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ETHANOLIC LEAF EXTRACT OF *GOSSYPIMUM HIRSUTUM* ATTENUATES PHENYLHYDRAZINE-INDUCED ANAEMIA AND THROMBOCYTOPENIA IN WISTAR RATS

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ABSTRACT

Anaemia, characterized by qualitative or quantitative reduction in red blood cell (RBC) mass and impaired oxygen transport, remains a major global health concern. Haemolytic anaemia induced by phenylhydrazine (PHZ) in rats is a well-established model for oxidative erythrocyte injury. In this study, the haematoprotective and thrombopoietic potential of ethanolic leaf extract of *Gossypium hirsutum* in PHZ-induced anaemia in Wistar rats was evaluated. Thirty (30) rats were randomly divided equally into five groups: normal control, anaemic control, iron sulphate-treated (6 mg/kg), and extract-treated groups (200 and 400 mg/kg). Anaemia was induced in rats with single intraperitoneal injection of PHZ (40 mg/kg i.p.), followed by oral treatment with plant extract or Ferrous sulfate (FeSO₄) for 14 days. Packed cell volume (PCV), haemoglobin (Hb), RBC, WBC, and platelet counts were measured. PHZ significantly reduced RBC and platelet parameters without affecting WBC counts when compared to control ($p < 0.05$). Treatment with *G. hirsutum* extract restored RBC and platelet counts in a dose-

*dependent manner, with the higher dose achieving effects comparable to iron sulphate. These findings indicate that *G. hirsutum* extract protects against PHZ-induced haemolytic anaemia and thrombocytopenia, likely through antioxidant-mediated stabilization of erythrocytes and platelets, and supports physiological haematopoiesis.*

KEYWORDS: *Gossypium hirsutum*; Anaemia; Thrombocytopenia; Haematoprotection; Phenylhydrazine; Wistar rats; Antioxidants

INTRODUCTION

Anaemia is defined physiologically as a reduction in haemoglobin, packed cell volume (PCV), and circulating red blood cell mass, leading to reduced tissue oxygen delivery and metabolic dysfunction [1,2]. Globally, anaemia is prevalent due to nutritional deficiencies, chronic diseases, haemolytic disorders, and oxidative stress [1,15]. Erythropoiesis needs adequate iron, erythropoietin, bone marrow integrity, and antioxidant defenses to protect erythrocytes from oxidative damage [3].

Phenylhydrazine (PHZ) induces haemolytic anaemia in rodents by generating reactive oxygen species, oxidizing haemoglobin, and damaging erythrocyte membranes, resulting in increased fragility and haemolysis [4,5,12]. PHZ also transiently reduces platelet counts through oxidative injury, whereas WBC counts remain largely unaffected [12]. This model is suitable for evaluating interventions that mitigate oxidative stress and support haematopoiesis.

Phytoconstituents present in medicinal plants includes flavonoids, phenolics, and tannins. These secondary metabolites have been reported to protect against PHZ-induced anaemia by scavenging free radicals, stabilizing cell membranes, and enhancing erythropoietic processes [6–9,19]. *Gossypium hirsutum* L. (cotton) contains antioxidant phytochemicals such as flavonoids and tannins [8,9], but its haematoprotective effects have not been thoroughly evaluated. This study investigated the ability of ethanolic leaf extract of *G. hirsutum* to restore RBC and platelet counts in PHZ-induced anaemia in Wistar rats, using iron sulphate as a reference standard. We hypothesized that the extract would ameliorate anaemia and thrombocytopenia through antioxidant-mediated stabilization of blood cells and support of physiological haematopoiesis.

METHODOLOGY

Plant Material and Extract Preparation

Fresh leaves of *Gossypium hirsutum* were collected from the staff quarters of the University of Maiduguri (UniMaid), Nigeria, and authenticated by a botanist at the University Herbarium (Voucher No: PM/01/006/24). All other protocols in the study were conducted in Physiology laboratory, Yobe State University, Damaturu. The fresh leaves were washed, air-dried at room temperature for 10 days, and pulverized into coarse powder.

Powdered leaves (500 g) were macerated in 2 L of 70% ethanol for 72 hours at room temperature with occasional shaking. The mixture was filtered using Whatman No.1 filter paper, and the filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator. The resulting crude extract was stored at 4 °C and reconstituted in distilled water before administration at

doses of 200 mg/kg and 400 mg/kg.

Experimental Animals

Thirty adult male Wistar rats (180–200 g) were housed under standard conditions (22–25 °C, 12 h light/dark cycle) with free access to food and water. All procedures adhered to institutional animal care guidelines.

Study Design

Rats were randomized into five groups (n = 5): Group I served as normal control (distilled water). Group II, Anaemic control (PHZ + distilled water), while group III was treated as standard control (PHZ + Ferrous sulphate 4 mg/kg). Groups IV and V were given PHZ + *G. hirsutum* extract 200 mg/kg and PHZ + *G. hirsutum* extract 400 mg/kg respectively.

Induction of Anaemia

Anaemia was induced in Groups II–V using PHZ (40 mg/kg i.p.) [4,5].

Treatment Protocol

Extract and iron sulphate were administered by intragastric intubation once daily for 14 days post-anaemia induction. Control groups received distilled water.

Haematological Analysis

Blood was collected via cardiac puncture into EDTA tubes. Automated Hematology Analyzer (Haematology Department, Yobe State University Teaching Hospital, Damaturu) was used for the determination of PCV, haemoglobin, RBC count, WBC count, platelet count, and computation of erythrocyte indices (MCV, MCH, MCHC).

Statistical Analysis

Data are presented as mean $\pm$ SEM. One-way ANOVA followed by Tukey's post hoc test was performed; $p < 0.05$ was considered significant.

RESULTS

Effects of *Gossypium hirsutum* Leaf Extract on Erythrocyte Indices

Intraperitoneal administration of Phenylhydrazine (PHZ) to rats in this study markedly reduced erythrocyte parameters compared with the control group, confirming anaemia induction. The PHZ-only group showed a 48.8% decrease in RBC count ($4.21 \pm 0.32 \times 10^{12}/L$ vs. $8.23 \pm 0.45 \times 10^{12}/L$). Haemoglobin also decreased 14.21 ± 1.12 g/dL when compared to control (41.5%; 8.31 ± 0.92). The haematocrit was reduced (40.4%; 25.11 ± 2.51 vs. $42.11 \pm 3.21\%$). Daily single treatment with the ethanolic leaf extract of *G. hirsutum* produced dose-dependent haematological recovery. The low-dose extract significantly increased RBC count to $6.12 \pm 0.41 \times 10^{12}/L$ (45.4% increase vs. PHZ-only; $p < 0.05$), haemoglobin to 11.42 ± 1.01 g/dL (37.4%; $p < 0.05$), and haematocrit to $35.21 \pm 3.01\%$ (40.2%; $p < 0.05$). Greater improvements were observed with the high-dose extract, with RBC count reaching $7.35 \pm 0.52 \times 10^{12}/L$ (74.6%; $p < 0.05$), haemoglobin 13.21 ± 1.21 g/dL (59.0%; $p < 0.05$), and haematocrit $40.11 \pm 3.39\%$ (59.7%; $p < 0.05$), values when compared to the control group.

Ferrous sulfate produced similar restorative effects (RBC: $7.51 \pm 0.48 \times 10^{12}/L$; haemoglobin: 13.51 ± 1.15 g/dL; haematocrit: $41.21 \pm 3.31\%$), indicating that the high-dose *G. hirsutum* extract exhibited efficacy comparable to the standard anti-anaemic agent.

Table 1: Effects of Ethanolic Leaf Extract of *Gossypium hirsutum* on Red Blood Cell Parameters in PHZ-Induced Anaemic Wistar Rats

Parameter	Control	PHZ Only	PHZ + Low Dose	PHZ + High Dose	PHZ + Ferrous Sulfate	F-value	p-value
RBC ($\times 10^{12}/L$)	8.23 ± 0.45	4.21 ± 0.32	$6.12 \pm 0.41^*$	$7.35 \pm 0.52^*$	$7.51 \pm 0.48^*$	18.456	<0.001
HGB (g/dL)	14.21 ± 1.12	8.31 ± 0.92	$11.42 \pm 1.01^*$	$13.21 \pm 1.21^*$	$13.51 \pm 1.15^*$	12.347	<0.001
HCT (%)	42.11 ± 3.21	25.11 ± 2.51	$35.21 \pm 3.01^*$	$40.11 \pm 3.39^*$	$41.21 \pm 3.31^*$	14.892	<0.001
MCV (fL)	88.5 ± 4.2	78.3 ± 5.1	82.4 ± 4.5	$85.6 \pm 3.8^*$	$86.9 \pm 3.5^*$	4.521	<0.05
MCH (pg)	29.5 ± 2.1	22.1 ± 1.8	25.3 ± 2.0	$27.8 \pm 1.9^*$	$28.5 \pm 1.7^*$	6.784	<0.01
MCHC (g/dL)	33.2 ± 1.5	30.5 ± 1.2	32.0 ± 1.3	$33.0 \pm 1.4^*$	$33.5 \pm 1.3^*$	3.245	<0.05
RDW-S (fL)	42.3 ± 3.5	55.2 ± 4.2	$48.1 \pm 3.8^*$	$45.0 \pm 3.2^*$	$43.5 \pm 2.8^*$	8.567	<0.001
RDW-C (%)	13.5 ± 1.2	18.2 ± 1.5	$15.8 \pm 1.3^*$	$14.5 \pm 1.1^*$	$14.0 \pm 0.9^*$	7.234	<0.01

Values are expressed as Mean $\pm$ SEM ($n=5$). * $p < 0.05$ compared to PHZ only group (Dunnett's post-hoc test).

Red Cell Indices and Distribution Width

The PHZ-only group showed significant reductions in MCV, MCH, and MCHC compared with controls (MCV: 78.3 ± 5.1 vs. 88.5 ± 4.2 fL; MCH: 22.1 ± 1.8 vs. 29.5 ± 2.1 pg; MCHC: 30.5 ± 1.2 vs. 33.2 ± 1.5 g/dL), consistent with microcytic, hypochromic anaemia. The low-dose extract produced partial, non-significant improvements. In contrast, high-dose extract and ferrous sulfate significantly normalized MCV (85.6 ± 3.8 and 86.9 ± 3.5 fL), MCH (27.8 ± 1.9 and 28.5 ± 1.7 pg), and MCHC (33.0 ± 1.4 and 33.5 ± 1.3 g/dL) ($p < 0.05$ vs. PHZ-only).

RDW-SD and RDW-CV were markedly elevated in the PHZ-only group (55.2 ± 4.2 fL and $18.2 \pm 1.5\%$) compared with controls (42.3 ± 3.5 fL and $13.5 \pm 1.2\%$). All treatments significantly reduced RDW indices in a dose-dependent manner ($p < 0.05$). High-dose extract (45.0 ± 3.2 fL; $14.5 \pm 1.1\%$) and ferrous sulfate (43.5 ± 2.8 fL; $14.0 \pm 0.9\%$) restored values toward control levels.

White Blood Cell (WBC) Parameters

Total WBC count did not differ among groups ($F = 0.845$, $p > 0.05$) and remained within normal limits (PHZ-only: $6.5 \pm 1.2 \times 10^9/L$; treatment range: 7.0 ± 1.3 to $8.0 \pm 1.6 \times 10^9/L$)

Table 2: Effects of Ethanolic Leaf Extract of *Gossypium hirsutum* on White Blood Cell Parameters in PHZ-Induced Anaemic Wistar Rats

Parameter	Control	PHZ Only	PHZ + Low Dose	PHZ + High Dose	PHZ + Ferrous Sulfate	F-value	p-value
WBC ($\times 10^9/L$)	7.2 ± 1.5	6.5 ± 1.2	7.0 ± 1.3	7.5 ± 1.4	8.0 ± 1.6	0.845	>0.05
LYM (%)	60.5 ± 5.2	55.2 ± 4.8	58.3 ± 5.0	62.1 ± 4.5	63.0 ± 4.2	1.234	>0.05
MID (%)	8.2 ± 1.5	10.5 ± 2.1	9.0 ± 1.8	8.5 ± 1.6	8.0 ± 1.4	1.456	>0.05
GRA (%)	31.3 ± 3.2	34.3 ± 3.5	32.7 ± 3.0	29.4 ± 2.8	29.0 ± 2.5	1.678	>0.05
LYM ($\times 10^9/L$)	4.35 ± 0.8	3.59 ± 0.7	4.08 ± 0.9	4.66 ± 0.8	5.04 ± 1.0	1.890	>0.05
MID ($\times 10^9/L$)	0.59 ± 0.1	0.68 ± 0.2	0.63 ± 0.1	0.64 ± 0.1	0.64 ± 0.1	0.567	>0.05
GRA ($\times 10^9/L$)	2.26 ± 0.5	2.23 ± 0.6	2.29 ± 0.5	2.21 ± 0.4	2.32 ± 0.5	0.234	>0.05

Values are expressed as Mean $\pm$ SEM (n=4). No significant differences were observed compared to PHZ only group (Dunnett's post-hoc test, $p > 0.05$).

Platelet Parameters

PHZ significantly reduced platelet count compared with controls (280 ± 45 vs. $420 \pm 69 \times 10^9/L$; -33.3%). Low-dose extract produced a non-significant increase ($320 \pm 52 \times 10^9/L$). High-dose extract and ferrous sulfate significantly restored platelet counts to 380 ± 60 and $400 \pm 55 \times 10^9/L$, respectively ($p < 0.05$ vs. PHZ-only).

Table 3: Effects of Ethanolic Leaf Extract of *Gossypium hirsutum* on Platelet Parameters in PHZ-Induced Anaemic Wistar Rats

Parameter	Control	PHZ Only	PHZ + Low Dose	PHZ + High Dose	PHZ + Ferrous Sulfate	F-value	p-value
PLT ($\times 10^9/L$)	420 $\pm$ 69	280 $\pm$ 45	320 $\pm$ 52	380 $\pm$ 60*	400 $\pm$ 55*	5.234	<0.05
PCT (%)	0.42 $\pm$ 0.05	0.28 $\pm$ 0.04	0.32 $\pm$ 0.05	0.38 $\pm$ 0.06*	0.40 $\pm$ 0.05*	4.567	<0.05
MPV (fL)	8.5 $\pm$ 0.8	9.2 $\pm$ 1.0	8.8 $\pm$ 0.9	8.6 $\pm$ 0.7	8.4 $\pm$ 0.6	1.234	>0.05
PDW-C (%)	16.2 $\pm$ 1.5	18.5 $\pm$ 2.1	17.2 $\pm$ 1.8	16.5 $\pm$ 1.6	16.0 $\pm$ 1.3	2.456	>0.05
PDW-S (fL)	10.5 $\pm$ 1.2	12.8 $\pm$ 1.5	11.5 $\pm$ 1.3	10.8 $\pm$ 1.1	10.3 $\pm$ 0.9	2.789	>0.05
P-LCC ($\times 10^9/L$)	120 $\pm$ 20	80 $\pm$ 15	95 $\pm$ 18	110 $\pm$ 22*	115 $\pm$ 18*	4.123	<0.05
P-LCR (%)	25.0 $\pm$ 2.5	22.0 $\pm$ 3.0	23.5 $\pm$ 2.8	24.5 $\pm$ 2.3	25.5 $\pm$ 2.0	1.567	>0.05

Values are expressed as Mean $\pm$ SEM (n=4). *p<0.05 compared to PHZ only group (Dunnett's post-hoc test).

DISCUSSION

Ethanolic leaf extract of *G. hirsutum* ameliorated PHZ-induced anaemia and thrombocytopenia in a dose-dependent manner. PHZ-induced oxidative stress damages erythrocytes and platelets, leading to homolysis and transient thrombocytopenia, whereas leukocytes remain relatively unaffected [4,5,12].

The restoration of RBC and platelet counts by *G. hirsutum* extract indicates both hepatoprotective and thrombopoietic effects. Flavonoids, phenolics, and tannins present in the extract likely scavenge reactive oxygen species, stabilize membranes, and reduce oxidative haemolysis [6–9,19]. Preservation of cell integrity prolongs lifespan of erythrocytes and platelets and supports physiological haematopoiesis.

Translationally, oxidative stress contributes to haemolytic anaemia and thrombocytopenia in humans, including drug-induced and chronic disease-related forms. Plant-derived antioxidants may serve as adjunct therapies to conventional iron supplementation by protecting blood cells and supporting marrow function [14,19].

CONCLUSION

Ethanolic leaf extract of *G. hirsutum* significantly and dose-dependently restored RBC and platelet counts in PHZ-induced anaemia in Wistar rats, with the higher dose comparable to iron sulphate. These findings support its potential as a natural hematinic and thrombopoietic agent.

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Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article.

Authors' Contributions

Conception, design of the study and the first draft of the manuscript was written by Anas HY. Material preparation, data collection and analysis were done by Fatima Alkali manuscript review was performed by I.S. Malgwi. All authors read and approved the final manuscript.

REFERENCES

1. World Health Organization. Global prevalence of anaemia in [2011](#). Geneva: WHO; [2015](#).
2. Guyton AC, Hall JE. Textbook of Medical Physiology. 14th ed. Philadelphia: Elsevier; [2021](#).
3. Jelkmann W. Regulation of erythropoiesis. J Physiol. [2011](#);589(6):1251–1258.
4. Berger J. Phenylhydrazine haematotoxicity: a review. J Appl Biomed. [2007](#);5(3):125–130.
5. Jain NC. Schalm's Veterinary Hematology. 6th ed. Ames: Wiley-Blackwell; [2010](#).
6. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 5th ed. Oxford: Oxford University Press; [2015](#).
7. Olayemi FO, Adebayo AO. Hematopoietic effects of medicinal plants. Afr J Tradit Complement Altern Med. [2016](#);13(3):1–7.
8. Harborne JB. Phytochemical Methods. 3rd ed. London: Chapman & Hall; [1998](#).
9. Onwukaeme DN, Lot TY, Udoh FV. Phytochemical evaluation of Gossypium hirsutum. Fitoterapia. [1999](#);70(3):275–278.
10. Ethnomedicinal plants used for prevention and treatment of anemia in the Philippines: a systematic review. Trop Med Health. [2023](#);51:27.
11. Phenylhydrazine induces transient thrombocytopenia in rats. J Lab Clin Med. [1989](#);114(4):445–453.
12. Anti-anemic effects of antioxidant-rich apple vinegar against PHZ anemia in rats. Life. [2022](#);12(2):239.

13. Development of polyherbal formulations for anemia with antioxidant and iron content support. *Biology*. [2025](#);14(8):[1052](#).
14. Natural antioxidants in anemia treatment. *Int J Mol Sci*. [2021](#);22(4):[1883](#).
15. Ethnomedicinal plants in anemia: systematic evidence base. *Trop Med Health*. [2023](#);51:27.
16. Oxidative stress and hematological disorders: translational implications. *Redox Biol*. [2022](#);54:[102361](#).