

Anti-anaemic Potential of Aqueous Extract of *Spinacia oleracea* Leaf in Phenylhydrazine-treated Rats¹Luka, C.D., ¹Abdulkarim, M., ¹Adoga, G.I., ²Tijjani, H. and ¹Olatunde, A.¹Department of Biochemistry, University of Jos, P.M.B. 2084, Jos, Plateau State, Nigeria²DBR Laboratory, Department of Biochemistry, Bauchi State University, P.M.B. 65, Gadau, Nigeriaolatundeahmed@gmail.com

Abstract: The effects of oral administration of aqueous extract of *Spinacia oleracea* leaf on some haematological indices and body weight changes in phenylhydrazine-treated rats as well as its phytochemical constituents were investigated. Extract was administered to anaemic and normal rats at 100 mg/kg bwt. once daily for 28 days. Extract was found to be rich in phytochemicals and improved the body weight of anaemic and normal treated rats. No significant ($p > 0.05$) change was observed in PCV of anaemia treated group and normal treated group compared with control. However, there was a significant ($p < 0.05$) increase in level of Hb of the normal treated group. The PCV, Hb and RBC level of the anaemia treated group increased ($p < 0.05$) significantly when compared with that of the anaemia untreated group. Aqueous extract of *S. oleracea* leaves reversed anaemic conditions in the phenylhydrazine-treated rats; these may be attributed to its rich phytochemical, nutrient and vitamin contents.

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1.0 Introduction

Anaemia is a common blood disorder that affects people of all ages, with the elderly, young women of childbearing age and infants at greater risk. The condition is not a disease but could develop as a result of various diseases. There are over 400 types of anaemia, many of which are rare but in all cases there is lower than normal number of circulating red blood cells (Duff, 2008). Anaemia is characterised by the decrease of the haemoglobin rate less than 13 g/dl in male or 12 g/dl in female (Kanfer and Nicol, 1997). It remains a major public health concern in many developing and under-developed countries with all age groups at risk, because it causes varying degrees of lowered work capacity, impairment in cognitive performance, lowered immunity to infections, pregnancy complications, reduced psychomotor skills, and poor learning capacity (Oladiji *et al.*, 2007).

Over the years, folklore medicine has grown to be recognized as of great health importance to the health of individuals and communities (Oladiji *et al.*, 2007). In Nigeria, folklore medicine plays an important role in health care delivery and about 70-80% of the population depends on it for their ailments (Akah *et al.*, 1998). A good number of medicinal plants are traditionally employed to alleviate anaemia. Some of these plants include *Telfeiria occidentalis*, *Combretum dolichopetalum*, *Psorospermum ferbrifugum*, *Jatropha curcas*, *Flacourtia flavescens*, *Brillantasia nitens* (Alada, 2000; Dina *et al.*, 2006), *Brillantasia nitens* (Akah *et al.*, 2009), *Sphenocentum jollyanum* seed oil (Mbaka

and Owolabi, 2011), *Telferia occidentalis* (Alada, 2000), *Waltheria indica* (Oladiji *et al.*, 2005), *Hygrophila spinosa* (Gomes *et al.*, 2001), *Hibiscus sabdariffa* (Adigun *et al.*, 2006), *Sorghum bicolor* (L.) moench stem bark (Oladiji *et al.*, 2007).

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family of Chenopodiaceae (Gaikwad *et al.*, 2010). *S. oleracea* Linn, is well-known for its vitamin and mineral contents (Hanif *et al.*, 2006; Gaikwad *et al.*, 2010). The alkaline vegetable contains carotene (the precursor of vitamin A), calcium, iodine and ferrous ions, and is considered suitable for children and pregnant women (Maeda *et al.*, 2005). Additionally, the vegetable has beneficial effects on anemia and constipation, due to its saponin, dietary fiber, ferric salt, and folic acid content. Folic acid and ferric salts in spinach have a potent anti-proliferation to combat stomach, colon, and lung cancer (Maeda *et al.*, 2005; Matsubara *et al.*, 2005). A recent study shows folic acid in spinach exerts anti-hyperglycemic effects by removing homocysteine in the blood. Moreover, the vegetable not only inhibits DNA breakage due to oxidative stress, but also reduces damage to ischemic brains (Wang *et al.*, 2005).

The aim of this study is to evaluate the potential of aqueous extract of *S. oleracea* leaf in the treatment of anaemia in phenylhydrazine-treated albino rats.

2.0 Materials and Methods**2.1 Chemical and Reagents:**

Phenylhydrazine used was products of Sigma, St. Louis, USA. All other chemicals were of

analytical grades and prepared in glass apparatus using distilled water.

2.2 Laboratory Animals

Wistar rats (16) of both sexes weighing between 133-138g were obtained from the Animal Breeding Unit of the National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria.

2.3 Plant Material

The leaves of *Spinacia oleracea* (spinach) were collected at Massallacin-Jumma'a Street, Jos, Plateau State, Nigeria in July 2010 and were authenticated by Dr. M.C. Okonkwo of Department of Forestry Technology, FCF Jos, Plateau State, Nigeria.

2.4 Preparation of Extract

The method described by Yakubu *et al.* (2005) was adopted for the preparation of the aqueous extract of *Spinacia oleracea* leaves. Briefly, the leaves were separated from the stem and oven dried at 40°C for 72 hours to a constant weight. The dried pieces were then pulverized using a blender. The powdered material was stocked in a plastic container from which 40 g was extracted in 200ml of cold distilled water for 48 hours at 37°C. This was then filtered with Whatman No. 1 filter paper. The filtrate was concentrated on a steam bath to give the extract (brownish black slurry). The extract was then reconstituted in distilled water to give the required dose of 100 mg/kg body weight was used in this study.

2.5 Induction of Anaemia

Anaemia was induced in rats by intraperitoneal administration of phenylhydrazine (60 mg/kg body weight) daily for 2 days, according to Roque *et al.* (2008). Phenylhydrazine hydrochloride solution was prepared in 0.1 M potassium phosphate buffer, pH 7.4 Confirmatory test using 3 rats was carried in a trial experiment, after which the plasma hemoglobin level was determined to verify that the rats were anaemic.

2.6 Animal Grouping and Administration of Plant Extract

The animals were housed in standard plastic cages and were acclimatized for a week. They were maintained under standard conditions of 12 hours light and dark cycle and were fed with standard pelleted feed and distilled water *ad libitum*. The animals were grouped as follows:

Group A: Normal Control (1ml distilled water/day)

Group B: Normal + Extract (100 mg/kg body weight/day)

Group C: Anaemic Control (1ml distilled water/day)

Group D: Anaemic + Extract (100 mg/kg body weight/day)

Each group consists of four animals (n = 4).

All administration was done orally using oropharyngeal cannula once per day for 28 days.

2.7 Phytochemical Screening (Qualitative)

The presence of alkaloids, flavonoids, tannins, terpenes, steroids, phenolics, cardiac glycosides, resins, balsam and saponins were determined by the methods described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

2.8 Collection of Blood Sample

The methods described by Yakubu *et al.* (2005) were used for blood collection. In brief, with the animal under diethyl ether anesthesia, the neck area was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were then sharply cut with a sterile scalpel blade and about 3cm³ of blood was collected in an EDTA sample bottle for the haematological assay.

2.9 Determination of Haematological Parameters

Packed Cell Volume (PCV), Haemoglobin (Hb) and Red Blood Cell (RBC) values were determined using Mindray Haematology Analyser (Mindray BC-2300, Guangzhou Shihai Medical Equipment Co., Ltd, China).

2.10 Statistical Analysis

The data were expressed as Mean \pm Standard Error of Mean. Statistical analysis was performed using analysis of variance (ANOVA) and Duncan multiple range test at 5% level of confidence ($p < 0.05$).

3.0 Results

Phytochemical screening of the aqueous extract of *Spinacia oleracea* leaf revealed the presence of flavonoids, saponins, cardiac glycosides, terpenes, steroids and resins (Table 1). The presence of alkaloid, tannins, phenolics and balsam were not detected.

Table 2 shows the initial body weight, final body weight and the percentage weight gain of phenylhydrazine-induced anaemic rats administered with aqueous extract of *Spinacia oleracea* leaf for 28 days. Results showed that there was no significant ($p > 0.05$) difference in the initial weights of rats in all groups. However, there was a significant ($p < 0.05$) decrease in the anaemia treated and anaemia non-treated group at the 28th day. Results also show that anaemia untreated group has 6.88% weight gain

compared with that of the control which was 16.67%, while normal treated group and anaemia treated group has 18.82% and 11.21% weight gain respectively.

Haematological indices (Hb, PCV and RBC) of the four experimental groups showed that there was a significant ($p < 0.05$) decrease in PCV, Hb and RBC levels of the anaemia untreated group when compared with the control group (Table 3). No

significance ($p > 0.05$) change was observed in the PCV of anaemia treated group and normal treated group compared with the control. However, there was a significant ($p < 0.05$) increase in the level of Hb of the normal treated group compared with the control. The PCV, Hb and RBC level of the anaemia treated group increased ($p < 0.05$) significantly when compared with that of the anaemia untreated group at the 28th day.

Table 1: Qualitative phytochemical screening of aqueous extract of *Spinacia oleracea* leaf

Phytochemicals	Status
Alkaloids	-
Flavonoids	+
Tannins	-
Saponins	+
Cardiac glycoside	+
Terpenes	+
Steroids	+
Phenolics	-
Resins	+
Balsam	-

Key= + present; - absent

Table 2: Body weight and percentage weight gain of phenylhydrazine-induced anaemic rats administered with aqueous extract of *Spinacia oleracea* leaf

Groups	Initial weight (g)	Final weight (g)	% weight gain
Control	135.00 \pm 4.10 ^a	157.50 \pm 6.50 ^a	16.67
Normal + Extract	138.75 \pm 4.70 ^a	165.00 \pm 4.10 ^a	18.92
Anaemic Control	138.75 \pm 4.70 ^a	148.30 \pm 2.80 ^b	6.88
Anaemic + Extract	138.75 \pm 4.30 ^a	148.75 \pm 4.80 ^b	11.21

Values are expressed as Mean \pm SEM (n = 4) except for % weight gain. Values in each column with different superscript (a-b) are significantly different ($p < 0.05$).

Table 3: Some haematological parameters of phenylhydrazine-induced anaemic rats administered with aqueous extract of *Spinacia oleracea* leaf

Groups	PCV %	Hb (g/dl)	RBC ($10^6/\mu\text{l}^3$)
Control	37.80 \pm 2.20 ^a	13.25 \pm 0.34 ^a	5.40 \pm 0.20 ^a
Normal + Extract	40.80 \pm 1.70 ^a	15.13 \pm 0.34 ^b	5.85 \pm 0.22 ^a
Anaemic Control	30.70 \pm 1.50 ^b	10.20 \pm 0.26 ^c	3.15 \pm 0.06 ^c
Anaemic + Extract	36.00 \pm 1.80 ^a	12.70 \pm 0.24 ^a	4.15 \pm 0.05 ^b

Values are expressed as Mean \pm SEM (n = 4). Values in each column with different superscript (a-c) are significantly different ($p < 0.05$). PCV=Packed Cell Volume, Hb= Haemoglobin, RBC=Red Blood Cell

4.0 Discussion

Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005). Diets containing an abundance of fruits and vegetables are protective against a variety of diseases, particularly cardiovascular diseases (Okogun, 1985). An example of such plant is *Spinacia oleracea*. *Spinacia oleracea* has been used in most tropical countries as both vegetable and herb due to its high nutritional content

and medicinal value in the alleviation of many ailments due to unaffordability of the cost of modern health care and orthodox medicine.

In our present study of *S. oleracea* leaves, the phytochemical investigation indicates the presence of flavonoids, saponins, cardiac glycosides, terpenes, steroids and resins (Table 1). Saponins and alkaloids have been reported to possess anti-anaemic potentials (Falcone *et al.*, 1997). Saponins are also known to inhibit platelet aggregation and thrombosis. Saponin containing herbs have been successfully used in the

management of liver inflammation, as tonic sedative formulas and to promote and vitalize blood circulation (Shi *et al.*, 2004; Wang *et al.*, 2004). Since saponins are membrane active agents that lyse red blood cells or other wall, it is possible that the red blood cells were initially lysed by this plant; the cells overcome this inhibition by producing glycosidic enzyme which cleaves some of the terminal sugars from the saponin, thereby detoxifying it (Pathirana *et al.*, 1990). This detoxification of saponins, thus enhanced the proper utilization of the iron contained in the aqueous extract of *Spinacia oleracea* to synthesize heme/haemoglobin for new red blood cells thus leading to an improved Hb, PCV and RBC. Interestingly, saponins especially terpene glycosides enhance natural resistance and recuperative powers of the body (Singh *et al.*, 1991). The anti-anaemic potential and haemoglobin restoring effect of aqueous extract of *Spinacia oleracea* leaf as suggested by the data in the present study could be attributed in part to its saponins contents.

It has been demonstrated previously that intraperitoneal administration of phenylhydrazine decreased haemoglobin concentration, red blood cell number and haematocrit (Ebuehi and Mbara, 2011) as also observed in this study. Roque *et al.* (2008) demonstrated that intraperitoneal administration of 60 mg/kg phenylhydrazine for 2 days reduce haematological indices. Their method was adapted for this study, which is aimed at evaluating the effect of *S. oleracea* leaves extracts on the haemolytic anaemia induced by phenylhydrazine in albino rats.

Anaemia is very common and the incidence is likely to increase in future, there is a need to prevent it or seek for more cost-effective and better treatment strategies (Ogbe *et al.*, 2010). Some of the signs of anaemia in rats include hair loss or alopecia, which could be apathetic and lethargic to the rats. This could be due to the inability of the blood which carries oxygen and nutrients to the various body tissues and to the hair follicles (body cells), resulting in a less effective circulatory system (Ebuehi and Mbara, 2011). Furthermore, there was a reduction in the body weight of the anaemic rats when compared with control and normal/extract treated rats (Table 2). This observation is in agreement with previous report of Saimak (2009). The reduction in body weight in anaemic rats has reportedly been linked to reduced disaccharidases (enzymes that catalyse the last stage of carbohydrate digestion) activities in anaemic rats (Gudmand-Hoyer and Skovbjerg 1996; Vieira *et al.*, 2000). Aqueous extract of *Spinacia oleracea* leaf improved the % weight gain of normal treated and anaemic rats at the end of the study period.

The results of this study indicated that the aqueous extract of *Spinacia oleracea* increased

significantly the concentration of haemoglobin, red blood cell count and the packed cell volume after of the treatment regime (Table 3). The increase in the haematological indices exhibited by *Spinacia oleracea* extract may be due to the presence of vitamin and mineral contents of the leaves of *Spinacia oleracea*. These constituents are well known haemopoietic factors that have direct influence on the production of blood in the bone marrow. Also, the significant increase in the blood parameters (Hb, PCV, and RBC) of the anaemic rats following the administration of *Spinacia oleracea* leaf may indicate that the plant extract has the ability to stimulate the erythropoietin release into the kidney which is the humoral regulator of RBC production (Sanchez-Elsner *et al.*, 2004). Erythropoietin increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to red blood cell and subsequently to mature erythrocytes (Ganong, 1997). Aqueous extract of *Spinacia oleracea* leaf at 100 mg/kg body weight significantly increase ($p < 0.05$) only the haemoglobin concentration of normal treated rats when compared to the control and other parameters.

4.1 Conclusion

This study provides scientific evidence and lends credence to the efficacy of *Spinacia oleracea* extract in the management of anaemia in phenylhydrazine-treated rats. The haematinic potential of the plant might have been brought about by the phytochemicals which increase the haematological indices (PCV, Hb and RBC). Also, this may be made possible by vitamin and mineral constituents of the plant needed in the restoration of normal haematological conditions.

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