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Original Research Article

Chronic toxicity study of *Pterocarpus santalinoides* leaf extract in albino rats

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Abstract

Purpose: *To investigate the possible toxic effects associated with prolonged use of Pterocarpus santalinoides leaf extract.*
Methods: *Methanol leaf extract of Pterocarpus santalinoides (MEPS) was incorporated in rat feed at different doses (2.5, 5.0 and 10.0 mg/kg) for 90 days. On days 30, 60 and 90, blood was collected from the retro-orbital plexus of four rats that were randomly selected from each group (n = 14). Full blood count was done using an auto hematological analyzer, liver marker enzymes (AST, ALT and ALP) and kidney function parameters (serum urea and creatinine) were determined following standard methods as contained in Randox® test kits. The histopathological examination of the liver, kidney, lung and heart was also carried out.*
Results: *MEPS did not cause significant ($p > 0.05$) changes in the body weight, relative organ weights and hematological indices of treated rats when compared with control. The extract (5.0 and 10.0 mg/kg) significantly ($p < 0.05$) increased the AST activity of the rats on day 90. Total bilirubin concentration was significantly ($p < 0.05$) reduced by all doses of MEPS, while serum urea and creatinine were significantly ($p < 0.05$) increased. Degeneration of hepatocytes and tubular epithelial cells of the kidney were observed in rats treated with MEPS at a dose of 10.0 mg/kg on days 60 and 90 of the study.*
Conclusion: *MEPS does not cause significant toxicity in albino rats, when administered for a short duration. However, long term therapy with the extract precipitates liver and kidney damage.*

Keywords: *Pterocarpus santalinoides; toxicity; antioxidant; Liver and kidney damage*

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INTRODUCTION

Interest in using medicinal plants for the treatment of diseases is on the rise globally. The World Health Organization (WHO) estimates that about 80 % of the human population particularly in developing countries rely on herbs for their primary health challenges [1]. Apart from the fact that they are cheap, always available and are well acceptable both by rural and urban dwellers, herbal remedies are generally perceived to be safer (less toxic) than orthodox medication [2]. However, there are reports of adverse effects after administration of herbal formulations, hence the need for pre-clinical toxicological evaluations be carried out with herbal and potential herbal drugs [3].

Pterocarpus santalinoides is a shade-tolerant tree commonly found along riverine forests in tropical South America and West Africa. The common names of the plant in some ethnic groups in Nigeria include: *nturukpa* (Igbo), *gunduru* (Hausa), *gbengbe* (Yoruba), *nja* (Efik), *ikyarakwa* or *kereke* (Tiv), *maganchi* (Nupe), okumeze (Edo) and *piegwu* or *uturukpa* (Igede) [4]. Among the Igbo-speaking communities in southeastern Nigeria, decoctions of the leaves and bark of the plant are taken orally as remedy for stomach ache, diarrhea and diabetes [5]. The Igede people in North central Nigeria also consume decoctions of the leaves of *P. santalinoides* as remedy for some infectious diseases [4].

Antimicrobial [4], hematopoietic [6], hepatoprotective [4], antidiarrheal [5], hypolidemic and hypoglycemic activities [7] of *P. santalinoides* have been reported. However, there is no documented evidence of comprehensive toxicological evaluation of the plant. This necessitated the present study.

EXPERIMENTAL

Plant collection and extraction

Leaves of *P. santalinoides* were harvested from its habitat in Amaokwe Ugwu Nkpa, in Abia State, Nigeria. Identification of the plant was done at the Bioresources Development and Conservation Program, Nsukka. A representative voucher specimen (no. MOUAU/VPP/2014/017) was deposited in the Institution's herbarium. The leaves were air-dried, pulverized and extracted with methanol (80 %), by cold maceration method. The extract was oven-dried (40 °C) after passing through a rotary evaporator, and stored as methanol extract of *Pterocarpus santalinoides* (MEPS) at 4 °C until time of use [5].

Animals

Mature albino rats bred in the Laboratory Animal Unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the experiments. The animals weighed 117.4 ± 4.25 g and were housed in stainless steel cages. They were given standard pelleted feed (Vital feed®, Nigeria) and clean drinking water *ad libitum*. The experimental protocols were approved by the institution's Ethics Committee for use of Laboratory Animals (approval no. MOUAU/CVM/REC/19008), and the rats were handled in strict compliance with NIH Guidelines for Care and Use of Laboratory Animals [8].

Preparation of experimental feed

The extract (MEPS) was incorporated in feed at varying concentrations (2.5, 5.0 and 10.0 mg/kg feed). For each concentration the amount of extract required was weighed out (based on the duration of treatment, mean body weight and feed consumption rate of rats) and dissolved in 20 ml of water. The dissolved extract was uniformly mixed with feed by adding additional 810 ml of water for every 1 kg of feed. The mixture was then pelleted and dried in hot air oven (40 ºC). The feed was kept in a dry environment throughout the duration of the experiment [9].

Experimental design

Fifty-six mature albino rats were randomly assigned into 4 groups *(n = 14)*, with male and female rats housed in separate cages to prevent breeding. Group 1 rats served as control and received feed without extract. Rats in groups 2 - 4 were given MEPS 2.5, 5.0 and 10.0 mg/kg in feed respectively. All the rats were allowed free access to drinking water, but fed with 0.1 g feed/g b. wt./day, which is the normal feed consumption rate for rats [9]. On days 30, 60 and 90, four rats were randomly selected from each group and weighed.

Blood was collected from the retro-orbital plexus into EDTA and plain sample bottles for hematological and serum biochemical analysis, respectively. Thereafter, the rats were lightly anaesthetized using petroleum ether and sacrificed by cervical dislocation. Vital organs were harvested for histopathological examination.

Determination of weight parameters

The weights of the rats as well as the relative weights of some vital organs: kidney, liver, lung and heart were determined at weekly intervals, using a digital balance.

Hematological studies

An auto hematological analyzer (BC-2800, Shenzhen Midray Bio-Medical, China) was used to determine the following parameters: packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell (RBC) count, total white blood cell (WBC) count, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

Assay of liver marker enzymes

Randox® test kits were used to estimate the concentrations of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin and total protein following the manufacturer's instructions.

Kidney function assessment

Randox® test kits were used to determine the serum concentrations of urea and creatinine, following the instructions of the manufacturer.

Histopathological studies

Tissue samples from the liver, kidney, lung and heart were fixed in 10% formalin. After 24 h they were processed in ascending concentrations of ethanol, cleared with xylene and embedded in paraffin wax.

The paraffin-embedded tissues were sectioned using a microtome, after which they were stained with hematoxylin and eosin (H & E). An Olympus photomicroscope was used to examine the slides and capture photomicrographs of histopathological changes [10].

Statistical analysis

Data obtained from the study are presented as mean ± S. E. M. and analyzed using one-way analysis of variance (ANOVA). Least significance difference (LSD) of the different groups was used to separate the variant means. Significance was accepted at the level of $p < 0.05$.

RESULTS

Visual appearance

The rats appeared healthy and showed no signs of toxicity throughout the 90-day experimental period.

Weight parameters

There were continuous increases in body weight of rats across the groups, however, significant ($p > 0.05$) difference was not observed between the control and the MEPS-treated groups throughout the duration of the study.

The relative weight of the vital organs (liver, kidney, heart and lung) did not also show significant difference ($p > 0.05$) between the control and the groups treated with MEPS.

Hematology

The hematological indices (HB, PCV, RBC, MCH, MCHC and MCV) of rats treated with different doses of MEPS (2.5, 5.0 and 10.0 mg/kg feed) did not vary significantly ($p > 0.05$) from those of the control group.

Liver marker enzymes

Alanine aminotransferase

The results showed that throughout the duration of the study, there was no significant difference ($p > 0.05$) in ALT activity between the control group and the extract-treated groups (Figure 1).

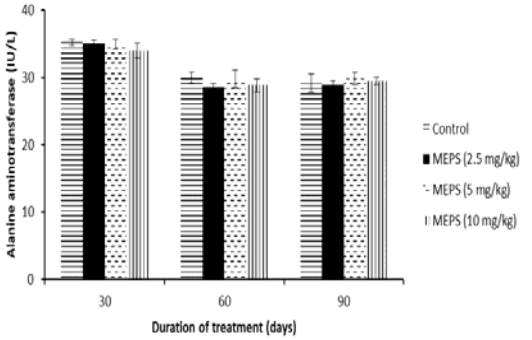

Figure 1: Effect of 90 days administration of MEPS on Alanine aminotransferase of rats

Aspartate aminotransferase

On the 30th and 60th days AST values in MEPS-treated groups did not vary significantly ($p > 0.05$) from those of the control group. However, on day 90, MEPS (5.0 and 10.0 mg/kg feed) significantly ($p < 0.05$) increased AST activity of the extract-treated groups with respect to the control (Figure 2).

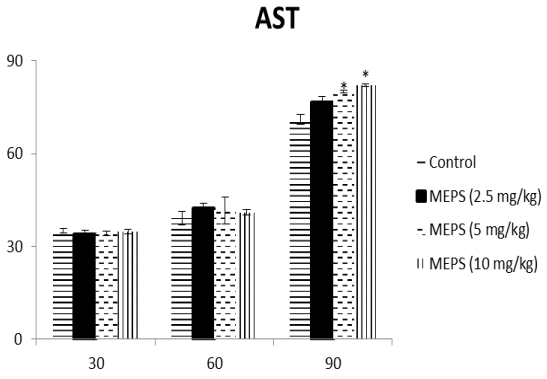

Figure 2: Aspartate aminotransferase of rats fed with MEPS for 90 days. *$P < 0.05$ when compared with control

Alkaline phosphatase

Chronic (90 day) administration of MEPS did not produce any significant difference ($p > 0.05$) in the level of ALP in the treated rats, when compared to control (Table 1).

Total bilirubin

The extract, at all the doses tested, caused significant ($p < 0.05$) reduction in total bilirubin in the treated groups, when compared to control on days 30, 60 and 90 (Figure 3).

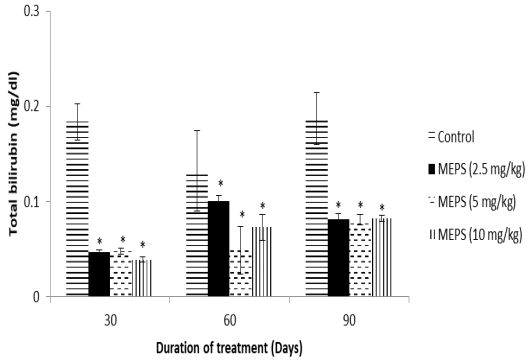

Figure 3: Effect of MEPS on total bilirubin of rats following chronic administration**.** **P < 0.05* when compared with control

Serum total protein concentration

The result revealed that throughout the duration of the study, there was no significant difference ($p > 0.05$) in total protein between the control group and the groups treated with MEPS (Table 2).

Serum urea

The result showed that on day 30, the extract at all doses tested did not produce any significant difference ($p > 0.05$) in the level of urea in the treated groups with respect to the control. However, on days 60 and 90, MEPS (5.0 and 10.0 mg/kg) significantly ($p < 0.05$) increased urea concentration in the treated groups. (Figure 4).

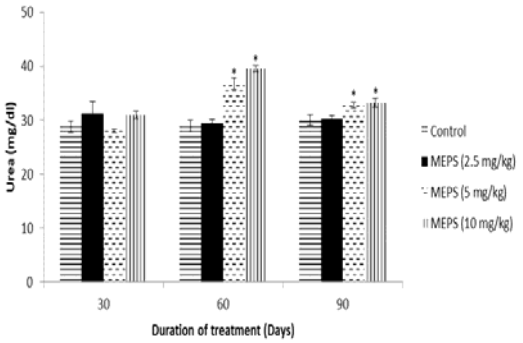

Figure 4: Effect of chronic administration of MEPS on serum urea of albino rats; **p < 0.05* when compared with control

Serum creatinine levels

There was no significant ($p > 0.05$) difference on days 30 and 60 in the level of creatinine between the control and the MEPS-treated groups. However, on day 90, all doses of MEPS caused significant ($p < 0.05$) increase in creatinine concentration in the treated groups, with respect to the control (Table 3).

Table 1: Alkaline phosphatase of rats given MEPS in feed for 90 days

Group	Treatment	ALP (IU/L)		
		Day 30	***Day 60***	***Day 90***
1	Control (Feed without extract)	28.19 ± 0.70	30. 53 ± 1.05	28.51 ± 2.54
2	MEPS (2.5 mg/kg in feed)	29.06 ± 0.70	30.30 ± 0.77	27.87 ± 1.13
3	MEPS (5.0 mg/kg in feed)	30.68 ± 0.31	31.26 ± 0.87	28.78 ± 1.34
4	MEPS (10.0 mg/kg in feed)	28.83 ± 0.31	28.44 ± 1.22	26.85 ± 0.77

Table 2: Total protein of rats treated with MEPS for 90 days

Group	Treatment	Total protein (g/dL)		
		Day 30	***Day 60***	***Day 90***
1	Control (Feed without extract)	6.99 ± 0.64	6.97 ± 0.03	6.43 ± 0.14
2	MEPS (2.5 mg/kg in feed)	6.73 ± 0.55	7.46 ± 0.18	6.89 ± 0.03
3	MEPS (5.0 mg/kg in feed)	7.12 ± 0.70	7.20 ± 0.51	6.14 ± 0.04
4	MEPS (10.0 mg/kg in feed)	6.85 ± 0.47	7.33 ±0.07	6.86 ± 0.28

Table 3: Creatinine levels of rats fed with MEPS for 90 days

Group	Treatment	Creatinine (mg/dL)		
		Day 30	***Day 60***	***Day 90***
1	Control (Feed without extract)	1.29 ± 0.12	0.97 ± 0.10	0.95 ± 0.06
2	MEPS (2.5 mg/kg in feed)	0.94 ± 0.09	0.74 ± 0.01	1.42 ± 0.06*
3	MEPS (5.0 mg/kg in feed)	1.01 ±0.09	0.92 ± 0.07	1.12 ± 0.01*
4	MEPS (10.0 mg/kg in feed)	1.11 ± 0.14	0.85 ± 0.05	1.84 ± 0.05*

*$P < 0.05$ when compared to control

Histopathology

Throughout the 90 days treatment period, 2.5 mg/kg of MEPS produced no histopathological changes in any of the organs examined. The extract, at 5.0 mg/kg feed als caused no lesion on days 30 and 60 of the study, but on day 90, it caused degeneration of hepatocytes (Figure 6C) and tubular epithelial cells of the kidney (Figure 5 C). In rats treated with 10.0 mg/kg of MEPS, none of the organs showed any histopathological lesion on day 30. However, on day 60, there were pyknotic cells and necrosis of tubular epithelial of the kidney (Figure 5 B) and mild degeneration of hepatocytes (Figure 6 B), and on day 90, severe degeneration and necrosis of hepatocytes (Figure 6 D) and tubular epithelial cells (Figure 5 D) were observed.

DISCUSSION

The non-significant variation in hematological parameters of treated rats when compared to the control indicate that the extract, at the doses tested had no effect on the haematopoietic system of the rats. The body weight gain recorded in all the MEPS-treated groups as well as the control group is attributable to normal growth of the rats with age.

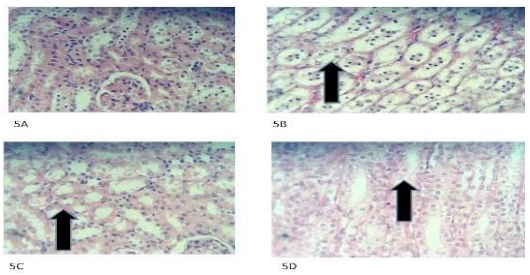

Figure 5: Photomicrograph of sections of the kidney of rats following 90 days treatment with MEPS (H&E, ×400). A: Normal kidney, control (day 90); B: Histologic section of the kidney showing necrosis of tubular epithelial cells with pyknotic cells (arrow) in rats treated with MEPS (10 mg/kg), on day 60; C: Arrow pointing at degeneration and necrosis of tubular epithelial cells of kidney in rats that received MEPS (5.0 mg/kg), on Day 90. D: Histologic sections of the kidney showing severe degeneration and necrosis of tubular epithelial cells (arrow) in rats treated with MEPS (10.0 mg/kg), on day 90

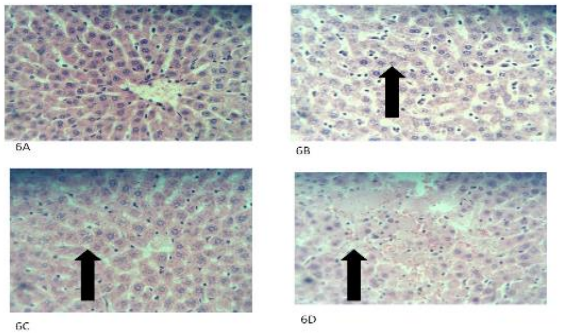

Figure 6: Photomicrograph of sections of the liver of rats after 90 days treatment with MEPS (H & E X400). **A:** Histologic section of the liver showing normal liver architecture of rats in control group on day 30. **B:** Arrow pointing at mild degeneration of the hepatocytes in rats treated with MEPS (10 mg/kg), on day 60. **C:** Histologic section of the liver showing mild degeneration of hepatocytes (arrow) in rats that received MEPS (5.0 mg/kg), on day 90. **D:** Severe degeneration and necrosis of the hepatocytes (arrow) in rats treated with MEPS (10.0 mg/kg), on day 90

The liver is the principal organ responsible for detoxification of drugs and xenobiotics [11], hence the levels of serum liver biomarker enzymes (ALT, AST and ALP) are vital indicators of hepatotoxicity. For instance, increase above the normal range of ALT and AST indicate damage of hepatic tissue and compromised permeability of hepatocytes membranes [12,13]. On day 90 of this work, MEPS (5.0 and 10.0 mg/kg) provoked significant increase in AST level, indicating that chronic administration of MEPS at high doses may lead to hepatocellular damage. This is supported by the degeneration of hepatocytes observed in the histopathology.

It is known that bilirubin is a product of the heme component of red blood cells and that the liver plays the role of conjugation and eventual excretion of bilirubin [14]. In this study, total bilirubin was significantly lowered in the treated groups by all doses of MEPS. It may have been that MEPS enhanced hepatic function with respect to conjugation and excretion of bilirubin. It may also be that the extract caused reduction in the rate of heme degradation, hence lowering the concentration of unconjugated (indirect) bilirubin in the rats.

Proteins are synthesized in the liver and form the major portion of dissolved substances in the plasma. Serum albumin or total protein level therefore reflects the liver's synthetic function [15]. In this study, the concentration of total protein in the extract-treated rats did not vary significantly from that of the control, suggesting that the live synthetic function was not significantly affected by MEPS throughout the experimental period.

Creatinine and urea are important indicators of kidney function [16]. The significantly higher levels of urea and creatinine on day 90 of the study, coupled with the observed histopathological changes in the rats' kidney suggest that chronic administration of MEPS may interfere with renal function.

CONCLUSION

The results of this work reveal that prolonged administration of *Pterocarpus santalinoides* methanol leaf extract at a low dose of 2.5 mg/kg feed causes no toxic effect in albino rats. However, there are indications that higher doses (5.0 and 10.0 mg/kg feed) of the extract, when given to albino rats beyond 30 days leads to nephropathy and hepatocellular damage. Further studies are required to unravel the effect of the plant on the functions of other organs/systems.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Isaac U Asuzu conceived and designed the study. Aruh O Anaga collected and analysed the data, while Kelechi G Madubuike wrote the manuscript. All authors read and approved the manuscript for publication.

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