

# Ameliorative effects of the hydromethanol leaf extract of *Craterispermum schweinfurthi* on phenyl hydrazine induced anemia in male wistar rats

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**Abstract:** Introduction: Anaemia is a clinical condition characterized by a reduction in the normal values of haemoglobin concentration, circulating erythrocytes and other erythrocyte indices per unit of blood.

**Aim:** The present study evaluates the potential ameliorative effects of the hydromethanol extract of the leaves of *Craterispermum schweinfurthi* following phenyl hydrazine induced anemia in male wistar rats.

**Methodology:** A total of 40 male wistar rats weighing between 100-250 g were randomly divided into 8 groups of 5 rats each. Phenyl hydrazine was administered in 3 separate doses: 9am on day 0; 9am and 6pm on day 1 to all rat groups except groups 1 and 8. The rat groups were subsequently treated as follows: Group 1 (negative control) received extract vehicle only; Group 2 received 40 mg/kgbw of phenyl hydrazine only; Groups 3-5 received 250, 500 and 750 mg/kgbw respectively of hydromethanol leaves extract of *Craterispermum schweinfurthi*; Group 6 received 0.23ml/kgbw of Bioferon®; both Groups 7 and 8 received 2000mg/kgbw of phytosterol. The extract, Bioferon and phytosterol were administered once daily using oro-gastric cannula for 14 days. On day 15, the rats were placed under chloroform anaesthesia and blood samples collected by direct cardiac puncture into appropriate sample tubes for estimation of erythrocyte count, haemoglobin concentration, haematocrit, platelet count, total white blood cell count and differential counts using automated methods.

**Results:** Amongst Group 2 rats, values of erythrocyte, platelet and leucocyte indices were significantly reduced ( $p < 0.05$ ) following phenyl hydrazine administration compared to Group 1 rats. However, administration of the extract, significantly increased ( $p < 0.05$ ) these parameters in a dose dependent manner amongst Groups 3, 4 and 5 rats. A similar increase was observed amongst Groups 6 and 7 rats administered Bioferon® and phytosterol respectively: suggesting a potential ameliorative effects of the extract comparable to the effects of Bioferon®.

**Conclusion:** Apparently the hydromethanol leaf extract of *Craterispermum schweinfurthi* exhibits potential beneficial dose dependent effects on haematopoiesis in experimental animals. These validates the use of the leaves of the plant in our environment as a tonic. Our findings are preliminary requiring further investigations.

**Keywords:** *Craterispermum schweinfurthi*, phenyl hydrazine, phytosterol.

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## 1. INTRODUCTION

Anaemia is a disease characterized by a reduction in the normal values of haemoglobin, circulating erythrocytes and other erythrocyte indices per unit of the blood [1-3]. The main function of erythrocytes is the transport of oxygen from the lungs to the tissues. Consequently, any pathological or physiological condition that affects the erythrocyte or alters its function may be detrimental to health [3]. Phenylhydrazine (PHZ) via its ability to induce haemolysis in experimental animals and humans decreases haemoglobin concentration, erythrocyte count and haematocrit values [4-6]. A hydrochloride derivative PHZ hydrochloride, has long been used to treat polycythaemia vera [7, 8] but has been shown to induce tumour formation in mice [9]. PHZ is commonly used worldwide mainly as a chemical intermediate in the pharmaceutical, agrochemical and chemical industries.

There is an increasing dependence on native African (medicinal) plants for the prevention and treatment of various illnesses around the world [10]. Furthermore, it has been estimated that a large proportion of the population in developing countries depend mainly on medicinal plants of therapeutic value and the services of traditional medicine practitioners [10]. The usefulness and importance of medicinal plants is derived from their accessibility, affordability and minimal side effects compared to orthodox medications amongst other benefits [11]. The active compounds identified in most medicinal plants are effective in the treatment, management, and prevention of disease conditions. These bio-active compounds are frequently characterised, extracted and used as raw materials in the production of many drugs [10]. *Craterispermum schweinfurthii* species are shrubs or small trees with axillary or supra-axillary inflorescences, paired at the nodes and often condensed; they are widely distributed in tropical Africa, Madagascar, and the Seychelles [12-13]. The anecdotal applications of *Craterispermum schweinfurthii* in traditional medicine are numerous. For instance, in traditional folklore medicine, the seed, leaves, and inner bark have been described to have a beneficial effect in cases of stomach afflictions, ulcer, diabetes and fever in central West Africa [14]. However, scientific reports validating these anecdotal benefits of the leaves of *Craterispermum schweinfurthii* are relatively scanty. Furthermore, identification of the bioactive compounds present in the leaves has become imperative to enable further studies. An earlier report by the authors identified phytosterol, amongst other compounds, as a major constituent of the hydromethanol leaf extract of *Craterispermum schweinfurthii* using GCMS characterisation [15].

The present study describes potential ameliorative effects of hydromethanol extract of *Craterispermum schweinfurthii* leaves following phenyl hydrazine induced haemolytic anemia using male wistar rats as models. This is with the view of evaluating the anecdotal use of the leaves of *Craterispermum schweinfurthii* as a tonic in our environment. In addition, attempt was made to comparatively evaluate phytosterol a major bioactive compound previously identified in the leaves of *Craterispermum schweinfurthii*. This is with the view to properly identify the potential bioactive compounds responsible for any of these effects.

## 2. MATERIALS AND METHODS

### *Collection, Identification and Extraction of Plant Materials*

Fresh leaves of *Craterispermum schweinfurthii* were obtained from the Botanical Garden, University of Port Harcourt, Nigeria. Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port-Harcourt, Nigeria identified and authenticated the specimen and assigned a reference code: UPH/V/296. Voucher specimen was subsequently deposited in the University Herbarium for future reference. The plant leaves were gathered, and all extraneous materials carefully removed. The leaves were air dried at room temperature for a minimum of 7 days after which it was pulverized into powder and the weighed quantity of 670.6g dissolved using Soxhlet device in 390ml of water-methanol mixture (25:75% v/v BDH) for three days in a jar. It was filtered and concentrated using a rotary evaporator at 40°C and the yield was 73%. Obtained extract was preserved in airtight containers and stocked at room temperature prior administration.

### *Procurement and Handling of Experimental Animals*

Male wistar rats weighing between 100–250g were used for the study. Animals were kept at the Animal House, Department of Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were acclimatized for two weeks and subsequently grouped for the study.

### ***Ethical Approval and Acute Toxicity Test***

Ethical approval was sought and obtained from our Institutional Ethical Committee vide a communication referenced: UPH/CEREMAD/REC/MM82/024 dated 23<sup>rd</sup> November, 2021. The acute toxicity of the hydromethanol extract of *Craterispermum schweinfurthi* leaves was determined using Karber's method as modified by Aliu and Nwude, 1982 [16]. Lethal dose (LD<sub>50</sub>) of the extract was found to be 3968mg/kg bw. The study was conducted in accordance with the guidelines for the care and use of laboratory animals [17].

### ***Phenyl hydrazine (PHZ), Drug and Phytosterol Procurement***

Phenyl hydrazine (PHZ) was purchased from JHD Co., LTD, 618, Qingshan Road, Licang Dist., Qingdao, Shandong, China; Bioferon was obtained from Biopharm Quality and Tradition, 12 Klemenova Dacha Street, Apt. 11, Kharkiv, 61033, Ukraine. Phytosterol was obtained from Wakunaga of America Co., LTD. Mission Viejo, CA92691 U.S.A.

### ***Experimental Design***

A total of 40 male wistar rats weighing between 100-250g were used for the study. The rats were randomly divided into 8 groups of 5 rats per group and designated Group 1 to Group 8. Anaemia was induced in all rats except Group 1 and Group 8 rats via intraperitoneal injection of PHZ at a dose of 40mg/kg. This was administered at 9am on day 0 and at 9am and 6pm on day 1 of the experiment. This was as previously described [18, 19]. Each rat group was subsequently treated as follows for 14 days:

**Group 1:** Control group. Rats in this group received 2ml/kg bw of extract vehicle only.

**Group 2:** PHZ only (Negative control group). Rats in this group were left untreated after receiving the doses of PHZ.

**Group 3:** PHZ + Low dose extract group. Rats in this group were treated with 250mg/kg bw of the leaf extract of *Craterispermum schweinfurthi* after receiving the doses of PHZ.

**Group 4:** PHZ + Medium dose extract group. Rats in this group were treated with 500mg/kg bw of the leaf extract of *Craterispermum schweinfurthi* after receiving the doses of PHZ.

**Group 5:** PHZ + High dose extract group. Rats in this group were treated with 750mg/kg bw of the leaf extract of *Craterispermum schweinfurthi* after receiving the doses of PHZ.

**Group 6:** PHZ + Bioferon® group. Positive control group. Rats in this group were administered 0.23ml/kg bw of Bioferon® after receiving the doses of PHZ [20].

**Group 7:** PHZ + Phytosterol group. Rats in the group received 2000mg/kg bw of Phytosterol after receiving the doses of PHZ.

**Group 8:** Phytosterol only group. Rats in this group received only 2000mg/kg bw of Phytosterol.

The extract vehicle, extract, Bioferon®, phytosterol were administered daily using an oral canula.

### ***Collection of Blood Samples and Determination of Hematological Parameters***

On day 15, the rats were placed under chloroform anaesthesia and blood samples collected via direct cardiac puncture. The blood samples were immediately transferred into EDTA sample tubes for estimation of erythrocyte count, haemoglobin concentration, haematocrit scores, platelet count and total white blood cell (WBC) and differential counts. All parameters were determined using a reflotron manufactured by Boehringer Mannheim.

### ***Statistical Analysis***

Results are as presented in Table 1 and 2 as Means  $\pm$  standard error of means (SEM). Significant differences were determined using one-way ANOVA and LSD Post Hoc test. A p value of less than 0.05 was considered statistically significant.

### 3. RESULTS AND DISCUSSION

#### ***Values of erythrocyte parameters following PHZ administration and treatment with Craterispermum schweinfurthi leaf extract, Bioferon and phytosterol***

Predictably, administration of PHZ amongst group 2 (Negative control) rats caused significant reduction ( $p < 0.05$ ) of the values of erythrocyte count, haemoglobin concentration and haematocrit scores as compared to group 1 (Control) rats. However, groups 3, 4 and 5 rats administered low, medium, and high doses respectively of the hydromethanol leaf extract of *Craterispermum schweinfurthi* (following the administration of PHZ) demonstrated significant and dose dependent increases ( $p < 0.05$ ) in the values of these parameters compared to both groups 1 and 2 rats. These findings suggest a possible amelioration of the effects of phenyl hydrazine on erythrocytes by the hydromethanol leaf extract of *Craterispermum schweinfurthi*. Similar ameliorative effects have been described from our centre for *Citrullus lanatus* by Kolawole *et al.*, [19] and for *Cnidioscolus aconitifolius* by Ezebuoro *et al.*, [21]. Also, significantly higher ( $p < 0.05$ ) erythrocyte count, haemoglobin concentration and haematocrit scores were observed amongst group 5 (High dose extract) rats compared to groups 6 rats suggesting a possible greater ameliorative effect of the extract compared to group 7 rats suggesting synergism with other bioactive compounds in the extract. Predictably, significantly higher ( $p < 0.05$ ) values were observed for erythrocyte count, haemoglobin concentration and haematocrit scores amongst Group 6 rats compared to Group 2 (Negative control) rats. Amongst Group 7 rats, significantly higher ( $p < 0.05$ ) values were observed in erythrocyte parameters compared to group 2 (Negative control) rats. Finally, significant increases in erythrocyte count, haemoglobin concentration and haematocrit scores were observed amongst group 8 (Phytosterol only) rats, compared to group 2 (Negative control) rats. The assessment of haematological indices remains valuable indicators to determine the functional capacity of the haemopoietic system of experimental animals [22]. Several studies confirm that decreases in erythrocyte parameters is suggestive of either increased erythrocyte destruction or decreased production resulting in the development of anaemia [22-23]. The observed significant increases in erythrocyte parameters following the administration of the extract and phytosterol suggest that the extract has a beneficial effect on erythropoiesis [24-25].

#### ***Values of platelet and leucocyte parameters following PHZ administration and treatment with Craterispermum schweinfurthi leaf extract, Bioferon® and phytosterol***

Administration of phenyl hydrazine at a dose of 40mg/kg to group 2 (Negative control) rats significantly decreased ( $p < 0.05$ ) platelet count, white blood cell count, and the differential counts of neutrophil, lymphocyte, monocyte, eosinophil, and basophil compared to group 1 (Control) rats. However, the extract administered at low, medium, and high doses to rats in groups 3-5 respectively significantly increased ( $p < 0.05$ ) platelet count white blood cell count, and the differential counts of neutrophil, lymphocyte, monocyte, eosinophil, and basophil values in a dose dependent manner compared to both groups 1 and 2 rats. These findings suggest a possible reversal of the effects of phenyl hydrazine administration in the platelets and leucocyte indices by the extracts of the leaves of *Craterispermum schweinfurthi*. These findings are consistent with the reports of Mohammad *et al.* (2019) [26], Anslem *et al.* (2017) [27] and Switti *et al.*, (2011) [28] in which extracts of plants reversed the hematological effects of phenyl hydrazine in laboratory animals. The effects of Bioferon administration observed amongst Group 6 (Positive control group) rats is comparable to the effect of *Craterispermum schweinfurthi* leaf extract administration; however, significantly higher platelet count, white blood cell count, neutrophil, eosinophil and basophil values were observed amongst group 5 (High dose extract group) rats compared to groups 6 (Positive control group) and 7 (Phytosterol and PHZ) rats. Suggesting a possible greater ameliorative effects of the extract for platelet and some leucocyte parameters. But the values of lymphocyte and monocyte were higher in group 6 (Positive control group) than observed in group 5 (High dose extract group) and 7 (Phytosterol and PHZ) rats. Similarly, Group 7 (Phytosterol and PHZ group) rats showed significantly higher ( $p < 0.05$ ) platelet and leucocyte values compared to group 2 (PHZ only group) rats. Phenyl hydrazine (PHZ) causes haemolysis in humans and rodents [5, 25]. Introduction of PHZ to rodents causes bone marrow depression, anaemia (reduced RBC counts), reduced platelet count and leucocyte indices as observed in this study [29].

Phytosterol administration at a dose of 2000mg/kg to rats in group 8 (Phytosterol only group), caused a significant increase in platelet count, white blood cell count, neutrophil, lymphocyte, monocyte, eosinophil and basophil counts compared to group 2 (PHZ only group) rats. The positive effect of the extract and phytosterol may be due to a possible stimulation of erythropoiesis, leucopoiesis and thrombopoiesis. The precise mechanism of action is presently unclear and would require further investigations. Phytochemical screening of *Craterispermum schweinfurthi* has shown the presence of several

compounds including tannins, glycosides, flavonoids, neophytadiene, phytosterol and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol amongst others [15]. These compounds have been shown to contribute to possibly ameliorating potential assaults on the haematological system [30].

**Table 1: Values of erythrocyte parameters in phenyl hydrazine induced anemic rats treated with extract and phytosterol**

Groups	Erythrocyte count (X10 <sup>12</sup> /L)	Haemoglobin concentration (g/dl)	Haematocrit (%)
Group 1: Negative control	9.34±0.00 <sup>b</sup>	18.21±0.00 <sup>b</sup>	54.78±0.00 <sup>b</sup>
Group 2: PHZ only group	5.31±0.00 <sup>a</sup>	9.40±0.01 <sup>a</sup>	28.80±0.00 <sup>a</sup>
Group 3: Low dose extract group	8.92±0.00 <sup>ab</sup>	11.77±0.00 <sup>ab</sup>	44.24±0.03 <sup>ab</sup>
Group 4: Medium dose extract group	9.55±0.00 <sup>ab</sup>	16.30±0.01 <sup>ab</sup>	51.20±0.01 <sup>ab</sup>
Group 5: High dose extract group	10.35±0.01 <sup>ab</sup>	19.10±0.00 <sup>ab</sup>	59.30±0.01 <sup>ab</sup>
Group 6: Positive control group	7.90±0.01 <sup>ab</sup>	19.30±0.01 <sup>ab</sup>	58.50±0.00 <sup>ab</sup>
Group 7: Phytosterol + PHZ group	9.38±0.00 <sup>ab</sup>	11.45±0.00 <sup>ab</sup>	43.00±0.03 <sup>ab</sup>
Group 8: Phytosterol only group	9.85±0.00 <sup>ab</sup>	18.90±0.00 <sup>ab</sup>	54.90±0.002 <sup>ab</sup>

Values are shown as Mean ± SEM; n=5; <sup>a</sup> Significant at P<0.05 compared to Group 1 (Negative control). <sup>b</sup> Significant at p<0.05 compared to Group 2 (PHZ only group).

**Table 2: Values of Platelet and Leucocyte parameters in phenyl hydrazine induced anemic rats treated with extract and phytosterol**

Groups	Platelet count (X10 <sup>9</sup> /L)	White blood cell count (X10 <sup>9</sup> /L)	Neutrophil count (%)	Lymphocyte count (%)	Monocyte count (%)	Eosinophil count (%)	Basophil count (%)
Group 1: Negative control	282.20±0.00 <sup>b</sup>	20.26±0.01 <sup>b</sup>	18.02±0.01 <sup>b</sup>	70.60±0.00 <sup>b</sup>	4.50±0.0 <sup>b</sup>	3.05±0.06 <sup>b</sup>	0.80±0.00 <sup>b</sup>
Group 2: PHZ only group	189.02±0.00 <sup>a</sup>	10.20±0.00 <sup>a</sup>	11.65±0.00 <sup>a</sup>	65.00±0.00 <sup>a</sup>	3.00±0.0 <sup>a</sup>	2.40±0.00 <sup>a</sup>	0.48±0.00 <sup>a</sup>
Group 3: Low dose extract group	242.21±0.00 <sup>ab</sup>	17.20±0.00 <sup>b</sup>	19.00±0.00 <sup>ab</sup>	74.20±0.00 <sup>ab</sup>	4.58±0.00 <sup>ab</sup>	3.20±0.00 <sup>ab</sup>	0.40±0.00 <sup>ab</sup>
Group 4: Medium dose extract group	289.20±0.00 <sup>ab</sup>	21.20±0.00 <sup>b</sup>	21.50±0.00 <sup>ab</sup>	69.00±0.00 <sup>ab</sup>	4.40±0.00 <sup>ab</sup>	4.00±0.00 <sup>ab</sup>	1.00±0.00 <sup>ab</sup>
Group 5: High dose extract group	310.20±0.00 <sup>ab</sup>	21.07±4.60 <sup>b</sup>	22.40±0.00 <sup>ab</sup>	73.00±0.01 <sup>ab</sup>	7.00±0.00 <sup>ab</sup>	4.60±0.00 <sup>ab</sup>	1.60±0.00 <sup>ab</sup>
Group 6: Positive control group	251.00±0.00 <sup>ab</sup>	15.45±0.01 <sup>ab</sup>	18.81±0.00 <sup>ab</sup>	79.30±0.00 <sup>ab</sup>	7.01±0.00 <sup>ab</sup>	4.00±0.00 <sup>ab</sup>	1.30±0.00 <sup>ab</sup>
Group 7: Phytosterol + PHZ group	300.00±0.00 <sup>ab</sup>	22.00±0.00 <sup>b</sup>	17.50±0.00 <sup>ab</sup>	75.20±0.00 <sup>ab</sup>	4.50±0.0 <sup>b</sup>	2.90±0.00 <sup>ab</sup>	0.39±0.00 <sup>ab</sup>
Group 8: Phytosterol only group	283.50±0.00 <sup>ab</sup>	20.72±0.00 <sup>b</sup>	18.30±0.00 <sup>ab</sup>	73.00±0.01 <sup>ab</sup>	4.41±0.00 <sup>ab</sup>	3.00±0.00 <sup>ab</sup>	0.41±0.00 <sup>ab</sup>

Values are shown as Mean ± SEM; n=5; <sup>a</sup> Significant at P<0.05 compared to Group 1 (Negative control). <sup>b</sup> Significant at p<0.05 compared to Group 2 (PHZ only group).

#### 4. CONCLUSION

In conclusion, this study reports that administration of hydromethanol extracts of the leaves of *Craterispermum schweinfurthi* caused a significant and dose dependent increase in erythrocyte count, hemoglobin concentration, haematocrit scores, platelet count and leucocyte indices following PHZ induced anemia in male wistar rats. The results suggest a potential ameliorative effects of the extract of the leaves of *Craterispermum schweinfurthi* on haematological parameters following PHZ administration in experimental animals. The precise mechanism of action of the extract is presently unclear requiring further investigation.

#### Competing interest

Authors have declared that no competing interests exist.

#### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

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