The effect of Solenostemma argel on anemia-related parameters in Albino Rats and Rabbits

H. M. Osman, M. E. Shayoub, E. M. Babiker

Departments of Biochemistry, University of National Ribat, 1Pharmaceutics, and 2Zoology, University of Khartoum, Sudan

Address for correspondence: Hisham Mohamed Osman, Department of Biochemistry, Faculty of Pharmacy, University of the National Ribat, Khartoum, Sudan. E-mail: hisham1212ribat@yahoo.com.

ABSTRACT

Background: The plant has been used for treatment of number of diseases in folklore medicine but not used for anemia. The present study is aimed to investigate its effect on anemia and related blood indices and body weights (BW). Materials and Methods: Two sets of Albino rats each of four groups of six animals were used in this study. In each set one group for the control and the three groups for experimental doses. Two groups of rabbits each of five animals were also used for control and experiment. In one set of Albino rats, leaves of Solenostemma argel extracted in ethanol were used to test for its effect alone although in the second set, Albino rats were used to test for anemia. However, the rabbits were tested for the effect of S. argel on blood indices and BW. Blood parameters were measured by Sysmex, electrolytes by spectrophotometer, and flame photometer and BW by Mettler balance. Measurements were conducted after 30 days. Results: The present study revealed significant increases (P<0.05) in mean cell hemoglobin concentration (MCHC) and packed cell volume (PCV) in rats provided with 600 mg/kg and PCV in the group provided with 300 mg/kg, whereas no significant changes in the group provided with 150 mg/kg. For rabbits, significant increase (P<0.05) was restricted in mean cell volume (MCV). However, no significant changes (P>0.05) occurred in BWs of experimental animals. In the meantime, significant decreases (P<0.01) in red blood cell counts, Hb, MCHC, PCV and Fe as well as increase in MCV occurred in those treated with Aluminum chloride (AlCl3) alone (Al) group or Aluminum chloride co-administered with 600 mg/kg of S. argel extract (AlS) group. The results also showed significant increase (P<0.01) in PCV and MCHC and significant decrease in MCV in group treated with AlCl3 co-administered with plant when compared to those provided with AlCl3 alone. Moreover, administration of plant extracts alone (S) group lead to significant increase (P<0.05) in MCHC, PCV, K, P, and Ca when compared to the control group (CG). However, the present study concludes that S. argel is of high nutritional values and of significance in mitigating anemia.

Key words: Aluminum chloride, blood parameters, body weights, medicinal plant, nutritional anemia, Solenostemma argel

INTRODUCTION

The plant is indigenous to northern Sudan between Berber and Abu Hamad, and found in North Africa.[10] It belongs to the Asclepiadaceae family. It is an erect shrub, reaches a maximum height of 100 cm, with many velvety, pubescent branches from the base. It is known locally as “Hargal” and widely used in traditional folkloric medicine as antispasmodic,[2] anti-inflammatory,[1] anti-rheumatic as well as anti-syphilitic agent.[3,4] Smoke inhalation and infusion of the whole Solenostemma argel is also used in treatment of hypercholesterolemia, diabetes mellitus, cold, cough, jaundice and measles. Moreover, it is described for treating gastrointestinal cramp, urinary tract infection and the disturbance of the menstrual cycle.[4]

The plant also has antimicrobial activity,[5] it possesses insecticidal effect and hence was used to combat insect pests.[8] In this context, it was used against mosquito species, the causative agent of malaria in Sudan.[7-9] Moreover, it was reported to have antimicrobial properties, as well as antibacterial and antioxidant activity.[10,11] Moreover, chemical investigations, chromatographic screening and phytochemical as well as tissue culture studies of S. argel leaves, stems, and flowers revealed the presence of numerous biochemical...
ingredients such as pyrgene glycosides, flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids. Of these, pyrgene glycosides were reported to reduce cell proliferation. The leaves of this plant, however, are characterized by having a high percentage of carbohydrates (64.8%), slightly low percentage of protein (15%), low percentage of crude fiber (6.5%), crude oil (1.6%), about 7.7% as ash, and 4.4% as moisture. This in addition to lower percentage of minerals namely: potassium (0.54%), calcium (0.06%), magnesium (0.03%), sodium (0.01%), copper (0.001%), ferrous (0.002%), manganese (0.002%), and lead (0.001).

On the other hand, aluminum (Al), although in its ionic form has no known biological role, but when accumulates in the body it can induce several clinical disorders such as neurotoxicity, hepatotoxicity, bone diseases and anemia. It also has a direct effect on hematopoiesis and its toxic effects can be manifested as neurotoxicity, hepatotoxicity, bone diseases and anemia. On the other hand, aluminum (Al), although in its ionic form has no known biological role, but when accumulates in the body it can induce several clinical disorders such as neurotoxicity, hepatotoxicity, bone diseases and anemia.

The present paper is aimed to find out the effect of the ethanolic leaves extract of S. argel on the status of blood parameters, Al-induced anemia as well as on the body weights (BW) of albino rats and rabbits.

**MATERIALS AND METHODS**

The leaves of S. argel were bought from local market, and placed in ethanol (80%) for 3 days in Soxhlet apparatus. Then, the ethanol extract was dried in Rotary Evaporator apparatus, and dispersed in distilled water to give the final concentration of 150 mg extract/kg of BW, 300 mg extract/kg of BW and 600 mg extract/kg of BW.

For measuring the effect of S. argel on blood parameters and BWs, Albino rats (n = 24, average 235 g) and rabbits (n = 10, local breed, average 530 g) were used. Albino rats were divided into four groups of six animals; first group to act as a control group (CG) and the other three groups to act as experimental groups and denoted (G₁, G₂, and G₃). Similarly, rabbits were divided, but into two groups of five animals; one to act as a control (RG₁) and the other as an experimental group (RG₂). The control group of Albino rats (CG) was provided with normal diet concentrate (dried meat, milk powder, oil, and flour in some water) without S. argel although the experimental groups of Albino rats were provided, in addition to the concentrate of normal diet, with doses of 150, 300 and 600 of S. argel leaves extract for the three experimental groups of Albino rats (G₁, G₂, and G₃) respectively. The doses were administered orally by Gavage daily for 30 days. For the rabbits, the control group (RG₁) was provided with clover leaves only, whereas the experimental group (RG₂) was fed with both leaves of clover and of S. argel (2.5 BW) daily for 30 days. For these groups, BW of each animal before and after being experimented was measured using the Mettler sensitive balance.

For of Al-induced anemia, only albino rats (n = 24, average BW = 284 g) were used. The rats were also divided into four groups each of six. The 1st group denoted (Al) was provided with Aluminum chloride (AlCl₃) (0.5 mg/kg BW) The 2nd group denoted (AIL) was provided with AlCl₃ and 10 min later with S. argel extract (600 mg/kg BW). The 3rd group denoted (S) was provided with S. argel extract (600 mg/kg BW) alone. The 4th group denoted (C) was provided with the physiological normal saline and used to act as a control group. All experimental doses were administered orally by Gavage and were given daily for 30 days. These groups were measured for the status of blood as well as for electrolytes.

Both blood parameters and electrolytes were measured. For blood parameters about 2 ml of blood sample was collected by hematocrit capillary tube from retro-orbital plexus of each rat, although about 2 ml from jugular vein of each rabbit. Subsequently, each blood sample was placed in ethylene diamine tetra-acetic acid (EDTA) tube and used immediately for measurement of blood parameters using automated coagulating Sysmex apparatus of the type 8999. The blood parameters included: Hemoglobin (Hb), mean cell volume (MCV), red blood cells count (RBCs), white blood cells count (WBC), mean cell hemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LYM) and packed cell volume (PCV). For electrolytes, additional 2 ml of blood sample was taken only from each rat of those treated with aluminum alone (Al), with aluminum and plant (AIL), with plant alone (S) and from the control group (C). Each sample was then placed in a plane tube, left to coagulate, centrifuged at 1000 rpm and the supernatant (serum) was employed in measuring Na, K, Ca, P and Fe. For Na and K, aliquot for each element was placed in a cuvette and measured using flame photometer apparatus, and the same carried out for measuring Ca, P and Fe using Spectrophotometers apparatus of the type (30122). Both instruments were adjusted to zero by blanks brought with the kit for each element.

**Statistical Analysis**

Student t-test was used for paired comparison of mean values (mean ± SD) between the control and the experimental groups in both rats and rabbits, between the control (C) and Al-treated groups (Al) as well as between Al-treated and those
provided with Al co-administered with the plant (AlS). The significance is taken at $P < 0.05$ and $P < 0.01$.

RESULTS

The results of blood parameters in rats and rabbits are shown in Tables 1 and 2, respectively. For the rats, the results showed only PCV and MCHC in group (G3) and PCV in group (G4) that had increased significantly ($P < 0.05$) [Figure 1], whereas for the rabbits, only Hb and MCV values had increased significantly ($P < 0.05$) [Figure 2].

Mean values of BWs of rats and rabbits are shown in Tables 3 and 4, respectively. The results showed that there were no significant changes ($P > 0.05$) observed in the BWs of the two groups of animals.

The results of blood parameters in group (Al), group (AlS), group (S) and group (C) are shown in Table 5. The results showed RBCs, Hb, PCV and MCHC of group (Al) and group (AlS) were each significantly low when compared to those in group (C) and group (S) whereas in these two groups each pair was not significantly different ($P > 0.05$) [Figure 3 and 4]. On the other hand, WBC count, PLT and Lym of group (Al) were not significantly affected ($P > 0.05$) when each compared to that in group (C), group (AlS) and group (S) and only MCHC and PCV of group (S) were significantly higher ($P < 0.05$) than those in the control group (C).

The results showing the mean values of electrolytes; namely: Na, K, Ca, P and Fe are shown in Table 6. The results revealed the levels of K, Ca and P in group (S) to increase significantly ($P < 0.05$) when each compared to those in group (C). On the other hand, the level of K and P in group (Al) and K, Ca and P of group (S) were found significantly higher ($P < 0.01$) when each compared to that in group (Al).

For Fe concentration, it was significantly reduced ($P < 0.01$) in group (Al) and group (AlS) when each compared to its levels in group (C). Moreover, the level of Fe was found significantly higher ($P < 0.01$) when each compared to that in group (Al).

DISCUSSION

The blood parameters; RBCs count, Hb, PCV, MCHC, MCV together with the level of Fe are indicative indices of anemia and could be used to indicate nutritional values of
ingested diets as well.[26,27] Therefore, occurrence of anemia is attributable to lowered values of such indices.

However, the observed increase in PCV and MCHC in tested rats of this study might be due to stimulating effect of the *S. Argel* on erythropoietin, a hormone leading to red blood production.[28] Moreover, the increase in PCV appeared to be dose-dependent and to some extent for MCHC.

In the meantime, the increase of Hb and MCV in rabbits following intake of *S. argel*, could be due to high nutritional values of this plant particularly in vitamins and minerals such as Fe and copper.[13] In this context, Fe is reported to be necessary for the formation of the heme part of the hemoglobin and copper for the absorption of Fe from the gastrointestinal tract.[29]

These results, probably were expected as phytochemical screening of leaves of this shrub had revealed the presence of plenty of biochemical nutritive components as well as minerals.[13] Unexpected to occur, however, was that such finding not to be reflected in marked increase in BWs of the experimental animals. However, insignificant changes in BWs were probably owing to the fact that the animals were only fed for a month and progressed in weight with slightly slow rate of increase. On the other hand, persistence of feeding for prolonged periods might lead to overwhelmed increase in the BWs of experimental animals.

Similar to its toxic effects reported elsewhere Martinez et al.,[18] Vota et al.,[19] Tomljenovic and Shaw[21] Newairy et al.,[20] Al administration by rats in the present study had also induced significant reductions in the blood indices of anemia. However, the significant increase in MCV in Al-treated group could be attributed to reduced level of RBCs count in the same group where MCV is usually calculated by dividing PCV by RBCs count. In this context, several mechanisms have been proposed for the Al-induced anemia, but the exact mechanism is unknown. The proposed mechanisms appear to involve reduction in heme biosynthesis and Fe metabolism in rats[31,32] moreover, the increased Al levels in serum of patients with chronic renal failure on hemodialysis were reported to associate with impaired erythropoiesis and Fe metabolism.[31] Other studies conducted by Farina et al.,[33] Vittori et al.,[34] Willhite et al.,[37] confirmed AlCl 3 involvement in reduction of RBCs synthesis in bone marrow.

In the present study, uptake of ethanolic extract of *S. argel* co-administered with AlCl 3 seems to mitigate Al-induced anemia and to raise the same values of blood indices of anemia but not to significant levels except for MCHC, MCV and PCV when compared with those of (Al) alone. As inferred from other reports, two mechanisms could be suggested for the prevention of Al toxicity by *S. argel*: First, the plant inhibited or reduced Al absorption from intestine and the second: Aluminum overload might modulate gastrointestinal Fe absorption and hinder the cellular uptake of Al.[35] Moreover, insignificant changes of WBCs, PLT and LYM prior and after treatment with Al toxicity could confirm further that the effect of Al was associated with Fe metabolism.

On the other hand, concurrent fluctuated levels of Fe with administration of AlCl 3 alone and with *S. argel* could furtherly confirm the claim of the mitigative effect of *S. argel* against anemia. Moreover, having other electrolytes unaffected by Al toxicity taken with their levels being improved by *S. argel*, alone or mixed with AlCl 3, would probably support the claim that the plant was effective as anti-inflammatory agent,[40] against adverse effect that might be caused by Al and hence enhanced absorption of these minerals via intestinal tract.

REFERENCES

17. Willhite CC, Ball GL, McElhaney CJ. Total allowable concentrations of monomeric inorganic aluminum and hydrated aluminum silicates in...


32. Han J, Han J, Dunn MA. Effect of dietary aluminum on tissue nonheme iron and ferritin levels in the chick. Toxicology 2000;142:97-109.


How to cite this article: ???

Source of Support: Nil, Conflict of Interest: None declared.

Author Queries???
AQ1: Kindly provide running title missing???
AQ2: Kindly cite the reference 24 and 25 citation in the text
AQ3: Please provide Conclusion in abstract???
AQ4: Tables 1-6 are cited in text, but tables not provided. Please provide tables???