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Assessment of Serum Parameters After 28-day Oral Daily Administration of Methanol Leaf Extract of *Laggera aurita* (Linn) in Wistar Rats

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ABSTRACT

Lagerra aurita is widely used in traditional medicine to treat various ailments in African countries like Nigeria, Senegal and Ghana. It has been reportedly used for the treatment of fever, pain, epilepsy, dyspepsia and indigestion. The present study is aimed at assessing the sub-chronic toxicity of *Laggera aurita* crude extract in rats. A total of fifty rats were employed and divided into 5 groups of 10 animals each (5 males and 5 females). The animals were orally administered the extract at doses of 75, 150, 300 and 600 mg/kg daily for 28 days, thereafter, biochemical assay was conducted. Liver and kidney function tests revealed some significant ($p < 0.05$) changes especially at higher doses. Similarly, lipid profile indices and oxidative stress markers were not significantly changed as compared to the control. Also, estrogen and testosterone levels in male rats were not significantly ($p > 0.05$) different from the control. In female rats, while the estrogen and progesterone levels were also not significantly different from the control, there was a significant ($p < 0.05$) elevation of the testosterone level in all the treatment groups. Although some signs of toxicity and relative safety has been observed with the short-term use of *Laggera aurita* extract, its use should be recommended at lower doses.

Keywords: *Laggera aurita*, Relative safety, Toxicity, Serum parameters.

Introduction

Medicinal plants and their extracts provide limitless opportunities for discovery of new drugs.[1] However, the issue of standardization and the knowledge of extent of adverse effects associated with their use has been a major challenge.[2] Such plants have potentially unexpected effects and toxicity;[3] it is therefore important to obtain information through toxicity studies of these plants in order to improve on the safety and development of new drugs.[4]

Laggera aurita is an annual plant found mostly in tropical Africa and Southeast Asia; it is widely used in African countries like Nigeria, Senegal and Ghana. It has been reportedly used in traditional medicine for the treatment of fever, pain, epilepsy, dyspepsia and indigestion.[5] Preliminary anti-tuberculosis screening suggests some activity in *aurita* species.[6] Membrane stabilizing effect of the ethanol crude extract of *L. aurita* has been established.[7] The anti-hyperalgesic activities of *Lagerra aurita* extract [8] and its mosquito repellent properties[9] have been reported; analgesic and anti-inflammatory properties of the methanol extract were also reported.[10] Results from the anticonvulsant studies on the methanol leaf extract of *Laggera aurita* suggest its anti-epileptogenic properties.[5] *Lagerra aurita* is widely used in traditional medicine to treat various ailments, hench, the documentation of its toxicity and safety profile could provide additional information for its traditional use. Therefore, this research seeks to assess sub-chronic toxicity of methanol extract of *L. aurita* in rats.

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Materials and Methods

Plant material and preparation of extract

The leaves of *Laggera aurita* were collected from Dajin Kudingi Zaria LGA, Kaduna state and authenticated by Mallam Namadi Sanusi of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The sample was compared with a previously deposited specimen and a voucher number (No. 2002) was collected. The leaves were washed and air dried until a constant weight was obtained. They were then powdered after which 500 g of the powdered leaves was weighed and extracted with 2 L of methanol by soxhlet extraction. The extract was evaporated to dryness with a thermostat oven (DHG/910/1SA) at 40°C. The dried extract was weighed and stored in an air-tight container.

Animals

Wistar rats of both sexes (110 – 130 g) were acquired from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU), Zaria. The rats were kept under normal laboratory conditions with access to food and water. The animals were randomly selected, marked to allow individual identification and kept in their cages for 5 days prior to the experiment in order to allow for acclimatization to the laboratory conditions. Animals were used in compliance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (Publication no. 85-23, revised 1985).

Sub-chronic toxicity studies

Wistar rats were divided into five groups of ten animals each (five males and five females). Group I, II, III, and IV were orally administered the freshly prepared extract daily at doses of 75, 150, 300 and 600 mg/kg, respectively while group 5 which served as the control was administered distilled water. This was done continuously at about the same time daily for 28 days while the animals were observed for signs of toxicity and mortality. At the end of the experiments, animals were fasted overnight, weighed and anaesthetized with the aid of chloroform after which they were sacrificed by decapitation and blood samples were collected from the jugular vein of each rat in plain bottles for biochemical analysis.

Biochemical analysis

Blood samples collected in the plain bottles were centrifuged and the serum was analysed for liver enzymes, urea, creatinine, electrolytes, cholesterol, triglyceride, High-density lipoprotein (HDL), Low-density lipoprotein (LDL), testosterone, estrogen and progesterone with the aid of commercially available assay kits for each analysis. Glutathione peroxidase was determined using the method described by Rotruck,[11] sodium dismutase was determined using the method described by Martin,[12] malondialdehyde was estimated using the method of Farage[13] and catalase was determined by the method described by Aebi.[14]

Statistical analysis

Data were presented as Mean ± standard error of mean. The results were analyzed using one-way ANOVA and Dunnett's post hoc test with significance at $P < 0.05$.

Results and Discussion

Daily administration of the methanol leaf extract of *Laggera aurita* (MLLA) for 28 days did not produce observable signs of toxicity as well as mortality.

There was no significant change in the levels of creatinine, bicarbonate, chloride, catalase, malondialdehyde and sodium dismutase. However, there was a significant ($P < 0.05$) elevation of alkaline phosphatase (ALP) level in all treatment groups; alanine aminotransferase (ALT) was significantly ($P < 0.05$) lowered in all treatment groups; a significant ($P < 0.05$) reduction in aspartate aminotransferase (AST) level was recorded at 600 mg/kg and 300 mg/kg. Drug-induced liver damage can be classified as hepatocellular, cholestatic or mixed based on the pattern of biochemical injury.[15] The effect of the extract showed significant elevation of (ALP); lowering of AST and ALT. Liver plays an important role in metabolic processes as well as maintaining homeostasis.[16] Hepatic injury due to some toxic phytochemical constituents of plants and failure of the liver to eliminate these metabolic products results in distortion of the normal functioning of the liver.[16] Acute and chronic liver damage is often reflected by the abnormal liver enzyme level which could also indicate obstruction of bile flow.[15] Serum ALT and AST are associated with hepatocellular damage while serum ALP is a sensitive indicator in biliary cirrhosis, hepatitis and disease characterized by inflammation and bile obstruction.[16] A rise in serum ALP levels is usually a characteristic of cholestatic liver disease.[17] Therefore, the rise in ALP suggested that a possible cholestatic liver damage occurred at the doses tested. Cholestatic liver damage is a subtype of liver injury that is characterized by predominant elevation of alkaline phosphatase and bilirubin, thus, drug-induced cholestasis is secondary to administration of hepatotoxic agent.[18] Although elevation of liver enzymes occurs in diseased liver, these elevations can also be secondary to enzyme induction without pathology,[19] thus the lowering of the AST, ALT and elevation of ALP could be due to enzyme induction or inhibition.

Urea level was significantly ($P < 0.05$) lowered at 600 mg/kg, also sodium and potassium levels were elevated at 300 mg/kg and 600 mg/kg, respectively, although it was not dose-dependent. Evaluation of the kidney markers showed no significant change in creatinine level which is one of the main indicators of kidney damage; however, there was a significant decrease in the urea level at the highest dose. Urea is the major nitrogenous end product of protein and amino acid catabolism which is produced by the liver and distributed throughout the intracellular and extracellular spaces and subsequently filtered by the kidney.[20] High levels of blood urea nitrogen (BUN) is an indication of kidney damage whereas low levels of urea are seen in malnutrition, low protein diet, fluid excess, trauma, surgery,

opioids and anabolic steroids.[20] There was elevation of potassium and sodium levels at higher doses and could be due to dehydration. Potassium and sodium are principal electrolytes that maintain the acid-base balance and normal distribution of water in the cells; elevated level of sodium (hypernatremia) causes the intracellular fluid to move to the extracellular spaces leading to dehydration.[21] Potassium is the principal cation in the intracellular fluid while a small portion can be found in the extracellular fluid; one of its functions is maintenance of the acid-base balance. Elevated levels (hyperkalemia) are associated with kidney failure.[21] In this case animals were starved overnight and had no access to water thus the elevation of both sodium and potassium.

A significant ($P < 0.05$) reduction in both cholesterol and triglyceride levels were observed at 600 mg/kg and 300 mg/kg. HDL (high density lipoprotein) was significantly ($P < 0.05$) elevated at 75 mg/kg while LDL (low density lipoprotein) was significantly ($P < 0.05$) lowered at 75 mg/kg. Cholesterol and triglycerides levels were significantly reduced at higher doses, suggesting the extract's potential of reducing both cholesterol and triglyceride level. High levels of cholesterol and triglycerides are predisposing factors to cardiovascular diseases.[22] The low levels observed suggest that the extract has little or no adverse effect on the heart. Elevation of HDL and lowering of LDL further suggest that the extract may not have adverse effect on the heart.

A significant ($P < 0.05$) rise in glutathione peroxidase was observed at 300 mg/kg and 75 mg/kg which was not dose-dependent. Oxidative stress test indicated that there was no significant change in the oxidative markers compared to the control except in glutathione peroxidase (GPX). GPX is an enzyme dependent on micronutrient selenium; it plays a critical role in the reduction of lipid and hydrogen peroxide to their corresponding lipid alcohol and water, respectively. If GPX activity is reduced, more water will be present leading to tissue damage.[23] There are studies that suggest the association between diabetes and glucose intolerance with increase GPX activity and other studies suggest it is associated with decrease GPX

Table 1: Effects of Methanol Leaf extract of *Laggera aurita* on Liver Enzymes in Wistar Rats.

Treatment	Enzymes		
(mg/kg)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)
Distilled/water	7.70 ± 0.98	14.60 ± 1.05	28.10 ± 1.55
75	12.55 ± 1.84*	12.30 ± 0.91	22.20 ± 1.73*
150	14.55 ± 0.81*	12.20 ± 1.10	21.80 ± 1.39*
300	12.49 ± 1.58*	11.00 ± 0.93*	20.50 ± 1.83*
600	14.71 ± 0.99*	11.00 ± 0.79*	18.70 ± 1.43*

Data was analysed using one-way ANOVA and Dunnett's post hoc test.
*The mean difference is significant at $P < 0.05$, n = 10.
Dunnett tests treat one group as a control and compare all other group against it.
ALP = Alkaline phosphatase, ALT = Alanine transaminase, AST = Aspartate transaminase.

Table 2: Effects of Sub-Chronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Kidney Parameters of Wistar Rats.

Treatment	Parameter					
(mg/kg)	Urea (mg/dL)	Creatinine (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mg/dL)	Bicarbonate (meq/L)
Distilled/water	54.23 ± 1.69	1.24 ± 0.12	137.41 ± 5.28	10.73 ± 0.76	103.90 ± 4.72	25.80 ± 1.44
75	46.98 ± 2.06	1.19 ± 0.11	133.69 ± 4.58	13.06 ± 1.04	115.40 ± 3.65	24.00 ± 1.72
150	49.09 ± 2.97	1.35 ± 0.14	145.99 ± 5.45	11.88 ± 0.48	97.70 ± 2.63	26.00 ± 2.01
300	47.43 ± 2.07	1.11 ± 0.08	163.60 ± 6.14*	11.63 ± 0.65	108.30 ± 4.82	31.30 ± 2.43
600	45.50 ± 2.58*	1.10 ± 0.11	139.85±11.78	13.95 ± 0.73*	95.00 ± 3.12	25.90 ± 2.43

Data was analysed using one-way ANOVA and Dunnett's post hoc test.
*The mean difference is significant at $P < 0.05$, n = 10.
Dunnett tests treat one group as a control and compare all other group against it.

Table 3: Effects of Sub-Chronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Lipid Profile of Wistar Rats.

Treatment (mg/kg)	Parameter			
	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Distilled/water	229.37 ± 18.44	220.96 ± 39.67	110.88 ± 14.17	84.40 ± 10.94
75	252.29 ± 11.32	216.05 ± 38.08	153.80 ± 8.76*	36.16 ± 7.88*
150	250.29 ± 13.28	172.47 ± 28.91	143.59 ± 10.90	62.97 ± 11.54
300	157.90 ± 9.30*	53.74 ± 17.51*	111.01 ± 8.69	66.28 ± 11.03
600	176.89 ± 16.18*	34.52 ± 10.50*	86.27 ± 11.74	83.77 ± 11.68

Data was analysed using one-way ANOVA and Dunnett's post hoc test.
*The mean difference is significant at $P < 0.05$ level. n = 10.
Dunnett t-tests treat one group as a control, and compare all other groups against it.
HDL = low density lipoprotein, LDL = high density lipoprotein.

Table 4: Effects of Sub-Chronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Oxidative Stress Markers of Wistar Rats.

Treatment (mg/kg)	Markers			
	SOD (u/mL)	CAT (u/mL)	GPX (u/mL)	MDA (mmol/mgpr)
Distilled/water	25.53 ± 1.65	48.20 ± 4.10	39.0 ± 3.08	301.58 ± 30.4
75	29.40 ± 2.91	58.95 ± 4.33	47.64 ± 3.04	349.45 ± 64.1
150	31.71 ± 3.07	60.61 ± 5.28	53.68 ± 3.00*	342.74 ± 73.44
300	37.68 ± 2.88	62.46 ± 3.68	56.98 ± 4.01*	342.74 ± 36.52
600	38.33 ± 8.08	44.38 ± 4.03	38.12 ± 3.39	362.84 ± 30.26

Data was analyzed using one-way ANOVA and Dunnett's post hoc test.
*The mean difference is significant at $P < 0.05$ level. n = 10.
Dunnett tests treat one group as a control, and compare all other groups against it.
SOD = Superoxide dismutase, CAT = Catalase, GPX = Glutathione peroxidase, MDA = Malone dialdehyde.

Table 5: Effects of Sub-Chronic Oral Administration of Methanol Leaf Extract of *Laggera aurita* on Hormones of Wistar Rats

Treatment mg/kg	**Estrogen (pg/mL)**	**Testosterone (ng/mL)**	**Progesterone (ng/mL)**
Male			
Distilled/water	119.00 ± 1.15	12.60 ± 1.13	
75	119.00 ± 7.51	11.92 ± 0.36	
150	137.67 ± 23.95	12.53 ± 0.15	
300	143.33 ± 9.06	10.80 ± 1.75	
600	164.00 ± 1.15	14.18 ± 1.14	
Female			
Distilled/water	176.67 ± 19.33	2.10 ± 0.42	7.30 ± 2.50
75	171.67 ± 20.33	11.92 ± 0.17*	66.37 ± 2.77*
150	179.67 ± 19.33	13.65 ± 0.30*	20.75 ± 11.93
300	157.67 ± 12.67	11.28 ± 2.02*	19.83 ± 9.23
600	118.33 ± 6.33	11.55 ± 2.20*	4.67 ± 0.133

Data was analyzed using one-way ANOVA and Dunnett's post hoc test,
*. The mean difference is significant at $P < 0.05$ level. n = 5
Dunnett t-tests treat one group as a control, and compare all other groups against it.

activity.[23] High level of GPX increases antioxidant capacity which was observed in many tumour cells.[24] This suggests that the plant extract may have some antioxidant properties and could also have some potential for causing glucose intolerance rather than triggering oxidative stress damage. There were no significant changes observed in estrogen and testosterone levels in the male rats as well as estrogen and progesterone level in the female rats. Furthermore, the testosterone level of the female rats was significantly elevated in all treatment groups. Hormonal analysis was conducted to test for the effect of the plant extract on hormones in both male and female rats. High testosterone level in females is associated with conditions such as polycystic ovarian syndrome,[25] which is commonly associated with infertility in women, menstrual problems like amenorrhea, oligomenorrhea, and hair loss. This suggests that the extract can possibly have adverse effect associated with high testosterone level in females.

Conclusion

Assessment of the serum parameters of Wistar rats after 28-day oral administration of methanol extract of *Laggera aurita* suggests that the extract is relatively safe.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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