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HAEMOPOIETIC EFFECTS OF ETHANOLIC EXTRACT OF *Stellaria media* (L.) LEAVES ON ALBINO MICE.

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ABSTRACT: The effect of the ethanolic extract of the leaves of *Stellaria media* on haemoglobin (Hb), Red blood cell (RBC), Mean Corpuscular haemoglobin concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin (MCH), Platelet (PLT), Haematocrit (HCT) and minerals such as calcium (Ca) magnesium (Mg), potassium (K) and iron (Fe) were investigated in 24 albino mice. The mice were in 4 groups of 6 animals. The first group was the control and the three other groups were the experimental. The latter groups were given oral treatment of *Stellaria media* (L.) in concentration of 273.86mg/kg, 537.72mg/kg and 821.58mg/kg respectively for 14 days. Blood analysis was done at the end of 14 days. The extract of *Stellaria media* induced in a dose dependent increase in Hb (P < 0.05), RBC (P < 0.05), HCT (P < 0.05), MCV (P < 0.05) and MCH (P < 0.05), PLT (P < 0.05) and MCHC (P < 0.01) and a significant increase in all the minerals tested. The preliminary phytochemical screening showed the presence of saponins, alkaloids, flavonoid, tannins and cardiac glycosides. The result of this study supports the traditional use of *S. media* leaves as a remedy for anaemia.

INTRODUCTION

Stellaria media (L.) vill is in the family of Caryophyllaceae, with a cosmopolitan distribution. The common names include starwort, stitchwort and chickweed. It is an annual plant, about 3 to 8 inches tall, forms mat up to 16 inches long. A fine line of hair extends along the length of the slender, delicate, stem. Five green sepals (modified leaves) grow as long as the petals they underlie (Wildman *et. al*, 2008).

The stems are green, occasionally purple with slightly thickened nodes. The stems also make the plant easily identifiable with its singular longitudinal line of pubescence which runs along one side of the stem. The leaves are mostly ovate in shape 1.0cm across the stem from *S. media* concealed by a shell which is covered in teeth pubescence, which stick to animals or clothing. Chickweed is a tonic, diuretic, demulcent, expectorant, and mildly laxative. It's often recommended for asthma, bronchitis or congestion. The decoction is also used extremely to treat rheumatic pains wounds and ulcers (Sofowora, 1993 and Stray, 1998).

Anaemia is the condition characterized by abnormality low level of red blood cells. All cells in the body of animals require constant supply of oxygen to stay healthy. Oxygen is delivered to cells by red blood cells which pick them up in the blood streams. The oxygen carrying molecule is the haemoglobin. Therefore, anaemia developed as the result of insufficient supply of red blood cells and haemoglobin which carried the oxygen. When this happens cells do not get oxygen required and they begin to die off. This study is aimed at evaluating the bioactive components of *S. media* leaves and to validate the claims by traditional medicine practitioners of its efficacy in treating anaemia.

MATERIALS AND METHODS

Extraction

The fresh leaves (1kg) of *S. media* were collected in April, 2012, air-dried for a week and reduced to powder which yields 350g. About 300g of the powder was macerated in 50% ethanol (300ml) for 72 hours. The liquid extract obtained was concentrated in vacuo at 40°C to yield 60g dry ethanol extract.

Phytochemical Screening

The methods of Sofowora (1993) and Edeoga *et. al.*, (2005) were employed to determine the presence of secondary metabolites such as alkaloids, saponins, tannins, phlobatannin, flavonoid and cardiac glycosides.

Test Organism

The organisms in this study were 24 albino mice. They were obtained and maintained under standard animal house conditions in the animal house of the Department of Pharmacology and Toxicology, University of Uyo, Uyo. The mice were housed in cages and allowed free access to food (layer mash) and water for 1 week for acclimatization.

Administration of *Stellaria media* Extract

The 24 albino mice were put into 4 groups (I, II, III, and IV) of six animals per group. Group I mice were fed with the standard diet and served as the control. Groups II, III, and IV were administered with different concentrations of the extract 273.86mg/kg, 547.72mg/kg and 821.58mg/kg respectively.

LD₅₀ Determination (Toxicity Test)

Twenty four (24) mice weighing between 98g – 131g were kept in 4 cages (6per cage). The animals were maintained with standard animal diet and water. However, food was withdrawn 18 hours before the start of the experiment (Amresh *et. al.*, 2008). Groups I, II, and III were administered with 3000mg/kg, 2500 mg/kg and 2000mg/kg of the extract respectively and were observed for mortality within 24 hours, the fourth group, control was fed with animal feed and water. The LD₅₀ was calculated using the modified Lorke (1983) method.

Haemopoietic Test

All the animals were sacrificed at the end of 14 days of treatment with *Stellaria media* and whole blood was obtained via cardiac puncture centrifuged for 20 minutes to separate other blood cells from the serum and the blood samples were taken to the University of Uyo Teaching Hospital (UUTH) for determination of white blood cell (WBC), red blood cell (RBC), Haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet (PLT). Analysis of variance was calculated according to Ahmadiani *et al.*, (1998).

RESULTS

The *S. media* leaves extract contained alkaloids, saponins and flavonoid in high concentration, tannin in trace amount while phlobatanin was absent (Table 1).

Table 1: Phytochemical composition of *Stellaria media*

Metabolites	Concentration (ug/l)
Alkaloids	+++
Saponins	+++
Tannin	++
Phlobatannin	–
flavonoid	+++
Cardiac glycosides	+++

Key:

+++ = High concentration ++ = Moderate concentration
 + = Trace concentration - = Absent

The LD₅₀ Toxicity test revealed that *Stellaria media* has an LD₅₀ of 273.86mg/kg. Treatment of mice with ethanolic extract of *Stellaria media* produce dose dependent changes in mineral ions needed for the production of blood (Table 2).

Table 2: Mean values of calcium, potassium, magnesium, iron levels in mice blood

Parameters	Group 1 control	Group 2 273.86mg/kg	Group 3 547.72 mg/kg	Group 4 821.58mg/kg
Calcium	11.83±0.67	1.74±0.82*	20.44±0.29*	23.49±0.58*
magnesium	2.33±0.09	1.83±0.05*	3.88±0.03*	4.84±0.04*
Iron	1.74±0.33	3.84±0.28*	4.00±0.003*	3.4±0.003*
Potassium	8.05±0.62	11.25±0.07*	13.93±0.40	15.40±0.608*

Values expressed are mean ± SE 4 replicates and were significant at p < 0.05

The volume of red blood cells (RBC) increased in groups 2, 3 and 4 which received treatment doses of 273.86mg/kg, 547.72mg/kg and 821.58mg/kg respectively. There was a rise in mean corpuscular volume (MCV) in group 3 which received 547.72mg/kg preparation of the diet. There was a marked increase in mean corpuscular haemoglobin (MCH) in groups 3 and 4 and a high increase of platelet (PLT) in groups 2 and 4. However, there was no increase in white blood cell (WBC) and haemoglobin (Hb) (Table 3).

Table 3: Effect of *Stellaria media* diet preparation on haematological indices

Group	Treatment mg/kg	WBC 10 ³ /UL	RBC 10 ⁸ /UL	Hb/dl (%)	HCT (%)	MCV FL	MCH pg	MCHC g/dl	PLT UL
1	Control	16.10± 0.40	5.36±1. 75	13.96± 0.26	46.36± 1.70	57.83± 112	17.70± 0.12	3.00±0. 4	749.66 ±93.03
2	273.86	8.69±1. 62	7.36±1. 75	12.6±0. 67	40.70± 3.08	55.50± 1.01	17.26± 0.52	31.16± 0.52	762.30 ±36.6
3	547.72	0.70±1 2.10	7.07±0. 13	12.86± 0.4	42.06± 0.62	59.46± 0.18	17.9±0. 00	30.00± 0.10	704.66 ±25.71
4	821.58	2.93±0. 12	6.39±1. 27	13.46± 0.51	46.76± 1.77	57.50± 1.11	17.90± 0.23	30.80± 0.05	941.6± 147.15

Values expressed are mean ± SE of 4 replicates and were significant at p < 0.05.

DISCUSSION

The result of the haematological test significantly (p < 0.05) increase in the Red Blood Cell count (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet (PLT) while the White Blood Cell (WBC) and Haemoglobin (Hb) did not change when compared with the control group. This could be due to the abundance presence of alkaloids which has been known to exhibit pharmacological effect on humans and other animals. Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medical agents for their analgesic, antispasmodic and bactericidal effects (Stray, 1998). They exhibit marked physiological activity when administered to animals. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane (Iwu, 1986). The presence of tannin in *S. media* strongly supports their use in treating wounds, varicose ulcers and burns in herbal medicine (Duke, 1992).

The haemoglobin (Hb) and white blood cell counts (WBC) were significantly lower in groups 2, 3, and 4 as compared to control. This suggests that other factors, not immediately determinable, were responsible for the variation observed in the Haemoglobin (Hb) and White Blood Cell (WBC). The amount of haemoglobin determines how much oxygen the RBC's would be capable of carrying. Thus, other factors, like the antioxidant properties of bioactive

components present in the plant, aside from low haemoglobin and white blood cell contents may have been responsible for the observations. Saponins and flavonoids which are present in this plant have been shown to possess antioxidant properties in the plant material used in this study (Liener, 1980). These groups of compounds often lead to inferior haematological profile.

The haemopoietic studies showed that minerals essential for haemopoiesis (iron, magnesium, potassium and calcium) showed a dose dependent increase in the mice; this could be as a result of the presence of ions in the leaves of *S. media* (Ajayi et. al, 2000; Ogumike, 2002).

The part of the plant under investigation had an LD₅₀ of 273.86mg/kg. This value rates it as a mild toxic substance (Gosselin et. al, 1984). Though the haemopoietic effect it exerts is beneficiary there should be caution in the amount of the decoction of plant taken because of long term effect as a result of toxicity.

CONCLUSION

This study has shown that *Stellaria media* plant materials have potential for use in the treatment of anaemia, burns and sores. The isolation and characterization of active principles in the plant materials would provide greater understanding of their role in biological system.

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