RJMP Research Journal of Medicinal Plants

OPEN ACCESS

Ameliorative Effect of Methanol Leaf Extract of *Phyllanthus nirurion* Anaemic Male Wister Rats

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ABSTRACT

Background and Objective: Anaemia is a major public health problem which affects people of all ages, especially pregnant women and children. It is effectively treated by blood transfusion but a less expensive and easy approach for remedying anaemia is by the administration of oral supplements, for example, the use of plant extract with active secondary metabolites possessing anti-anaemic properties most especially in cases of iron deficiency anaemia. This study was aimed at investigating the ameliorative effect of methanol extract of *Phyllanthus niruri* on anaemic male Wister albino rats. **Materials and Methods:** Samples of fresh *Phyllanthus niruri* whole plant (roots, stems, leaves and seeds) were collected from Wukari, Taraba State, Nigeria. The plant samples were shade dried for 10 days. The dried samples were ground to powder with the aid of a mortar and pestle. The coarse materials were then sieved. Extraction of *P. niruri* was done by cold maceration with 100% methanol. Twenty male Wister rats were grouped into four groups (n = 5) and acclimatized for 7 days. Anaemia was induced by intraperitoneal injection of phenylhydrazine at 40 mg/kg/b.wt., on days 0 and 1. Packed cell volume (PCV) was determined to ensure induction of anaemia. Group 1 received no treatment, group 2 received ferrous sulphate at 75 mg/kg/b.wt. and groups 3 and 4 received 100 mg/kg/b.wt. and 200 mg/kg/b.wt., of methanolic extract of *Phyllanthus niruri*, respectively. **Results:** A significant ($p<0.05$) decrease in weight in groups 1 and 2 animals was observed. Animals whose PCV decreased by 29% were considered anaemic. The PCV levels on days 1 and 2 showed no significant ($p>0.05$) difference between groups, while a statistically significant ($p<0.05$) difference in PCV levels was observed on day 19. There was a significant ($p<0.05$) increase in PCV, platelet and eosinophil in groups 3 and 4. Other haematological parameters were not significantly different ($p>0.05$) between groups. **Conclusion:** The results suggested that the methanolic 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 drugs for the treatment of heamolytic anaemia.

KEYWORDS

Phyllanthus niruri, anaemia, phenylhydrazine, phytomedicine, Wister rats, ameliorative, anti-anaemic

ISSN: 2151-7924 (Online)
ISSN: 1819-3455 (Print)
https://doi.org/10.17311/rjmp.2023.32.41

Received: 08 Nov. 2022
Accepted: 24 Mar. 2023
Published: 28 Apr. 2023

INTRODUCTION

Anaemia is a common nutritional deficiency disorder and global public health problem which affects both developing and developed countries with major consequences for human health and their social and economic development[1]. One-third of the global population (over 2 billion) is anaemic due to an imbalance in their nutritious food intake[2]. Anaemia affects people of all ages, especially pregnant women and children[3]. It affects 41.8% of pregnant women and 47.4% of preschool children[4]. Its negative effect is seen on health and development, neonatal and perinatal mortality, low birth weight[5], premature birth[6,7] and retardation in thedevelopment of children[8]. The disorder is characterized by fatigue, dizziness, shortness of breath andweakness, among others[9]. Anaemia exists in various forms based on its underlying cause(s), viz: Sickle cell anaemia (synthesis of abnormal haemoglobin), iron deficiency anemia (defects in haemoglobin synthesis), thalassaemia (genetic defects of haemoglobin maturation), haemolytic anaemia(physical loss of red cells) etc[10]. As a response, poor management of anaemia can promote decreased cognitive ability, weakened immune system and increased mortality[11].

Plant materials as sources of medical compounds continue to play a dominant role in the maintenance of human health since antiquity[12]. Over 50% of all modern chemical drugs are of natural plant product origin[13]. *Phyllanthus niruri* or "stone breaker tea" is one of such plants that have been reported to possess anti-inflammatory, anti-hyperuricemic and diuretic properties[14]. *Phyllanthus niruri* is a widespread tropical plant found in coastal areas, known by the common names "gale of the wind", "stonebreaker" and "seed-under-leaf". It is in the genus *Phyllanthus* of the family *Phyllanthaceae*. It grows 50-70 cm tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds. Lignans, flavonoids, triterpenes, sterols, alkaloids and essential oils are found in this plant. The plant has been used in traditional medicine for treating various illnesses such as urinary stones[15]. Extracts of *Phyllanthus niruri* are reportedly used in herbal medicine to ameliorate the effect of anaemia[16].

Anaemia is effectively treated by blood transfusion which is quite an expensive approach[17]. However, a less expensive and easy approach for remedying anemia is the administration of oral supplements most especially in cases of iron deficiency anemia. This is achieved by the use of plant extract with active secondary metabolites possessing anti-anaemic properties. The global prevalence of anaemia and the need to develop less expensive approaches to cure this diseasehavecalled for an awakening in phytomedicine research. This study was aimed at investigating the ameliorative effect of methanol extract of *Phyllanthus niruri* on anaemic male Wister albino rats.

MATERIALS AND METHODS

Study area: The study was carried out in the Central Research Laboratory located at Federal University Wukari, Taraba State, Nigeria, between November and March, 2021.

Plant collection: Fresh *Phyllanthus niruri* whole plant (roots, stems, leaves and seeds) samples (weighing up to 200 g) were collected from Wukari, Taraba State, Nigeria, in the month of November 2021 (Fig. 1). The plant samples were shade dried for 10 days. The dried samples were ground to powder with the aid of a mortar and pestle. The coarse materials were sieved.

Preparation of methanolic extract of *Phyllanthus niruri*: Cold maceration technique was employed for the extraction. The method of Evans and Trease[18] was adopted with little modification. To 250 g of the coarsely powdered crude sample (*P. niruri*), 500 mL of 100% methanol was added. The mixture was sealed with foil paper and allowed to stand at room temperature for 7 days with frequent. The mixture was sieved, strained and the marc (damp solid material) waspressed. The solvent extract was filtered using Whatman No. 1 filter paper as fine solvent extract was obtained. The methanolic extract was concentrated

Fig. 1: *Phyllanthus niruri* plant[18]

by evaporating the solvent (100% methanol) at 50°C using arotary evaporator and vacuum oven (Manufactured by SH SCIENTIFIC, Yaba, Lagos State, Nigeria) to obtain the oily-sticky extract. It was dissolved in 80% for an aqueous extract of *Phyllanthus niruri* (AEPN), the AEPN was stored in the refrigerator prior to the commencement of treatment.

Experimental design: A total of twenty male albino rats weighing 130-191 g (8 weeks old) were obtained. They were assigned into four groups, five animals per group (n = 5). The animals were maintained under standard laboratory conditions and had free access to standard finisher feeds and water for one week for acclimatization before the commencement of the experiments. Anaemia was induced by intraperitoneal phenylhydrazine injection (40 mg/kg/b.wt.) during two days (Day 0 and Day 1) based on the method described by Cyril *et al.*[19].

Experimental treatment:

Group I: Anaemic control (AC) received distilled water and normal feed for 19 days
Group II: Standard control (SC) received ferrous sulphate and normal feed for 19 days
Group III: Animals received AEPN (100 mg/kg/b.wt.) for 19 days
Group IV: Animals received AEPN (200 mg/kg/b.wt.) for 19 days

Determination of haematological parameters: The Wister rats were sacrificed under chloroform upon completion of the experimental period. A blood sample was collected by cardiac puncture. About 3 mL of blood was collected into an EDTA sample bottle for haematological assay and sample bottles was labelledaccordingly for all 4 groups. The PCV, MCHC, RBC, MCH, MCV, platelets, WBC, Eosinophil, Neutrophil and Lymphocyte levels were determined using an auto Hematology analyzer.

Ethical consideration: All ethical matters as concerned animal handling were observed following the animal ethical policies of the Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria.

Statement of informed consent: Participants of this study provided their written informed consent to participate in this study.

Statistical analysis: The values expressed as Mean±Standard Deviation (SD) from 4 animals. In each group, the various means were compared with those of day 0 (D0) than day 2 (D2) by using a One-Way Analysis of Variance (ANOVA) followed by Dunnett's Test. The statistical difference was considered significant at $p<0.05$.

RESULTS

Effect of methanolic extract of *P. niruri* on the body weight of phenylhydrazine (PHZ) induced anaemic Wister rats: *Post hoc* comparison using the Dunnett t-test for groups after treatment on day 19 showed that there was no significant (p = 0.941) reduction in the body weight of animals treated with ferrous sulphate, but a significant dose-dependent increase was observed in the body weight of animals (113.33±17.21 g) in groups treated with methanolic extract of *Phyllanthus niruri* at 100 mg kg^{-1} and 200 mg kg^{-1} with significance at p = 0.012 and p = 0.005 and mean±SD of body weight of animals, 167.33±8.38 g and 175.66±15.30 g, respectively when compared with the untreated group (anaemic control), 124.00±19.69 g as seen in Fig. 2.

Effect of methanolic extract of *P. niruri* on PCV levels of phenylhydrazine(PHZ) induced anaemic Wister rats: *Post hoc* comparison test using the Dunnett t-test showed a statistically significant difference in the PCV values of animals in the group treated with ferrous sulphate (100 mg kg^{-1} and 200 mg kg^{-1}) methanolic extract of *Phyllanthud niruri* compared with the untreated group with p values significant at p = 0.046, p = 0.013 and p = 0.014 with corresponding mean±SD PCV values of 41.33±2.08%, 43.00±1.73% and 44.33±2.08%, respectively against 34.33±1.52% of the untreated group as presented in Fig. 3.

Effect of methanolic extract of *P. niruri* on RBC and WBC of phenylhydrazine (PHZ) induced anaemic Wister rats: One-way analysis of variance revealed that there was no statistically significant difference in the RBC and WBC counts between at least two groups (F (3,8) = [1.60], p = 0.264) and (F (3,8) = [0.66], p =0.597), respectively (Fig. 4).

Effect of aqueous extract of *P. niruri* on platelet count in phenylhydrazine (PHZ) induced anaemic Wister rats: The result for platelet countwas shown in Fig. 5. One-way ANOVA revealed that there was a statistically significant difference in mean±SD PLC between at least two groups (F (3, 8) = [6.30],

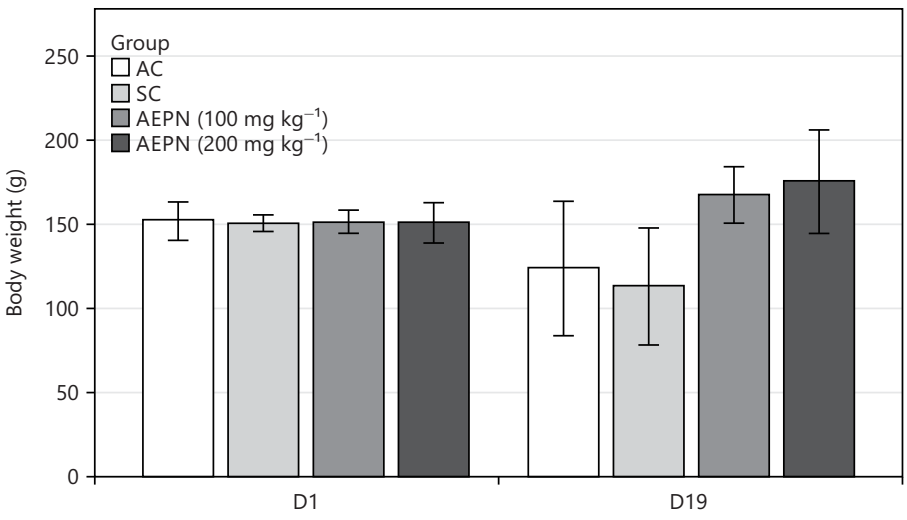

Fig. 2: Effect of methanolic extract of *P. niruri* on the body weight of phenylhydrazine (PHZ) induced anaemic Wister rats pre and post treatment of anaemia after 19 days

Values are Mean±Standard Deviation, D1: Day one, before treatment was administered, D19: Day 19, body weight before animals were sacrificed, AC: Anaemic control group, received no treatment, SC: Standard control group, received ferrous sulphate, AEPN (100 mg kg^{-1}) = Group treated with 100 mg kg^{-1} aqueous extract *Phyllanthus niruri* and AEPN (200 mg kg^{-1}) = Group treated with 200 mg kg^{-1} aqueous extract of *Phyllanthus niruri*

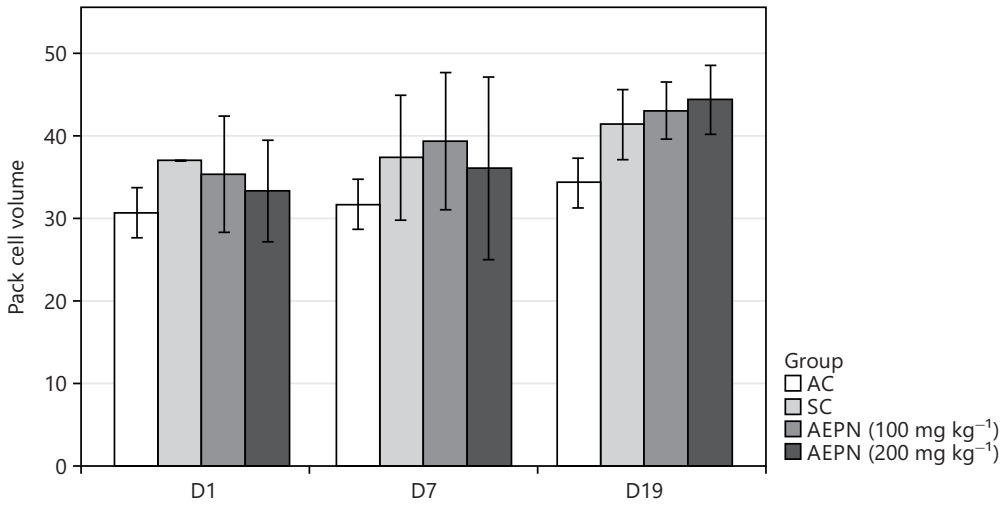

Fig. 3: Effect of methanolic extract of *P. niruri* on PCV levels of phenylhydrazine (PHZ) induced anaemic Wister rats on day 1, day 7 and day 19

Values are Mean±SD, AC: Anaemic control group, SC: Standard control group, received ferrous sulphate, AEPN (100 mg kg^{-1}) = Group treated with 100 mg kg^{-1} aqueous extract *Phyllanthus niruri*, AEPN (200 mg kg^{-1}) = Group treated with 200 mg kg^{-1} aqueous extract of *Phyllanthus niruri*, D1: Day one before extract was administered, D7: Seventh days of treatment and D19: Day 19, after animals were sacrificed

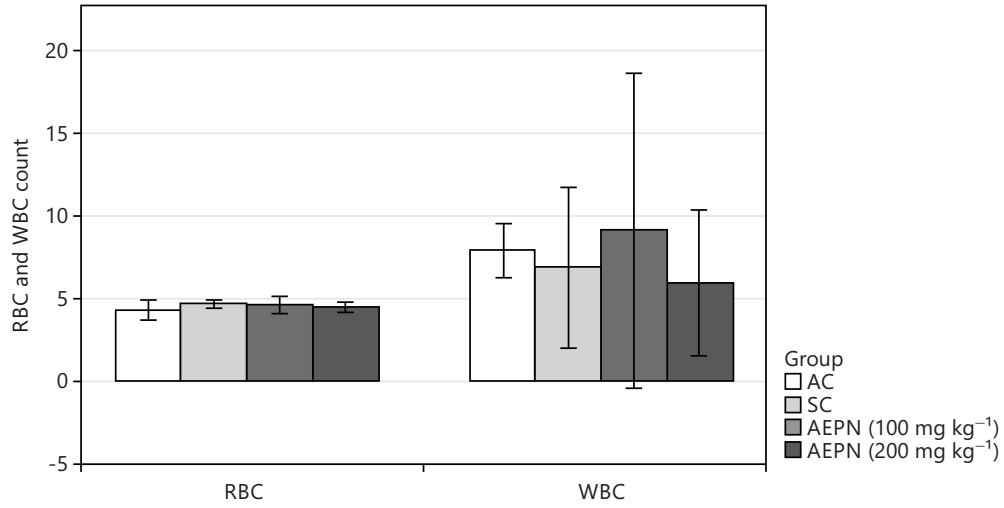

Fig. 4: Effect of methanolic extract of *P. niruri* on RBC and WBC of phenylhydrazine (PHZ) induced anaemic Wister rats

Values are Mean±SD, p<0.05 is considered as significant, RBC: Red Blood Cell count, WBC: White Blood Cell count, Ac: Anaemic control group, SC: Standard control group treated with Ferrous sulphate, AEPN: Groups treated with aqueous extract of *Phyllanthus niruri* at 100 mg kg^{-1} and 200 mg kg^{-1}

p = 0.017). Dunnett t-test for multiple comparisons showed a level of statistically significant difference for p = 0.010, p = 0.034 and p = 0.028 correspondently with groups treated with Ferrous sulphate, 100 mg kg^{-1} and 200 mg kg^{-1} aqueous extract of *Phyllanthus niruri*.

Effect of methanolic extract of *P. niruri*on MCV, MCH and MCHC in phenylhydrazine (PHZ) inducedanaemic rats: Results for MCH, MCV and MCHC respectively showed no significant difference (F (3, 8) = [0.79], p = 0.535), (F (3, 8) = [0.32], p = 0.811) and (F (3, 8) = [1.92], p = 0.205) between at least two groups treated according to one-way ANOVA (Fig. 6).

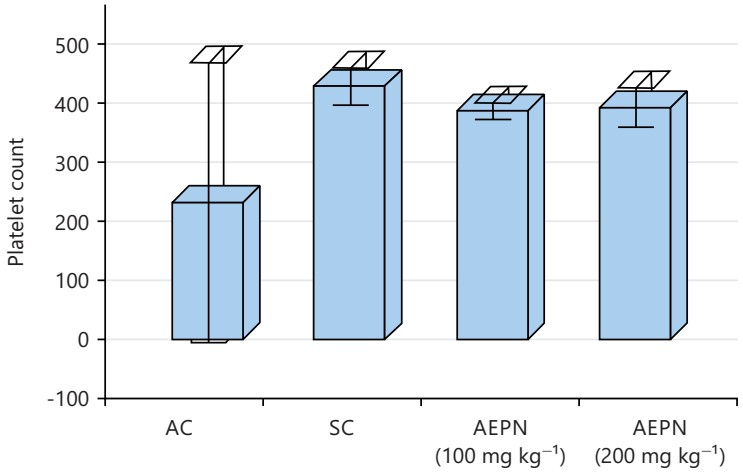

Fig. 5: Effect of methanolic extract of *P. niruri* on platelet count in phenylhydrazine (PHZ) induced anaemic Wister rats

Values are Mean±SD, p<0.05 is considered as significant, Ac: Anaemic control group, SC: Standard control group treated with ferrous sulphate, AEPN: Groups treated with aqueous extract of *Phyllanthus niruri* at 100 mg kg^{-1} and 200 mg kg^{-1}

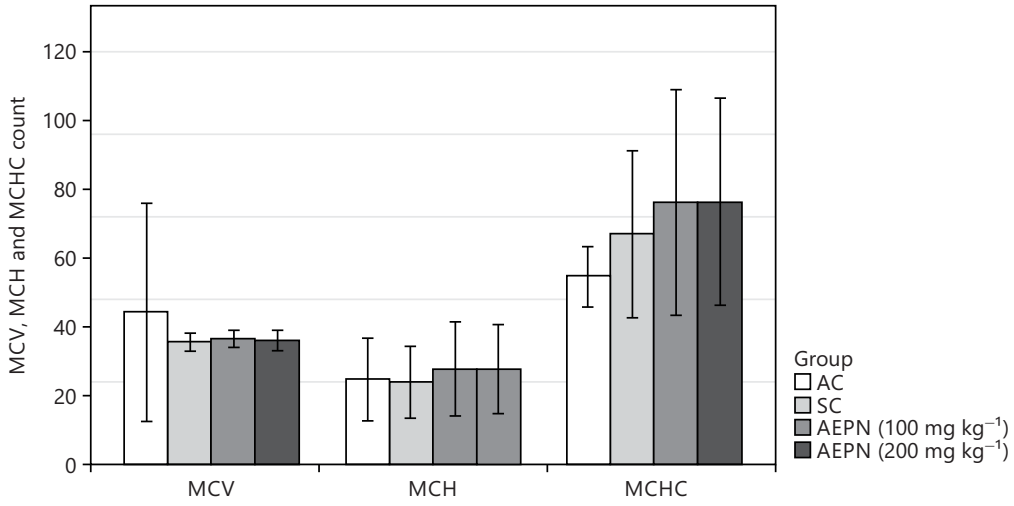

Fig. 6: Effect of aqueous extract of *P. niruri* on MCV, MCH and MCHC in phenylhydrazine(PHZ) induced anaemic Wister rats

Values are Mean±SD, p<0.05 is considered as significant, Ac: Anaemic control group, SC: Standard control group treated with ferrous sulphate, AEPN: Groups treated with aqueous extract of *Phyllanthus niruri* at 100 mg kg^{-1} and 200 mg kg^{-1}. MCH: Mean corpuscular haemoglobin, MCV: Mean cell volume and MCHC: Mean cell haemoglobin concentration

Effect of aqueous extract of *P. niruri* on neutrophil, eosinophil and lymphocyte count in phenylhydrazine (PHZ) induced anaemic Wister rats: The treatment of PHZ induced anaemic rats with aqueous extract of *P. niruri* for 19 days disclosed a significant difference (F (3, 8) = [6.04], p = 0.019) in eosinophil count between at least two groups, there was a significant p = 0.022 increase in eosinophils in the group treated with 100 mg kg^{-1} aqueous extract of *P. niruri* as compared to the untreated group. Also, neutrophil and lymphocyte counts showed no significant difference (F (3, 8) = [2.11], p = 0.178) and (F (3, 8) = [1.34], p = 0.327) accordingly between at least two of the groups treated against the untreated group as shown in Fig. 7.

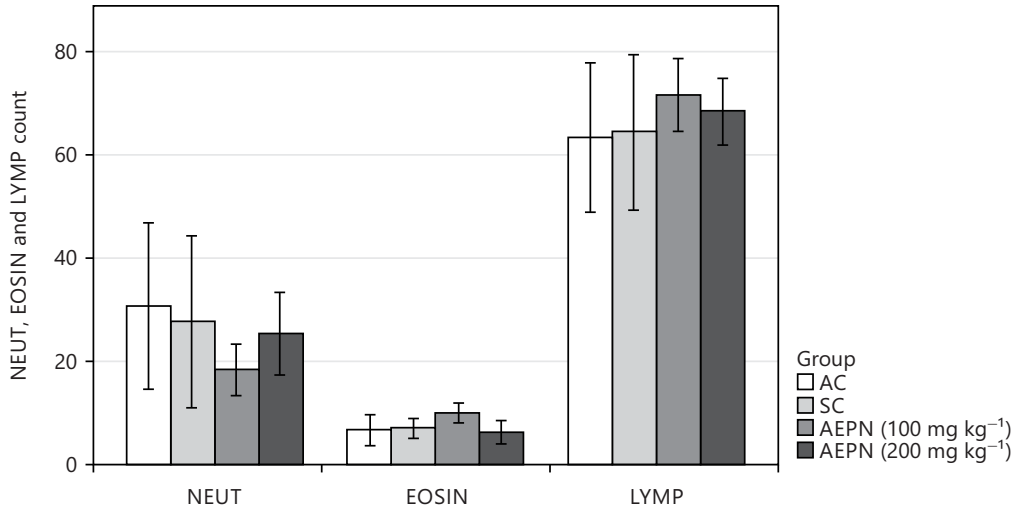

Fig. 7: Effect of aqueous extract of *P. niruri* on neutrophil, eosinophil and lymphocyte count in phenylhydrazine (PHZ) induced anaemic Wister rats

Values are Mean±SD, p<0.05 is considered as significant, Ac: Anaemic control group, SC: Standard control group treated with ferrous sulphate, AEPN: Groups treated with aqueous extract of *Phyllanthus niruri* at 100 mg kg^{-1} and 200 mg kg^{-1}, NEUT: Neutrophil count, EOSIN: Eosinophil count and LYMPH: Lymphocyte count

DISCUSSION

The results of this study revealed a significant (p<0.05) reduction in the weight of rats after the induction of anaemia which could be due to the effect of oxidative damages (erythrocytes haemolysis) induced by phenylhydrazine. Phenylhydrazine is a non-immunogenic chemical that induces ahemolytic type of anemia by selectively destroying mature RBCs through oxidative stress, denaturation of red cell Hb, membrane phospholipids and enzymes involved in energy metabolism[20]. Haemoglobin (Hb) is a protein within the cytoplasm of the red blood cells which is composed of the protoporphyrin ring heme and globin. It plays a role in tissue perfusion. It is the most commonly used marker of anaemia[20]. The red cell count on the other hand reflects the number of circulating red blood cells. The red cell count is particularly useful in identifying erythrocytosis, a normal red cell count with elevated haemoglobin/haematocrit suggests relative erythrocytosis (dehydration), while an elevated red cell count suggests absolute erythrocytosis (polycythaemiavera). A decrease in the red cell count and/or haemoglobin is an indication of anaemia and depending on the red cell indices values (MCH, MCV and MCHC), the aetiology of the anaemia can be inferred[21].

The reduction in body weight of the Wister rats improved in the group of Wister rats treated with *P. niruri* methanolic extract after 14 days probably due to thereversal of the oxidative damage by the extract. This finding was in agreement with studies reported by researchers[16,22-24], where the body weights of rats significantly reduced after induction of anaemia with PHZ but were improved after treatment with 200 mg/kg/b.wt., of methanolic extract of *Juncus repens* and *Corchorus fascicularis* L. and 400 mg/kg/b.wt., *Ziziphus jujube* fruits aqueous extract as well as *Phyllanthus niruri* 250 mg kg^{-1}, 500 mg kg^{-1} and 1000 mg kg^{-1} body weight for 2 weeks, respectively.

In the present study, the administration of PHZ in rats produces a decreased PCV level which agrees with previous reports by Bhave and Neilsonr[25] and Marrelli[26]. They reported a reduction in PCV in hemolytic anaemia induced by 2, 4-DPHZ that led to oxidation of HB and sulfhydryl groups of the erythrocytes membrane and enzymes resulting in the haemolysis of erythrocytes. However, oral administration of *P. niruri* whole plant methanolic extract produced asignificant (p<0.05) dose-dependent increase in PCV, platelets and eosinophils and a significant (p<0.05) decrease in WBC, MCV, MCH, after 19 days of

treatment. This agrees with the findings of Nadro and Modibbo[27], where oral administration of 250 mg kg^{-1} and 500 mg kg^{-1} 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. This could probably be due to the ability of AEPN to protect the RBC against oxidative haemolysis induced by 2,4-DNPH.

White blood cells are cellular elements which play a role in humoral and cell-mediated immunity. They help the body fight infections. A white blood count is most often used to help diagnose disorders related to having a high white blood cell count (leukocytosis) or low white blood cell count (leucopenia). In the present study, the increase in WBC level seen after induction of anaemia might be due to the immune-stimulatory ability of the chemical, similar to what was reported by Turaskar *et al*.[28] following the administration of ethanolic extract of *Picrorhiza kurroa* leaves extracts at 100 mg kg^{-1} and 200 mg kg^{-1}, to PHZ induced anaemic rats which caused increased the level of RBC, PCV and HB in rats. Another report by Ologundudu *et al*.[29] which involved the treatment of 2,4-DPHZ-inducedanaemic rabbits with *Hibiscus sabdarifa* anthocyanin extract produced a significant ($p<0.05$) increase in, RBC counts, PCV and Hb and a decrease in WBC count. Elevated WBC count is considered a risk marker for cardiovascular disease incidence and mortality[30]. White blood cell (WBC) subsets such as neutrophils, lymphocytes, monocytes, or ratio of neutrophil to lymphocyte counts (N/L) have been identified as an easy, simple, inexpensive and reliable prognostic index to determine host immunity[31].

This study revealed the anti-anaemic property of the methanolic extract of *P. niruri* such that conventional anaemia drugs which are expensive may be replaced by cheaper anemia drugs of plant origin as shown by this study. Patients suffering from anaemia who are not financially buoyant may resort to using anaemia drugs of plant origin. A limitation of this research work is that possible effects that may result from prolonged use of the methanolic extract of *P. niruri* were not looked into. However, researchers interested in this field may explore this gap.

CONCLUSION

The results of the present study revealed that the methanolic extract of *P. niruri*in this study showed a significant positive effect on animal body mass. This suggests that the methanolic extract of *P. niruri* plant possesses a significant anti-anaemic property and is relatively safe. Therefore, it may be a potential lead in the discovery of a drug for the treatment of haemolytic anaemia. Researchers interested in this field may explore the possible effects that may result from the prolonged use of the methanolic extract of *P. niruri* in curing anaemia as this present study did not address it.

SIGNIFICANCE STATEMENT

This study discovered the ameliorative effect of methanolic extract of *Phyllanthus niruri* on male anaemic Wister rats. This study will help the researchers to uncover the critical areas of phytomedicine in the *Phyllanthus niruri* plant that many researchers were not able to explore. Thus it may be a potential lead in the discovery of a drug for the treatment of haemolytic anaemia.

ACKNOWLEDGMENT

We would like to thank Dr. Ayo Victoria Ifeoluwa for initiating the concept of this research work and spearheading it and also other co-researchers that contributed to the success of this work.

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