https://dx.doi.org/10.4314/dujopas.v11i1a.29
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 11 No. 1a March 2025*

Evaluation of Histological Changes and Liver Function Parameters in Rats Exposed to Aqueous Extract of *Evolvulus alsinoides* Linn

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Abstract
The liver is a multilobed, largest organ in the body. Its functions include haematopoiesis, detoxification, productions of bile, plasma proteins and cholesterol, and storage of glycogen. Fresh Evolvulus alsinoides (dwarf morning glory) was procured, authenticated, dried under shade, pounded roughly and extracted in water using soxhlet extraction method. Fourty (40) young adult male albino Wister rats (weighing 110g-140g) were randomly divided into four (4) groups (I-V) of 10 rats each. Group I was designated as the control group. Groups II, III and IV were administered with 150 mg/kg, 250 mg/kg and 350 mg/kg respectively. After 28 days, the rats were euthanized using ketamine injection 75mg/kg. Blood samples were collected in plain bottles by cardiac puncture and liver function parameters were estimated from sera. While livers were fixed, processed, sectioned and mounted on glass slide for histopathological analyses. Data from this study were analysed using GraphPad Prism Software, version 9 (La Jolla, CA, USA) and presented as mean ± standard error of the mean. At 350 mgkg^{-1} significant decrease ($P<0.05$) was observed in plasma concentrations of ALP, ALT and AST when compared with the control group. Histologically, no conspicuous changes were observed when the livers of the treated groups were compared with that of the control group. Further research should be conducted at different doses of aqueous extract of Evolvulus alsinoides Linn in the rats and other species to ascertain such a significant decrease in plasma concentration of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase.

Keywords: ***Evolvulus alsinoides*, histology, liver function, rat**

INTRODUCTION
The liver is a multilobed, largest organ in the body (Bazira, 2023). It is one of the major target organs of toxicity (Diallo, 2020). Structurally, the parenchymal cells of the liver are arranged in sheets forming sinusoids radiating from the central vein (Liedtke *et al.*, 2013). Functions of the liver include haematopoiesis (during foetal period), detoxification, productions of bile, plasma proteins and cholesterol, and storage of glycogen (Giancotti *et al.*, 2019). The liver's rich blood supply and detoxification function make it a popular target organ for toxicity (Gu and Manautou, 2012). Liver function test involves the evaluation of plasma levels of the

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following parameters: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein (TP) (Sharma, 2022).

Alanine aminotransferase (ALT) is primarily produced by the cells of the liver alongside the cells of the heart, kidney and muscles. It catalyzes the reaction by which an amino group is transferred from an amino acid to a keto acid (Koper *et al*., 2022). High concentrations of serum ALT levels maybe an indicative of liver injury (Newsome *et al*., 2018; Sinn *et al*., 2022). Aspartate aminotransferase (AST) is an enzyme that is found in the cells of the liver, and also in the cardiac muscle, renal tissue, skeletal muscle and brain tissue (Kalas *et al*., 2021). Alkaline phosphatase (ALP) is present in the cytosol of hepatic cells. It is also found in cells of bone, kidney and placenta (Green and Sambrook, 2020). Increased plasma concentration of ALP enzyme results from heightened synthesis (Lowe *et al*., 2023). Albumin is the most abundant plasma protein produced by the liver, its physiologic functions, include anti-thrombotic actions, molecular transport, anti-inflammation, endothelium stabilisation, anti-oxidation, and the adjustment of capillary permeability (Sun *et al*., 2019). Albumin concentration falls in a variety of liver disorders (Jagdish *et al*., 2021).

Evolvulus alsinoides Linn is a member of convulvulacaea family. It is widely distributed in the tropical and subtropical regions throughout the world (Hyde *et al*., 2024) and, is called dwarf morning glory (English), kaafi malam (Hausa), ndottiyel (Fulfulde) and efunle in Yoruba (Austin, 2008; Isa and Garba, 2022). *Evolvulus alsinoides* Linn is used for the treatment of asthma, amnesia, cough, cold, fever, neurodegenerative diseases and venereal disease (Kuppusamy *et al*., 2021). It also exhibited antioxidant, anti-inflammatory, anti-haemorrhagic, and nootropic properties (Andrade *et al*., 2012). However, Madhuranga and Samarakoon (2022) reported that aqueous extract of *Evolvulus alsinoides* was toxic to brine shrimp algae. Therefore, there is need to evaluate this plant material thoroughly on mammalian species at different doses to ascertain its safety level as per oral administration.

Medicinal plants have been in use for thousands of years, some of them are safe while others are toxic (Altaf *et al*., 2019). One of the organs that are first attacked by toxicity in mammalian body is the liver. Even though, some plants have hepato-protective effects, others have hepatotoxic effects (Ugwu and Suru, 2021). The aim of this research was to evaluate the effects of oral administration of aqueous extract of *Evolvulus alsinoides* Linn on the histology and functions of the liver in albino Wister rats.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh whole plant *Evolvulus alsinoides* was obtained from a herb seller at Maiduguri Monday market in Borno State, Nigeria. The plant was identified and authenticated by an experienced plant taxonomist from the Department of Botany, Faculty of Life Science, University of Maiduguri, Nigeria. The plant material was dried under shade, pulverized and subjected to exhaustive soxhlet aqueous extraction.

Animal Husbandry

The rats were obtained from the animal house of the Department of Biochemistry, Faculty of Life Science, University of Maiduguri, Nigeria. Following two weeks of acclimation, the research was conducted at the animal house, Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Maiduguri, Nigeria. The rats had access to feed (Vital Feed Growers, Grand Cereals Ltd., Jos-Nigeria) and water *ad libitum*, and were maintained

under natural 12-hour light and dark cycles. The rats were handled and cared for according to the requirements of University of Maiduguri Ethical Committee on use of animals for research.

They were acclimatized for two weeks after which they were screened for body weight gain and any signs of diseases. The rats were kept in plastic cages at room temperature of 32 ± 4 ˚C and < 30% relative humidity with a 12-hours light/dark cycle naturally.

Experimental Design

Forty (40) young adult male albino Wister rats (weighing 110g-140g) were used for this study. The rats were divided into four (4) groups (I-V) of 10 rats each. Group I was designated as the control group. Groups II, III and IV were administered with 150 mg/kg, 250 mg/kg and 350 mg/kg respectively. The extract was administered to the treatment groups daily (at approximately the time) by gavage for 28 days. On the last day, all the rats were euthanized by intraperitoneal injection of ketamine chloride 75mg/kg (Hospira Inc. Lake Forest, USA).

Biochemical Studies

Liver function parameters were analysed from blood serum. Blood samples were collected in plain bottles by cardiac puncture. The samples were centrifuged at 3500 rpm for 10 minutes (using 896 MSE minor centrifuge, England) to obtain the serum. Liver function parameters analysed include: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein (TP).

Histological Analyses

Liver tissues were histologically processed as was performed by Isa et al (2023). Liver from each rat was dissected out and cleared from other attached tissues, weighed and cut within the range of 2-3 mm thick and fixed in 10% formalin (about 10 times the volume of the tissue). Fixed tissues were dehydrated in ascending grades of alcohol, starting from 50%-100%. After dehydration, the tissues were impregnated and embedded in paraffin, sectioned at 5 µm, cleared in xylene, rehydrated in descending grades (100%-50%) of alcohol and stained in haematoxylin and eosin. Tissue slides were photomicrographed at ×100 using Olympus biological microscope CX23, Japan.

Data Analyses

Statistical analyses were performed as was previously used by Isa et al (2023). Data from this study were analysed using GraphPad Prism Software, version 9 (La Jolla, CA, USA) and presented as mean ± standard error of the mean. *P*-values less than 0.05 are considered statistically significant.

RESULTS

Effects of the Extract on Body and Liver Weights

Table 1 shows the results of body weight (initial and final), body weight difference and organ weight (absolute and relative). Results of comparison between the mean body weight difference of control group and the group administered with 150 $mgkg^{-1}$ showed no significant difference (P=0.3113). No significant difference was observed when the mean body weight difference in the control group was compared with the mean body weight difference in groups administered with 250 $mgkg^{-1}$ (P=0.2207) and 350 $mgkg^{-1}$ (P=0.3575). Based on the results, there was no body weight loss or reduced growth in all the treatment groups. Absolute

liver weight of the group administered with 350 $mgkg^{-1}$ showed significant increase ($P<0.05$) when compared with the control group.

Table 1: **Body Weight and Liver Weight**

	Body weight (g)			Liver weight	
Doses ($mgkg^{-1}$)	**FBW**	**IBW**	**BWD**	**Absolute (g)**	**Relative (%)**
0	161.72±1.37	118.56±2.10	43.16±1.60	6.22±0.29	3.85±0.18
150	167.02±1.58	116.96±1.16	50.06±1.94	5.22±0.34	3.42±0.09
250	172.82±2.48	120.26±1.13	52.56±2.73	6.49±0.13	3.75±0.06
350	186.90±5.63	137.78±3.15	49.12±5.89	7.31±0.17*	3.93±0.17

IBM= Initial Body Weight; FBW=Final Body Weight; BWD= Body Weight Difference
*=p<0.05

Effects of the Extract on Liver Function Parameters

Table 2 shows results of effects of the extract on plasma concentrations of albumin (AL), alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and total protein (TP). At doses of 150 $mgkg^{-1}$ and 250 $mgkg^{-1}$ of the extract, no significant changes were observed in all the liver function parameters evaluated. At 350 $mgkg^{-1}$ significant decrease ($P<0.05$) was observed in plasma concentrations of ALP, ALT and AST when compared with the control group. However, albumin and total protein did not differ significantly from those of the control group.

Table 2**: Biomarkers of Liver Function Test**

Doses ($mgkg^{-1}$)	ALB (g/dL)	ALP (g/dL)	ALT (IU/L)	AST (IU/L)	TP (g/dL)
0	4.82± 0.78	31.63±3.10	3.65±0.46	3.75±0.22	5.36±0.16
150	3.96± 0.48	33.03±1.93	3.39±0.39	3.14±0.34	5.50±0.27
250	3.28±0.34	35.18±1.65	2.73±0.17	3.18±0.29	5.16±0.17
350	4.51±0.88	23.92±1.33*	2.55±0.09*	3.13±0.16*	4.97±0.14

*=p<0.05

Effects of the Extract on Histology of the Liver

Figure 1 shows the photomicrographs of liver sections from the control group (Plate A), while Plates (B), (C) and (D) were from groups administered with 150 $mgkg^{-1}$, 250 $mgkg^{-1}$ and 350 $mgkg^{-1}$ respectively. Plate (A) revealed a normal structure of the liver. The central vein (CV) appeared normal with hepatocyte (H) plates (laminae) radiating radially from it. Sinusoids (S) can be seen between hepatocyte plates. Photomicrographs of livers from groups administered with 150 $mgkg^{-1}$, 250 $mgkg^{-1}$ and 350 $mgkg^{-1}$ showed no conspicuous difference when compared to that of the control group.

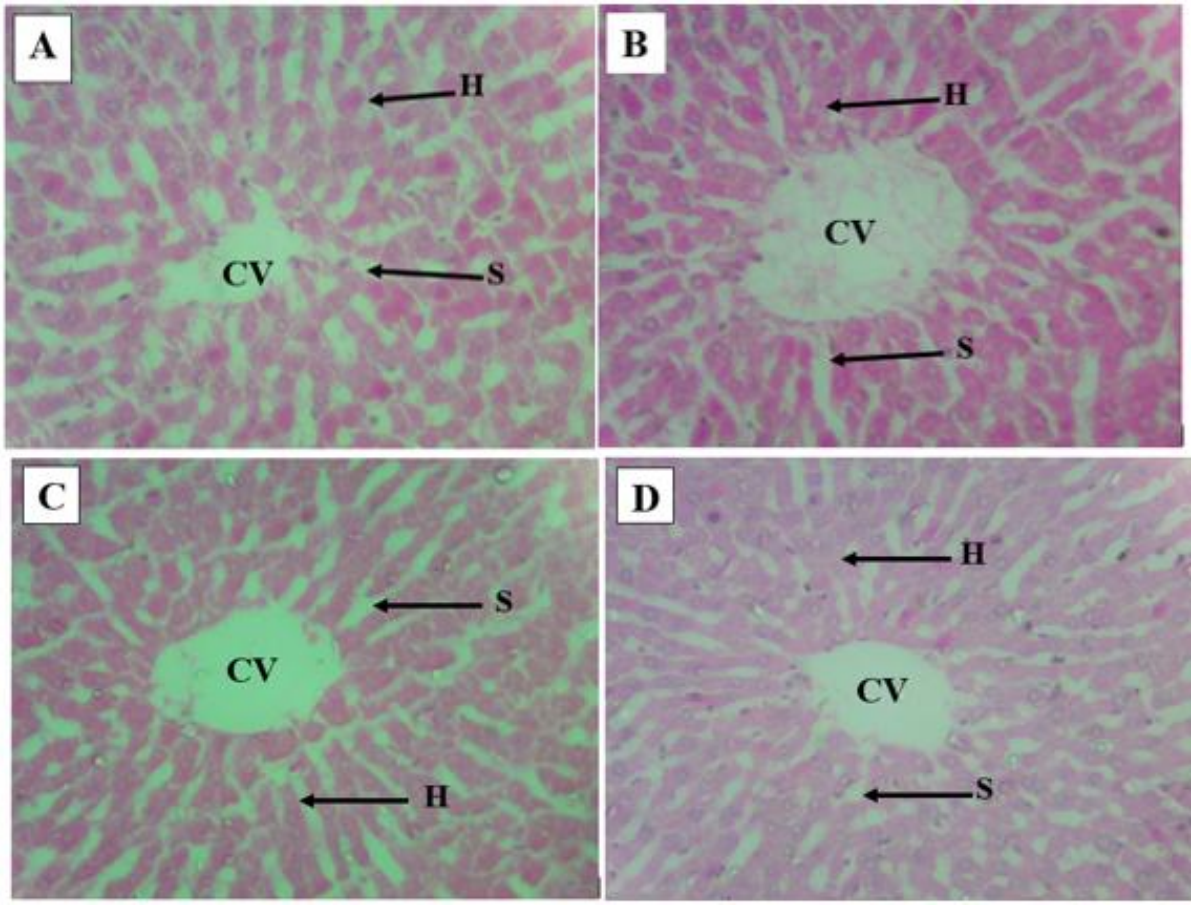

Plate 1**:** shows liver sections (**A**) from control group, (**B**) from group administered with 150 mgkg^{-1}, (**C**) from group administered with 250 mgkg^{-1} and (**D**) from group administered with 350 mgkg^{-1}. Hepatocyte (**H**) plates form Sinusoids (**S**) which are shown radiating from the Central Vein (**CV**) which is located at the centre of hepatic lobule.

DISCUSSION

In relation to body weight change, it can be stated that at the dose levels used in this research, *Evolvulus alsinoides* Linn has no effect on body weight change in rat model. This finding is similar to the findings of Isa and Garba (2022) who reported that oral administration of aqueous extract of *Evolvulus alsinoides* Linn has no effects on body weight of albino Wister rats. Similarly, Yadav et al (2019), also reported that *Evolvulus alsinoides* has no effect on body weight of mice.

The significant increase in absolute liver weight might not be treatment-related. It could be as a result of variation of body weight in the groups. It may also be as a result of water retention or hyperplasia in the livers of that group.

Significant decrease in alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase suggest that *Evolvulus alsinoides* might be good to liver functions and enhanced hepatic detoxification process. Such a decrease in the plasma levels of these enzymes suggest that *Evolvulus alsinoides* might have improved the integrity of plasma membranes of the hepatocytes at 350 mgkg^{-1} thereby significantly reducing the leakage of those enzymes to the extracellular environment. Such a property could be as a result of various phytochemicals present in the extract of the plant material. One of the phytochemicals responsible for causing significant decrease of these hepatic enzymes include flavonoids (Qin *et al*., 2022), which was found in high amounts in the aqueous extract of *Evolvulus alsinoides* L. (Padi *et al*., 2022). Flavonoids are strong antioxidants, scavenging free radicals to overcome

oxidative stress. It improves the integrity of cell membranes of hepatocytes, in this way, inhibiting the leakage of enzymes indicative of liver injury. This finding is in line with the findings of Chander and Reddy (2014), who reported significant decrease of these hepatic enzymes after administering ethanolic extract of *Evolvulus alsinoides* L. in rats.

Results on histopathological study of the liver in this research rhymed with that of other researchers. Chander and Reddy (2014) reported that ethanolic extract of *Evolvulus alsinoides* Linn revealed improvement of hepatic cells cyto-architectural organization in CCl_4-induced hepatotoxicity in albino Wister rats. The results of the present research are also in line with the findings of Yadav et al (2019) who reported that ethanolic extract of *Evolvulus alsinoides* showed no morphological changes in the liver of mice.

CONCLUSION

At the end of this research, histology and functions of the liver were evaluated after exposure of the rat model to oral administration of aqueous extract of *Evolvulus alsinoides* Linn. The histology of the liver might have not been affected. However, significant decrease in alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase was observed. The plant material evaluated might have enhanced liver function. Further research is recommended to ascertain the effects of this plant on the liver.

Acknowledgements

We acknowledge the technical assistance received from Mr. Ephraim Ayuba and Sunday J. Manye both of the Histology laboratory, Department of Human Anatomy, University of Maiduguri.

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