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TOXICOLOGICAL STUDIES OF ETHANOLIC LEAVES EXTRACT OF *SARCOSTEMMA VIMINALE*

Moussa Yahaya, *Angela Nnenna Ukwuani, Caleb Francis

Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.

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***Correspondence for Author**
Angela Nnenna Ukwuani
Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria.

ABSTRACT

The effects of *Sarcostemma viminale* leaves extract on the haematological, toxicological and histopathological indices were evaluated in rats. The extracts at a single dose of 5000 mg/kg did not produce any behavioural sign of toxicity or mortality in any of the animals tested during 14 days observation period and as such, LD_{50} of this plant was estimated to be greater than 5000 mg/kg. In the 28days repeated dose oral sub-chronic toxicity study, administration of 250, 500 and 1000 mg/kg body weight *Sarcostemma viminale* leaves extracts revealed no significant difference ($p>0.05$) in the haematological parameters. Analysis of biochemical parameters reveals no significant difference ($p>0.05$) in the extract treated groups when compared to the control group. The histopathological examination of the treated groups showed no visible lesions or signs of the liver damage while the kidney revealed tubular congestion and necrosis, focal haemorrhage and hyaline degeneration. These results suggest that the sub-chronic administration of ethanolic extracts of *Sarcostemma viminale* leaves has no marked toxic effect on the liver while unmasking the possible dangers of this plant to the kidney.

KEYWORDS: Toxicity, *Sarcostemma viminale*, leaves extracts, ethanolic.

INTRODUCTION

Herbal remedies have a therapeutic effect and are acceptable interventions for diseases and symptoms. Interestingly, demand for medicinal plants is progressively rising in industrialized nations as it is in developing countries.[1] The World Health Organisation (WHO) estimates that about 80% of the developing world‘s population meets their primary healthcare needs

through traditional medicine.[1,2,3,4] African indigenous herbal medicines are widely used throughout the African continent, despite an apparent lack of scientific evidence for their quality, safety and efficacy.[5] Most plants consumed locally in Nigeria have not been thoroughly evaluated for their toxicity profiles [6] including the plant of this study.

Though, herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life-style related disorders.[7] However, relatively very little knowledge is available about their mode of action and safety. The study of toxic or adverse effect of crude drugs of plant origin is essential in order to prove a guide to their safe usage and eventual standardization. This is especially important as traditional medicine practitioners often administer such preparations without regards to their possible adverse effects. The leaves of *Sarcostemma viminale* is known in Nigerian traditional medicine to promote milk production in animals and for treating dysentery, indigestion, and haemorrhoid in man. However, there is no scientific work on safety or toxicity of this plant. In this study, ethanolic leaves extract of *Sarcostemma viminale* (ELSV) is assessed *in vivo* for its toxicity.

MATERIALS AND METHOD

Identification of the plant

Identification of *Sarcostemma viminale* was done in the field by a botanist. The plant parts (leaves, stems-bark and roots) were collected from the wilds of Aliero Local Government Area of Kebbi State, Nigeria. Herbarium specimens (Voucher specimens No. 165) were prepared and deposited in the Herbarium, Botany Unit, Biological Science Department, Kebbi State University of Science and Technology, Aliero, Nigeria, where identity of the plants was confirmed by comparison with available voucher specimens.

Extraction of plant materials

The plant materials were open air dried under the shade and chopped into smaller pieces. The dried leaves were pulverized into moderately coarse powder. The powdered plant material (100 g) was macerated in ethanol in an air tight aspirator bottle for 72 hours. This was then filtered with the aid of sterile sieving cloth and evaporated using a Water bath at 45^{o}C. The dried extract collected was weighed, labelled and stored in an air tight bottle container.

Animals

White Wistar strain albino rats of both sexes, weighing averagely 100 - 210g were used for these study. The rats are purchased from the Animal House, Department of Biochemistry,

Ahmadu Bello University, Zaria and were transported to the Department of Biochemistry Laboratory, Kebbi State University of Science and Technology, Aliero, Kebbi State. They were allowed to acclimatise to 2 weeks with free access to drinking water and standard diet (Vital feeds, Jos, Nigeria).

Acute Oral Toxicity Study (LD_{50})

After acclimatization period, the acute oral toxicity study as described by Dixon.[8] was performed as per the OECD-423 guidelines (acute toxicity class method). Five (5) rats of either sex selected by random sampling technique were used for this study. The animals were fasted over night providing only water, after which ELSV was administered orally at a dose of 5000 mg/kg body weight to each rat at 48 hours interval respectively and subsequent observed for 14 days. The behavioral changes (abdominal constriction, hyperactivity, sedation, grooming), and body weight were observed for 14 days.

Sub-chronic Toxicity Study

Rats were divided into five (4) groups of five (5) rats each for sub-chronic toxicity study. ELSV extract was orally administered daily for 28 days. Group 1 serve as control receiving normal saline (5ml/kg) while Groups 2 to 4 served as ELSV treated Groups receiving (250, 500 and 1000mg/kg) bodyweight, respectively. The weights of the rats were recorded weekly. The rats were fasted overnight on the 28th and on the 29th day, weights were taken and blood samples were collected via cardiac puncture for further analysis.

Markers of toxicity

The following parameters were analyze from the blood samples collected at the end of the sub-chronic toxicity studies; Aspartate aminotransferase (AST).[9] Alanine aminotransferase (ALT).[9] Alkaline phosphatase (ALP).[10] Bilirubin.[11] Albumin.[12] Total protein.[13] and the haematological parameters were analyse using the method described by Dacie and lewis.[14]

Histopathological Studies

The liver and kidney were harvested, weighed and preserved in 10% formalin for histopathological analysis according to reported procedures of Aliyu *et al*.[15]

Statistics Analysis

Data collected in this study were expressed as Means ± Standard Deviation (SD) and subjected to analysis of variance (ANOVA) for accessing statistical significance. The

differences among experimental and control groups were determined using Turkey-Kramer and Dunnett Multiply Comparism Tests respectively.

RESULTS AND DISCUSSION

In this study, extraction process yielded 27.5% of ELSV. The limit dose of 5000 mg/kg body weight ELSV did not cause mortality nor show any clinical signs of acute toxicity in all of the five rats tested in the short term (48 hours) and long term (14 days) observatory period. The result showed the LD_{50} of ELSV is greater than 5000mg kg^{-1} suggesting that at the limit dose tested ELSV is essentially non-toxic and safe for oral formulation. Sub-chronic toxicity study after 28 days of daily administration of hydromethanolic extract of ELSV showed a non significant decrease in body weights of the control and treated animals of this study (Figure 1).

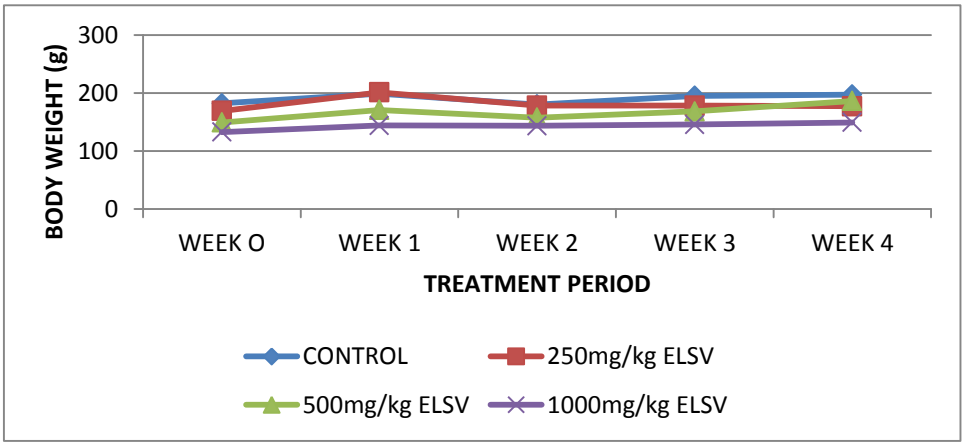

Figure 1: Effect of sub-chronic administration of ELSV on Body weight of rats.

Reductions in body and internal organ weights have been considered sensitive indices of toxicity after exposure to toxic substance.[16] In the present study, there was no significant difference ($p<0.05$) in the organ weight of extract treated groups compared to the control groups (Table 1).

Table 1: Effect of sub-chronic administration of ELSV on rat organ weight

Groups	Left Kidney	Right Kidney	Liver	Heart
Control	0.65±0.08	0.54±0.05	4.87±0.65	0.73±0.11
250mg/Kg	0.57±0.08	0.52±0.08	4.73±0.83	0.62±0.07
500mg/Kg	0.63±0.02	0.57±0.03	5.83±0.37	0.60±0.03
1000mg/Kg	0.53±0.12	0.51±0.10	5.55±1.73	0.56±0.12

Values are mean ± standard deviation (n = 4).

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals.[17,18] Assessment of the haematological parameters can be used to determine the extent of deleterious effect of extracts on the blood of an animal or determine blood relating functions of a plant extract or its product. Such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when the data are translated from animal studies.[19] In the present study, there were no significant differences ($p>0.05$) in the hematocrit, hemoglobin, Total Leukocyte Count (TLC), lymphocytes, neutrophils and platelets when compared to the control (Table 2). However, there was a non significant dose-dependent decrease in lymphocytes of the ELSV treated groups. Lymphocytes, the main effector cells of the immune system, usually show increase in activity in response to toxic environment.[20] Leucocytosis observed in the present study indicates a stimulation of the immune system which protects the rats against infection that might have been caused by chemical and secondary infections.[19]

Table 2: Effect of sub-chronic administration of ELSV on Hematological parameters.

Parameter	Control	ELSV 250 mg/kg	ELSV 500mg/kg	ELSV 1000mg/kg
Hematocrit (%)	39.75 ± 5.05	36.50 ± 0.57	39.00 ± 5.56	37.50 ± 13.12
Hemoglobin(g/dl)	13.25 ± 1.68	12.16 ± 0.19	13.00 ± 1.8	12.50 ± 4.37
TLC($X10^9$ cells /L)	2.45 ± 0.58	2.62 ± 0.45	2.86 ± 1.10	3.72 ± 1.28
Lymphocyte (%)	77.25 ±14.17	77.50 ± 1.91	74.00 ± 6.00	52.25 ± 19.50
Neutrophils(%)	22.00 ± 12.75	17.50 ± 3.78	20.66 ± 1.15	40.50 ± 22.17
Platelet($x10^9$/l)	150.00 ± 35.59	173.75 ±23.59	181.66 ±20.20	144.50 ±49.80

Values are Mean ± Standard deviation (n = 4). *Significant different ($p<0.05$) compared to control.

The result of hepatic biomarkers (AST, ALT and ALP, Bilirubin, albumin and protein) of liver function is shown in Tables 3. Biochemical indices monitored in the serum such as secretory substances of the liver can be used as 'markers' for assessing its functional capacities.[21] Plasma proteins can be used to examine specific biochemical functions and the general status of the body's protein metabolism while increased billirubin level reflects the depth of jaundice.[22] Increase in the level of AST, ALT and ALP reflects the structural and functional dysfunction of hepatocellular membrane or cell rupture, and thereby indicates liver damage. The results of this study revealed no significant difference ($P>0.05$) in all the biochemical parameters of ELSV treated groups as compared to the control respectively. Since serum biochemical parameters reflects an index of liver function, findings of this study

suggests that sub-chronic administration of ELSV has no hepatotoxic effects in treated animals.

Table 3: Effect of sub-chronic administration of ELSV on Biochemical parameters.

Parameter	Control	ELSV 250 mg/kg	ELSV 500mg/kg	ELSV 1000mg/kg
Total Bilirubin (mg/l)	0.65 ± 0.05	0.55 ± 0.12	0.67 ± 0.11	0.70 ± 0.12
Con. Bilirubin (mg/l)	0.15 ± 0.01	0.14 ± 0.02	0.17 ± 0.01	0.17 ± 0.01
Total protein(g/dl)	63.50 ± 3.41	53.25 ± 8.65	61.00 ± 2.64	60.20 ± 8.25
Albumin	29.50 ± 1.29	29.25 ± 7.36	30.00 ± 1.00	29.20 ± 3.63
ALP(m/l)	65.00 ± 8.16	59.25 ± 10.71	71.00 ± 2.65	73.20 ± 8.22
AST(U/l)	53.50 ± 8.06	50.25 ± 9.97	61.00 ± 2.00	65.00 ± 6.67
ALT(U/l)	59.00 ± 7.95	53.00 ± 6.97	67.00 ± 3.60	64.20 ± 8.07

Values are mean ± standard deviation (n =4). *Significant different (p>0.05) compare with control.

All groups treated with ELSV showed no significant damage of the liver while the kidney showed various degrees of tubular necrosis and congestion, glomerular and hyaline degeneration, and focal heamorrhage when compared with the control group (Figure 2 – 4). The result from histological screening was in agreement with the serum biomarkers as no apparent damage to the liver was observed in all the treated groups when compared with the control group. This further confirms the plant extract to be non toxic to the liver within the treatment durations. However, this finding also exposes the possible risk of kidney damage upon sub-chronic administration of ELSV.

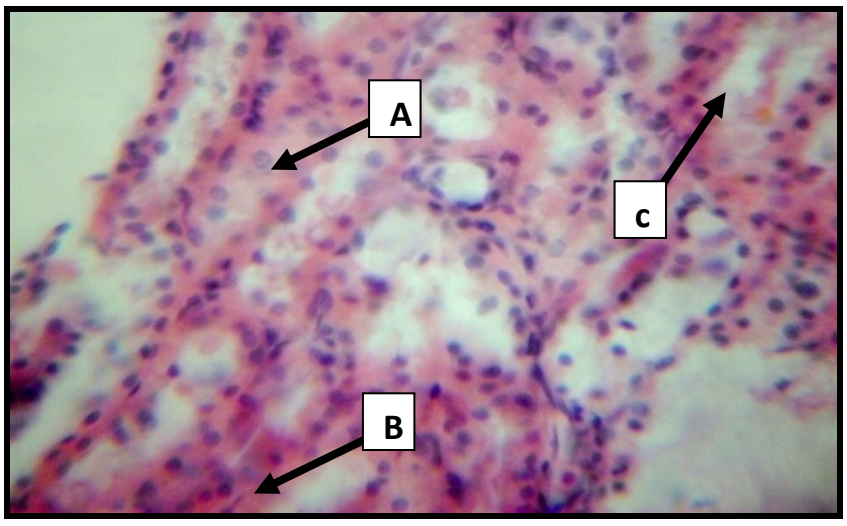

Figure 2: Photomicrograph of a group 2 (250mg/kg ELSV) rat's kidney. Showing Tubular necrosis (arrow A), focal haemorrhage (Arrow B) and slight congestion (arrow C). H&E Stained, Magnification x 40.

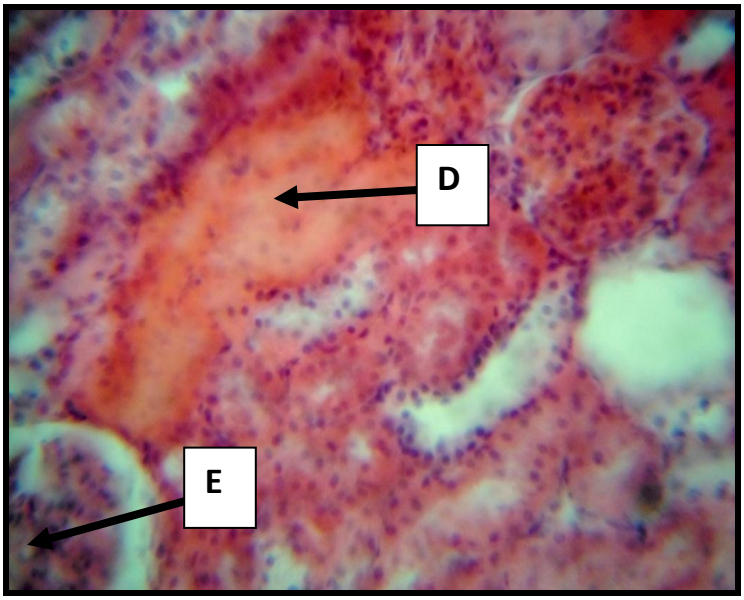

Figure 3: Photomicrograph of a group 3 (500mg/kg ELSV) Rat's Kidney.

Showing: Glomerular cellular degeneration (arrow D) and Tubular necrosis (Arrow E). H&E Stained, Magnification x 40.

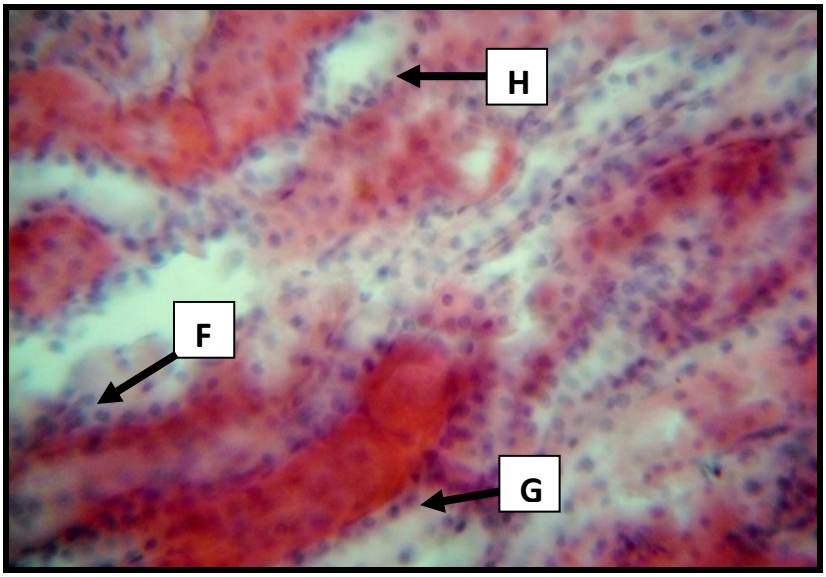

Figure 4: Photomicrograph of a group 4 (1000mg/kg ELSV) rat's Kidney.

Showing: Hyaline degeneration (arrow F), tubular congestion (Arrow G) and Vacuum (Arrow H). H&E Stained, Magnification x 40.

CONCLUSION

In conclusion, sub-chronic administration of ELSV showed no effect on the haematological indices, biochemical function and hepatic secretions while eliciting nephrotoxic effects.

However, further work is needed to confirm and justify the risk of kidney injury (via biochemical markers of toxicity) as this was not covered within the scope of this study.

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