

Full Length Research Paper

Effects of *Ambrosia maritima* (Damsissa) ethanolic extract on phenylhydrazine hydrochloride-induced anaemia in rabbits (*Lepus cuniculus*)

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This work was performed to evaluate the anti-anaemic activity of the ethanolic extract of *Ambrosia maritima* (Damsissa) in phenylhydrazine hydrochloride (PHZ)-induced anaemia in rabbits. Twenty-five adult rabbits of different sexes were equally divided into 5 groups. The induction of anaemia was performed by subcutaneous administration of PHZ at a dose of 30 mg/kg body weight and maintained a dose of 15 mg/kg body weight. Ethanolic extract of *A. maritima* was orally administered to the groups at different dose rates (250, 500 and 1000 mg/kg body weight) and blood samples were collected at different intervals of time for haematological examinations. The investigated haematological parameters included Hb concentration, total erythrocytes count (RBCs), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). The results revealed a significant ($p < 0.05$) decrease in Hb concentration, RBCs count and PCV as well as a significant increase in MCV and MCH in response to PHZ-treatment. No significant changes were observed in MCHC throughout the duration of the experiment. The data demonstrated that *A. maritima* ameliorated hemolytic anemia (anti-anaemic effect). Oral treatment with *A. maritima* extract did not demonstrate any toxic effect at the given doses as marked by hematological data.

Key words: Hemolytic anemia, phenylhydrazin, *Ambrosia maritima*, anti-anaemic effect.

INTRODUCTION

Medicinal plants, since time immemorial, have been used virtually in all cultures as a source of medicine. The widespread utilize of herbal remedies and health care preparations, as those described in ancient texts such as the Vedas and medicinal plants have been traced to the

occurrence of natural products with medicinal properties (Akerle, 1988).

Anaemia is a condition in which the number of circulating red blood cells is decreased, either the amount of haemoglobin, or the volume of packed red cells

causing a reduction in the blood's ability to provide enough oxygen to body tissues and organs. Certain diseases, such as malaria, malnutrition, protozoal infections and physiological conditions such as pregnancy are among the various conditions that may lead to anaemia in both the adults and children. WHO epidemiological studies revealed that almost more than 1/4 of the world population suffered from anemia (Adusi-Pokuyet al., 2008).

Ambrosia maritima is a member of the family Asteraceae. It is known in Sudan as "Damsisa", and is a widely distributed weed in Northern and Central Sudan especially near water catchment and Nile Bank (El Ghazali et al., 1994). Traditionally, the decoction of the whole plant is used to cure gastrointestinal disturbances, abdominal pain, kidney inflammation and renal colic, whereas the leaves are used for diabetes and hypertension cure. In addition, its curative properties are extended to include molluscicidal, antimalarial and antitumor activities (Diraret al., 2014).

As most plants of Asteraceae family, the plant is rich in sesquiterpene lactones, such as neoambrosin, ambrosin, and damsin which have molluscicidal and cytotoxic activities. In addition, this plant has been shown to contain several phytoconstituents such as coumarins, flavonoids, sterols and tannins, and exhibit considerable antioxidant activity (Said et al., 2018).

The current work aimed to investigate the effects of *A. maritima* (Damsisa) ethanolic extract on the Phenylhydrazine hydrochloride-induced anaemia and to determine the main active principles responsible for its anti-anaemic activity.

MATERIALS AND METHODS

Collection, identification and preparation of plant material

A. maritima shoots were collected from their natural habitat from the river bank of Elmatmma locality, River Nile State, Sudan. The plant was authenticated by Medicinal and Aromatic Plants Research Institute (MAPRI), Sudan. The plant material was cleaned from dirt, shade dried, and then ground into coarse powder.

Preparation of the ethanolic extract of *Ambrosia maritima*

Extraction was performed according to the method depicted previously by Sukhdev et al. (2008) as follows; 2000 g *A. maritima* shoots was ground using mortar and pestle and successively extracted by soaking in 80% ethanol for about 72 h with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the yield weight was 138.5 g.

Proximate and chemical analysis of *A. maritima*

A. maritima shoots were analyzed for their proximate composition and mineral contents. The proximate analysis was done for crude fibre, crude protein, dry matter, ash, ether extract, and nitrogen free extract. The mineral contents include: sodium, potassium, calcium, magnesium and iron.

Phytochemical screening of *A. maritima*

Phytochemical analysis tests were carried out using specific standard methods. Ethanolic extract of *A. maritima* was divided into several portions for identification of tannins, terpenoids, cardiac glycosides, alkaloids, saponins, and anthraquinones (Boham et al., 1994; Debiyi and Sofowora, 1978; Harborne, 1973; Obadoni and Ochuko, 2002; Sofowora, 1993; Wagner and Bladt, 1996).

Test for tannins: 5 ml of bromine water was mixed with 0.2 g ethanolic extract. Decoloration of bromine water demonstrated the entity of tannins (Boham et al., 1994).

Test for terpenoids: 2.0 ml of chloroform was added to 5 ml of the ethanolic extract, evaporated on a water bath and then heated with 3 ml of concentrated H_2SO_4 . The formation of a grey color is an indication of the presence of terpenoids (Debiyi and Sofowora, 1978; Sofowora, 1993).

Test for alkaloids: Crude extract (0.3 g) was added to 2 ml of concentrated HCl. A small amount of amyl alcohol was added to the mixture and filtered at room temperature. Few drops of Dragendorff's reagent (solution of potassium bismuth iodide) were added to the acid layer and a reddish-brown precipitate was observed (Harborne, 1973; Obadoni and Ochuko, 2002).

Test for cardiac glycoside (Kedde test): Part of the ethanolic extract (3 ml) was added to a small amount of Kedde reagent (Mix equal volumes of a 2% solution of 3, 5 dinitrobenzoic acid in menthol and a 7.5% aqueous solution of KOH). Appearance of a blue or violet color indicated the presence of cardinols (Debiyi and Sofowora, 1978; Sofowora, 1993).

Test for saponins (Froth Test): Crude extract (0.5 g) was dissolved in 5 mL distilled water. The mixture was shaken vigorously and stable persistent froth was obtained (Wagner and Bladt, 1996).

Test for anthraquinones (Borntrager's Test): Crude extract (0.5 g) was taken into the first test tube, and 5 ml chloroform was added while shaking for 5 min. The extract was filtered to the second test tube and shaken with an equal volume of 100% ammonia solution. The development of pink or red color in the ammonia layer (lower layer) indicated the presence of anthraquinones (Debiyi and Sofowora, 1978; Sofowora, 1993).

Experimental animals

Twenty-five apparently healthy adult rabbits (*Lepus cuniculus*) of different sexes weighing 0.9 to 1.7 kg were obtained from Omdurman Local Market, Sudan. The rabbits were identified by ear

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Table 1. Phytochemical screening of ethanolic extract of *Ambrosia maritima*.

Secondary metabolite	Results
Terpenoids	+
Alkaloids	-
Saponins	+
Anthraquinones	-
Cardiac glycosides	-

+, presence; -, absence.

tags, housed in cages in the Department of Nutrition, Faculty of Animal Production, University of Khartoum and maintained under standard environmental condition; controlled temperature, relative humidity with free access to water and *Medicago sativa* (Alfalfa) hay. The experiment in rats was done in accordance with the ethical principles in animal research, approved by the Committee for Ethics at Sudan Veterinary Council, Ministry of the Cabinet.

Experimental design

After adaption period for 30 days, the rabbits were weighed, and assigned randomly into five groups; A, B, C, D, and E (5 rabbits/each). Groups B, C, D and E were injected subcutaneously with a single dose of phenylhydrazine hydrochloride (30 mg/kg body weight) and with a maintained dose of 15 mg/kg body weight for 2 days after administration of the first dose. *A. maritima* ethanolic extract was administered orally to rabbits of groups B, C, at doses 250, 500 and 1000 mg/kg body weight/day, respectively starting from day 8. Animals in Group E served as phenylhydrazine control group while Group A served as control one (without phenylhydrazine and without *A. maritima*).

Blood samples collection

The area of blood sampling was shaved scrubbed by disinfectant (70% ethanol) before the jugular vein was punctured. The collection of blood samples was achieved at days 0, 7, 14, 22 and 30 of the experimental periods, for haematological analysis, which include: Hemoglobin concentration (Hb), Hematocrit (PCV), Erythrocyte count (RBCs), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC).

Statistical analysis

One-way analysis of variance (ANOVA) was utilized for the analysis of data. Duncan's multiple range test was used for determining the significance. A probability value of $p < 0.05$ was considered as significant (Snedecor and Cochran, 1989).

RESULTS

Phytochemical screening

The results of phytochemical constituents of *A. maritima* ethanolic extract are demonstrated in Table 1. The

phytochemical screening showed the entity of triterpenes, saponins and tannins as well as the absence of alkaloids, anthraquinones and cardiac glycosides.

Proximate analysis

The proximate analysis of the *A. maritima* ethanolic extract demonstrated the presence of all the macronutrients (Table 2).

Mineral content evaluation

Table 3 exhibited that major trace elements and minerals are present in *A. maritima*, in relatively high concentrations. The highest mineral concentration (141.50 ppm) was that of potassium (K), mean while, the lowest concentration (9.55 ppm) was that of magnesium (Mg).

Haematology analysis

The haematological values of Hb, RBCs, PCV, MCV, MCH, and MCHC, are presented in Figures 1 to 6, respectively. The data did not show any significant differences among the experimental groups at day zero of the experiment. At day 7, all groups except group A (negative control), of the haematological parameters revealed significant ($P < 0.5$) reduction in the values of Hb concentration (Figure 1), RBCs count (Figure 2) and PCV (Figure 3); meanwhile, there were significant ($p < 0.05$) increase in MCV (Figure 4) and MCH (Figure 5) in PHZ-treated groups compared to the negative control (Group A). However, these groups (B, C, D and E) did not exhibit any significant differences in MCHC (Figure 6) compared to the negative control (Group A).

At day 14, Groups B, C and D which were given *A. maritima* showed an increase in Hb concentration compared to Group E. Nevertheless, this increase was significant in Group D, in which the Hb concentration was not significantly different from that of Group A (Figure 1). The RBCs count also showed significant ($p < 0.05$)

Table 2. Percentage proximate composition of *Ambrosia maritima*.

Macronutrient	Composition (%)
Crude protein	6
Crude fiber	27.51
Ether extract	1.42
Ash	13.47
Nitrogen free extract	45.28
Dry matter	93.58

Table 3. Mineral contents of *Ambrosia maritima*.

Minerals	Concentration (ppm)
Sodium (Na)	57.50
Potassium (K)	141.50
Calcium (Ca)	25.86
Magnesium (Mg)	9.55
Iron (Fe)	19.7

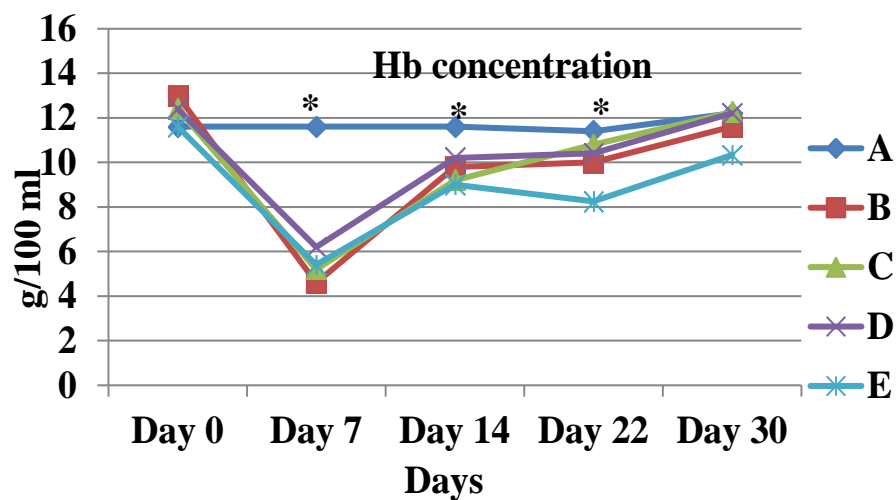


Figure 1. Effect of ethanolic extract of *Ambrosia maritima* on haemoglobin concentration (g/dl) in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean \pm SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritima* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritima* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritima* ethanolic extract/kg body weight. E: PHZ.

increase in all the *A. maritima* treated groups (B, C and D) compared to that of group E. Noteworthy, the *A. maritima* treated groups did not demonstrate any significant differences in RBCs count when compared to Group A (negative control group). The PCV values increased in Groups B, C and D to a level that was significantly higher than that of group E, but was not significantly different from that of group A. The MCV

values decreased significantly in Groups B, C and D in comparison to that of Group E. MCV values in these groups were comparable to that in control group and did not show any significant differences compared to Group A. The MCH values in *A. maritima* treated groups decreased to a level, which was not significantly different compared to that of Group A. On the other hand, group E still showed significant rise in MCH value compared to

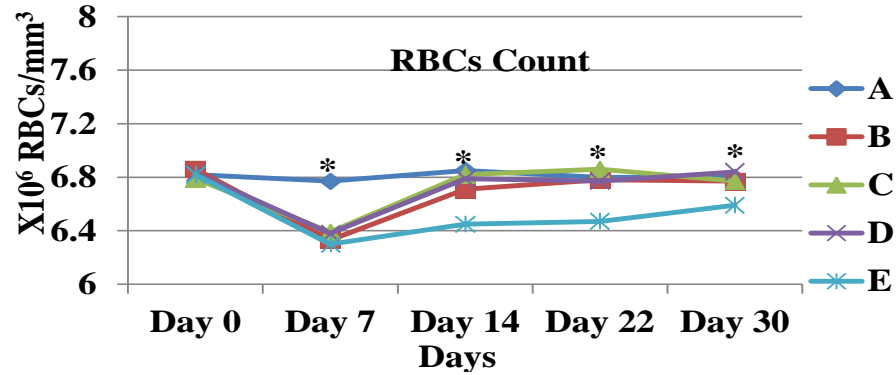


Figure 2. Effect of ethanolic extract of *Ambrosia maritima* on total erythrocyte count in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritima* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritima* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritima* ethanolic extract/kg body weight. E: PHZ.

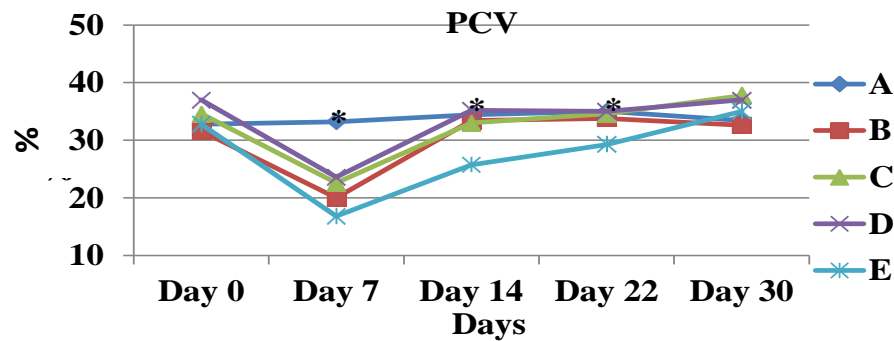


Figure 3. Effect of administration of ethanolic extract of *Ambrosia maritima* on packed cell volume (PCV %) in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritima* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritima* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritima* ethanolic extract/kg body weight. E: PHZ.

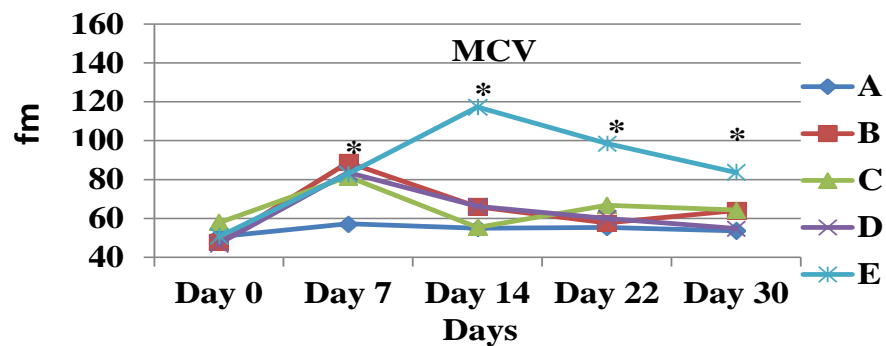


Figure 4. Effect of ethanolic extract of *Ambrosia maritima* on mean corpuscular volume (MCV) fm, in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritima* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritima* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritima* ethanolic extract/kg body weight. E: PHZ.

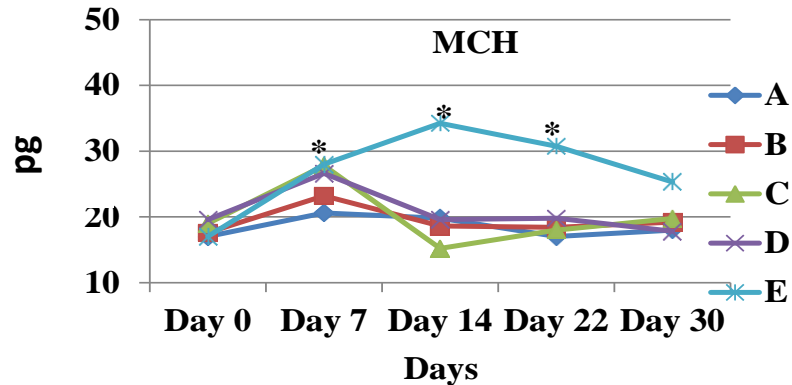


Figure 5. Effect of ethanolic extract of *Ambrosia maritima* on mean corpuscular haemoglobin (MCH) pg, in phenylhydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritima* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritima* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritima* ethanolic extract/kg body weight. E: PHZ.

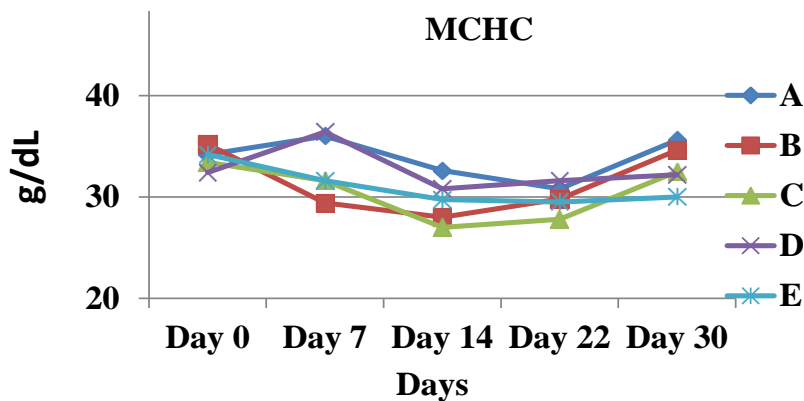


Figure 6. Effect of ethanolic extract of *Ambrosia maritima* on mean corpuscular haemoglobin concentration (MCHC) g/dl in phenyl hydrazine HCL (PHZ)-induced anaemic rabbits (Mean±SEM). A: Control without phenylhydrazine and without *Ambrosia maritima* extract. B: PHZ +250 mg *Ambrosia maritima* ethanolic extract/kg body weight. C: PHZ +500 mg *Ambrosia maritima* ethanolic extract/kg body weight. D: PHZ +1000 mg *Ambrosia maritima* ethanolic extract/kg body weight. E: PHZ.

that of the other experimental groups (A, B, C, and D). MCHC values at day 14 did not show any significant difference among all the experimental groups.

At day 22, no significant differences in Hb concentrations were noticed between the *A. maritima* treated groups (B, C, and D) and control group (Group A). However, the PHZ-treated group (group E) still showed significantly lower value compared with the control group (group A) and to the *A. maritima* treated groups, with the exception of group B (which was treated with 250 mg *A. maritima*). The same trend was noticed for RBCs count, the *A. maritima* treated groups did not show any significant difference compared to control

group (Group A). However, the PHZ-treated group (Group E) showed significantly lower value of RBCs count compared to control group (A) and to that of all *A. maritima* treated groups (B, C, and D). PCV value was not significantly different from that of all experimental groups. On the other hand, MCV in *A. maritima* treated groups did not show any significant difference compared to control (A). Same trend was noticed for MCH; no significant differences were noticed between *A. maritima* treated groups and the control, meanwhile PHZ-treated group (E) showed significantly higher MCH compared to control group and *A. maritima* treated groups (B, C, and D). Moreover, all experimental groups (including control

one) did not exhibit any significant differences in MCHC when compared to each other.

At day 30, all experimental groups did not show any significant differences in Hb concentration, PCV, MCH, and MCHC. However, RBCs count in Group E (phenylhydrazin group) was still significantly lower compared to negative control group (Group A) and *A. maritima* treated groups (Groups B, C, and D). MCV value in group E was significantly higher compared to Groups A and D; however, it did not demonstrate any significant difference compared to Groups B and C. Noteworthy among the treated groups, Group D had MCV which numerically coincided with that in control group and significantly different from that in Group E.

DISCUSSION

The data of the proximate analysis of *A. maritima* (Table 2) showed that crude protein content of the extract was 6% which is comparable to those reported for *Grewia tenax* fruits (Ali, 2009).

On the other hand, the data of the proximate analysis demonstrated that the crude fibre content (Table 2) was higher than the determined fibre content of *G. tenax* fruits (Ali, 2009) and *Telfairia occidentalis* (Ogbe et al., 2010).

This has been beneficial because food fibre has been documented to potentiate the absorption of trace elements in the intestine and decrease cholesterol absorption (Le-veille and Sanberlich, 1966). This makes the plant suitable for combating anaemia.

The ash content is an indicator for plant's mineral content. The higher ash content (13.47%) indicated that the plant contains appreciable amount of mineral elements. The value was higher than that reported for *Jatropha tanjorensis* leaves (Iduet et al., 2014) and *G. tenax* fruits (Ali, 2009).

Evaluation of the mineral content (Table 3) showed that *A. maritima* contains high values of iron. This is consistent with the results of Yagi et al. (2013) who reported that *A. maritima* leaves have higher value of iron (590 ± 1 ppm) compared to *G. tenax* (200 ± 4 ppm), which is used as anti-anaemic plant in Sudan. Iron is an essential element for human beings and animals for the synthesis of haemoglobin. It facilitates the oxidation of carbohydrates, proteins and fats to control body weight, which is very important factor in diabetes (Yagi et al., 2013).

On the other hand, *A. maritima* contains high level of calcium (Table 3). Calcium is the main constituent of the skeleton and is essential for regulating many vital cellular activities such as nerve and muscle functions, hormonal actions, blood clotting and cellular mortality (Yagi et al., 2013). Furthermore, the proximate analysis revealed high potassium content, which is essential for different physiological processes including enzyme activation and protein synthesis. Potassium also participates actively in

the maintenance of the cardiac rhythm (Martin et al., 1985).

Haemolytic anaemia could be induced in rabbits after subcutaneous administration of phenylhydrazine hydrochloride at a dose of 30 mg/kg body weight with maintained dose of 15 mg/kg body weight of the same drug 2 days after the administration of the first dose (Prasong and Maitree, 1994; Ogbe et al., 2010).

Phenylhydrazine hydrochloride has been earlier used to induce anaemia in rats (Bowman and Rand, 1980). These authors reported that anaemia was observed after 6 days of injection. The recovery from anaemia occurs at day 9. After six days of exposure, Phenylhydrazine was reported to cause the formation of Heinz bodies on RBC membranes (Bowman and Rand, 1980; Gordon-Smith, 1980). Akah et al. (2009) reported that oral administration of 10 mg/kg phenylhydrazine for 8 days reduces haematological indices by 50%. In further studies, phenylhydrazine was found to decrease haemoglobin concentration, red blood cell count and haematocrit (Agbor et al., 2005; Berger, 2007).

In the present study, the anemia developed by phenylhydrazine was macrocytic hypochromic anaemia, which is common in haemolytic anaemia (Lee et al., 2014).

The effect of administration of *A. maritima* ethanolic extract at 250, 500 and 1000 mg/kg body weight on haematological parameters in anaemic rabbits, clearly indicated that there were significant increase in the values of Hb, RBC and significant decrease in MCH, MCV values, which might indicate significant improvement of these parameters by *A. maritima* extract. Treatment of anaemia by plant extracts has been well documented by many research works. Oladijiet al., (2007) reported that administration of aqueous extract of *Sorghum bicolor* stem bark results in significant increase in haemoglobin concentration and PCV. Similarly, Pawar et al. (2010) reported significant increase in erythrocytes count, haemoglobin concentration, leukocytes count and haematocrit in anaemic rats treated with *Asteracantha longifolia*. Moreover, Akah et al. (2009) demonstrated previously that the orally administration of *Brillantai sianitens* extract to PHZ-treated rats elevated Hb, RBCs count, and PCV within one week.

Nevertheless, the extract did not show any alteration in MCHC values throughout this study. Thus, the MCHC values remain in the normal level which may be further evidence for macrocytic anaemia or due to releasing of reticulocytes. Similar result has been reported by Ali (2009) in the treatment of hemorrhagic anaemic rats with *Azanza garckeana* aqueous extract.

Furthermore, the PCV values increased in Groups B, C and D to a level which was significantly higher than that in Group E, but was not significantly different from that in Group A.

Nevertheless, a significant reduction in Hb and RBCs count in response to intramuscular injection with *A.*

maritima extract and feeding *A. maritima* to chicks in diet has been reported (Bakhiet and Adam, 1996; Azza, 2003). The discrepancy between the present results and the previous ones might be due to source of the plant, method of extraction, route of administration and animal species used; however, the oral route is the most common in folk medicine. Noteworthy, oral administration of water extract of *A. maritima* has been found to increase RBCs count and Hb concentration significantly in anaemic rats (Azza, 2003).

Conclusion

The study concluded that *A. maritima* ethanolic extract showed anti-anaemic effect on phenylhydrazine induced anaemia in rabbits and this was clearly proved by increasing the hematological values.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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