

Research Article

Antianaemic Activity of Aqueous Extract of *Phyllanthus niruri* Linn in 2, 4-Dinitrophenylhydrazine Induced Anaemic Rats

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Abstract

Background: *Phyllanthus niruri* is an annual plant that is widely used in Asia and Africa traditionally for the treatment of various ailments such as Jaundice, Asthma, Hepatitis, Flu, Dropsy, Diabetes, Malaria, Hemorrhages, Diarrhea, and Anaemia.

Objective: This study evaluates the haematological and biochemical parameters of *P. niruri* in 2,4-dinitrophenylhydrazine-induced haemolytic anaemia in Wister rats for possible antianemic potential.

Methods: This study evaluates the Antianaemic effect of the plant extract based on haematological and biochemical parameters and body weight in 2,4-dinitrophenylhydrazine-induced haemolytic anaemia in Wister rats.

Extract was administered in three different doses (250, 500, and 1000 mg/ kg b.wt) of the plant extract and folic acid was used as a positive control. Haematological parameters such as PCV, Hb, RBC, WBC, MCV, MCH, Reticulocyte count were evaluated. Oxidative stress markers (MAD, catalase and SOD) and bilirubin (total and unconjugated) were also analyzed.

Results: The results showed that, PCV, Hb, RBC concentration increased significantly ($P < 0.001$) at a dose of 500 and 1000 mg/kg b.wt respectively while no significant ($P > 0.05$) effect was observed at a dose of 250 mg/kg b.wt. WBC, MCV, MHC and reticulocytes was decreased significantly ($P < 0.001$) at doses of 500 and 1000 mg/kg b.wt while no significant ($P > 0.05$) effect was observed at a dose of 250 mg/kg b.wt. A significant ($P < 0.01$) decrease in MAD and bilirubin concentration and a significant ($P < 0.01$) increase in catalase and SOD activity was seen at doses of 500 and 1000 mg/kg b.wt, while no significant ($P > 0.05$) effect was observed at a dose of 250 mg/kg b.wt.

Conclusion: The results suggest that the aqueous whole plant extract of *P. niruri* plant is relatively safe and possesses a significant anti-anaemic property; therefore, it may be a potential lead in the discovery of drug for treatment of haemolytic anaemia.

Keywords: *Phyllanthus niruri*; Antianaemic; 2,4-dinitrophenylhydrazine; Haemolytic anaemia; Oxidative stress markers

Introduction

Anaemia is among the common blood associated ailments which affect people all around the globe [1]. It can emerge at all phases of the life process, but it occurs mostly in pregnant women and young children [2]. The occurrence of anaemia is higher in the tropics due to the high prevalence of malaria and other parasitic infections that could lead to decrease in circulating red blood cells or hemoglobin level [3]. The World Health Organization (WHO) categories anaemia as a dreadful public health problem (prevalence $> 40\%$) for children of not up to five years in 69 countries and for pregnant women in 68 countries. According to National Family Health Survey (NFHS-3), the occurrence of anaemia in the developed world is 71%, in the underdeveloped world it is 84% and the global occurrence is 79% [4] The major cause of anaemia is linked to low iron levels. However, anaemia exists in association with other causes, such as malaria, parasitic infection, nutritional deficiencies, drug toxicity as well as genetic or acquired defect [5].

Phyllanthus niruri is also known as 'Chanca piedra in Spanish, 'quebrapiedra' (stone breaker) in Brazil, *Pitirishi* or *Budhatri* in India [6]. In Nigeria, the Yorubas of the South-West called it *Iyin-olobe*, the Nupes in the Northern part called it *Egi kpeye kpezuma* and the Igbos of the South-East called it *Ngwu ite kwowa nasu* [7]. *P. niruri* is popularly used in Asia, Africa and South America [8] for the treatment of various ailments such as jaundice, asthma, hepatitis, flu, dropsy, diabetes, malaria [9] hemorrhages, diarrheas, dysentery, jaundice, cough and anaemia, It is also said to be effective in bronchitis, anaemia, leprosy, asthma, Urinary tract infections, stimulating liver, improving digestion, increase appetite and produce laxative effects [10]. *Phyllanthus niruri* is also traditionally used

in treating anaemia [7], tuberculosis, vaginitis, tumors, gonorrhoea, syphilis [8]. A study by Okoli *et al.* (2010) [8] reveals that the pharmacologically active compounds present in *P. niruri* consist of flavonoids, anthraquinones, alkaloids, saponins, steroids, tannins and terpenoids. Coumarins and lignans were also found to be present [6]. Harish *et al.*, 2006 reported the high antioxidant potentials *P. niruri* extracts (aqueous and methanolic) in vivo by their ability to inhibit CCl₄ – induced lipid peroxidation in rats. Thus, the plant is used in treating various diseases including anaemia across the globe.

Materials and Methods

Drugs and Reagents

2,4-dinitrophenylhydrazine (British drug house, UK), Folic acid (Emzor pharmaceutical industry, Lagos). Other chemicals used were of analytical grade purchased from BDH, Poole UK.

Plant Material and Extraction

Fresh *P. niruri* whole plant was collected from Minna in Niger state, Nigeria in the month of October 2018. The plant was identified by a Pharmacognosist from the Department of Ethnomedicine and Pharmacognosy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. A specimen was deposited at the herbarium of the faculty and a voucher number (PCG/UDUS/Euph/0011) was obtained.

The plant was carefully rinsed with water and then shed dried to a constant weight. Using a pestle and mortar the plant was grounded to a coarse powder. Using cold maceration method 2kg of the powdered plant was dissolved in 10liters of distilled water to give 200g/L. The homogenate was kept for 3 days at 40°C with daily stirring. The mixture was then filtered using a muslin cloth, then with a Whatman No. 1 filter paper. The filtrate was then concentrated to dryness using a freeze dryer and stored at 4°C until it was required for the study.

Animals Grouping and Experimental Procedure

Adult Wister albino rats of both sexes (males and females) of about 12 weeks of age weighing between 180-200g were used for the study. They were procured from the Faculty of Pharmaceutical Sciences, A.B.U Zaria, Nigeria. The animals were housed in standard cages at the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto and allowed to acclimatize to the new environment under optimum feeds (Standard commercial diet from Vital Feeds company, Jos, Plateau State Nigeria) with full access to drinking water for a period of 2 weeks prior to the commencement of the experiment. The animals were randomly divided using computer aided randomization (Decision analyst stats 2.0) into various groups and each rat was marked using a permanent marker for easier identification. The extract was administered orally using a feeding cannula. Each rat was weighed prior to extract administration to determine the appropriate dose in mg/kg body weight. The maintenance and handling of the animals were in line with the regulations of the Institutional Animal Ethics Committee. Weights of the rats were monitored through the period of the experiment.

Induction of Anaemia using 2,4-dinitrophenylhydrazine

A modified method described by Berger (2007) was used in this study. The experiment was performed for a period of three weeks. The animals were randomly divided into five groups of eight animals each (n=8). All the animals received 2,4-DNPH (20 mg/kg b.wt, p.o) once daily for 7 consecutive days using a feeding canula. On the 8th day, their blood sample was collected by sinus puncture at the tail veins of each rat into heparinized capillary tubes for hematological analysis. Rats with $\geq 30\%$ reduction in PCV were considered anemic and were used for the study [5].

PCV was determined pre and post induction of anaemia by obtaining blood from a slight cut around the tail and allowing the blood to flow through a capillary tube to more than two-thirds of the tube. The end of the tube was then sealed off using a Bunsen burner. The samples were centrifuged for 5 min at 3,000 rpm. The capillary tubes were placed on the microhaematocrit to determine the PCV.

Treatment with the extract commenced immediately after the establishment of anaemia. Treatment groups were given different doses (250, 500 and 1000mg/kg) of plant extract, positive control group was given Folic acid (75 μ g/kg) while the negative control was left untreated. All treatments were administered once daily for 14 consecutive days using oral feeding cannula.

Haematological Assay

After two weeks of treatment with the aqueous extract, blood samples were obtained through cardiac puncture and collected in EDTA bottles to prevent the blood from clotting. The samples were used for determination of haematological parameters (RBC, PCV, Hb, WBC, MCHC, MCV and reticulocyte count). All haematological parameters except reticulocytes were carried out using the automation method using sysmex Machine (Model No KX-21N) made by Sysmex Laboratories Ltd USA. The blood samples were centrifuged or mixed and put through a machine called Automated Analyzer which counts the numbers and types of different cells within the blood. Reticulocyte count was conducted using visual method [20].

Lipid peroxidation assay (Thiobarbituric acid method)

Principle

MDA react with thiobarbituric acid (TBA) to form an MDA-TBA₂ adduct that absorbs strongly at 532 nm.

Procedure

As described by (David et al., 1990) 150 μ L of the sample was diluted to 500 μ L with double deionized water. In test tubes, 250 μ L of 1.34% Thiobarbituric acid was added followed by the addition of equal volume of 40% trichloroacetic acid. The mixture was well shaken and incubated for 30 minutes in boiling water (temperature $>90^{\circ}\text{C}$). Tubes were allowed to cool down to room temperature and the absorbance of Malondialdehyde (MDA) formed was read at 532nm using 0 concentration as blank. The concentration of Malondialdehyde formed was calculated using the following formula.

$$\text{MDA } (\mu\text{mol/L}) = \text{Absorbance at 532} \times 1000/1.56.$$

Enzymatic Assay for Superoxide Dismutase (Kakkar *et al.*, 1984)

Principle

The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\bullet-}$ and the dismutation of this radical by SOD.

Procedure

Test tubes were labeled as the test and blank and 0.1mL of Buffer was added into test labeled tubes while 0.15mL was added into blank labelled test tube. 0.83mL of distilled water was then added to both blank and test. Serum (0.05mL) was added into test and incubated at 24⁰C for 10 minutes then transferred into cuvette. 0.02mL of Pyrogallol was added to both blank and test. The mixture is immediately mixed by inversion and change in absorbance at 240nm was recorded after 3 minutes approximately. The change in absorbance at 420nm/minute is calculated using the maximum linear rate for both test and blank.

$$\% \text{ inhibition} = \frac{\text{Abs at 420nm /min of blank} - \text{Abs at 420nm/min of sample} \times 100}{\text{Abs 420nm/min of blank}}$$

Abs – Absorbance

The number of units of SOD is calculated using the following formula:

$$\% \text{ inhibition} / (100 - \% \text{ inhibition}) = \text{Units/ml}$$

One unit of SOD activity was defined as the amount of enzyme that reduces the rate of auto-oxidation of pyrogallol by 50% at standard assay conditions.

Bilirubin

Principle

Bilirubin reacts with diazotized Sulphanilic acid to form a colored azobilirubin compound. The unconjugated bilirubin coupled with the Sulphanilic acid in the presence of caffeine produces a colour complex also. The intensity of the color is directly proportional to the concentration of Bilirubin in the specimen and is measured at 546 nm.

Total Bilirubin

Procedure

As described by (Olukunle *et al.*, 2010). Test tubes were labeled as blank and sample 200 μ L of reagent 1 was added to both sample and blank tubes followed by the addition of 50 μ L of reagent 2 to sample test tubes. Reagent 3 (100 μ L) was added to both sample and blank tubes followed by addition of 200 μ L of the sample (serum) to both sample and blank tubes. The mixtures were incubated at 20-25⁰C for 10 minutes followed by the addition of 1000 μ L of reagent. The content of the tubes were mixed and incubated at 25⁰C for 5-30minutes. The content of the tubes

was transferred into a Cuvette and absorbance was recorded at 546nm. The concentration of total Bilirubin was calculated using the following formula.

$$\text{Total bilirubin } (\mu\text{m/L}) = 185 \times \text{Absorbance of total bilirubin (546nm)}$$

Direct Bilirubin

Test tubes were labeled as blank and sample, 200 μ L of reagent 1 was pipetted into both blank and sample test tubes. Followed by the addition of 50 μ L of reagent 2 into sample test tubes. Addition of 0.9% NaCl (200 μ L) was done to both sample and blank test tubes followed by the addition of 200 μ L of the test sample (serum) into both sample and blank test tubes. The content of the tubes was mixed and incubated for 10min at 20-25⁰C. Absorbance was taken at 546nm. The concentration of direct Bilirubin was calculated using the following formula.

$$\text{Direct bilirubin } (\mu\text{mo/L}) = 246 \times \text{Absorbance of direct bilirubin (546nm)}$$

Statistical Analysis

The Data were expressed as mean \pm standard error and were analyzed using One-way Analysis of Variance (ANOVA). The comparison between untreated and Treatment groups was done using the Dunnet's multiple comparison tests. The level of significance was set at $P < 0.05$.

Results

Following the induction of anaemia, body weights of the rats pre and post induction of anaemia with PHZ was evaluated. There was a significant ($P < 0.05$) reduction in weight in the PHZ induced animals and a significant ($P < 0.01$) increase in weight the groups with various concentration of aqueous extract of *P. niruri* when compared with the untreated group as seen in Figure 1 below.

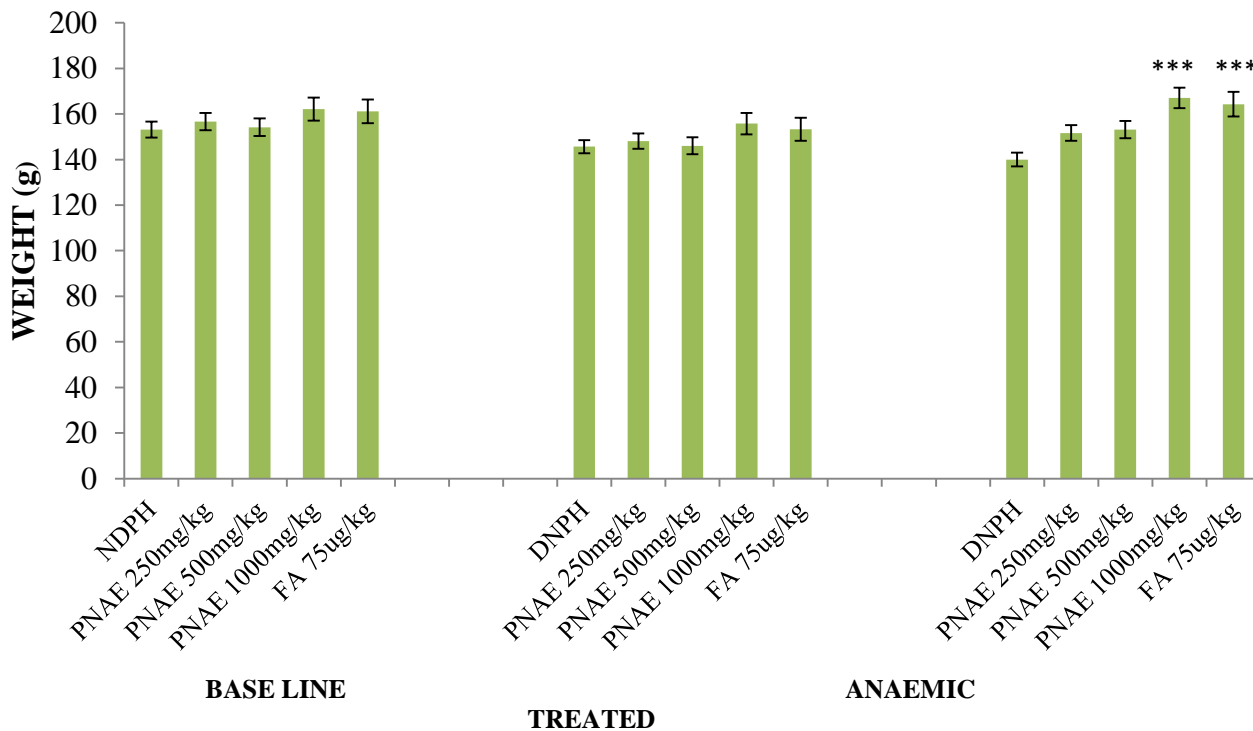


Figure 1: Effect of *P. niruri* Aqueous Extract on weight of Rats pre and post induction of anaemia 14 days after treatment with PNAE. Values represent the means \pm SE (n= 8 rats each for all the groups). P<0.05 and P<0.001 was considered significant when compared with untreated group. PNAE = *P. niruri* aqueous extract, DNPB = 2, 4-dinitrophenylhydrazine, FA = Folic acid.

Following induction of anaemia treatment, the result of PCV shows a significant ($P>0.001$) decrease in PCV in PHZ treated groups (at day 7) but a dose-dependent increase in PCV was observed after 14 days of treatment with *P. niruri* extract when compared with the untreated control group compared with the untreated normal control group as illustrated in Figure 2 below.

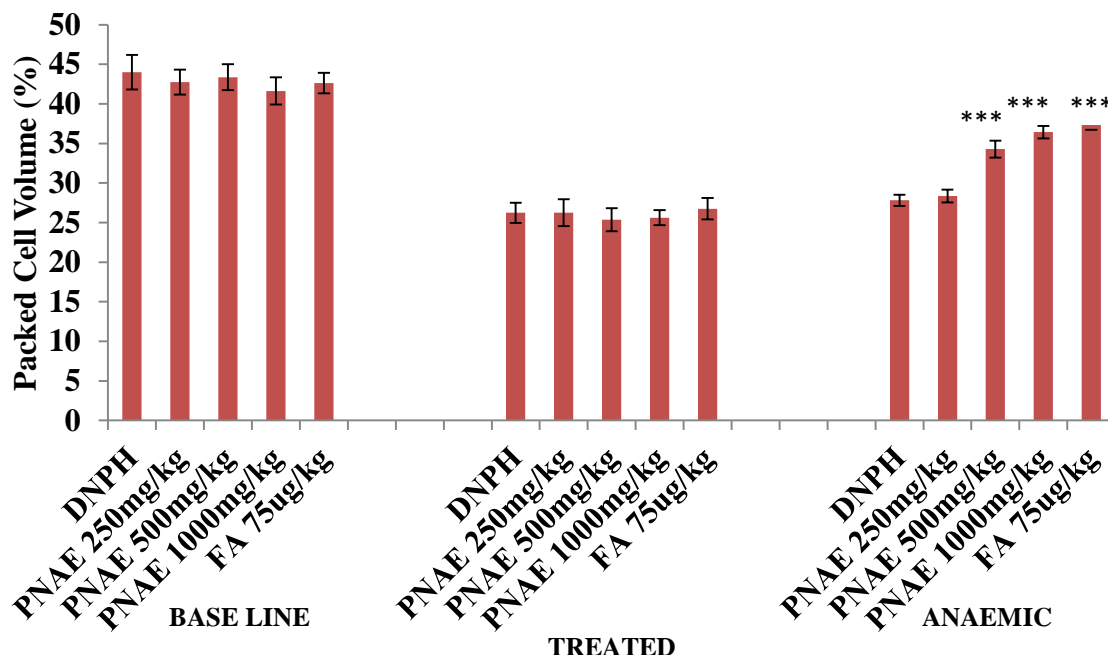


Figure 2: Effect of *P. niruri* aqueous extract on PCV in 2,4-DPHZ-induced anaemic rats at baseline, after induction of anaemia and after 14 days treatment with PNAE. Values represent the means \pm SE (n= 8 rats each for all the groups). One-way ANOVA with Dunnet’s posttest was used to arrive at the P values. * signifies $P < 0.05$ when compared with untreated group. PNAE = *Phyllanthus niruri* aqueous extract, DNPH = 2,4-dinitrophenylhydrazine, FA = Folic acid.

The treatment of PHZ induced anemic rats with PNAE for 14 days shows a significant ($P > 0.001$) and dose-dependent increase in Hb concentration and also a significant ($P < 0.001$) decrease in the untreated control group as illustrated in the Figure 3 below.

The result of RBC concentration following treatment of PHZ induced anemic rats with PNAE Produces a significant ($P < 0.001$) and dose-dependent increase in RBCs when compared with the untreated group. There was also a significant ($P < 0.001$) decrease when PHZ group was compared to the control after treatment as shown in Table 1 below.

The result of WBC concentration following treatment of PHZ induced anemic rats with PNAE Produces a significant ($P < 0.02$) and dose-dependent decrease in WBCs when compared with the untreated group. There was also a significant ($P < 0.001$) increase in WBC level when PHZ group was compared to the control after treatment as shown in Table 1 below.

Table 1: Effect of treatment of anemic rats with aqueous extract of *P. niruri* on HB, RBCs, and WBCs

Treatments	Indices		
	HB (g/dl)	RBC ($\times 10^6/\mu\text{l}$)	WBC ($\times 10^3/\mu\text{l}$)
DNPH PNAE	7.79 \pm 0.34	3.19 \pm 0.29	19.16 \pm 0.38
250	8.21 \pm 0.27 ^{ns}	3.51 \pm 0.21 ^{ns}	17.58 \pm 0.24*
500	10.43 \pm 0.54*	4.83 \pm 0.27*	12.65 \pm 0.44*
1000	11.31 \pm 0.28*	5.33 \pm 0.10*	11.81 \pm 0.32*
Folic acid (7.5 $\mu\text{g}/100\text{g}$)	11.55 \pm 0.36*	5.74 \pm 0.16*	10.95 \pm 0.39*

Values are expressed as mean \pm SEM, n=8. P<0.05 is considered as significant. Values with * are significant when compared to untreated groups.

Also, a significant (P<0.05) decrease in MCV, MCH and reticulocytes was observed at 500 and 1000mg/kg after 14 days treatment with 500 and 1000mg/kg body weight when compared to the 2,4-DNPH induced anemic group of the extract. No significant (P>0.05) effect was observed at a dose of 250 mg/kg body weight as shown in Table 2 below.

Table 2: Effect of treatment of anemic rats with aqueous extract of *P. niruri* on MCV, MHC, and Retics

Treatments	Indices		
	MCV (fl)	MHC (pg)	RETICULOCYTES (%)
DNPH PNAE	93.50 \pm 7.14	25.38 \pm 1.78	8.30 \pm 0.36
250	84.54 \pm 3.69 ^{ns}	23.06 \pm 0.79 ^{ns}	7.44 \pm 0.30 ^{ns}
500	71.59 \pm 1.78*	21.61 \pm 0.36*	5.67 \pm 0.35*
1000	68.63 \pm 0.598*	21.00 \pm 0.24*	5.35 \pm 0.26*
Folic acid (7.5 $\mu\text{g}/100\text{g}$)	65.35 \pm 1.61*	20.05 \pm 0.53*	5.11 \pm 0.29*

Values are expressed as mean \pm SEM, n=8. P<0.05 is considered as significant. Values with * are significant when compared to untreated group.

Biochemical Parameters

Oxidative Stress Markers

Malondialdehyde

The result of malondialdehyde following treatment of PHZ induced anemic rats with PNAE produces a significant ($P < 0.05$) and dose-dependent decrease in malondialdehyde when compared with the untreated group (PHZ). There was also a significant ($P < 0.001$) increase in MDA level when PHZ group was compared to the control after treatment as shown in Figure 3 below.

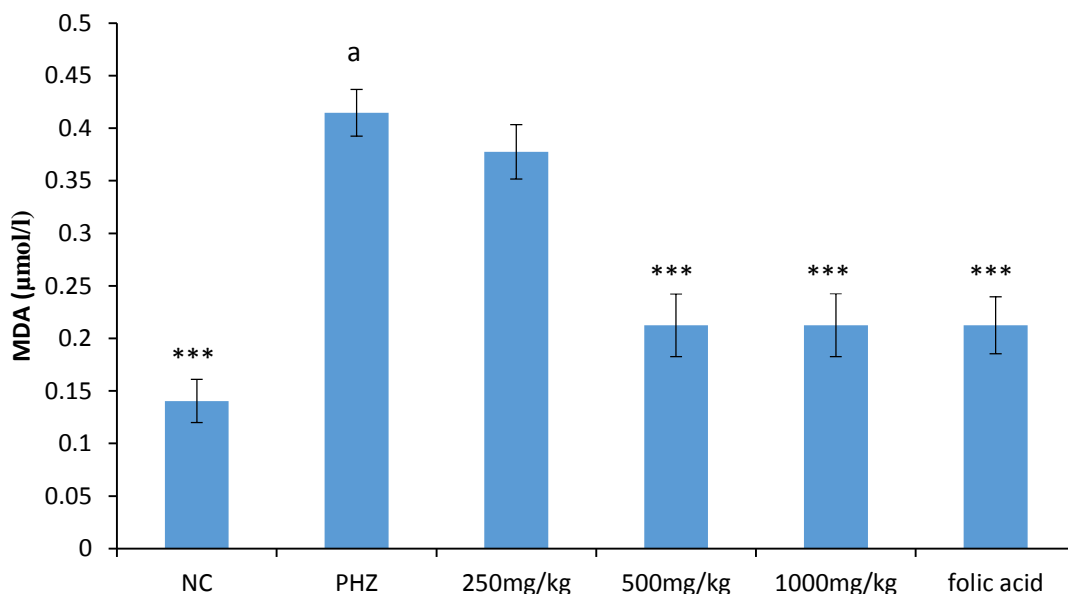


Figure 3: Effect of PNAE on MDA in PHZ-induced Anaemic Rats after 14 days treatment with PNAE. Values represent the means \pm SD (n= 8 rats each for all the groups). Oneway ANOVA with Dunnet’s post-test was used to arrive at the P values. *, ** and *** signifies $P < 0.005$, $P < 0.02$ and $P < 0.001$ respectively when compared with PHZ-treated group while ^a signifies $P < 0.001$ when compared with normal control.

Superoxide Dismutase Stimulatory Activity of PNAE

The result of SOD activity following treatment of PHZ induced anemic rats with PNAE shows a significant ($P < 0.01$) and dose-dependent increase in SOD activity when compared with the untreated group (PHZ). There was also a significant ($P < 0.001$) decrease in SOD activity level when PHZ group was compared to the control after treatment as shown in Figure 4 below.

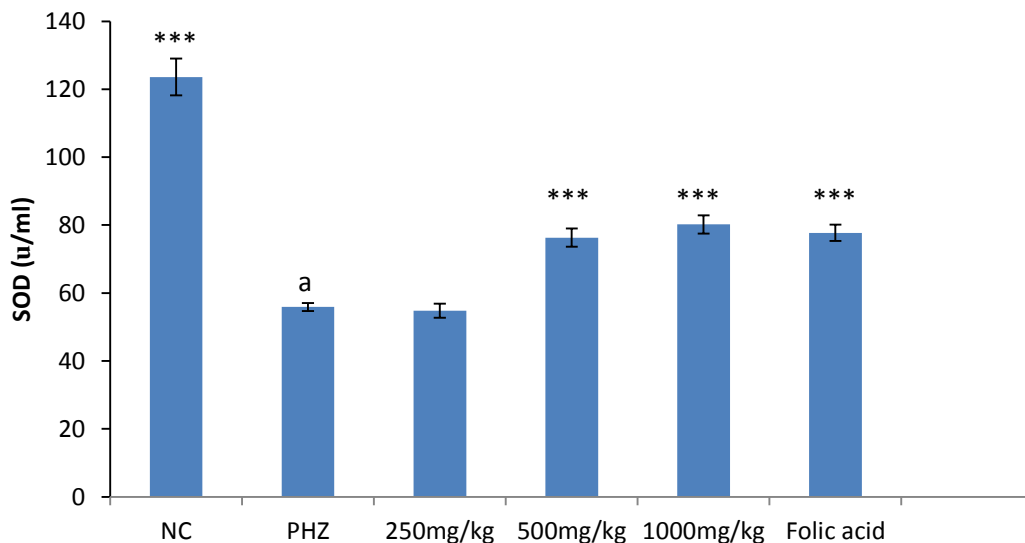


Figure 4: Effect of PNAE on SOD in PHZ-induced Anaemic Rats after 14 days of treatment with PNAE. Values represent the means \pm SD (n= 8 rats each for all the groups). One-way ANOVA with Dunnet’s post- test was used to arrive at the P values. *, ** and *** signifies P<0.005, P<0.02 and P<0.001 respectively when compared with PHZ-treated group while ^a signifies P<0.001 when compared with normal control.

Effect of treatment on catalase activity

The result of Catalase activity following treatment of PHZ induced anemic rats with PNAE shows a significant (P<0.01) and dose-dependent increase in catalase activity when compared with the untreated group (PHZ). There was also a significant (P<0.001) decrease in catalase activity when PHZ group was compared to the control after treatment as shown in Figure 5 below.

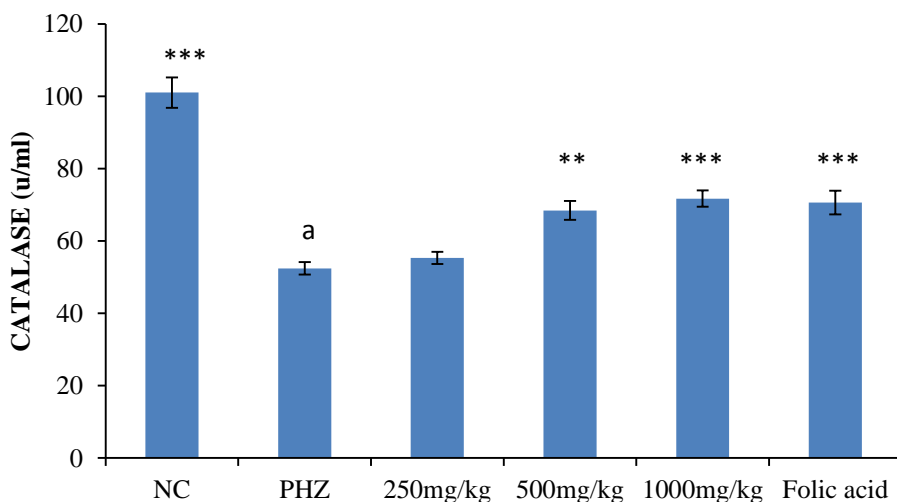


Figure 5: Effect of PNAE on Catalase in PHZ-induced Anaemic Rats after 14 days of treatment with PNAE. Values represent the means \pm SD (n= 8 rats each for all the groups). One-way ANOVA with Dunnet’s post-test was used to

arrive at the P values. *, ** and *** signifies P<0.005, P<0.02 and P<0.001 respectively when compared with PHZ-treated group. while ^a signifies p<0.001 when compared with normal control.

Biochemical Parameters

Oxidative Stress Markers

Malondialdehyde

The result of malondialdehyde following treatment of PHZ induced anemic rats with PNAE produces a significant (P<0.05) and dose-dependent decrease in malondialdehyde when compared with the untreated group (PHZ). There was also a significant (P<0.001) increase in MDA level when PHZ group was compared to the control after treatment as shown in Figure 6 below.

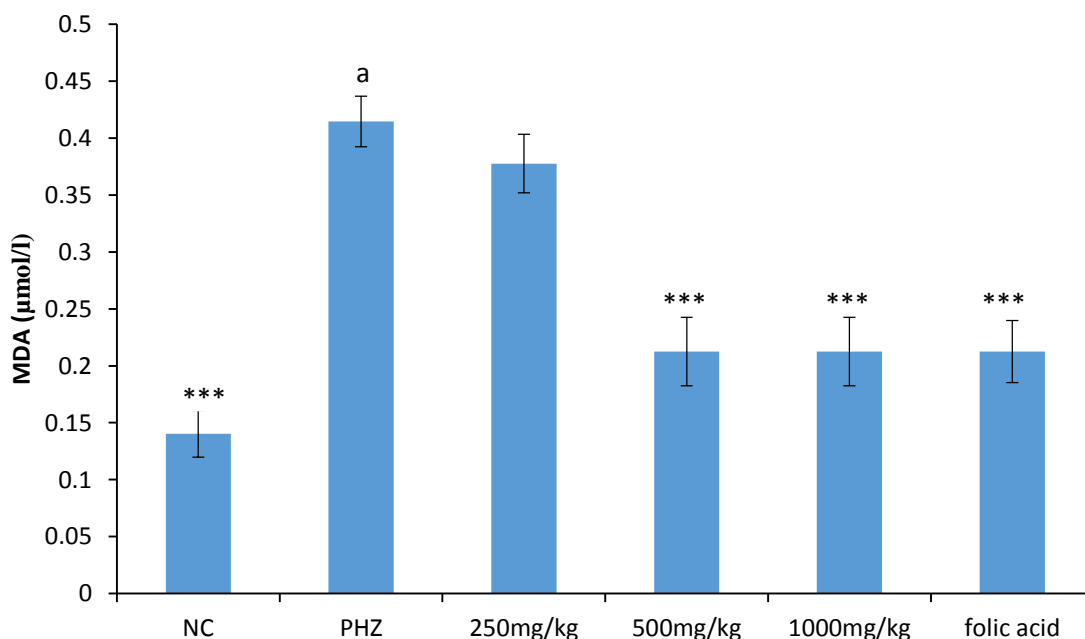


Figure 6: Effect of PNAE on MDA in PHZ-induced Anaemic Rats after 14 days treatment with PNAE. Values represent the means ± SD (n= 8 rats each for all the groups). One-way ANOVA with Dunnet’s post-test was used to arrive at the P values. *, ** and *** signifies P<0.005, P<0.02 and P<0.001 respectively when compared with PHZ-treated group while ^a signifies p<0.001 when compared with normal control.

Superoxide Dismutase Stimulatory Activity of PNAE

The result of SOD activity following treatment of PHZ induced anemic rats with PNAE shows a significant (P<0.01) and dose-dependent increase in SOD activity when compared with the untreated group (PHZ). There was also a significant (P<0.001) decrease in SOD activity level when PHZ group was compared to the control after treatment as shown in Figure 7 below.

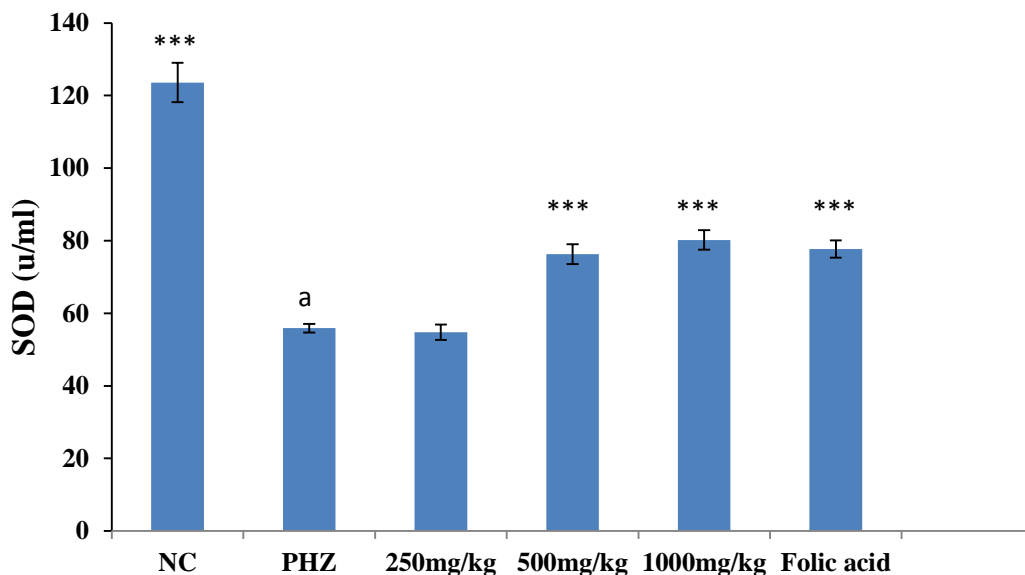


Figure 7: Effect of PNAE on SOD in PHZ-induced Anaemic Rats after 14 days of treatment with PNAE. Values represent the means \pm SD (n= 8 rats each for all the groups). One-way ANOVA with Dunnet’s post-test was used to arrive at the P values. *, ** and *** signifies $P < 0.005$, $P < 0.02$ and $P < 0.001$ respectively when compared with PHZ-treated group. While ^a signifies $P < 0.001$ when compared with normal control.

Effect of Treatment on Bilirubin

The result of bilirubin assay revealed a significant ($P < 0.001$) and dose-dependent reduction in total, direct and unconjugated bilirubin when PHZ induced anaemic rats were treated with PNAE compared with the untreated group. There was also a significant ($P < 0.001$) increase in bilirubin when PHZ group was compared to the control after treatment as shown in Figure 8 below.

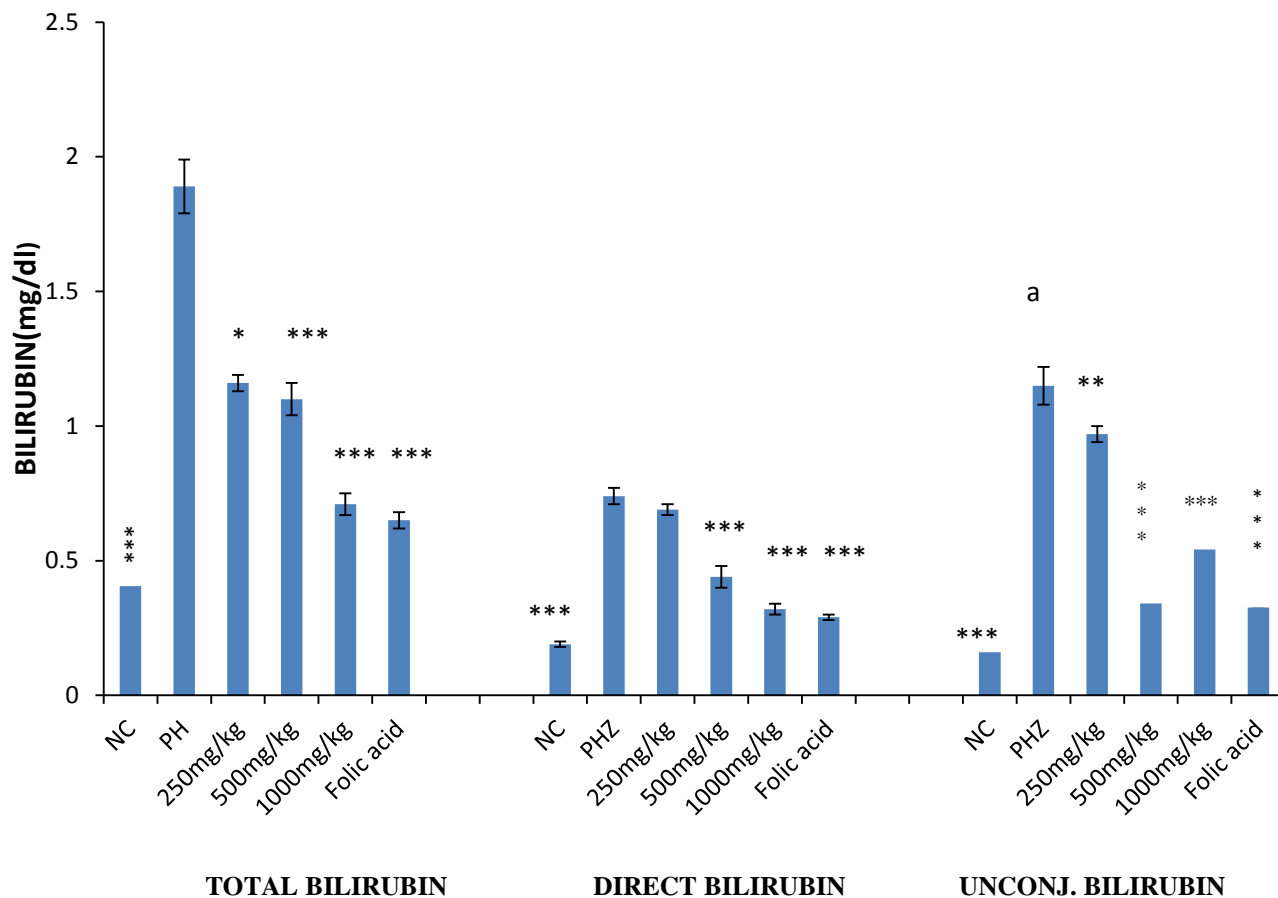


Figure 8: Values represent the means \pm SD (n= 8 rats each for all the groups). One-way ANOVA with Dunnet’s posttest was used to arrive at the P values. *, ** and *** signifies P<0.005, P<0.02 and P<0.001 respectively when compared with PHZ-treated group while signifies P<0.001 when compared with normal control.

Discussion

The present study evaluated the anti-anaemic activity of *P. niruri* in phenylhydrazine-induced hemolytic anaemia in rats. The results of the study revealed a significant reduction of the weight of rats after induction of anaemia which could be due to the effect of oxidative damages (erythrocytes haemolysis) induced by 2,4-DPH. The reduction in body weight was seen to improve in the group of rats treated with *P. niruri* aqueous extract after 14 days probably due to reversal of the oxidative damage by the extract. This finding is in agreement with some studies reported by [2,4,11]. 2015 where the body weights of rats significantly reduced after induction of anaemia with PHZ but were improved after treatment with 200mg/kg body weight of ethanolic extract of *J. repens* and *C. fascicularis* L and 400mg/kg body weight *Z. jujuba* fruits aqueous extract for 2 weeks respectively.

Berger in 2007 reported that, PHZ injected at a dose of 90 mg/kg body weight to 8 weeks old rats produced a reduction of normal RBC by 45% and PCV by 53% on day 3; reticulocytes by 47% on day 7; and a high MCV of 170% as well as a 60% increase in MCV on day 3. More than 30%, reduction in HB and RBCs was also reported by [4,5,12-16]. In the present study, administration of PHZ in rats produces a decreased PCV level which agreed with previous reports by [17], Maria Claro *et al.*, 2006; [5,13] that reported reduction in PCV in hemolytic anaemia induced by 2, 4-DPHZ caused that leads to oxidation of HB and sulfhydryl groups of the erythrocytes membrane and enzymes leading to haemolysis of erythrocytes [17]. However, Oral administration of *P. niruri* whole plant aqueous extract produces a dose-dependent increase in PCV, RBC, HB ($P < 0.05$) and a significant ($P < 0.05$) decrease in WBC, MCV, MCH and reticulocytes after 14 days treatment. This agreed with earlier findings by [13] where oral administration of 250 and 500mg/kg body weight aqueous extract of *P. erinaceus* Stem Bark significantly increased PCV, Hb, RBC and significantly decreased WBC, MCH and MCHC in PHZ induced anaemic rats probably due to the ability of PNAE to protect the RBC against oxidative haemolysis induced by 2,4-DNPH. The increase in WBC level seen after induction of anaemia might be due to immune stimulatory ability of the chemical similar to what was reported by [18] following administration of ethanolic extract of *P. kurroa* leaves extracts at 100 mg/kg and 200 mg/kg, to PHZ induced anaemic rats which caused increased the level of RBC, PCV and HB in rats. Similarly, treatment of 2,4-DPHZ induced anemic rabbits with *Hibiscus sabdarifa* anthocyanin extract produces a significant ($P < 0.05$) increase in, RBC counts, PCV and Hb and a decrease in WBC counts [19].

Conclusion

The results of the present study revealed a significant anti-anemic property of whole plant aqueous extract of *P. niruri* in 2,4-dinitrophenylhydrazine-induced haemolytic anaemia in experimental rats. The anti-anemic activity of the plant could be due to the presence of important phytochemicals such as flavonoids and alkaloids which are known to possess erythrocytes protective abilities hence, reversing the anaemia induced in the rats.

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