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Investigation of Hepatic Toxicity of *Salacia lehmbachii*



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ABSTRACT

Herbs have been used traditionally as therapeutic agents since the beginning of human civilization in the whole world. *Salacia lehmbachii* has a wide folkloric usage in Southeastern Nigeria and its toxic effects on hepatocytes are worth evaluating scientifically. This study assessed the toxic effects of aqueous (ASL) and ethanol (ESL) root bark extracts of *S. lehmbachii* on hepatocytes in albino rats. Defatted petroleum ether residue of the plant powder was Soxhlet extracted using water and ethanol. Thirty five healthy albino rats weighing 180-200g were randomly divided into seven groups, A-G (n=5). Group A (Control) received 2ml of distilled water, groups B, C and D received 250, 500 and 750mg/kg of ASL respectively while groups E, F and G received 250, 500 and 750mg/kg of ESL respectively. Administration was per oral using an orogastric tube for 28 consecutive days. At the end of treatment, blood samples were collected via cardiac puncture for biochemical analysis. Rats livers were excised, weighed and processed for histological examination. Data obtained were computed by one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison as post hoc. The results revealed that serum levels of ALT, AST, ALP, bilirubin, protein and albumin were not significantly ($P > 0.05$) changed as compared to control group. Liver cytoarchitecture was well preserved for lower doses except at 750mg/kg where there were mild changes which were again not significant. This study shows that the two extracts at the doses used were not hepatotoxic and lays credence to the plant being used in folkloric medicine in Southeastern Nigeria.

INTRODUCTION

The liver is the largest solid organ in the mammalian body, weighing on the average 1.2-1.5kg and receives about 30% of the body's cardiac output and thus serves as the largest reservoir for blood [1]. It performs diverse vital roles which include metabolic, biochemical, synthetic and excretory functions. The liver is often pruned to hepatobiliary pathology or hepatocellular damaged [2] and is ranked the 12th most common cause of death in adults in the U.S. [3]. Early stage of any hepatic disease of any origin is associated with inflammation which could be tender and enlarged [4].

Although research into herbal medicine is laudable because of its wide therapeutic window, easy accessibility and cheapness, some herbs have been known to damage the liver [5]. It then becomes imperative for serious scientific assessment of herbs on body organs to ascertain the level of toxicity before employing them therapeutically.

Salacia lehmbachii is a small woody plant of Celastraceae family with height of about 3m. The leaves are simple, ovate-oblong, acuminate, opposite and shining with yellowish flowers on auxiliary tubercles. The fruits are globose orange with unique large seed at the center and two to four seeds almost at the periphery of the pulp [6]. *S. lehmbachii* can be found in the tropical forest of Southeastern Nigeria and Cameroon [7] and water extract of root bark has been reported scientifically to possess analgesic and anti-inflammatory property [8], anti-abortifacient activities [9], antidiuretic property [10], and cholinergic property [11] in Wistar rats. Herbalists in Southeastern Nigeria use root decoction in curing gastrointestinal disorders and malaria fever. Considering the importance of liver in the human physiology, the organ was investigated in Wistar rats for potential toxic effect of *S. lehmbachii* treatment since there are great similarity and homology between the genomes of rats and humans [12].

MATERIALS AND METHODS

Collection and identification of plant material

The roots of *S. lehmbachii* were bought from Watt market in Calabar, Cross River State, Nigeria. The plant was identified in Cameroon National Herbarium (CNH), Yaounde, with Voucher No. 40730/SRF/CAM.

Preparation of the extract

The roots were washed with clean water to remove dirt and dried in an electric oven, thermostatically controlled at 40⁰C for 12 hours. The bark was obtained by striking the dry roots with a hammer and the pieces obtained were pulverized into a coarse powder with a hand operated grain mill (Corona[®], Columbia). The root bark powder was stored in an airtight container. The root bark powder was then Soxhlet extracted in a two-staged process starting with petroleum ether (99.9%, Sigma Chemical Limited, USA) at 65⁰C as the solvent for twelve hours to remove fat. The petroleum ether residue was dried, weighed and re-extracted with water at 100⁰C and ethanol at 60⁰C for 72 hours to give aqueous and ethanol extract solutions which were evaporated to dryness using a rotatory evaporator at a reduced temperature of 45⁰C in-vacuo. The solid extract in each case was weighed and conserved in a dried corner.

Experimental animals

Wistar rats weighing between 180-200g were gotten from the Animal House of the Department of Pharmacology, University of Calabar and used for this study. They were housed in plastic cages, each containing five rats. Each rat was given appropriate identification marks using diluted picric acid. The animals were acclimatized for seven days to normal laboratory conditions (relative humidity: 50±5%, temperature: 28±2⁰C and 12 hr of light-dark cycle) before the start of the experiment and maintained at the same conditions throughout the experimentation period. They had standard rat chow (Agro-Feeds, Calabar) and water *ad libitum*. The guidelines on Care and Use of Laboratory animals were observed (OECD, 2000).

Animal treatment

Thirty five rats were randomly divided into seven groups of five rats per group. The groups were labeled A to G. Rats in group A were the Control rats and received 2 ml of distilled water (vehicle), groups B-D received 250, 500 and 750mg/kg of ASL respectively while groups E-G received 250, 500 and 750mg/kg of ESL respectively. Administration was per oral via a gastric cannula carried out daily between 9am to 10am to play down the effect of circadian rhythm and lasted for 28 consecutive days. At the end of the experimentation period, the rats were weighed, anaesthetized in a chloroform fume chamber and blood collected by cardiac puncture into plain sample bottles. To obtain serum, the blood in plain sample bottles was left for 2 hours to clot

and then centrifuged at 3000 rpm for 10 minutes for the serum to separate. Serum was then extracted with syringe and 21G hypodermic needle and emptied into a clean tube for storage at -20°C. Stored frozen serum was used within 12 hours of preparation.

Evaluation of liver functions

Estimation of serum liver enzymes

Sera from rats in each experimental group were analyzed using commercial kits (Randox, England) for AST, ALT and ALP. The manufacturers' instructions in each case were followed. The serum levels of AST and ALT were estimated using the method of Reitman and Frankel (1957) with modifications while ALP level was determined using the colorimetric method of Deutsche [13].

Estimation of serum bilirubin levels

Diazo-sulfanilic acid method of determining total and direct serum bilirubin levels after Walters and Gerarde [14] was used.

Determination of total proteins and albumin concentration

Sera from rats in the different experimental groups were used for the determination. For total protein levels, Biuret method as earlier described by Reinhold, [15] was used while the Bromocresol Green method described by Spencer and Price [16] was used to estimate total serum albumin.

Determination of liver relative weight

The weight of rats was taken on the day of sacrifice. The liver was dissected out from the sacrificed rats, cleaned of blood in between filter papers and weighed. Relative liver weight was determined following the protocols of Yakubu *et al.* [17] as follows:

$$\text{Relative liver weight} = \frac{\text{Absolute liver weight (g)}}{\text{Weight of rat on sacrifice day (g)}} \times 100$$

Histological studies

The liver gotten as above was processed for histological examination following the methods of Avwioro [18]. Sections of 5 microns stained with Haematoxylin and eosin were examined using a light microscope (Olympus/3H, Japan). Photomicrographs of the slides were taken at different magnifications (100X and 400X).

Data analysis

SPSS version 20.0 software was used for data processing and values obtained from descriptive statistics expressed as means \pm standard error of mean (SEM). The data was analyzed adopting ANOVA with “Turkey’s multiple comparison post hoc tests to compare the level of significance between results from control and treated groups”. Differences were considered significant at P value of < 0.05 after Duncan et al.[19]

RESULTS

The effect of ASL and ESL on liver weights in Wistar rats are shown in Table 1. There was no significant ($P > 0.05$) change in the organ body weight ratio of treated rat liver as compared to control group. The serum liver enzymes AST, ALT and ALP in treated rats also showed no significant change as compared to control group.

The effect of ASL and ESL on rat total, direct and indirect bilirubin concentrations is shown in Table 2. Levels of albumin and total proteins in the treatment and control groups were not significantly different.

The effect of ASL on control rat liver tissue is shown in Plate 1. The liver cytoarchitecture was well preserved in all treated rats as revealed by normal hepatic cytoarchitecture with central vein (CV), hepatocytes (H) and sinusoids (S).

The effect of ESL on rat liver tissue treated with 750mg/kg is shown in Plate 2. Again normal liver cytoarchitecture was not much distorted.

All other plates analyzed showed no significant change in the cytoarchitecture of liver tissue and are not presented in this article.

Table 1. Effect of ASL and ESL on liver weights in Wistar rats

Study groups (n=5)	Weight of animal on sacrifice day (g)	Absolute liver weight (g)	Relative liver weight (g)
A (Control)	186.02±2.26	6.40±0.15	3.44±0.06
ASL:			
B (250mg/kg)	193.33±2.92	6.67±0.05	3.45±0.10
C (500mg/kg)	187.35±3.31	6.37±0.03	3.40±0.30
D (750mg/kg)	186.11±1.97	6.57±1.10	3.53±0.07
ESL:			
E (250mg/kg)	184.55±3.11	6.33±2.11	3.43±0.11
F (500mg/kg)	190.54±3.83	6.65±3.06	3.49±5.83
G (750mg/kg)	186.04±1.09	6.53±0.07	3.51±4.09

Values are mean ± SEM. ASL = Aqueous root bark extract of *Salacia lehmbachii*, ESL = Ethanol root bark extract of *Salacia lehmbachii*

Table 2: Effect of ASL and ESL on rat total, direct and indirect bilirubin concentrations in Wistar rats.

Study groups (n=5)	Total bilirubin (µmol/L)	Direct bilirubin (µmol/L)	Indirect bilirubin (µmol/L)
A (Control)	4.67 ±0.60	1.23 ±0.11	3.44 ±0.62
ASL:			
B (250mg/kg)	4.56 ±0.51	1.11 ±0.03	3.45 ±0.42
C (500mg/kg)	4.48 ±0.46	1.15 ±0.06	3.33 ±0.71
D (750mg/kg)	4.54 ±0.48	1.30 ±0.12	3.24 ±0.82
ESL:			
E (250mg/kg)	4.48 ±0.59	1.14 ±0.08	3.31 ±0.69
F (500mg/kg)	4.59 ±0.27	1.28 ±0.03	3.20 ±0.76
G (750mg/kg)	4.71 ±0.29	1.27 ±0.09	3.44 ±0.69

Values are mean ± SEM, ASL = Aqueous root bark extract of *S. lehmbachii*. ESL = Ethanol root bark extract of *S. lehmbachii*.

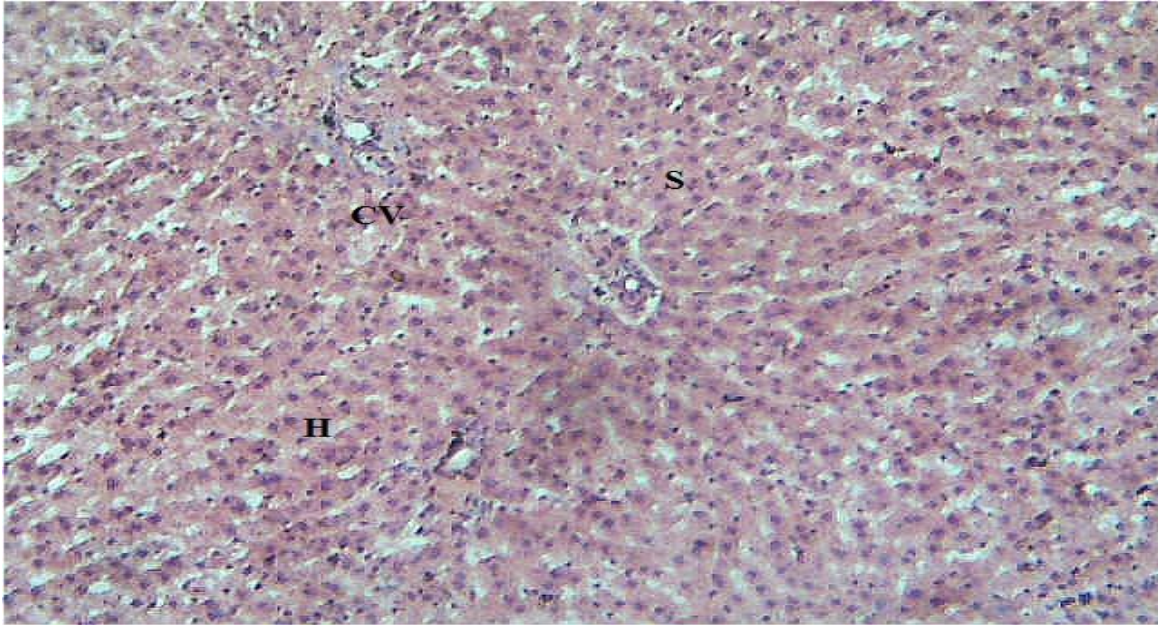


Plate 1: Photomicrograph of control rat liver tissue showing normal hepatic cytoarchitecture with central vein (CV), hepatocytes (H) and sinusoids (S).

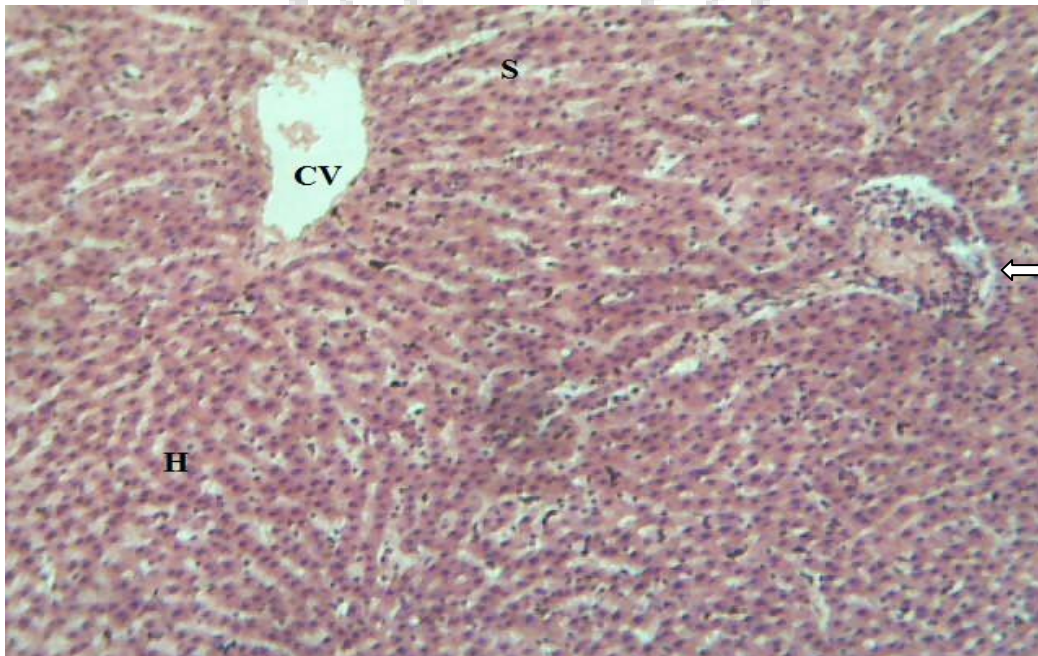


Plate 2 Photomicrograph of liver tissue of rat treated with 750mg/kg of ESL, x100. H & E stain. CV = Central vein, localized area of marked inflammatory cells (white arrow) H = Hepatocytes and S = sinusoids appear normal.

DISCUSSION

The liver is an important organ that regulates the body's metabolism particularly detoxification of medicines and other xenobiotics [20]. Some medicinal plants may affect the functions of the liver especially when they are orally administered. Liver function tests are helpful in establishing the presence of liver pathology, recognizing the type of disease, estimating the severity, assessing the prognosis and monitoring therapy. Serum levels of albumin assess the functional state of the liver, transaminases (ALT and AST) measure liver cellular integrity, ALP evaluates the connectivity with the biliary tract and the biosynthetic ability is evaluated with total protein, albumin and bilirubin levels [21]. In the present study, these parameters were not affected even after 28 days of treatment. It is known that if the ASL and ESL were hepatotoxic, they would have interfered with cytochrome P-450 oxygenase system thereby yielding free radicals which would bind to proteins or lipids abstracting hydrogen atoms from them and thus initiating lipid peroxidations. Lipid peroxidation would eventually damage hepatic plasmatic membrane resulting in leakage of hepatic biomarkers and thus increase in their numbers.

The relative weight of the liver also justified the non-hepatotoxic property of the *S. lehmbachii* extracts. This is because it has been observed that some plant extracts do cause cellular constriction and inflammation [22] resulting in increased relative weight. In the present study, no significant change in relative weight of the liver was observed. Most hepatoprotective plants have been shown to possess antioxidant property which mop up free radicals in organs. This, therefore, suggests the presence of alkaloids which have been identified in root bark of *S. lehmbachii* [8].

Adverse interaction of a plant extract with body organs could cause cellular constriction and inflammation which may be reflected in the relative weight (organ/body weight ratio) of the affected organs [22]. This study did not produce any alterations in the liver/body weight ratio suggesting that the extracts were nontoxic to the liver.

CONCLUSION

Therefore, aqueous and ethanol extracts of *S. lehmbachii* are not hepatotoxic. This corroborates the plant being used in folkloric medicine in Southeastern Nigeria.

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