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Research Article

Effect of Aqueous Extract of *Evolvulus alsinoides* Linn. on Some Blood Parameters and the Histology of the Spleen in Rats

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ABSTRACT

Evolvulus alsinoides Linn. (family Convolvulaceae) is used in folkloric medicine as adaptogenic, antihypertensive, antiphlogistic, antipyretic, antiseptic, aphrodisiac, and in the management of neurodegenerative disorders. The effect of the aqueous extract was investigated on some blood parameters and spleen histology in rats. Fifty young adult male Wistar rats were divided into five groups of ten rats each. Group I served as the control group; groups II and III and IV were administered with 150 mg/kg 250 mg/kg and 350 mg/kg respectively. Groups V animals were administered with 350 mg/kg each but allowed a post recovery period of 14 days to observe for reversibility or delayed effect. The plant extract was administered by gavage for 28 days. Blood parameters were evaluated manually. The extract contained carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids. Sections from the spleen of rats administered with 350 mg/kg revealed prominent germinal centers with expanded lymphoid cells. Significant increases in red blood cell ($p < 0.001$) and white blood cell ($p < 0.001$) counts were observed. These suggest that the plant has haematinic and immunostimulatory effects. These are important properties that can be of benefit medicinally. However, further research on the safety levels of oral consumption of *Evolvulus alsinoides* Linn. is recommended.

Keywords: *Evolvulus alsinoides*, Histology, Spleen, Blood, Rats.

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INTRODUCTION

Evolvulus alsinoides Linn. is a small perennial herb that belongs to the family convolvulaceae. It is one of the medicinal plants commonly used in Africa, India and Philippines (Singh, 2008). It is called dwarf morning glory in English (USDA, 2007). In Nigeria, *Evolvulus alsinoides* Linn. is called kaafi malam (Hausa), ndottiyel (Fulfulde) and efunle in Yoruba (Austin, 2008).

The spleen is an intra-peritoneal organ located directly below the diaphragm connected to the stomach (Silva *et al.*, 2015), it is the largest secondary lymphoid organ containing about one-fourth of the body's lymphocytes and initiates immune responses to blood-borne antigens (Cesta, 2006; Elmore, 2006). It is an important organ to evaluate for treatment-related lesions because it is the site for draining compounds administered intravenously (Cesta, 2006). The functional activities of the spleen range from successful induction of specific immunity through trapping, transportation, processing and presentation of antigens, recycling of iron and phagocytosis of senescent or damaged red blood cells (RBCs) and pathogens (Getu, 2019).

Its medicinal uses include use as adaptogenic, antihypertensive, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, and against asthma, bronchitis,

syphilis, epilepsy, insanity, nervous debility and loss of memory (Anis *et al.*, 2000; NRCSU, 2007; Austin, 2008; Siraj *et al.*, 2019). It is also used in the management of neurodegenerative disorders (as brain tonic), amnesia, epilepsy and as a hepatoprotective agent (Kumar *et al.*, 2010; Abubakar *et al.*, 2013; Chander and Reddy, 2014; Sethiya *et al.*, 2019). In Africa, it is used to treat low spirit and depression (Bussmann, 2006; Isa and Garba, 2017), while in Nigeria, it is used as stomachic, and against asthma and bronchitis (Singh, 2008; Indhumol *et al.*, 2013; Isa and Garba, 2017). Literature has revealed that *Evolvulus alsinoides* Linn has several medicinal uses. However, its effect on some vital organs has not been reported. The present study was designed to evaluate the effect of oral administration of aqueous extract of *Evolvulus alsinoides* Linn. on the histology of the spleen and blood parameters.

MATERIALS AND METHODS

Collection and Identification of Plant Materials: The whole plant (*Evolvulus alsinoides* Linn.) used in this study was obtained from an herb seller at Monday market, Maiduguri, Borno State. The plant was identified and authenticated by a plant taxonomist in the Department of Biological Sciences,

University of Maiduguri. A specimen voucher (EA.01) of the plant was prepared and deposited at the herbarium.

Extraction Procedures: Extraction of the plant material was conducted as described by the World Health Organization (WHO, 1998). In order to prevent ultra-violet rays from sunlight to affect the phytochemical constituents present in the plant material, the plant material was air-dried under shade for one week. Air-dried plant material was pulverized using mortar and pestle. One hundred grams (100g) of the pulverized plant material was subjected to exhaustive soxhlet extraction in distilled water (500ml) for 72h at 60 °C. The extract was further concentrated in a water bath at temperature of 40-60°C until a dark sticky residue with a constant weight was obtained. A mean extract yield of 12.3g w/w was obtained. The extract was stored in stoppered container in a refrigerator at -4 °C until required and stock solution (50 mg/ml) was prepared by dissolving 1g of the extract in 20 ml of normal saline.

Phytochemical Analysis: The phytochemical analysis was conducted at Chemistry Department (University of Maiduguri, Maiduguri-Nigeria) according to standard methods of Clarke (1975); Odebiyi and Sofowora (1978); Trease and Evans (1989).

Animal and Husbandry: A total of fifty (50) young adult male Wistar rats weighing (134-150g) were obtained from National Veterinary Research Institute (NVRI), Vom, and Plateau State, Nigeria. The rats were weighed and individually identified by colour tattoo. 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. Standard laboratory diet (pelletized grower feed from Grand Cereals and Oil Mills Ltd, Jos, Nigeria) and water was provided to the rats *ad libitum*.

Experimental Design: Fifty (50) young adult male Wistar rats were used for this study. The rats were divided into five (5) 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. While Group V was administered with 350 mg/kg and allowed a post recovery period of 14 days to observe for reversibility or delayed effect. The extract was administered to the treatment groups daily by gavage for 28 days.

Determination of Hematological Indices: Determination of red blood cell count, white blood cell count, platelet count, and haemoglobin concentration were all performed manually.

Determination of Red Blood Cell (RBC) Count: The RBC count was performed as described by Sood (2009) Doig and Zhang (2017). Each rat was placed firmly in a restrainer which allowed the passage of the tail through a hole. The tip of the tail was disinfected with methylated spirit and then bled using a sharp razor blade. Blood sample was then drawn using RBC pipette until it reached 0.5 mark on the pipette. To the blood sample in the pipette, a diluting fluid (Hayem's fluid) was

drawn up to 101 mark. This gives an exact dilution of 1:200 with Hayem's solution. The pipette was rocked gently for about 3 minutes. A drop of the mixture was released gently at an angle of 45° onto a clean counting chamber, pre-mounted with a cover slip. The chamber was then transferred to microscope stage and allowed for about 5 minutes for the cells to settle. The RBCs were then counted at magnification of ×40. Finally, the number of red blood cells was calculated using the formula below:

$$RBC\ count = N \times 10000\ cells/mm^3$$

Where N is the number of red blood cells counted from the 80 small squares of the five large squares of the haemocytometer.

Determination of White Blood Cell (WBC) Count: White blood cell count was determined as was described by Sood (2009) Doig and Thompson (2017). Blood sample was drawn to 0.5 mark of the white blood cell pipette, and then Turk's solution was drawn in the same pipette to the mark of 11.0; giving a ratio of 1:20. The two were mixed together gently. A drop of the mixture was gently released onto a counting chamber pre-mounted with a cover slip. The four outer groups of the haemocytometer were used and the number of the cells in each smaller square was counted. The formula below was used to calculate the white blood cell count:

$$WBC\ count = N \times 50\ cells/mm^3$$

Where N is the number of white blood cells counted from the small squares of the four large outer squares of the haemocytometer.

Determination of Platelet Count: Determination of platelet count was performed as was previously described by Sood (2009) and Doig and Thompson (2017). Using RBC pipette, blood sample was drawn to the mark of 0.5 and a diluting fluid (1% ammonium oxalate) was also added to the blood in the pipette until the volume of the two reached the mark 101 giving a dilution ratio of 1:200. The number of platelets can be calculated using the following formula:

$$Platelet\ count = N \times 10000 /L$$

Determination of Haemoglobin (Hb) Concentration: The aim of estimating hemoglobin concentration is to determine the oxygen-carrying capacity of the blood. In this study, cyanmethemoglobin method was employed. Drabkin's solution (5ml) was measured in a test tube, and a sample of blood (drawn with hemoglobin pipette to the mark of 0.02) was added. The mixture was thoroughly mixed and allowed to stand for about 5 minutes. The optical density of the mixture was read in a calorimeter at 540nm wavelength. The concentration of hemoglobin in grams per 100ml of blood was calculated as described by Ochei and Kolhatkar (2000) and Thakkar and others (2021) using the formula below:

$$\frac{\text{optical density of test Hb}}{\text{optical density of standard Hb}} \times \frac{\text{concentration of standard Hb per 100ml of blood}}{4}$$

The final concentration of hemoglobin is expressed in g/dl.

Histopathological Analyses: At the end of the experiment, the rats were anesthetized and the abdominal cavity was opened. Spleens of all the rats were procured, cleared of

adhering tissues, weighed and fixed in 10% formalin individually. The organs were dehydrated in a graded (70%, 80%, 90% and 95%) series of alcohol, infiltrated with, and embedded in paraffin wax. Tissue sections were cut at 5µm.

Statistical Analysis: Statistical analysis was performed as was previously employed by Balogun and others (2015). Data obtained were analysed using Statistical Package for Social Scientist version 16.0 (SPSS 16.0), and were expressed as mean± standard error of mean (SEM). *P*-values less than 0.05 were considered statistically significant.

RESULTS

Phytochemical Constituents of Aqueous Extract of *Evolvulus alsinoides* Linn: Results of the phytochemical analysis revealed the presence of carbohydrate, cardiac glycosides, flavonoids, saponins, tannins, and terpenoids (Table 1).

Effect of the Extract on body and organ weights: The body weight differences and spleen weight in groups administered with 150, 250 and 350 mg/kg body weight were compared with that of the control group (Table 2). While the post-recovery group was compared with the group administered with 350 mg/kg. No significant difference was observed in body weight and spleen weight.

Effect of the Extract on Hematological Indices: For all the blood parameters, the groups administered with 150 mg/kg, 250 mg/kg and 350 mg/kg body weight were compared with the control group while the post recovery group (350PRG) was compared with the group administered with 350 mg/kg body weight (Table 3). Red blood cell counts in groups administered with 150 mg/kg, 250 mg/kg and 350 mg/kg body weight showed significant (*p*<0.001) increase. A significant (*p*<0.001) increase in WBC counts was also observed in all the groups except the post recovery group. However, no significant change was observed in PCV, Hb concentration and platelet counts in all the groups.

Table 1: Phytochemical Constituents of the Aqueous Extract of *Evolvulus alsinoides* Linn.

Constituents	Inference
Alkaloids	–
Anthraquinone	–
Carbohydrates	+
Cardiacglycosides	+
Flavonoids	+
Phlobatannins	–
Saponins	+
Tannins	+
Terpenoids	+

+: present -: absent

Table 2: Effect of 28-Day Oral Administration of the Aqueous Extract of *Evolvulus alsinoides* Linn. on Mean Body Weight and Mean Spleen Weight in Male Rats

Parameters	Dosages administered (mgkg ⁻¹)				
	Control	150	250	350	350 ^{PRG}
Body Weight					
Day 0 (g)	138.70 ± 1.90	139.56 ± 1.07	140.00 ± 1.17	145.00 ± 0.60	142.00 ± 0.71
Day 28 (g)	175.30 ± 1.18	175.00 ± 0.95	173.70 ± 0.92	172.10 ± 0.60	173.20 ± 0.63
Weight difference (g)	36.60 ± 0.58	35.44 ± 0.44	33.70 ± 0.68	27.10 ± 1.02	31.20 ± 0.68
Absolute Spleen Weight (g)	0.39 ± 0.03	0.36 ± 0.02	0.36 ± 0.02	0.37 ± 0.01	0.37 ± 0.02
*Relative Spleen Weight	0.22 ± 0.00	0.21 ± 0.02	0.21 ± 0.01	0.20 ± 0.02	0.20 ± 0.02

N=10, Results are presented as Mean±SEM. PRG=Post Recovery Group: Sacrificed 14 days after the last administration of the extract.

$$*Relative\ Spleen\ Weight = \frac{Absolute\ Spleen\ Weight \times 100}{Body\ Weight}$$

Table 3: Effect of 28-Day Oral Administration of the Aqueous Extract of *Evolvulus alsinoides* Linn. on Hematological Indices of Male Rats

Parameters	Dosages administered (mgkg ⁻¹)				
	Control	150	250	350	350 ^{PRG}
RBC (×10⁶/mm³)	7.88 ± 0.16	8.60 ± 0.21***	10.26 ± 0.17***	10.48 ± 0.07***	10.28 ± 0.10
WBC (×10³/mm³)	11.56 ± 0.07	12.44 ± 0.05***	14.04 ± 0.12***	14.54 ± 0.05***	14.40 ± 0.14
PCV (%)	45.80 ± 0.53	46.30 ± 0.15	46.50 ± 0.26	46.70 ± 0.83	46.50 ± 0.43
Hb (g/dL)	10.50 ± 0.22	11.20 ± 0.33	11.30 ± 0.42	11.70 ± 0.46	11.50 ± 0.37
PLT (×10⁹/L)	534.00 ± 0.00	510.00 ± 12.47	535.00 ± 15.72	505.00 ± 0.02	489.00 ± 12.06

N=10, Results are presented as Mean±SEM. PRG=Post Recovery Group: Sacrificed 14 days after the last administration of the extract

****p*<0.001. **RBC**=Red Blood Cell, **WBC**=White Blood Cell, **PCV**=Packed Cell Volume, **Hb**=Hemoglobin, **PLT**=Platelet

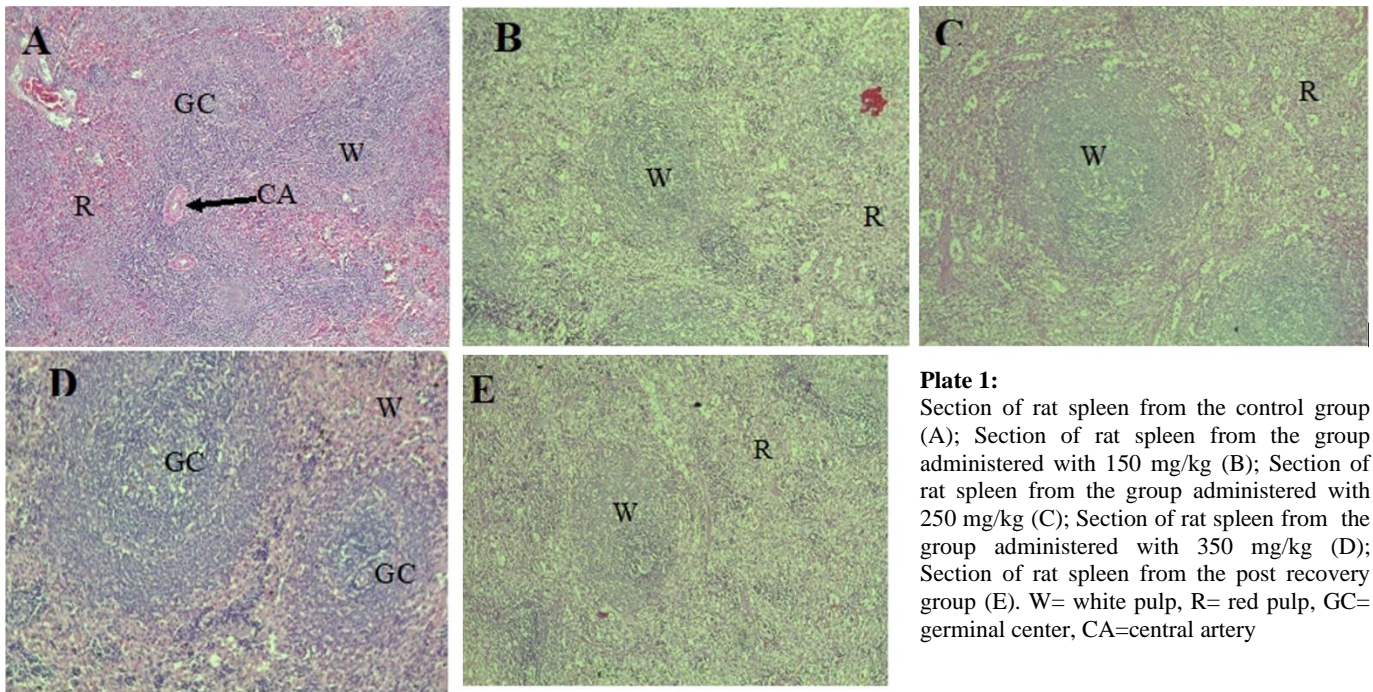


Plate 1:
Section of rat spleen from the control group (A); Section of rat spleen from the group administered with 150 mg/kg (B); Section of rat spleen from the group administered with 250 mg/kg (C); Section of rat spleen from the group administered with 350 mg/kg (D); Section of rat spleen from the post recovery group (E). W= white pulp, R= red pulp, GC= germinal center, CA=central artery

Effect of the Extract on the Histology of the Spleen

Normal histology of rat spleen (Plate 1A) shows the germinal center (GC), red pulp (R), white pulp (W) and central artery (CA). Spleen sections from rats administered with 150 and 250 mg/kg body weight showed no conspicuous difference from the control group (Plates 1B and C). Section from the group administered with 350 mg/kg body weight revealed prominent germinal centers with expanded lymphoid cells (Plate 1D). Section from post-recovery group showed no recovery (Plate 1E).

DISCUSSION

The various pharmacological effects of the plant extract could be a result of various phytochemicals present. Many beneficial biological activities such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported (Sasidharan *et al.*, 2011). The presence of prominent germinal centers with expanded lymphoid cells from spleen sections administered with 350 mg/kg body weight is an indication of increases in the population of proliferating B-lymphocytes and stimulation of humoral response (Salvador-Membreve *et al.*, 2018). This suggests that the extract of *Evolvulus alsinoides* ingested orally at such a high dose, has immunostimulatory effect. Hence, the plant extract may serve as an immune booster. Sections from post-recovery group showed no recovery. That means, prominent germinal centers with expanded lymphoid cells were also observed in the post-recovery group 14 days after extract withdrawal. Other medicinal plants with immunostimulatory effects include *Actinidia macrosperma*, *Aesculus indica*, *Allium sativum*, *Andrographis paniculata* (Kumar *et al.*, 2011), and *Dendranax moribifera leveille* (Birhanu *et al.*, 2018).

The present study revealed that the oral administration of aqueous extract of *Evolvulus alsinoides* has haematinic activity. However, the work of Yadav and others (2019) reported a result that differed from ours. Variations in such situations could be as a result of geographical distribution, climate change, nature of the soil and its nutrient content, specie differences, and extraction methods and media of extraction used (Dhami and Mishra, 2015). Increase in red blood cell count suggests that the plant extract stimulates the production of erythropoietin or mimic the action of that hormone. Significant increases in blood parameters in this research is worth considering because it is dose-dependent. Several medicinal plants have haematinic effects, for instance, Momoh and others (2019) reported that the extracts of *Ficus exasperata* and *Jatropha curcas* exhibits additive haematinic effect when co-administered.

In conclusion, oral administration of aqueous extract of *Evolvulus alsinoides* was shown to increase red blood cell and white blood cell counts in albino Wister rats. If these actions can be tested on other species and the toxicity of this plant is further studied, safe doses can be deduced for human consumption.

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