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Haematological Effect of a Bi-Herbal Composition on Phenyl Hydrazine-Induced Anaemia in Albino Wistar Rats

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Abstract

Picralima nitida and Cymbopogon citratus are plants known for their anti-inflammatory, antioxidant, and haematopoietic properties. This study aimed to determine the haematological effect of a dual herb composition of these plants on phenylhydrazine-induced anaemia in albino Wistar rats. Sixty adult male rats were divided into six groups: control, phenylhydrazine group, ferrous sulphate group, and three groups receiving phenylhydrazine plus 100 mg/kg, 200 mg/kg, and 400 mg/kg of the extract. Haematological parameters were measured using an autoanalyzer and analyzed with SPSS software V22. The red blood cell count was significantly higher in the control group compared to other groups ($p<0.05$). Haemoglobin was significantly higher in the control, ferrous sulphate, 100 mg/kg, and 400 mg/kg groups compared to the phenylhydrazine and 200 mg/kg groups ($p<0.05$). Mean cell volume was significantly lower in the control group compared to all other groups ($p<0.05$). Mean cell haemoglobin was significantly lower in the control group compared to other groups while MCHC was higher in the control group compared to phenylhydrazine, ferrous sulphate, 100mg/kg and 200mg/kg groups ($p<0.05$). RDWSD was significantly lower in the control group compared to all other groups ($p<0.05$). Total white blood cell count was significantly lower in the control and phenylhydrazine groups compared to others ($p<0.05$). Lymphocyte was significantly higher in the ferrous sulphate group compared to others, while monocyte count was significantly lower in the control, phenylhydrazine, and ferrous sulphate groups ($p<0.05$). Neutrophil was significantly higher in the control and phenylhydrazine groups ($p<0.05$). Platelet was significantly lower in the control group compared to ferrous sulphate, 200 mg/kg, and 400 mg/kg groups ($p<0.05$). Mean platelet volume was significantly lower in the control, phenylhydrazine, ferrous sulphate, and 100 mg/kg groups compared to the 400 mg/kg group ($p<0.05$). The study concludes that the biherbal formulation of the plants caused significant alterations in haematological parameters.

Keywords: Albino wistar rats, *Cymbopogon citratus*, Haematological parameters, *Picralima nitida*

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INTRODUCTION

Medicinal plants have been vital to human healthcare since ancient times, offering a rich source of bioactive compounds with therapeutic potential (Ramawat *et al*., 2009). These bioactive compounds, such as alkaloids, flavonoids, and terpenoids, exhibit diverse pharmacological activities including anti-inflammatory, anti-oxidant, antimicrobial, and anti-cancer effects (Li and Weng, 2017). Combining two or more medicinal plants to form a dual herb formulation is usually employed to synergistically enhance therapeutic effects while minimizing adverse reactions. This practice, known as polyherbalism, capitalizes on the complementary actions of different plants, optimizing their combined efficacy (Karole *et al*., 2019). For instance, pairing plants with complementary mechanisms of action can target multiple pathways involved in a disease process, leading to improved treatment outcomes (Yuan *et al*., 2017). A dual herb formulation presents a promising avenue for the development of safer and more effective treatments in modern medicine (Kumar and Sharma, 2018).

Picralima nitida commonly known as *Akuamma*, is called *Abere* in Yoruba, and *Osu* in Edo. It is a tropical plant native to West Africa, valued for its numerous therapeutic applications strongly established in traditional healing practices (Ndukwu *et al*., 2019). Research has provided information regarding its potential haematopoietic effects. Studies investigating *Picralima nitida's* influence on haematologic parameters, including red blood cell count, haemoglobin levels, haematocrit, white blood cell count, and platelet parameters, have showed positive findings (Unakalamba *et al*., 2023). These results revealed that *Picralima nitida* supplementation may hold therapeutic promise in addressing haematologic diseases (Nwankwo *et al*., 2017). *Cymbopogon citratus* generally known as lemongrass is called *Koko-oba* in Yoruba, *Achara- ehi* in Ibo, *Ikonti* in Efik and *Myoyaka* in Ibibio. It is an herb from the *Poaceae* family, originating in tropical places including Southeast Asia, Africa, and Australia. Lemongrass has long been valued for its various therapeutic applications, addressing concerns from digestion to respiratory diseases and even fever management (Wifek *et al*., 2016). Rich in bioactive components such as citral, limonene, and geraniol, lemongrass demonstrates significant antibacterial, antioxidant, and anti-inflammatory effects, displaying its usefulness as a botanical treatment (Boukhatem *et al*., 2014). Lemongrass consumption may lead to considerable changes in several haematologic markers indicating its medicinal potential in addressing illnesses connected with haematologic disorders, presenting a natural alternative to conventional treatments (Nosiri *et al*., 2020). Haematological parameters are a range of measurements that provide insights into the composition and function of blood, acting as essential markers of overall health and physiological status (Etim *et al*., 2014). Phenyl-hydrazine administration triggers the destruction of red blood cells, leading to a decrease in red blood cell count (RBC), haemoglobin levels, and haematocrit. This haemolytic effect occurs through the compound's ability to oxidize hemoglobin, forming methemoglobin and denaturing the protein structure, ultimately resulting in the rupture of erythrocytes (Pandey *et al*., 2014). Beyond its impact on erythrocytes, phenylhydrazine-induced hemolysis may trigger alterations in white blood cell count (WBC) and platelet parameters, indicative of inflammatory responses and thrombotic events, respectively (Zhu *et al*., 2022).

Haemolytic anaemia, characterized by the accelerated destruction of red blood cells, poses a significant health burden globally. By administering a dual herb formulation of *Picralima nitida* and *Cymbopogon citratus* aqueous leaves extracts to phenylhydrazine-induced anaemic rats, this study aimed to evaluate its potential therapeutic efficacy in ameliorating anaemia and restoring haematological parameters to normal levels. Assessment of key haematological parameters such as red blood cell count, haemoglobin concentration, haematocrit, white blood cell count, and platelet parameters will provide valuable insights into the formulation's effects on erythropoiesis, haemoglobin synthesis, immune response, and coagulation pathways.

MATERIALS AND METHODS

Study Area
This study was carried out in the University of Benin, Ovia North-East Local Government Area, Edo State, Nigeria, located along latitude: 6° 20' 1.32" N longitude: 5° 36' 0.53" E.

Collection of Samples
A total of thirty (30) of the Albino Wistar strain were purchased from the animal holdings of the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed at the animal housing wing of the Department of Anatomy, University of Benin (Obazelu and Faluyi, 2023). *Cymbopogon citratus and Picralima nitida* leaves were collected around a community in Ovia North-East Area, Edo State, Nigeria. The leaves were then identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city; with ID Number: UBH-C451 and UBH-P424 for *Cymbopogon citratus and Picralima nitida* leaves respectively.

Laboratory Procedures
The procedure began by removing any unhealthy leaves from the samples. Subsequently, the leaves underwent a thorough washing process followed by drainage. To facilitate proper grinding, the leaves were air-dried under shade for duration of two weeks. Further drying was then carried out using a hot air oven at 50^{o}C for 24 hours. This ensured that the leaves were adequately dried and prepared for grinding. The grinding process itself was conducted using a high-speed grinding machine, specifically an industrial 1000A high-speed grinder. Finally, 250 grams of each leaf were precisely weighed for subsequent usage (Obazelu and Williams, 2024).

Preparation of Plants Extract
About 250 grams of ground powder were mixed with 2.5 liters of distilled water. Subsequently, the mixture was left to soak for duration of 24 hours under constant storage conditions. After the specified duration, the mixture underwent filtration using Whatman's (Nitro cellulose 45; 0.45µm pore size) filter paper, with the residue being discarded. Following filtration, the resulting filtrate was subjected to concentration in a Water bath maintained at 45°C until it reached a paste-like consistency. The paste obtained from this process was then accurately weighed and subsequently dissolved in distilled water to achieve the recommended concentrations for administration (Obazelu and Williams, 2024).

Animal Care
Animals were housed in a cross ventilated room in the animal holdings of the department of anatomy, University of Benin, Benin City. Animals were exposed to 12 hours dark and light cycles with access to feed and water *ad libitum*. The rats were acclimatized for a period of two (2) weeks before commencement of the experiment (Obazelu and Williams, 2024).

Ethical Consideration
Ethical approval was obtained from Research Ethics Committee on animal subjects from Edo State Ministry of Health, Benin City (Ref Number: HA/737/23/B.200600195 issued on 14th, December, 2023).

Preparation of Phenyl-hydrazine and Ferrous Sulphate Drug Solution
Phenyl-hydrazine solution was prepared by combining phenyl-hydrazine (Sigma-Aldrich, Batch Number: PHZ789001) with distilled water v/v and 2-propanol in a ratio of 1:5:5. This entailed mixing 1 part of phenyl-hydrazine with 5 parts of distilled water v/v and 5 parts of

2-propanol. Subsequently, 0.2ml of this phenyl-hydrazine solution was administered to each animal in the various test groups, with an average weight of 150g, every 48 hours for duration of 28 days (Obazelu and Williams, 2024).

Ferrous Sulphate Drug Solution was made by mixing 1000mg of the powdered drug in 50ml of distilled water. 0.3ml of this drug solution was administered orally to each animal in group C of an average weight of 150g every 48 hours for 28 days (Obazelu and Williams, 2024).

Laboratory procedures and Analysis

Thirty (30) Mature Wistar rats weighing 150-200g were randomly selected and divided into six groups (n = 5 per group). The Groups were the Group A to Group F. Group A (control) received only standardized feed (Manufactured by Karma Agric Feeds and Food Limited, Oyo State) and clean water *ad libitum*. Group B (phenyl-hydrazine treated group) were administered 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days. Group C (ferrous sulphate drug solution treated group) were administered 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 6mg/ml of ferrous sulphate 48 hourly for 28 days. Group D were administered with 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days and treated with 0.15ml of 100mg/kg body weight of bi-herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaf extracts orally using a gavage tube every 24 hours for 28 days. Group E were administered with 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 200mg/kg body weight of bi-herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally using a gavage tube every 24 hours for 28 days. Group F were administered with 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days and treated with 0.6ml of 400mg/kg body weight of bi-herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally using a gavage tube every 24 hours for 28 days.

At the end of the experimental period, the animals were grossly observed for general physical characteristics. A midline incision was made through the ventral wall of the rats after anaesthetizing (using chloroform) and cervical dislocation. Five milliliters (5ml) of blood were collected from each rat using a sterile syringe and placed in an Ethylene Diamine Tetra-acetic Acid (EDTA) container for full blood count analysis.

The full blood count parameters were analysed immediately after sample collection using the automated three parts ERMA Haematology Auto analyser PCE-210N (Diamond Diagnostic; Holliston, USA). Whole blood was properly mixed and inserted into the probe. Then 20 µL of the blood was aspirated into the instrument. The analysis was immediately done and the results displayed on the screen after about 1-2 minutes, which was printed by the printer.

Statistical Analysis

Data obtained from this research was presented and analyzed using statistical package for social sciences (SPSS) version 21.0 (IBM Inc. USA). Analysis of variance (ANOVA) was used to compare treatment groups of continuous variables. Tukey HSD *post hoc* was applied where a significant difference was observed in the ANOVA. A p-value of ≤0.05 was considered statistically significant.

RESULTS

The results showed the comparison of Mean±SEM of red blood cell count, Haemoglobin concentration, haematocrit and red cell indices of six groups namely; groups A, B, C, D, E and F, representing control, phenyl-hydrazine group, ferrous sulphate group, phenyl-hydrazine

+ 100mg/kg bi-herbal formulation of *Picralima nitida* and *Cymbopogon citratus*, phenyl-hydrazine + 200mg/kg bi-herbal formulation of *Picralima nitida* and *Cymbopogon citratus* and phenyl-hydrazine + 400mg/kg bi-herbal formulation of *Picralima nitida* and *Cymbopogon citratus* respectively.

Red blood cell count (RBC) (µL) of groups A (4.43±0.45) was significantly higher than groups B (2.19±0.31), C (3.41±0.10), D (3.49±0.10), E (2.67±0.08) and F (3.30±0.03) ($p<0.05$). Haemoglobin concentration (g/dL) was significantly higher in groups A (10.69±0.65), C (9.79±0.09), D (9.95±0.27) and F (10.10±0.10) when compared to groups B (9.79±0.09) and E (10.10±0.10) ($p<0.05$). There was no statistically significant change in haematocrit across all the groups ($p>0.05$). Mean cell volume (MCV) (μm^3) was significantly lower in group A (68.62±1.21) when compared to groups B (106.04±0.71), C (108.36±0.34), D (109.89±1.95), E (112.00±1.25) and F (104.92±0.53) ($p<0.05$). Mean cell haemoglobin (MCH) (pg) was significantly lower in group A (20.13±0.09) when compared to groups B (29.27±0.44), C (28.81±0.65), D (28.48±0.17), E (27.73±0.31) and F (29.35±0.27) ($p<0.05$). Mean cell haemoglobin concentration (MCHC) (g/dL) was significantly higher in group A (29.41±0.45) when compared to groups B (27.68±0.33), C (26.60±0.52), D (26.01±0.43) and E (24.82±0.07) ($p<0.05$). RDWSD (%) of group A (55.13±2.96) was significantly lower when compared to groups B (87.26±2.43), C (88.50±1.39), D (90.26±1.96), E (91.95±0.72) and F (91.90±1.85) ($p<0.05$). There were no significant changes in RDWCV (μm^3) across all the groups ($p>0.05$) (Table 1).

Total white blood cell count (TWBC µL) was significantly lower in group A (3.75±0.28) when compared to group C (13.87±1.35), D (19.06±1.81), E (22.89±2.41) and F (12.62±0.98) ($p<0.05$). Lymphocyte count (%) was significantly higher in group C (95.23±0.09) when compared to groups A (93.41±0.65), B (92.37±0.39), D (92.46±0.36), E (91.05±0.39) and F (92.09±0.49) ($p<0.05$). Monocyte count (%) was significantly lower in groups A (3.40±0.53), B (4.40±0.24) and C (3.63±0.07) when compared to groups D (6.09±0.32), E (6.85±0.21) and F (5.79±0.30) ($p<0.05$). Neutrophil count (%) was significantly higher in groups A (3.19±0.18) and B (3.23±0.63) when compared to groups C (1.14±0.03) and D (1.45±0.04) ($p<0.05$). Platelet count was significantly lower in group A (451.2±28.5) when compared to group C (896.5±103.6), E (1167.1±123.9) and F (1078.3±116.0) ($p<0.05$). Mean platelet volume (MPV) was significantly lower in groups A (10.99±0.20), B (10.81±0.41), C (10.74±0.41) and D (10.28±0.16) when compared to group F (12.56±0.30) ($p<0.05$). PDW was significantly lower in group B (12.34±1.45) when compared to group E (17.03±0.60) and F (18.02±1.60) ($P<0.05$). PCT was significantly lower in groups A (0.50±0.04), B (0.75±0.17) and D (0.72±0.03) when compared to groups E (1.33±0.14) and F (1.37±0.17) (0.467±0.024) ($p<0.05$). PLCR was significantly higher in group A (28.51±1.48) when compared to group D (20.22±0.98) ($p<0.05$) (Table 2).

Table 1: Mean Comparison of Red Blood Cell Count, Haemoglobin Concentration and Red Cell Indices of Studied Groups

Parameter	Group A (Control)	Group B (PHZ only)	Group C (PHZ + Ferrous sulphate)	Group D (PHZ + 100mg/kg)	Group E (PHZ + 200mg/kg)	Group F (PHZ + 400mg/kg)	F value	p value
RBC ($\times10^6$/µL)	4.43±0.45bcdef	2.19±0.31acdf	3.41±0.10ab	3.49±0.10ab	2.67±0.08a	3.30±0.03ab	10.81	0.001
Haemoglobin (g/dL)	10.69±0.65be	6.72±1.00acdf	9.79±0.09be	9.95±0.27be	7.42±0.14acdf	10.10±0.10be	10.12	0.001
HCT (%)	36.24±2.06	23.87±3.25	36.89±1.05	38.14±0.41	37.56±0.21	36.05±1.12	0.96	0.450
MCV (fl)	68.62±1.21bcdef	106.04±0.71ae	108.36±0.34a	109.89±1.95a	112.00±1.25abf	104.92±0.53ade	208.19	0.001
MCH (pg)	20.13±0.09bcdef	29.27±0.44a	28.81±0.65a	28.48±0.17a	27.73±0.31a	29.35±0.27a	92.67	0.001
MCHC (g/dL)	29.41±0.45bcde	27.68±0.33ade	26.60±0.52ae	26.01±0.43abf	24.82±0.07abcf	28.05±0.38de	16.95	0.001
RDW-SD (%)	55.13±2.96bcdef	87.26±2.43a	88.50±1.39a	90.26±1.96a	91.95±0.72a	91.90±1.85a	59.03	0.001
RDW-CV (µm³)	26.46±3.84	21.20±0.60	19.98±0.30	20.04±0.18	20.42±0.10	23.38±0.53	0.88	0.501

p≤0.05- Significant; p ≥ 0.05- Not significant, PHZ=Phenylhydrazine. a: significance with control, b: significance with PHZ group, c: significance with PHZ + Ferrous sulphate group, d: significance with PHZ + 100mg/kg, e: significance with PHZ + 200mg/kg, f: significance with PHZ + 400mg/kg.
RBC: Red Blood Cell, HCT: Haematocrit, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, MCHC: Mean Cell Haemoglobin Concentration, RDWC: Red cell distribution width – Coefficient of Variation, RDWS: Red cell distribution width – Standard Deviation.

Table 2: Mean Comparison of White Blood Cell and Platelet Parameters of Studied Groups

Parameter	Group A (Control)	Group B (PHZ only)	Group C (PHZ + Ferrous Sulphate)	Group D (PHZ + 100mg/kg)	Group E (PHZ + 200mg/kg)	Group F (PHZ + 400mg/kg)	F value	p value
TWBC ($\times10^6$/µL)	3.75 ± 0.28^{cdef}	3.14 ± 0.67^{cdef}	13.87 ± 1.35^{abe}	19.06 ± 1.81^{abf}	22.89 ± 2.41^{abcf}	12.62 ± 0.98^{abde}	30.66	0.001
Lymphocyte (%)	93.41 ± 0.65^{ce}	92.37 ± 0.39^{c}	95.23 ± 0.09^{abdef}	92.46 ± 0.36^{c}	91.05 ± 0.39^{ac}	92.09 ± 0.49^{c}	11.09	0.001
Monocyte count (%)	3.40 ± 0.53^{def}	4.40 ± 0.24^{def}	3.63 ± 0.07^{def}	6.09 ± 0.32^{abc}	6.85 ± 0.21^{abc}	5.79 ± 0.30^{abc}	20.64	0.001
Neutrophil count (%)	3.19 ± 0.18^{cd}	3.23 ± 0.63^{cd}	1.14 ± 0.03^{ab}	1.45 ± 0.04^{ab}	2.10±0.18	2.12±0.19	8.82	0.001
PLT ($\times10^3$/µL)	451.2 ± 28.5^{cef}	694.1 ± 136.8^{e}	896.5 ± 103.6^{a}	705.7 ± 17.8^{e}	1167.1 ± 123.9^{abd}	1078.3 ± 116.0^{a}	7.22	0.001
MPV (µM³)	10.99 ± 0.20^{f}	10.81 ± 0.41^{f}	10.74 ± 0.41^{f}	10.28 ± 0.16^{f}	11.58±0.32	12.56 ± 0.30^{abcd}	6.47	0.001
PDW (%)	13.96±0.37	12.34 ± 1.45^{ef}	14.81±1.29	12.65 ± 0.27^{f}	17.03 ± 0.60^{b}	18.02 ± 1.60^{bd}	4.63	0.001
PCT (%)	0.50 ± 0.04^{ef}	0.75 ± 0.17^{ef}	0.97±0.13	0.72 ± 0.03^{ef}	1.33 ± 0.14^{abd}	1.37 ± 0.17^{abd}	7.88	0.001
PLCR (%)	28.51 ± 1.48^{d}	25.62 ± 2.26^{f}	23.24 ± 2.50^{f}	20.22 ± 0.98^{af}	27.92±2.20	34.92 ± 1.74^{bcd}	6.81	0.001

p≤0.05- Significant; p ≥ 0.05- Not significant, PHZ=Phenylhydrazine. a: significance with control, b: significance with PHZ group, c: significance with PHZ + Ferrous sulphate group, d: significance with PHZ + 100mg/kg, e: significance with PHZ + 200mg/kg, f: significance with PHZ + 400mg/kg.
TWBC=Total white blood cell count, PLT: Platelet Count, MPV: Mean Platelet Volume, PCT: Platelecrit, PDW: Platelet Distribution Width, PLCR: Platelet-Large cell ratio.

DISCUSSION

This study presents a comparison of red blood cell, white blood cells and platelet parameters among different experimental groups. It is evident that phenyl-hydrazine (PHZ) administration alone significantly reduced red blood cell count and haemoglobin concentration compared to the control group which aligns with the findings of studies done by Criswell *et al*. (2001) and Meena *et al*. (2014). This reduction indicated the induction of anaemia by PHZ, consistent with its known haemolytic properties. The group treated with ferrous sulphate and various concentration of bi-herbal formulation of the extract showed a partial restoration of red blood cell count and haemoglobin concentration compared to the PHZ-only treated group, suggesting a potential beneficial effect of ferrous sulphate supplementation and the extract in mitigating PHZ-induced anaemia. One possible reason for the observed improvements in red blood cell count and haemoglobin concentration in groups treated with the bi-herbal formula could be attributed to the presence of bioactive compounds in *Picralima nitida* and *Cymbopogon citratus* extracts. Also, this extract possessed antioxidants properties which can reverse the oxidative damage brought about by phenyl-hydrazine administration (Vasquez *et al*., 2015; Feyisayo and Victor, 2019). The alterations in red cell indices, including increase in mean cell volume (MCV) and mean cell haemoglobin (MCH),

and decrease in mean cell haemoglobin concentration (MCHC), reflect changes in the size, haemoglobin content, and concentration of red blood cells, respectively. This finding is consistent with the study done by Zanganeh *et al*. (2018) however; the decrease in MCHC in this study is in contrast to the study of Criswell *et al*. (2000). The significant deviations observed in these parameters in the PHZ-only group compared to the control group indicated abnormal erythropoiesis and impaired red blood cell maturation, characteristic of anaemia. Nosiri *et al*. (2020) reported an increase in MCH and MCHC following administration of *Cymbopogon citratus* which slightly contradict this study solely due to the fact that MCHC was decreased. Also, in contrast to this study, Oyeleke (2020) reported that *Picralima nitida* did not have any effect or cause any change in MCH. The increase in standard deviation (RDW-SD), in the PHZ-only group compared to the control group indicates increase in variability in red blood cell size, characteristic of anaemia. The lack of normalization of RDW values in groups treated with the bi-herbal formula suggests an inability of the extract to improve red blood cell homogeneity and maturation.

This study also compared white blood cell parameters among different groups in the study. There was an increase in WBC count in groups treated with the bi-herbal formulation compared to the control group which aligns with the increase in TWBC observed by Criswell *et al*. (2000). This elevation indicates a potential role of the biherbal formulation in enhancing the body's defense mechanisms. Nosiri *et al*. (2020) has reported that rats administered with 600mg/kg body weight of *Picralima nitida* showed an increase in TWBC count while Nwankwo *et al*. (2017) reported that *Picralima nitida* increased total WBC count in Wistar rats. Lymphocyte was slightly reduced in groups treated with PHZ alone, compared to the control group, although this difference was not statistically significant. However, treatment with ferrous sulphate led to an increase in lymphocyte. This suggests a possible immuno-modulatory effect of the ferrous sulphate, leading to an enhancement of lymphocyte activity. There was no significant change in monocytes in the groups administered PHZ alone and ferrous sulphate compared to the control group. However, treatment with the bi-herbal formulation led to an increase in monocyte count. This suggests a potential regulatory effect of the bi-herbal formulation on monocyte production or activity, possibly through its anti-inflammatory properties. Neutrophil count showed a significant decrease in groups treated with ferrous sulphate and 100mg/kg biherbal formulation when compared to the control and PHZ treated groups. This suggests a potential immunomodulatory role of the biherbal formulation on neutrophil concentration. Previous studies have highlighted the immunomodulatory effects of herbal formulations on neutrophil function and inflammatory responses (Murunikkara and Rasool, 2014; Khazdair *et al*., 2018).

Findings from this study also revealed that platelet count (PLT) increased in groups receiving phenyl-hydrazine compared to the control which aligns with the study of Meena *et al*. (2014), indicating a potential effect of phenyl-hydrazine on platelet production. Interestingly, administration of the bi-herbal formulation at doses of 200mg/kg and 400mg/kg led to a further significant elevation in PLT compared to phenyl-hydrazine alone, suggesting a dose-dependent effect of the herbal formulation on platelet count. This increase in platelet count could be attributed to the stimulatory effects of the herbal components on bone marrow function or thrombopoiesis. Notably, previous studies have reported similar findings, where herbal extracts have demonstrated the ability to modulate platelet count through various mechanisms, including the regulation of hematopoietic stem cells and megakaryocytes (Nosiri *et al*., 2020, Oyeleke, 2020). The mean platelet volume (MPV), a marker of platelet size and activity, exhibited significant variations among the treatment groups. Interestingly, only 400mg/kg led to a notable increase in MPV compared to all other groups. This suggests that the herbal formulation may influence platelet activation and function, potentially enhancing

their thrombotic potential. Similarly, PDW, PCT and PLCR showed notable increase in the groups administered higher doses of the herbal formulation compared to control and PHZ groups.

CONCLUSION

Results from this study showed that phenyl hydrazine caused significant alterations in red blood cell parameters and indices. However, treatment with ferrous sulphate and the bi-herbal formulation resulted in restorations in red blood cell count, haemoglobin concentration, and red cell indices. This study also revealed that in response to phenyl hydrazine-induced anaemia, there was a decrease in white blood cell (WBC) counts and alterations in platelet parameters. The treatment with the bi-herbal formulation led to notable increase in total white blood cell count and platelet count.

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