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Effect of *Solanum nigrum* Methanol Leaf Extract on Phenylhydrazine Induced Anemia in Rats

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Abstract

Anemia is a global health problem affecting both developed and developing countries, characterized by low level of haemoglobin in the blood. The effect of *Solanum nigrum* methanol leaf extract on phenyl hydrazine induced anemia in rats was investigated using an automatic counter. Forty-two (42) Albino rats were induced anemia through intraperitoneal injection of phenylhydrazine at 10mg/kg for 8 days. Packed cell volume was taken after some hours to ensure that the rats were anemic; those with packed cell volume less than 29% were grouped into seven groups of six rats each. Methanol extract of *Solanum nigrum* was administered at 100, 200, 300 and 400 mg/kg/body weight to groups 4, 5, 6 and 7 for three weeks orally by gastric intubation. Result obtained revealed that oral administration of *S. nigrum* methanol leaf extract to rats previously treated with phenylhydrazine significantly (p<0.05) increased the packed cell volume, haemoglobin, red blood cells, mean corpuscular volume, mean capsulated haemoglobin, and platelets in a dose dependent manner but decreased the white blood cells, lymphocytes and neutrophils within three weeks. Phytochemical analysis of the plant revealed the presence of alkaloids, saponins, flavonoids, phenols and tannins. The extract also contains substantial amount of vitamins A, K, B_6, C, E, and folic acid. Mineral elements, such as iron, magnesium, calcium, zinc and copper, were also observed in the plant extract. Results obtained also revealed that the methanol leaf extract of *S. nigrum* exhibited strong antioxidant activity measured using 2, 2-Diphenyl-l-Picryl Hydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assay at different concentrations of the methanol extract (20, 40, 60, 80 and 100 mg/mL). The findings of the present study suggest that *S. nigrum* methanol leaf extract contains hematinic properties thus, justifying the use of the plant in the management of anemia in north eastern Nigeria.

Keywords: Anaemia, *Solanum nigrum*, Vitamins, Mineral elements, Phytochemicals, Phenyl hydrazine, Haematological Parameters.

1. Introduction

Anemia is a medical condition in which the red blood cells count is less than normal. It is evidenced by a reduced quality or quantity of red blood cells. It is devastating effects on health, physical and mental productivity affect the quality of life and translate in to significant economic losses for individuals and for countries with high anemia prevalence (Diallo *et al*., 2008). Anemia is one of the world's most widespread health problems. It affects more than one third of the world's population. In almost all the developing countries, between one third and one-half of the females and children are anemic. The prevalence among pregnant women and children under two years of age is typically more than fifty percent (WHO, 2002).

Anemia has multiple causes categorized as poor, insufficient or abnormal red blood cells production, excessive red blood cells destruction and excessive red blood cell loss (Dacia and Lewis, 2004). According to WHO 2005, several factors are associated with anemia; these are iron deficiency, micronutrient deficiency, malaria, parasitic infestation and HIV infection.

A good number of medicinal plants are traditionally employed to alleviate anemia. Some of these plants include *Spinacia oleracea*, *Telfeira occidetallis*, *Jatropha curcas*, *Waltheria indica and Spondias mombin* (Luka *et al*., 2014; Dina *et al*., 2006). *Solanum nigrum* is a species in the Solanum genus, native to Eurasia and introduced in the America, Australia, and South Africa. The plant has a long history of medicinal usage, dating back to ancient Greece. Plant parts are used in traditional medicine. The juice of the plant is used on ulcers and other skin diseases. The fruits are used as a tonic, laxative, appetite stimulant, and for treating asthma and "excessive thirst." The plant *Solanum nigrum* (black night-shade) commonly known as *kumbi* in *Hausa* is a widely used plant in oriental medicine where it is considered to be antitumor, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic (Jain *et al*., 2011). *Solanum nigrum* is also used in the

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north eastern Nigeria to treat anemia. The present study, therefore, seeks to scientifically look at the antianemic potential of *Solanum nigrum* on phenylhydrazine induced anemia.

2. Materials and Methods

2.1. Plant Material

Solanum nigrum leaf was collected in March from farm in vunoklang, Girei Local Government Area of Adamawa State and was authenticated by a Botanist in the Department of Plant Science, Modibbo Adama University of Technology, Yola. The fresh leaf sample was shade-dried for 7 days and milled into coarse powder using a manual blender. The coarse material was sieved using 0.3mm endicott test sieve to obtain a fine powder.

2.2. Preparation of Plant Extract

Powdered sample (1 kg) was extracted with 1.5 L of methanol by cold maceration for 48 h (Trease and Evans, 1989). The solvent extract was then concentrated by evaporating the solvent at 50° C using rotary evaporator and vacuum oven to obtain a dry powder.

2.3. Quantitative Analysis of Phytochemicals

The presence of alkaloids, saponins, flavonoids, total phenols and tannins were determined using the methods of Trease and Evans (1989), Harbone (1973) and Sofowora (1993); Alkaloid was determined using the method of Trease and Evans (1989); Saponin was determined using the method of Harbone (1973); Flavonoid was determined using the method of Harbone (1973); Total phenol was determined using the method of Sofowora (1993); Tannin was determined using the method of Harbone (1973).

2.4. Vitamin Analysis

Vitamin analysis was carried out for vitamin A, K, B_6, C, E and Folate using the method of AOAC (2000).

2.5. Elemental Analysis

The method of AOAC (1990) was used to determine iron, magnesium, calcium, zinc and copper.

2.6. Determination of Antioxidant Activity

2.6.1. Determination of DPPH (2, 2-diphenyl-l-picryl hydrazyl)

DPPH (2,2-diphenyl-l-picryl hydrazyl) radical scavenging assay was determined using the method described by Sasidharan *et al*. (2007). The free radical scavenging activity of the extract was measured by the decrease in absorbance of methanol solution of DPPH. Different concentration of the plant extracts (20, 40, 60, 80 and 100 mg/mL in methanol) was added at an equal volume (10 mL) to methanol solutions of DPPH (400 mg/mL) and incubated for 30 minutes. The absorbance was measured at 517 nm using spectrophotometer (VIS 721, PEC MEDICAL USA). A different concentration of L-ascorbic acid ((20, 40, 60, 80 and 100 mg/mL) was used as standard antioxidant. The antioxidant activity of the leaf extract was compared with L-ascorbic acid. Values obtained were converted in to percentage antioxidant activity using the equation below:

$$\% \text{ DPPH antiradical scavenging capacity} = \frac{\text{absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of blank}} \times 100$$

2.6.2. Determination of Ferric Reducing Antioxidant Power (FRAP Assay)

Ferric Reducing Antioxidant Power (FRAP Assay) was determined using the method described by Banerjee *et al*. (2008). Various concentration (20, 40, 60, 80 and 100 mg/100 mL of the methanol leaf extract was mixed with 1mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in a temperature-controlled water bath at 50 ^{0}C for 20 min, followed by the addition of 1 mL of 10 % trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 mL) was added with 1 mL of de-ionized water and 200 µL of 0.1 % $FeCl_3$. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. L-ascorbic acid was used as standard. The reducing power was expressed as an increase in $A_{700\ nm}$ after blank subtraction (Banerjee *et al*., 2008). Percentage inhibitory activity was calculated as follows:

$$\% \text{ inhibitory activity} = \frac{\text{absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.7. Experimental Animals

Forty-two male albino rats (90 ± 10 g) were obtained from National Veterinary Research Institute (NVRI) Vom, Plateau State. The animals were maintained under standard laboratory conditions and had a free access to standard finisher feeds and water for two weeks for acclimatization before the commencement of the experiments. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals.

2.8. Induction of Anemia

Anemia was induced in rats by daily injection (intraperitoneally) of Phenylhydrazine (PHZ) at 10 mg/kg for 8 days (Yeshoda *et al*., 1942). No death was recorded. Rats that developed anemia with PCV less than 29% were recruited for the study (Agbor *et al*., 2005).

2.9. Experimental Design

Forty-two (42) Albino rats weighing 90 ± 10g (eight weeks old) were assigned into 7 groups (n=6) animals per groups. The rats were administered different doses of the methanol extract of *S. nigrum* orally by gastric intubation daily for 3 weeks following Demo *et al*. (2007).

2.10. Collection of Blood Samples

GROUP	TREATMENT
I	Normal Control
II	Experimental Control (Anemia + No treatment)
III	Standard Control (feroton 10 mg/kg body weight)
IV	Treatment group (100mg\kg body weight)
V	Treatment group (200mg\kg body weight)
VI	Treatment group (300mg\kg body weight)
VII	Treatment group (400mg\kg body weight)

At the end of the three weeks' experimental period, the albino rats were sacrificed under chloroform. A blood sample was collected by cardiac puncture. About 3mL of blood was collected into an EDTA sample bottle for haematological assay and sample bottles labeled accordingly for all the 7 groups.

2.11. Determination of Hematological Parameters

The Red Blood Cell count (RBC), White Blood Cell count (WBC), Haemoglobin concentration (HGB), Mean Capsulated Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Platelets (PLT) were assayed using an automatic counter (Sysmex K21, Tokyo, Japan), as described by Dacie *et al*. (2001).

2.12. Statistical Analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) and LSD multiple range test to determine significant differences between means. The difference between the means was regarded as significant at $p<0.05$ and the differences of the mean was expressed using SPSS software version 23.

3. Results

3.1. Phytochemical Screening

Quantitative phytochemical screening of *S. nigrum* methanol leaf extract revealed the presence of alkaloids, saponins, flavonoids, total phenols and tannins. Total phenols and flavonoids were found to be highest while tannins, saponins and alkaloids were found to be lowest.

Table 1. Quantitative Phytochemical Content of *Solanum nigrum* Methanol Leaf Extract (mg GAE/g)

Parameters	Sample
Alkaloids	3.70 ± 0.03
Saponins	3.62 ± 0.06
Flavonoids	43.67 ± 1.08
Total Phenols	70.60 ±2.15
Tannins	1.89 ± 0.22

Values are Mean ± SD for 3 determinations

3.2. Vitamins Composition of Solanum nigrum Methanol Leaf Extract

The Vitamins composition of *S. nigrum* methanol leaf extract revealed the presence of high amounts of Vitamin C. The plant extract was also found to contain vitamin A, vitamin K, vitamin B_6, vitamin E and Folic acid in substantial quantities.

Table 2. Vitamins Composition of *Solanum nigrum* Methanol Leaf Extract

Vitamins	Concentration
Vitamin A (µg/100g)	400.66 ± 06.02
Vitamin K (µg/100g)	42.14 ± 0.10
Vitamin B_6 (Mg/100g)	14.23 ± 0.01
Vitamin C (Mg/100g)	45.18 ± 0.02
Vitamin E (IU/100g)	10.72 ± 0.02
Folic acid (µg/100g)	1100.61 ± 10.01

Values are mean ± SD for 3 determinations

3.3. Mineral Composition of Solanum nigrum Methanol Leaf Extract

Table 3 shows the level of some mineral elements in *S. nigrum* methanol leaf extract. Magnesium was found to be highest in the plant extract followed by calcium and iron while copper and zinc were found to be lowest.

Table 3. Mineral Composition of *Solanum nigrum* Methanol Leaf Extract in mg/100g

Mineral Element	Concentrations
Iron	13.01±0.01
Magnesium	247.59±4.12
Calcium	17.33±0.03
Zinc	0.07±0.01
Copper	2.12±0.12

All values are mean ± SD for 3 determinations

3.4. DPPH Radical Scavenging Activity Solanum nigrum Methanol Extract

The result of DPPH radical scavenging activity of the *S. nigrum* methanol extract is as presented in Table 4. The plant extract significantly ($p<0.05$) exhibited a high radical scavenging activity at the different concentrations of the plant extract (50, 100, 150, 200 and 250 mg/mL) compared to L-ascorbic acid at the same concentrations. The radical scavenging activity was also found to be dose-dependent. Significantly higher ($p<0.05$) radical scavenging activity was observed at the highest concentration of the plant extract (250 mg/mL) while the lowest activity was observed at 50mg/mL of plant extract.

Table 4. DPPH Radical Scavenging Activity of *S. nigrum* Methanol Leaf Extract in %

Concentration (mg/mL)	Methanol Extract	Ascorbic Acid
50	56.00 ±0.65 [a]	42±1.01
100	67.53±1.42 [a]	58±0.26
150	78.24±1.32 [a]	65±1.13
200	86.92±1.68 [a]	72±2.15
250	97.08±1.20 [a]	81±1.92

All values are mean ± SD for 3 determinations. [a]=Significantly ($p< 0.05$) higher compared to ascorbic acid

3.5. Ferric Reducing Antioxidant Power (FRAP) of Solanum nigrum Methanol Leaf Extract

The ferric reducing antioxidant power of *S. nigrum* methanol leaf extract revealed the antioxidant power of the plant extract in percentage. The antioxidant power of the plant was found to be dose-dependent. Significantly higher (p<0.05) antioxidant power was observed at 250 mg/mL while the least antioxidant power was observed at the lowest concentration (50 mg/mL) of both the plant extract and ascorbic acid. The ferric reducing antioxidant power of the methanol extract of *S. nigrum* was significantly (p<0.05) higher at 200 mg and 250 mg compared to the antioxidant power of ascorbic acid at the same concentration. No significant (p<0.05) difference was observed in the antioxidant power of the plant extract and ascorbic acid at the concentrations of 50, 100 and 150 mg/mL.

Table 5. Ferric Reducing Antioxidant Power (FRAP) of *Solanum nigrum* Methanol Leaf Extract in %

Concentration (mg/mL)	Methanol Extract	Ascorbic acid
50	39.22 ± 0.64	37.42±0.61
100	48.26±0.22	46.64±0.72
150	57.48±0.46	55.55±0.45
200	75.55±0.48 [a]	63.25±0.65
250	89.64±0.62 [a]	74.24±3.12

All values are mean ± SD for 3 determinations. [a]=significantly higher compared to Ascorbic acid

3.6. Effect of Solanum nigrum Methanol Leaf Extract on PCV

Table 6 shows the progressive effect of *S. nigrum* methanol leaf extract on PCV levels of rats in percentage. Administration of Phenylhydrazine to rat significantly reduced the PCV levels of rats. Administration of plant extract to rats revealed a dose dependent increase in PCV levels of rats compared to control. The PCV level of rats increased significantly (p<0.05) at 300 and 400 mg extract concentration compared to control. Results also indicate significantly (p<0.05) higher PCV levels in rats treated with 400 mg/kg *S. nigrum* (65.60±1.03 %) compared to rats treated with standard drug (59.50 ± 1.32 %). A progressive increase in PCV levels was observed with days of treatment. A significantly higher (p<0.05) increase in PCV was observed at day 21 of treatment in all the groups compared to day 0. However, rats in group 6, treated with 400 mg of the plant extract, had significantly higher (p<0.05) PCV at day 21 compared to other groups treated with different concentrations of the plant extract.

3.7. Effect of Solanum nigrum Methanol Leaf Extract on Different Hematological Indices

Table 7 shows the effect of *S. nigrum* methanol leaf extract on different hematological indices. Significantly (p>0.05) lower values of PCV, HGB, MCV, MCH, PLT and RBC were observed in the experimental control group compared to normal rats. The result of the study showed that rats treated with 400mg/kg had significantly (p>0.05) lower levels of WBC count, lymphocytes and neutrophils compared to Negative control. However, an increase in HGB, MCV, MCH, platelets and RBC values was observed in a dose dependent manner compared to negative control when the extract was administered to the different groups. The PCV was significantly (p>0.05) higher at 400 mg/kg body weight compared to groups administered 100, 200 and 300 mg/kg body weight. Significant (p>0.05) increase was also observed in PCV of rats in groups 3, 4, 5, 6 and 7 compared to control group. Values for MCV, PLT and RBC were found to be significantly (p>0.05) higher at 400 mg of *S. nigrum* extract compared to the different extract concentrations administered. The levels of PCV and RBC were observed to be significantly (p>0.05) higher at 400 mg of the extract compared to normal rats.

Table 6. Effect of *Solanum nigrum* Methanol Leaf Extract on PCV Levels of Rats (%)

GROUP	DAY 0	DAY 7	DAY 14	DAY 21
Normal	44.83 ± 1.38	45.67 ± 1.20	46.23 ± 1.15	47.33 ± 1.45 [b]
PHZ Negative control	28.33 ± 0.88[a]	30.67 ± 0.67[a]	31.00± 1.15[a]	36.33 ± 1.45[a]
Standard Control (feroton 10mg/kg/ body wt)	27.75 ± 0.25[a]	40.00 ± 3.19[b]	48.00± 1.73[b]	59.50 ± 1.32[b]
S.*nigrum* 100 mg/kg/ body wt	28.26 ± 0.62[a]	34.59 ± 0.46[a]	40.38± 1.6 [a b]	48.20± 2.15 [b]
S.*nigrum* 200 mg/kg/ body wt	27.67 ± 0.33[a]	36.67 ± 0.88[a]	44.67± 1.86[b]	55.30± 1.20 [b]
S.*nigrum* 300 mg/ kg/ body wt	28.25± 0 .48[a]	39.70 ± 0.85 [a b]	50.25±0.85[b]	59.00 ± 1.29[b]
S.*nigrum* 400 mg/ kg/ body wt	28.40 ± 0.51[a]	43.40 ± 1.63[b]	53.80± 0.58[b]	65.60 ± 1.03[bc]

Values are Mean ± SEM, (n = 6). [a=]Significantly (p< 0.05) lower compared to normal, [b=] Significantly (p< 0.05) higher compared to negative control, [c=] Significantly (p< 0.05) higher compared to different extract concentrations. Wt= body weight.

Table 7. Effect of *Solanum nigrum* Methanol Leaf Extract on Hematological Indices

GROUP	PCV (%)	WBC (*10)	HGB (g/dl)	MCV (fl)	MCH (g/dl)	PLT (*10)	LYM (%)	NEU (%)	RBC (*10)
Normal	47.33 ± 1.45 [b]	11.87 ± 2.02	14.15 ± 0.35 [b]	78.73 ± 0.32 [b]	30.20 ± 1.64 [b]	479.67 ± 3.84 [b]	28.33± 1.20	39.00 ± 3.61	5.77±0.02 [b]
PHZ Negative control	36.33 ± 1.45[a]	18.07 ± 0.27[c]	9.23 ± 0.32 [a]	43.27 ±0.85[a]	23.37 ± 0.83[a]	411.67 ±1.45[a]	8.30±0.88[c]	60.35 ±0.88[c]	3.27 ± 0.17 [a]
Standard control	59.50 ± 1.32[b]	11.40 ± 0.78	12.15 ± 0.26 [b]	74.33 ± 0.27[b]	31.60 ± 1.27[b]	479.30 ± 3.52[b]	41.00±0.91	47.25 ± 0.75	7.40 ± 0.08[b]
S.*nigrum* 100 mg /kg / body wt	42.30 ± 2.10	14.00 ± 0.80	10.20 ± 0.16 [a]	62.74 ± 0.44 [b]	26.43 ± 0.20	424.13 ± 2.12[a]	45.04±1.28	57.10 ± 3.42	4.50 ± 0.30 [b]
S.*nigrum* 200 mg /kg / body wt	55.30 ± 1.20 [b]	8.00 ± 0.10 [a]	12.80 ± 0.15 [b]	72.87 ± 1.94 [b]	29.43 ± 0.19 [b]	454.33 ± 3.21[a b]	40.40±2.22	52.20 ± 3.93[c]	6.50 ± 0.10 [b]
S.*nigrum* 300 mg / kg / body wt	59.00 ± 1.29[b]	7.93 ± 1.63[a]	13.87 ± 0.51[b]	74.42 ± 2.83 [b]	30.10 ± 0.88 [b]	474.67 ± 4.25 [b]	34.25±1.03	47.67± 1.83	7.13 ± 1.63 [b]
S.*nigrum* 400 mg / kg per body weight	65.60 ± 1.03[bc]	6.30 ± 1.47[a]	14.26 ± 0.27 [b]	79.40 ± 0.66[bc]	31.24 ± 0.71 [b]	481.60 ± 3.11[bc]	34.67± 2.53	36.25 ± 1.11	7.51 ± 0.07[bc]

Values are Mean ± SEM, (n = 4). [a=]Significantly (p< 0.05) lower compared to normal; [b=]Significantly (p< 0.05) higher compared to negative control; [c=]Significantly (p< 0.05) higher compared to different extracts concentration

4. Discussion

The phytochemical analysis carried out on the methanol leaf extract of *S. nigrum* revealed the presence of high content phenols and flavonoids as well as alkaloids saponins and tannins. Results of phytochemical analysis seems to be in agreement with the findings of Temitope and Omotayo (2012). It has been reported that Phenylhydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species (Clemens *et al*., 1984). These phytochemicals protect cells as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species. Adewoye *et al*. (2012) stated that some of the biological functions of flavonoids include protection against allergies, free radicals, platelet aggregation microorganisms, ulcers, hepatotoxins and tumors. The presence of these phytochemicals might have contributed to the antihaematinic activity of *Solanum nigrum* observed in the present study.

The vitamins content of the plant (Table 2) revealed the presence of an appreciable amount of some haematinic vitamins, such as folic acid, vitamin A, vitamin C, vitamin K, vitamin $B_{6,}$ and vitamin E. Deficiency of folic acid and other vitamins constituents in erythropoiesis has been reported to cause macrocytic, megaloblastic and pernicious anemia (Chanarin *et al.*, 2004). These haematinic agents have been found to be effective in relieving the symptoms of anemia in pregnancy and infancy. Vitamins A and C contribute to the uptake of iron while vitamin C enhances the intestinal absorption of non-haem iron by reducing ferric ion to a ferrous form or by forming a soluble complex in the alkaline PH of the small intestine thereby increasing /enhancing iron absorption (Demodara, 2013). This probably was the reason for the observed increase in haemoglobin observed in the present study. The methanol extract of *S. nigrum* has contributed in the faster reversal of the phenylhydrazine induced anemia in rats treated with the extract for three weeks. A similar outcome was observed when anemic rats were treated with *Tectona grandis* (Diallo *et al*., 2008).

Table (3) shows the mineral composition of *S. nigrum.* Calcium and magnesium are useful in the formation of blood and intracellular and extracellular fluids of body cells. They also function as constituents of bones, teeth and in regulation of nerve and muscle function (Brody *et al*., 2004; Ogbe *et al*., 2010). The value of iron obtained in the present study (15.01±0.03 mg/100g) is higher than the values reported for some selected leafy vegetables in Nigeria. Iron is a part of the haemoglobin, myoglobin and

cytochrome. Findings by Nasima *et al*. (2004) reported that zinc plays a major role in the synthesis of haemoglobin. Zinc deficiency has been associated with anemia and erythrocyte fragility. Zinc is also a cofactor for RBC-SOD thereby protecting the integrity of the cell and oxidative stress (El-Nawawy *et al*., 2002). Copper is also an active agent in haemoglobin synthesis. Copper containing enzymes catalyze the oxidation of ferrous iron to ferric iron. It is necessary for the absorption and use of iron in the formation of haemoglobin (Whitney and Rolfes, 2001). Potassium is necessary in the management of sickle cell anemia. It plays a major role in heart beat and assist in nerve impulse transmission. Abnormal activation of potassium chloride co transport system was found to be involved in cell potassium loss and dehydration seen in sickle cell anemia (Agoreyo and Nwaeze, 2009). While magnesium is part of the protein making machinery, together with calcium, magnesium is involved in muscle contraction and blood clotting (Demo *et al*., 2007)

The radical scavenging activity of the plants revealed that both DPPH and FRAP activity of the methanol extract of *S. nigrum* exhibited significantly higher ($p< 0.05$) antioxidant activity compared to L-ascorbic acid. A study by Turaaskar (2013) revealed that most anti-anemic compounds are known for their free radical scavenging activity that reverses anemic conditions. The scavenging activity of free radicals and reactive oxygen species is in a dose dependent manner with reference to DPPH and FRAP antioxidant determination. This probably is due to the presence of phytochemicals in the leaf extract. According to Lv *et al*. (2013), good antioxidant activities exhibited by plants extracts are due to the presence of poly-phenolic compound. Administration of the methanol extract of *S. nigrum* significantly ($p<0.05$) increased the haematological parameters in the experimental groups in a dose dependent manner. A significant increase was observed in the levels of PCV, HB, platelets, MCV, MCHC, neutrophils and RBC. A similar result was obtained by Asuquo (2013) when ethanol leaf extract of yellow mombin was administered to rats. However, a significant decrease ($p<0.05$) was observed in the levels of WBC, lymphocytes and neutrophils. The white blood cells, lymphocytes and neutrophils are indices of immunology of the body against infection; thus, a significant decrease was seen in these parameters when methanol leaf extract of *S. nigrum* was administered. Treatment with 400 mg/kg/body weight was found to be more effective in ameliorating the effect of phenylhydrazine than other doses. A similar result was obtained by Vamsee *et al*. (2004) when a curry leaf was administered to anemic rats at 400 mg/kg body weight.

5. Conclusion

In conclusion, results obtained from the present study indicate that the methanol leaf extract of *S. nigrum* possesses anti-anemic potentials and this may be attributed to the phytochemicals, antioxidant vitamins, such as folic acid, vitamin C, and minerals, such as iron, zinc and calcium content of *S. nigrum* leaf. The present study, therefore, supports the therapeutic use of the plant in the traditional medicine for the treatment of anemia.

References

Adewoye E O, Salami AT and Emikpe B O. 2012. *Chrysophyllum albidum* bark on hematological indices in mice with experimental hemorrhagic anemia. *Afrircan*.*J Biomedical Res*.,**15**: 85-911.

Agbor A, Oben E and Ngogang Y. 2005. Haematinic activity of *Hibiscus cannabinus*. *African J Biotechnol*., **4(8):** 833-837.

Agoreyo F O and Nwanze N. 2010.Plasma sodium and potassium changes in sickle cell patients. *Inter J Genetics and Mol Biol*., **2(2)**:14-19.

A.O.A.C. 1990. Association of Official Analytical Chemists. 15th ed., Williams S.(Eds). Official methods of food analysis, Washington D.C., 152-164.

AOAC. 2000. Association of Official Analytical Chemists. 18th ed. Williams S. (Eds). **Official Methods of Analysis**. Washington D.C., pp.18-62.

Asuqua R O, Ekanem B T, Udoh B P, Mesenbe E P and Ebong E P. 2013. Haematinic potential of *Spondias mombin* leaf extract in wistar rats. *Advance*. *Bioresearch* **4** (2): 53-56.

Banerjee D, Chakrabarti S, Hazra A K, Banerjee S, Ray J and Mukherjee B. 2008. Antioxidant activity and total phenolics of some mangroves in underbans. *African J Biotechnol*., **7**: 805-810.

Brody H, Robert M, Bert L, Panja K, Claus L and Olson R. 2004. Changes in the red blood cell membrane in protein-calorie malnutrition. *Am J Clin Nutri*., **31**: 574-578.

Chanarin T, MacGibbon BM, O'sullivan WJ and Mollin DJ 2004. Folic acid deficiency in pregnancy. *Lancet* **69**: 634-635.

Clemens MR, Remmer H and, Waller H D. 1984. Phenylhydrazine-induced lipid peroxidation of red blood cells: *in vitro* and *in vivo* monitoring by the production of volatile hydrocarbons. *Biochem Pharmacol.*, **33**:1715-1718.

Dacie AD, Lewis M, Brain B J, Bates I and Lewis T. 2001. **Practical Haematology**. 9th Edn. Elsevier Publishers London, New York: 9-40.

Dacie V and Lewis M. 2004.**Practical Haematology**, 8th edn.*Churchill Livingstone London*, 49-59.

Damodara K M. 2013. Ethanolic extract of *Nardostachys jatamansi* potentiates haematopoietic system in albino wistar rats. Nitte *Universal J Health Sci*., **3**(1): 25-29.

Demo T, Mtopi O, Nguelefack B, Kamtchoung P.2007. Vasorelaxant effect of extracts on isolated rat vascular smooth muscle. *J Ethnopharmacol*.,**111 (1):** 104-109.

Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokou K. Agbonon A, Abena A, Souza C, Akpagana K. 2008. Effect of *Tectona grandis* phenylhydrazine induced anemia in rats. *Fitoterapia* **79**: 332-336.

Dina O A, Adedapo A A, Oyinloye O P and Saba A B. 2006. Effect of *T.occidentalis* extract on experimentally induced anaemia in domestic rabbits. *African. J Biomedical Res.*, **3**: 181-183.

El-Nawawy A, Barakat S, Elwalily T, Abdel- Moneim Deghady A and Hussein M. 2002. Evaluation of anemia in severe under nutrition in protein energy malnutrition, *East Mediterranean Health J*.,**8** (2-3):281–9.

Harborne JB. 1973. ***Phytochemical Methods***, London: Champan and Hall Ltd, 49: 188.

Jain R, Sharma A, Gupta S, Sarethy P and Gabrani R. 2011. *Solanum nigrum*: Current perspectives on therapeutic properties. *Alternative Medical Rev*., **16(1):** 78-85.

Luka C D, Abdulkarim M, Adoga G I, Tijjani H and Olatunde A. 2014. Antianaemic potential of aqueous extract of *Spinacia*

oleracea leaf in phenylhydrazine-treated rats. *N Y Sci J*., **7(6):** 14-18.

Lv H, Zhang X, Chen X, Xie X, Hu C, Wen C and Jiang K. 2013. Phytochemical compositions and antioxidant and anti-inflammtory activities of crude extracts from *Ficus panduraa* H. (Moraceae), *Evidenced-Based Comlementary Alternat Med.*, **2(4):** 1-8.

Nasima A, Raghib A, Abdul W M, Mostafa C G M, Sakhi C and Hai M A. 2003. Relationship between zinc and anaemia in chronic haemodialysis patients *J Teachers Asso RMC*. **16** (1): 1019-8555.

Ogbe R J, Adoga G I and Abu A H. 2010. Antianaemic potentials of some extract on phenylhydrazine-induced anaemia in rabbits. *J of Medi Plants*, **4(8):** 680-684.

Sasidharan S, Darah I, Mohammed J and Noordin M K. 2007. Free radical scavenging activity and total phenolic compounds of *Gracilaria changii*. *Inter J National and England Sci.*, **1(1):** 15-117.

Sofowora A. 1993. **Medicinal Plants and Traditional Medicines in Africa**. Spectrum Books Ltd, Ibadan Nigeria. P, 289.

Temitope IB and Omotayo F O. 2012. Comparative phytochemical analysis of selected medicinal plants in Nigeria. *Inter J Advanced Chem Res.*, **1**(1): 011-018.

Trease GE and Evans WC. 1989. **Textbook of Pharmacognosy**. 12th edition Balliere, Tindall London P, 45-50.

Turaskar A. 2013. Inhibitory potential of *Picrorrhiza kurroa royle* ex. extract on phenyhydrazine induced reticulocytosis in rats. *J Pharma Clin Res.*, **6 (2):** 215-216.

Vamse,e V A. 2014. Comparative anti anaemic activity of *Murraya koenigii* (inn.) leaves and its combination with *Emblica officinalis* in aluminum chloride induced anaemia using rodents. *Inter J Advance Res Med Sci.*, **1**(1): 15-24.

Whitney E N and Rolfes S R. 2001. **Understanding Nutrition** 9th edition Wadsworth/Thomson learning. Belmony. USA, 355-457.

World Health Organization. 2002. Preventing and Controlling Iron Deficiency Anaemia through Primary Health Care.*WHO Publications* 8.

World Health Organization. 2005. Global Database on Iron Deficiency and Anaemia, Micronutrient Deficiency Information System Geneva: *World Health Organisation*.

Yeshoda M. 1942. Phenylhydrazine anemia in rats. *Current Sci*., **11**: 360-363.