkaloids, anthraquinones, glycosides, saponin, tannins and vitamin C (61.4 $\mu\text{g dL}^{-1}$) were the substances detected in the herbal syrup. The pH of the extract was 10.6 (alkaline). Flavonoids, cardiac glycosides, steroids and reducing sugar were absent.

Acute Toxicity of NPS

In the acute oral toxicity test of NPS, there was no death recorded among the animals treated with 0.5-1.75 mL/100 g (b.wt.) during the 72 h observed. However, the rats treated with 1.5 and 1.75 mL/100 g (b.wt.) of the herbal syrup showed signs of hypo-activity immediately after the treatment but became active again after 20-30 min.

Subchronic Toxicity of NPS

The results of the observations recorded after 28 days oral administration of varying doses (0.5, 1.0 and 1.5 mL/100 g) of NPS to the rats are presented as follows:

Physical Changes and Mortality

The rats administered with 1.0 and 1.5 mL/100 g (b.wt.) of NPS showed obvious changes in hair coats after 10 days treatment. The hair removed easily from the dorsal surface of the animals especially after being handled. Bare areas (alopecia) can be seen on the back of the animals. Also, the rats in these same groups showed signs of progressive hypo-activity when compared to those in the control and 0.5 mL/100 g groups. Overall, no death was recorded among the control group. One animal (10%) died from group A (0.5 mL/100 g) on the 17th day of treatment. Four rats (40%) died from group B (1.0 mL/100 g) starting from the 10th to 21st days of NPS administration, while 70% of the animals died from group C treated with 1.5 mL/100 g of the extract. They started dying from the 7th day.

Effect of NPS on Feeding and Water Intake

There was a progressive reduction in both the feed and water consumption by the rats treated with 1.0 and 1.5 mL/100 g (b.wt.) of NPS. However, the animals treated with 0.5 mL/100 g of the NPS and those in the control group did not show any change in their feeding and drinking pattern.

Effect of NPS on Body and Organ Weights of Rats

Table 1 shows the initial mean body weight, final mean body weight and mean weight changes of the rats treated with different concentrations of the extract. Both the control and the 0.5 mL/100 g extract treated groups showed progressive weight gain, while the 1.0 and 1.5 mL/100 g (b.wt.) treated groups showed a progressive weight loss. NPS produced a dose dependent significant ($p < 0.05$) increase in the absolute weight of the heart and kidneys of the rats after 28 days treatment as compared to the control group (Table 2). There was a non significant ($p > 0.05$) increase in the liver weights of the rats treated with NPS when compared to the control.

Hematological Analysis

There were no significant changes in the PCV and RBC of the rats in the treatment and control groups. But the extract at a concentration of 1.0 and 1.5 mL/100 g doses produced a significant ($p < 0.05$) increase in leucocyte cell count (Table 3).

Biochemical Analysis

NPS produced a significant increase in the serum cholesterol levels of rats after 28 days treatment. There was no apparent effect on random blood sugar levels between the control and the treated groups

Table 1: Effect of 28 days administration of NPS on the body weight of rats

Interval of weighing (week)	Control	Dose of NPS (mL/100 g)		
		0.5	1.0	1.5
0	85.8±3.6	87.9±6.1	129.9±1.3	116.6±1.7
1	92.2±4.3	95.9±6.9	121.6±2.9	116.6±0.9
2	112.4±3.9	108.9±3.7	106.2±1.4	101.6±1.2
3	127.2±3.2	123.9±2.6	96.1±3.6	89.4±3.9
4	132.5±3.8	130.2±1.8	89.9±2.7	82.6±1.4

n = 10, NPS: Nasara pile syrup

Table 2: Effect of NPS on organs weight in the rats

Organ (g)	Control	Concentration of NPS (mL/100 g)			F and p-values
		0.5	1.0	1.5	
Kidney	1.21±0.1	1.10±0.1	1.48±0.2	1.8±0.0	$F_{3,20} = 3.293, p = 0.042^*$
Liver	5.86±0.5	6.14±0.7	8.03±0.9	7.7±0.0	$F_{3,20} = 1.772, p = 0.185$
Heart	0.50±0.1	0.59±0.1	0.85±0.1	0.8±0.0	$F_{3,20} = 8.601, p = 0.001^*$

n = 10, NPS: Nasara pile syrup, $p < 0.05$, *Significant value

Table 3: Effect of NPS on hematological indices of albino rats

Parameters	Concentration of NPS (mL/100 g)				F and p-values
	Control	0.5	1.0	1.5	
TLC	8.7±0.6	3.9±0.7	9.4±2.6	13.9±0.5	F _{3,14} = 15.27, p = 0.60
RBC	5.2±0.5	5.1±0.5	5.9±0.7	5.3±0.3	F _{3,14} = 0.51, p = 0.68
PCV (%)	27.2±0.6	26.4±1.6	31.8±2.2	24.7±1.2	F _{3,18} = 2.40, p = 0.10

n = 10, p<0.05, TLC: Total leucocytes count, RBC: Red cell count, PCV: Pack cell volume, F and p: Degree of freedom and probability values

Table 4: Effect of NPS on biochemical parameters in albino rats

Parameters	Concentration of NPS (mL/100 g)				F and p-values
	Control	0.5	1.0	1.5	
Cholesterol (mmol L ⁻¹)	118.80±8.1	133.30±13.2	133.30±6.2	180.00±152	F _{3,22} = 3.11338, p = 0.04607*
RBS (mmol L ⁻¹)	6.20±0.5	5.90±0.3	6.10±0.2	5.00±1.0	F _{3,21} = 1.0802, p = 0.37899
TP (g L ⁻¹)	5.60±0.6	5.50±0.4	4.20±0.2	5.90±0.4	F _{3,22} = 2.601, p = 0.0777
Albumin (μmol L ⁻¹)	3.50±0.1	3.50±0.3	3.10±0.1	4.00±0.7	F _{3,22} = 11.3719, p = 0.2774
TB (μmol L ⁻¹)	0.35±0.1	0.34±0.1	0.29±0.1	0.22±0.0	F _{3,18} = 0.218, p = 0.8665
ALT (UL)	20.10±4.3	21.90±4.0	21.20±1.6	22.00±3.2	F _{3,22} = 0.0551, p = 0.9825
AST (U L ⁻¹)	20.10±4.5	21.90±4.0	4.70±0.4	7.30±1.8	F _{3,21} = 1.3581, p = 0.2748
AP (U L ⁻¹)	76.10±8.4	68.10±5.9	74.80±9.1	118.70±4.7	F _{3,20} = 3.4886, p = 0.0348*

n = 10, p<0.05, RBS: Random blood sugar, TB: Total bilirubin, TP: Total protein, AP: Alkaline phosphate, *Significant value

Table 5: Electrolytes, urea and creatinine levels following 28 days treatment with NPS in rats

Parameters	Concentrations of NPS (mL/100 g)				F and p-values
	Control	0.5	1.0	1.5	
Sodium (mmol L ⁻¹)	128.10±3.5	123.30±7.8	127.80±1.6	130.70±1.7	F _{3,22} = 0.2553, p = 0.8567
Potassium (mmol L ⁻¹)	4.30±0.2	4.10±0.2	4.10±0.3	4.40±0.2	F _{3,22} = 0.2234, p = 0.82927
Bicarbonate (mmol L ⁻¹)	27.30±0.7	27.50±0.3	26.80±0.5	27.30±0.7	F _{3,22} = 1.4939, p = 0.2593
Urea (mg dL ⁻¹)	13.40±1.3	12.20±1.3	14.30±1.2	10.40±1.7	F _{3,22} = 0.9472, p = 0.4349
Creatinine (mg dL ⁻¹)	0.76±0.2	0.93±0.2	0.70±0.1	0.73±0.2	F _{3,20} = 0.4389, p = 0.7277

n = 10, p<0.05

(Table 4). The herbal syrup caused no significant change in the total protein, albumin and transaminases enzymes of the treated rats as compared to the control group. But the alkaline phosphatase level increased significantly in the rats treated with the highest dose. There was no significant change in the sodium, potassium, bicarbonate, urea and creatinine levels between the different groups (Table 5).

Histopathological Analysis

Physical examination of the liver, heart and kidneys of the animals immediately after laparotomy following 28 days oral administration of NPS revealed no obvious change in the morphology of the organs. However, histological examination of the liver revealed a dose dependent pathological lesions comprising of minimal portal inflammation, presence of acidophilus bodies and ballooning degeneration (Fig. 1a-d). The sections of the renal tissues in the control group, 0.5 mL/100 g and 1.0 mL/100 g groups showed normal architecture. But the transverse sections from the kidneys of the rats treated with the highest dose of the extract (1.5 mL/100 g) showed mild glomerular hypercellularity and lobular accentuation (Fig. 2a-d). The histology of the heart tissues of the animals treated with 0.5 mL and 1.0/100 g of the extract showed no significant changes. However focal myocardial necrosis was observed in the group receiving 1.5 mL/100 g of the extract (Fig. 3). Overall, the extract at the dose of 1.5 mL/100 g (b.wt.) produced pathological lesions in the liver, kidney and heart tissues of the rats after 28 days oral administration.

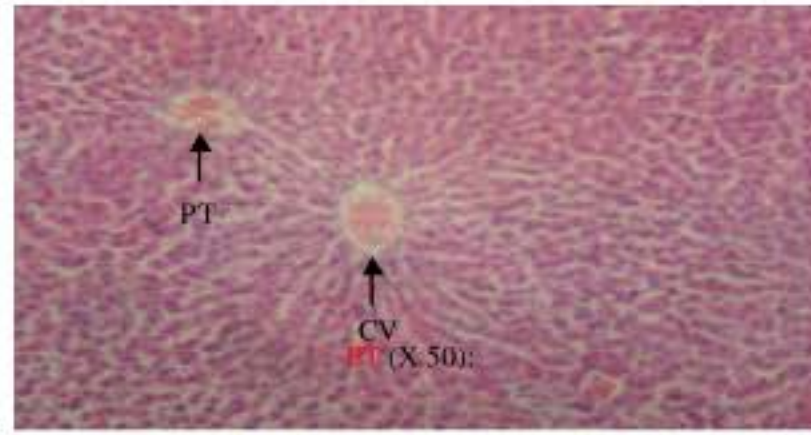


Fig. 1a: The photomicrograph of rat liver in control group, PT: Portal tract, CV: Central venule (x10)

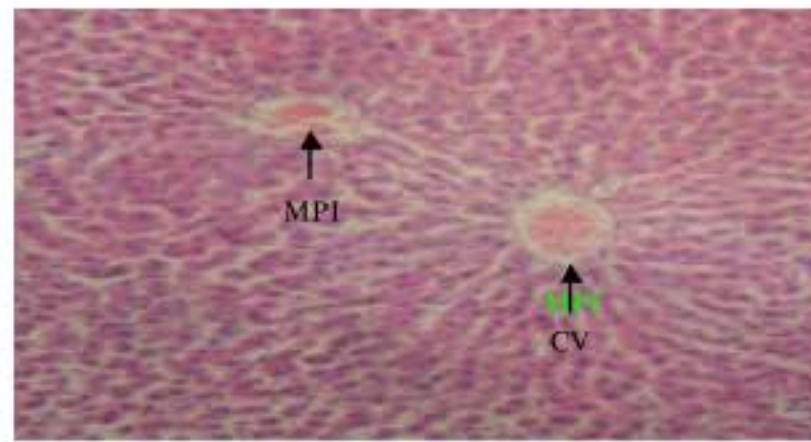


Fig. 1b: Photomicrograph of rat liver administered 0.5 mL/100 g, MPI: Minimal portal inflammation (x50)

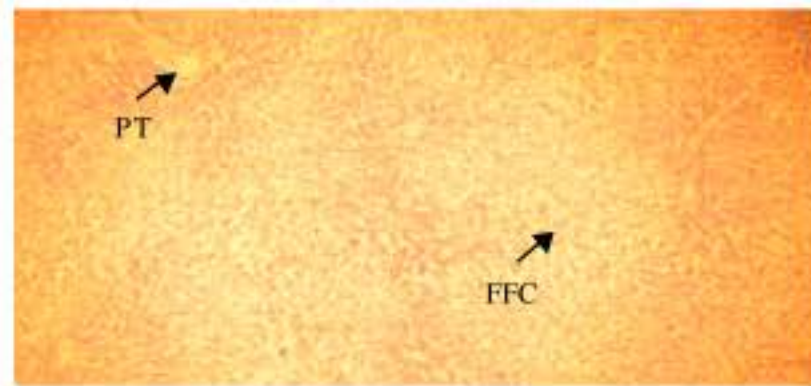


Fig. 1c: Photomicrograph of rat liver administered 1.0 mL/100 g, FFC: Focal fatty change, PT: Portal tract (x50)

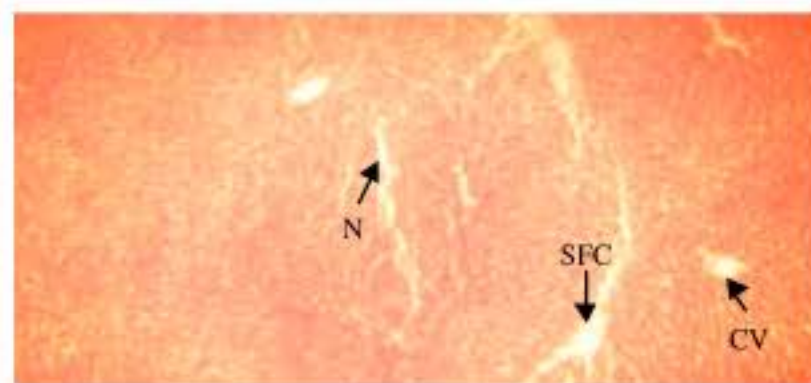


Fig. 1d: Photomicrograph of rat liver administered 1.5 mL/100 g, N: Necrotic area, SFC: Severe fatty change, CV: Central vein (x50)

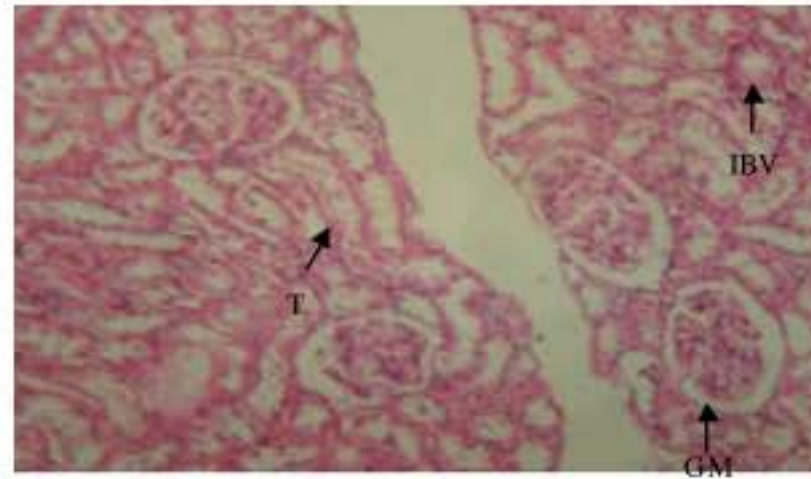


Fig. 2a: The photomicrograph of rat kidney control in group, T: Tubules, GM: Glomeruli, IBV: Interstitial blood vessels (x10)

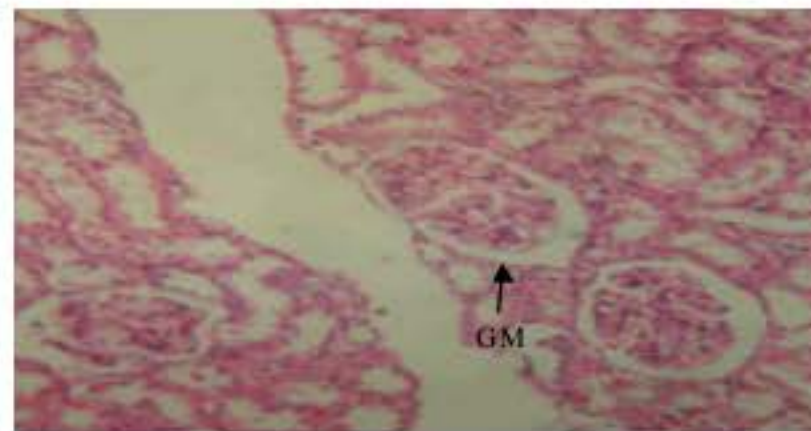


Fig. 2b: The photomicrograph of rat kidney administered 0.5 mL/100 g, GM: Glomeruli (x10)

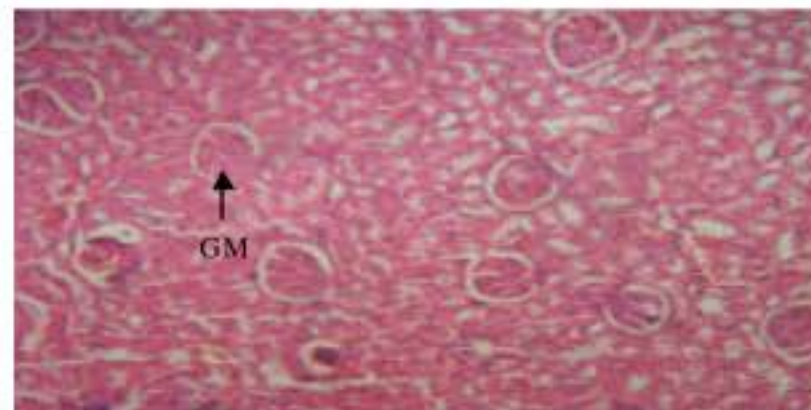


Fig. 2c: The photomicrograph of rat kidney administered 1.0 mL/100 g, (x10)

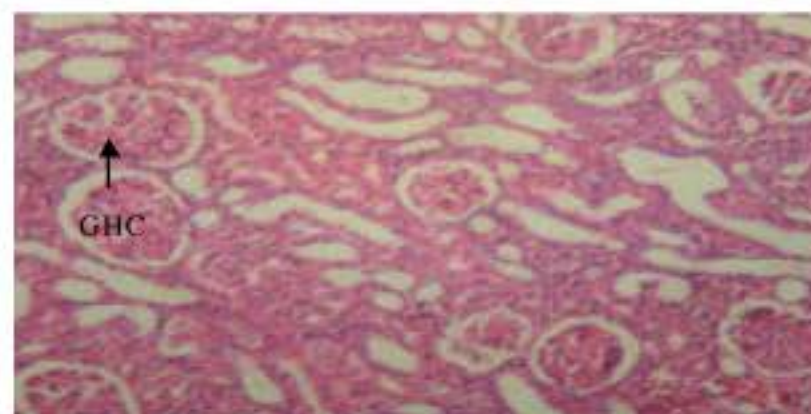


Fig. 2d: The photomicrograph of rat kidney administered 1.5 mL/100 g, GHC: Glomerular hypercellularity (x40)

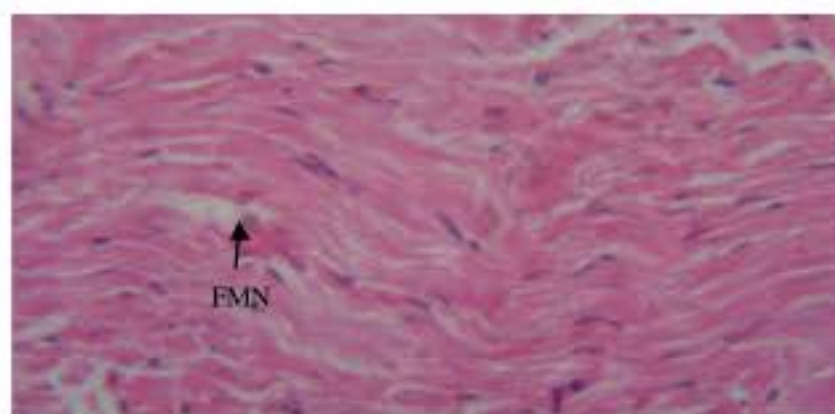


Fig. 3: The photomicrograph of rat myocardium administered 1.5 mL/100 g, FMN: Focal myocardial necrosis (x40)

DISCUSSION

Acute oral toxicity testing of NPS indicates that the extract is safe when taken for a short period of time. The maximum dose tested (1.75/100 g b.wt.) exceeds the daily recommended dose (2.5 mL kg⁻¹) by the herbal medicine's manufacturer.

However, the result was different in the repeated dose study where the extract produces a significant weight loss and mortality among the animals. The weight loss may be as a result of reduction in water and feed consumption by the animals as recorded in this study. Parveen *et al.* (2003) reported a significant weight loss in male rats after toxicity by *Quassia amara* extract. The number of deaths caused by a substance in experimental animals is always an indicator of its toxicity (Shankar *et al.*, 1980). Herbal medicine toxicity in experimental animals has been previously reported by Rao (1998).

The serum urea, creatinine, electrolytes, proteins and hepatic transaminases (except alkaline phosphatase) were normal while histological lesions were observed in the liver, heart and kidneys of the rats treated with 1.5 mL/100 g (b.wt.) of NPS for 28 days. This may suggest that, the liver and the kidney may be in early disease state. The liver like the kidney has ability to compensate functionally for initial damages. Derangement of hepatic and renal indices may not be noticed until a significant fraction of its structure has been damaged. Cell membrane destabilizations do result in increase serum hepatic enzymes levels associated with hepatotoxicants. The presence of vitamin C in high quantity in this extract might have offered some protection to the liver cells.

Administration of NPS for 28 days produced increase in absolute weight of the heart and kidney in the animals. Increased organ weight (either absolute or relative) has been observed as a sensitive indicator of organ toxicity by known intoxicants (Raffaele *et al.*, 2000). The demonstrable pathological lesions in the liver, kidney and heart of the rats caused by high dose of the extract may be as a result of high concentrations of heavy metals or inherent toxic phytochemicals present in the extract. A previous study conducted in our laboratory showed that, Nasara Pile Syrup (NPS) contained lead, nickel, cadmium, chromium, copper and manganese in excess of WHO permissible maximum limit for heavy metals in consumable items (Etuk *et al.*, 2008). Nasara pile syrup as shown in this study can cause hypercholesterolemia in rats' dose dependently. Elevated plasma cholesterol level may cause coronary heart disease (Mahley and Bersot, 2006). This may be responsible for the heart disease as evidenced by a significant increase in absolute heart weight and histopathological lesions. Also alkaline phosphatase the only enzyme raised after administration of high dose of NPS is known to be an indicator of myocardial infarction (Pennington *et al.*, 1978). The haematological parameters were apparently normal except for the increase in leucocyte count which may be a response by the body immune system to contaminants in the extract.

CONCLUSION

This study has shown that, NPS contains alkaloids, anthraquinones, glycosides, saponin, tannins and vitamin C. The pH of the extract was 10.6 (alkaline). The herbal medicine may be relatively safe when taken orally for a short period of time but prolonged administration may result in renal, heart or liver diseases. The public should be aware of this risk and exercise caution in the manner they use the extract. And the drug regulatory authorities should restrict the distribution of unevaluated and unregistered herbal medicine.

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