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Pharmacological studies on the renal and hepatic effect of methanol leaf extract of *Lupinus arboreus* in rats.

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ABSTRACT

Objective: To investigate the renal and hepatic effect of methanol leaf extract of *Lupinus arboreus* using experimental rats.

Methods: Three groups comprising five rats each were used.

Group II and III received 50 and 100 mg/kg of extract respectively. Group I served as negative control and received only normal saline (5 ml/kg). All administration was done once daily for 28 days. Urea and creatinine for renal effect were determined using Quimica Clinica applicado (QCA Test Kit, Spain);

while hepatic marker enzymes were evaluated using Assay Kits (Randox Laboratories Ltd., United Kingdom BT 294 QY). Histopathological evaluation was carried out using light microscopy. Student's t-test, ANOVA and Turkey-Kramer test were employed to assess significance of difference due to administration of extract and the control. Results: Treatment with extract did not produce significantly ($P > 0.05$) changes in the hepatic marker enzymes when compared with the control. The mean creatinine levels showed non-significantly ($P > 0.05$) differences when compared with the control. At 100 mg/kg, the extract exhibited significantly ($p < 0.05$) elevation of blood urea levels, as well as urea: creatinine ratio, compared with the negative control. Histopathological assessment revealed that the liver architecture was preserved and no occurrence of structural changes in the kidneys. Conclusion: The leaf extracts of *Lupinus arboreus* are devoid of deleterious renal and hepatic effects.

Keywords: *Lupinus arboreus*, Chikadoma, leaf extract, renal and hepatic effects.

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Introduction

Age-long use of medicinal plants especially in developing countries has encouraged research into pharmacologic activities of plants secondary metabolites. No doubt, the practice has gained continued grounds making herbal medicine an inevitable global discourse^[1], and has improved modern pharmacotherapeutics around the world^[2].

The phytomedicines are now available in most countries as food supplements^[1]. Studies have established indeed, that despite essential contribution to healthcare delivery particularly in developing countries for reasons mainly of low cost and availability, some plants species are potentially deleterious to vital organs such as kidneys and liver^[3,4,5]. Regarding the potential utility of plants for disease prevention and treatment, it is only when toxicity study data are compared to the evidence for beneficial health effects can a balanced judgement be made^[1]. The versatility of the use of *Lupinus arboreus* in the management of various disease conditions and safety/toxicity consideration, informed the choice for this study.

Lupinus arboreus referred to as Yellow bush belongs to the family Fabaceae^[6] It is known as "Chikadoma"^[7] in Igbo, South-eastern Nigeria, named after a lead researcher Dr. Chika Ohadoma, who pioneered extensively work on the novelty study of this plant^[8] A bushy shrub up to 1.8 m tall, *L. arboreus* is an ornamental plant recognisable with purple white colours blended with the bright yellow, sweet-welling flowers^[9]. The methanol leaf extract and fractions, have been reported to have a plethora of phytochemicals^[10,11,8] including flavonoids, saponins, tannins, glycosides, steroids, alkaloids and terpenoids; exerts antinociceptive and anti-inflammatory effect^[7] as well as antimicrobial effect^[12]. This present work investigated the renal and hepatic effects of the leaf extract of *L. arboreus* in rodents.

Materials and Methods

Plant material

Fresh *L. arboreus* leaves were obtained from Owerri, Imo State, Nigeria. The authentication of the leaves were done in the Department of Pharmacognosy, Madonna University, Elele, Nigeria by Osuala FN. A voucher specimen (number M/PC.193/10) was deposited in the Department's herbarium. The leaves

were air-dried at room temperature for 28 days. The leaves ground to fine powder (2 kg) were extracted using absolute methanol (Sigma Aldrich, Germany) by cold maceration for 48 h. The crude methanol extract (CME), after filtration (whatman No. 1 filter paper) was concentrated using a rotary evaporator (RV 05 Basic IB, IKA, Staufen, Germany); further oven-dried and stored in a refrigerator (4 °C).

Animals

Fifteen (15) adult Wister rats (150-220 g) kept in the Laboratory Animals facility of Department of Pharmacology and Toxicology, Madonna University, Elele, Nigeria, were used in this study. The rats were maintained under standard laboratory conditions and had free access to standard rat chow (Vital Feeds Plc, Nigeria) and clean water. Prior to experimental use, the rats were transferred to work area and allowed two weeks of acclimatization. In this study, all animals were handled according to international guidelines and ethics^[13]

Experimental design

The animals were randomly selected into three groups (I-III) comprising five rats each.

Group I received normal saline (5 ml/kg) only and served as normal control.

Group II were treated with 50 mg/kg of extract

Group III were treated with 100 mg/kg of extract

The period of administration through orogastric cannula was once daily for 28 days. At the end of 28 days, blood was collected from all the animals by retro-orbital puncture under either anesthesia. Blood was put into plain tubes for serum biochemistry in which sera were separated as soon as possible and stored frozen prior to analysis. Under ether anesthesia, the kidneys and liver were excised, washed in cold physiological saline and fixed with 10 % formalin for histopathological investigation.

Biochemical assay

For renal activity, urea and creatinine levels were evaluated using the protocol in Quimica Clinica aplicado (QCA) Test kit.

For liver activity, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphate (ALP) levels were evaluated using Assay kits (Randox Laboratory Ltd, United Kingdom BT 294 QY). The principle was based on colorimetric measurement.

Histopathological evaluation

The formalin-fixed liver and kidney tissues were processed, paraffin wax embedded and microtome sections (5-6m) made from them. These sections were stained with Haematoxylin and Eosin for light microscopy.

Statistical Analysis

Data were analyzed with Student's t-test from statistical package for social science (SPSS) version 15. The results were expressed as mean standard error of mean (SEM), (n=5). Mean values of test groups were compared with those of control groups and the values were considered statistically significant when p-value is less than 0.05 ($p < 0.05$).

Results

Biochemical assay

Treatment with methanol extract of *L. arboreus* leaf (100 mg/kg) showed a significantly ($p < 0.01$) elevation of blood urea levels when compared with the negative control (Table I). There were no significantly ($p > 0.05$) differences in the mean creatinine levels of treated groups compared with the negative control. The urea: creatinine ratio was significantly ($p < 0.05$) increased in the 100 mg/kg doses of the extract.

Table II showed the mean serum liver enzyme levels of the treated groups and that of the baseline control. Treatment with 50 mg/kg of extract for alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) revealed mean enzyme levels of non-significantly ($p > 0.05$) difference when compared with the control. With 100 mg/kg of extract, there were non-significantly ($p > 0.05$) mean differences in liver enzymes compared to the negative control.

Histopathological evaluation

Histopathological assessment of liver tissues of the animals treated with extract of *L. arboreus* showed that the liver architecture was preserved. Similarly,

the histology of the kidneys did not reveal structural changes.

Discussion

The results obtained in this study showed that methanol extract of *L. arboreus* at 100 mg/kg dose increased blood urea levels significantly ($p < 0.01$) and produced significantly ($p < 0.05$) elevation of blood urea: creatinine ratio as well as normal non-significantly ($p > 0.05$) decrease in blood creatinine levels. Though the most common cause of an elevated blood urea, azotemia, (> 60 mg/dL) is poor kidney function, yet a serum creatinine level is a more specific measure of renal function^[14]. An elevated blood urea level in the presence of a relatively normal creatinine level may reflect a physiological response to a relatively decreased of blood flow to the kidney (Such as in dehydration or heart failure) without indicating any true injury to the kidneys. Also, an isolated elevation of blood urea may reflect excessive formation of urea without necessarily any compromise to the kidneys. It is probable that the extract could have a diuretic effect which could be a setting for dehydration and subsequent haemoconcentration. Enhanced metabolism of proteins may also increase urea production, as may be seen with high protein diets. *L. arboreus* has been reported to be rich in proteins and amino acids. It is highly nutritive and wholesome hence grown for fodder and come close to soybean in protein content^[15] It is possible therefore, that the extract increased the nitrogen balance of treated rats positively. The methanol leaf extract of *L. arboreus* at doses of 50 and 100 mg/kg exhibited no deleterious effects on the liver of rats. The presence of flavanoids and antioxidants corroborate previous studies which have shown that plants extracts containing such phytochemicals do possess hepatoprotective activities^[14, 18]. ALT is an enzyme present in hepatocytes which leaks into the blood, when a cell is damaged, where they can be measured^[16] AST is another enzyme associated with liver parenchymal cells. Though AST is raised in acute liver damage, yet not specific to the liver but present also in red blood cells, cardiac and skeletal muscles^[17] It is rather used as a cardiac marker^[18] ALP is an enzyme in the cells lining the bile ducts of the liver. Its level increases with large bile duct obstruction, intrahepatic cholestasis or infiltrative disease of the liver^[16]

The antioxidants property of *L. arboreus* has been reported^[7]. The ability of the extract to preserve liver and kidney histoarchitecture could be due to their ac-

tivity to stabilize cell membranes through free medical scavenging activities. This is in consonance with many plants in Fabaceae family^[14]. Natural flavanoids and polyphenolic compounds have been reported to exhibit protective and strengthening tendencies on the liver cells^[19, 1]

Conclusion

The leaf extract of *L. arboreus* was not deleterious to renal and hepatic activities of the experimental rats.

Conflict of interest

No conflict of interest declared.

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Table I: Blood urea and creatinine concentration in rats treated with methanol extract of *L. arborescens* leaves.

Group	Medication	BU (mg/dL)		
		SC (mg/dL)	urea	CR
I	Normal Saline (5 ml/kg)	16.503.48	0.900.11	18.31.34
II	Extract (50 mg/kg)	18.252.95	1.150.15	15.91.06
III	Extract (100 mg/kg)	26.752.29**	0.850.15	31.51.14*

Table II: Liver marker enzyme levels in rats treated with methanol extract of *L. arborescens* leaves.

Group	Medication	ALT (IU/L)		
		AST (IU/L)	ALP (IU/L)	ALT (IU/L)
I	Normal Saline (5 ml/kg)	19.008.15	31.009.05	56.409.95
II	Extract (50 mg/kg)	25.004.65	50.503.70	62.604.65
III	Extract (100 mg/kg)	30.803.50	41.605.45	51.207.45

n = 5, ALT = alanine transaminase, AST = aspartate transaminase, ALP = alkaline phosphate.