



Research Article

Sub-acute Toxicity Profile of Methanol Leaf Extract of *Lophira lanceolata* (Ochnaceae) in Rats

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Abstract

Toxicological profiling is a crucial component of plant product evaluation. The aim of this study was to examine the sub-acute toxicity of the methanol leaf extract of *Lophira lanceolata*. *L. lanceolata* is a plant native to Africa and is widely used in Nigeria to relieve a wide variety of symptoms. Fresh leaves were collected, dried, ground and extracted by maceration in 70 % methanol for 72 hr. The oral LD₅₀ testing was evaluated and sub-acute toxicity studies were carried out using haematological parameters and liver enzyme markers as indices. Blood samples were collected by retro-bulbar route after fourteen days' administration of the extract and analyzed for alteration in haematological parameters and serum liver enzymes level. Results revealed no significant ($p > 0.05$) alteration in the levels of serum liver enzymes, viz; Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST) after 14 days of administration of the extract. The lymphocytes and total white blood cell counts were increased in a non-dose dependent manner. The weights of the liver, kidney and heart were not significantly ($p > 0.05$) altered. The body weights, food and water consumption did not change significantly following the extract administration. The study revealed the relative safety of the leaf extract of *Lophira lanceolata*.

Keywords: *Lophira lanceolata*; Liver Enzymes; Lymphocytes; Sub-Acute Toxicity; White Blood Cells

Introduction

An estimated 80 % of the world population depends on traditional medicine and plant-derived medicines for health care [1]. Intensive research on medicinal plants for bioactivity and lead compounds that could be developed into pure drugs for the treatment of ailments plaguing humans are being carried out in many laboratories worldwide [2]. The increasing consumption of natural products from plants as medicines and health supplements emphasizes the need for toxicity evaluation of medicinal plants. Efforts are being made in the area of herbal medicine to identify the bioactive compounds, elucidate their molecular structures, and establish their mechanisms of action and potential toxicological profile.

Lophira lanceolata is a wild oil seed plant from savannah regions which grows up to 12 m tall with twisted short branches. The fruits and seeds are rich in oils mainly polyunsaturated fatty acids such as α -linoleic (>30 % w/w) and arachidonic (>14%

w/w) acids [3] used as edible oils, in cosmetics, soap making and for medicinal purposes. Traditionally, its leaves are used to treat stomach pain and to control cough [4]. The leaves are natural aphrodisiac and fertility enhancer in males [5], and the infusion is used in treating malaria and jaundice [6]. The plant has been reported to have antidiabetic and antilipidemic [7], antiplasmodial and antioxidant [8], and anthelmintic. [9] activities. The leaves were found to contain flavonoids, anthraquinones, carbohydrate, glycoside, phenols, saponins, steroids, tannins, and free reducing sugar [10]. Considering the wide folkloric uses, as well as the reported biological activities of the leaf, this study was aimed at investigating sub-acute toxic effect of leaf extract of *Lophira lanceolata* employing some biochemical and hematological parameters.

Materials and Methods

Chemicals, Solvents and Reagents

All the chemicals and reagents used for this experiment were of analytical grade products of May and Baker (England) and Merck, Darmstadt (Germany).

Animals

Adult albino rats of either sex (100-150 g), obtained from the Animal House Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka were used for this study. Animal studies were conducted in compliance with the National Institute of Health Guide for Care and use of Laboratory Animals (Pub. No 85-23, revised 1985), and in accordance with the University of Nigeria Ethical Committee on the use of laboratory animals. The animals were housed in a well-ventilated room with a 12/12 h light/dark condition and ambient room temperature. They were maintained on standard feed pellets and water ad libitum throughout the duration of the experiment.

Equipment

Heparinized and non-heparinized capillary tubes, plastacine, test tubes, sample bottles, UV Spectrophotometer (Merck, Germany), Incubator, Micro capillary tube (Marrenfeld, Germany), Microheamatocit Centrifuge (Hawksley, England), Microheamatocit reader (Hawksley, England), Heamocytometer Set, Diluting Pipette (Hawksley, England), Automatic pipette (Superfit equipment Anies), Haemoglobinometer kit (Morienfeld, Germany), Laboratory Tally Counter (Clay Adams, New Jersey), light Microscope (Leica Inc, USA), Differential Cover Slips (Surgifriend Medicals, England).

Collection

Fresh leaves of *Lophira lanceolata* were collected in May 2013 from Nsukka, Nsukka Local Government Area of Enugu State, Nigeria. The plant was identified by Mr. Alfred Ozioko of International Center for Ethnomedicine and Drug Development (Inter CED) Nsukka.

Extraction

The leaves of the plant were air-dried at room temperature and ground into powder using a grinder (ADDIS Nigeria). The powdered material (2.37 kg) was macerated with 4.5 liters of 70 % methanol for 72 h with constant shaking. The resultant mixture was filtered using Whatman No. 1 filter paper and the filtrate was concentrated to dryness in vacuum at 40°C using rotary evaporator. This gave a yield of 108.81g (4.59 % w/w).

Sub-acute Toxicity Study

The animals were fasted overnight and were divided into four groups of five per group and treated as follows. Group 1 rats served as control and were given distilled water (10 ml/kg). Groups 2, 3 and 4 received 100, 400 and 1000 mg/kg of the extract administered orally for two weeks and the animals were regularly checked for any signs of toxicity. On day 0 and 14, blood was collected and subjected to hematological and biochemical tests.

The total white blood cell counts, and differential white blood cell counts were carried out according to the method of Docie and Lewis [11] while the assays of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were performed as described by Retaman and Frankel [12], while Alkaline Phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method for *in vitro* determinations using Quimica Clinica Applicada (QCA) test kit, Spain [12].

Body Weight, Food Intake, Water Consumption and Mortality

Body weights were measured before the treatment, and on days 7 and 14 post treatment. Food and water intake were monitored. The animals were observed for possible physical and behavioural changes and mortality within the experimental period [13].

Organ Weights

The heart, liver and kidneys were carefully excised, examined macroscopically and weighed.

Statistical Analysis

Results were expressed as mean \pm SEM. Data obtained were analyzed by one-way ANOVA and subjected to Dunnet Post Hoc test using Graph Pad Prism Version 5. Differences between means were accepted significant at $p < 0.05$.

Results

Effect of *L. lanceolata* Leaf Extract on ALT, AST and ALP in Treated Groups of Rats

Treatment with the methanol leaf extract of *L. lanceolata* did not cause any significant ($p > 0.05$) increase in serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase (Table 1).

Treatment Group	Dose (mg/kg)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group 1	-	34.87 \pm 1.24	81.13 \pm 1.28	305.3 \pm 23.19
Group 2	100	36.27 \pm 0.97	80.86 \pm 2.73	343.4 \pm 5.88
Group 3	400	40.87 \pm 1.68	84.27 \pm 1.22	348.2 \pm 6.37
Group 4	1000	37.19 \pm 2.9	81.28 \pm 2.71	319.3 \pm 45.99

The results are expressed as mean \pm standard error of mean (S.E.M), n=5.

Table 1: Effect of the extract on ALT, AST and ALP.

Effect of the Extract on Total White Blood Cell Count and Differential Leucocytes Counts

Hematological analysis revealed no significant ($p>0.05$) change in the hematological parameters of the treatment groups as compared to the control group (Tables 2 and 3).

Treatment Group	Dose (mg/kg)	TWBC Count ($10^9/L$)
Group 1	-	19460 ± 2573
Group 2	100	17738 ± 1274
Group 3	400	20400 ± 2042
Group 4	1000	22025 ± 2236
The results are expressed as mean ± standard error of mean (S.E.M), n=5.		

Table 2: Effect of the extract on Total White Blood Cell Count.

Treatment Group	Differential Leucocytes Counts ($10^9/L$)					
	Dose (mg/kg)	Basophils	Eosinophils	Neutrophils	Monocytes	Lymphocytes
Group 1	-	0.4000 ± 0.2449	2.200 ± 0.3742	16.80 ± 2.0100	1.400 ± 0.2449	79.20 ± 1.9340
Group 2	100	0.2500 ± 0.2500	1.750 ± 0.2500	13.50 ± 1.3230	1.250 ± 0.4187	83.25 ± 1.3770
Group 3	400	0.4000 ± 0.2449	2.600 ± 0.5099	14.20 ± 2.0830	1.600 ± 0.2449	81.20 ± 2.0350
Group 4	1000	0.5000 ± 0.2887	2.000 ± 0.4082	21.50 ± 3.4280	1.500 ± 0.2887	74.50 ± 3.7530
The results are expressed as mean ± standard error of mean (S.E.M), n=5.						

Table 3: Effect of the extract on Differential Leucocytes Counts.

Effect of the Extract on Gross Morphology

The animals were healthy with no differences being noted with respect to the control group. No significant changes were observed in the body weight of treated groups as compared to control and no mortality was observed during the experimental procedures. Oral administration of the extracts did not produce any symptoms of toxicity in rats. There were no deaths or any obvious signs of toxicity within the period. There was no evidence of changes in the skin, fur, eyes, sleep, salivation, faecal output and gross behaviour. Feed and water consumptions did not significantly alter, and the liver, kidney and heart weights were not significantly affected by treatment with the extract (Table 4).

Extract	Dose (mg.kg)	RLW	RKW	RHW
Group 1	-	4.50 ± 0.41	0.49 ± 0.01	0.45 ± 0.03
Group 2	100	3.86 ± 0.12	0.45 ± 0.02	0.42 ± 0.02
Group 3	400	4.00 ± 0.40	0.45 ± 0.26	0.44 ± 0.04
Group 4	1000	4.13 ± 0.33	0.47 ± 0.28	0.48 ± 0.05
The results are expressed as mean ± standard error of mean (S.E.M) RLW= Relative liver weight; RKW= Relative kidney weight; RHW= Relative heart weight				

Table 4: Effect of the extract on gross morphology.

Discussion

Liver function tests are useful in the evaluation of hepatic dysfunction. Some of the biochemical markers usually considered are serum bilirubin, alanine aminotransferase, aspartate aminotransferase, and ratio of aminotransferases, alkaline phosphatase, gamma glutamyl transferase, 5' nucleotidase, ceruloplasmin and α -fetoprotein [14]. These enzymes and the end products of metabolic pathways are very sensitive and their elevated levels in the serum may serve as indication of liver damage. Predominantly raised alkaline phosphatase represents the cholestatic pattern of biliary pathology, while predominantly raised alanine and aspartate aminotransferases represent the hepatocellular pattern of hepatocellular pathology [15]. Beyond the liver function tests, prothrombin time provides another marker of liver synthetic function and a low platelet count suggests portal hypertension.

In many developing countries, herbal medicines continue to receive attention as alternatives to synthetic Pharmaceutical products [16] and are generally considered safe and effective. In addition, bioactive compounds isolated from these herbal products are generally considered safe and used as over-the-counter products. Administration of these herbal medicines for a long period without expert delineating their potential side effects might be hazardous. [17].

In the present study, oral administration of the methanol leaf extract of *L. lanceolata* for 14 days did not elicit any signs of toxicity. Acute toxicity study of stem bark of *L. lanceolata* revealed oral LD₅₀ greater than 5, 000 mg/kg. [18] indicating the safety of this plant.

Drug induced toxicity is one of the leading causes of hepatotoxicity and the assessment of liver function serves as a diagnostic tool [19]. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are commonly assayed for in the serum to assess liver damage [20]. Unfortunately, extra hepatic injury such as muscle injury, can also lead to elevations in ALT, making ALT not entirely hepato-specific [21]. Despite the fact that extrahepatic injury can lead to increase in ALT, serum ALT remains the most widely used and universally accepted biomarker for liver damage, [22]. Oral administration of the methanol leaf extract of *Lophira Lanceolata* did not exert any significant change in the activities of AST, ALT and ALP enzymes suggesting no deleterious effect on the liver.

The extract did not have any significant effect on the lymphocytes and total white blood cell counts, thus, the extract is devoid of any serious inflammation or damage to body cells, tissues and organs.

Organ weight changes can be sensitive indicators of target organ toxicity, and significant changes in organ weights may occur

in the absence of changes in other pathologic parameters [23]. In this study, the extract did not cause any significant change in the weight of the three vital organs, indicating the absence of target organ toxicity. From the previous studies 100 mg/kg of the extract has been found effective in malaria [8] and can be used for clinical studies and treatment. Moreover, since the LD₅₀ is above 5 g/kg, a dose up to 200 mg/kg is still acceptable.

Conclusion

These results strongly suggest that the leaf extract of *L. lanceolata* is safe and well-tolerated and devoid of deleterious effects on the vital organs.

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