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# Evaluation of Antioxidant and Anti-anaemic Potential of *Moringa oleifera* Parts on Phenylhydrazine-induced Heamatotoxicity in Male Wistar Albino Rats

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**ABSTRACT:** In recent years, scientific interest in *Moringa oleifera* has surged due to its potential healthpromoting properties in traditional healthcare services. Hence, the objective of this paper was to evaluate the antioxidant activities of ethanol extract of different parts of *Moringa oleifera* tree and the anti-anaemic potential of the parts in phenylhydrazine-induced heamatotoxicity in forty-eight (48) male Wistar albino rats using appropriate standard techniques. Qualitative phytochemical screening showed *that Moringa oleifera parts contain flavonoids, saponins, and steroids and have* antioxidant capabilities. The IC<sub>50</sub> values for *M. oleifera* leaves and flowers DPPH were MOL 24.26  $\pm 2.92 \mu$ g/ml, MOF 43.69  $\pm 2.68 \mu$ g/ml, and gallic acid 16.71  $\pm 0.63 \mu$ g/ml. There was a significant increase in WBC, MCH, MCHC, and NEU in anaemic rats (14.07  $\pm 0.64 \times 10^3$ , 22.6 $\pm 0.44 \mu$ g, 32.5 $\pm 0.85 g/dl$ , 33 $\pm 1.00$  %, respectively) compared to the normal control (9.98  $\pm 0.92 \times 10^3$ , 18.98  $\pm 0.42 \mu$ g, 30.45  $\pm 0.5 g/dl$ , 29.05  $\pm 1.80$  %, respectively) but lowered significantly with the treatment of different parts of *Moringa oleifera*. Histopathology showed moderate myeloblastic and lymphoblastic cellular traits in anaemic rats. The groups treated with hydroxyurea and *Moringa oleifera* parts showed varying frequencies of mild myeloblastic and lymphoblastic cellular traits, indicating improvement in the bone marrow. Findings from this study showed that *Moringa oleifera* parts have antioxidant capabilities and anti-anaemic potentials in a rat model of phenylhydrazine-induced heamatoxicity. However, the leaf is the most potent and efficient part based on the results of these findings.

#### DOI: https://dx.doi.org/10.4314/jasem.v27i10.25

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**Cite this paper as:** OLUKANNI, AT; MINARI, JB; OKPUZOR, J. (2023). Evaluation of Antioxidant and Antianaemic Potential of Moringa oleifera Parts on Phenylhydrazine-induced Heamatotoxicity in Male Wistar Albino Rats. *J. Appl. Sci. Environ. Manage.* 27 (10) 2323-2329

Received: 27 August 2023; Revised: 25 September 2023; Accepted: 04 October 2023 Published: 30 October 2023

Keywords: anti-anaemic; haematotoxicity; phenylhydrazine; Moringa oleifera; antioxidant

*Moringa oleifera* (Lam), commonly known as the "drumstick tree" or "horseradish tree," is a versatile plant with a rich history of traditional medicinal uses. This remarkable tree is celebrated for its nutritional value, as various parts of the Moringa tree, including the leaves, seeds, and roots, are known to be a source of essential vitamins, minerals, and antioxidants. In recent years, scientific interest in *Moringa oleifera* has surged due to its potential health-promoting properties, particularly its role in combating oxidative stress and anaemia (Gopalakrishnan *et al.*, 2016; Watanabe *et al.*, 2021). Oxidative stress, characterized by an imbalance between the production of harmful

free radicals and the body's ability to counteract their effects with antioxidants, is implicated in the pathogenesis of numerous health disorders, including anaemia (Forman and Zhang, 2021). Anaemia, a condition marked by a deficiency in the number of red blood cells or a decrease in their ability to carry oxygen, can result from various factors, including the destruction of red blood cells. Phenylhydrazine is a chemical compound commonly used to induce hemolytic anaemia in laboratory animals, making it a valuable model for studying anaemia-related pathophysiology (Gheith and El-Mahmoudy, 2018). Bone marrow plays a significant role in sickle cell

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anaemia (SCA), a hereditary blood disorder characterized by abnormally shaped red blood cells (sickle-shaped) that can lead to various health complications (Tanabe et al., 2019). These abnormal red blood cells are fragile, prone to breaking apart (hemolysis), and have a shorter lifespan than normal red blood cells. To compensate for the rapid destruction of sickle cells, the bone marrow must produce new red blood cells at an increased rate (Giordano et al., 2021). This puts a considerable demand on the bone marrow to maintain an adequate supply of red blood cells. SCA can lead to haematological stress on the bone marrow, as it must work harder to produce red blood cells to replace those continuously destroyed (Leonard et al., 2019). This increased workload on the bone marrow can contribute to fatigue, anaemia-related symptoms, and sometimes even bone pain.

This study delves into the antioxidant and antianaemic potential of different parts of the Moringa oleifera tree in a rat model of phenylhydrazineinduced hematotoxicity. By assessing the impact of Moringa oleifera on oxidative stress markers and haematological parameters, we aim to shed light on the therapeutic potential of this natural resource in ameliorating anaemia and oxidative damage. Understanding the mechanisms underlying Moringa oleifera's effects on haematological health can pave the way for novel therapeutic interventions and the development of functional foods or supplements to combat anaemia and related disorders. This research could contribute to the growing body of scientific evidence supporting the traditional uses of Moringa oleifera and highlight its potential as a valuable addition to modern healthcare practices. Hence, the objective of this paper was to evaluate the antioxidant activities of ethanol extract of different parts of the Moringa oleifera tree and the anti-anaemic potential of the plant parts on phenylhydrazine-induced heamatotoxicity in forty-eight (48) male Wistar albino rats.

#### **MATERIALS AND METHODS**

*Qualitative analysis of phytochemicals:* Methodologies for determining phytochemicals were determined using a standard assay reported by Ezeonu and Ejikeme, 2016.

In-vitro antioxidant assays: Determination of 1,1, diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities: The assay was carried out according to Vats and Gupta's (2017) described method. Plant extract (1 ml) was mixed with 1 ml DPPH (0.3 mM) and allowed to stand for 30 min at room temperature in the dark. The absorbance was taken at 517 nm, and  $IC_{50}$  (µg/ml) was extrapolated from the graph of the % DPPH scavenging activities plotted against the concentration of gallic acid.

*Ferric-reducing antioxidant power assay (FRP):* The procedure, as outlined by Oyaizu (1986), with slight modifications, was employed in determining the reducing power of the extract. A mixture of five different concentrations of ethanol extracts (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) and gallic acid at the same concentrations in 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% potassium ferricyanide (K<sub>3</sub>Fe (CN)<sub>6</sub>) was made. At 50 °C, the mixture was incubated for 20 minutes, and 10% trichloroacetic acid (TCA) 2 ml was added. This was followed by centrifuging the mixture at 1000 revolutions per minute (rpm) for 10 min. Briefly, 2 ml of the supernatant was aspirated and mixed with distilled water (2 ml) and 0.1% ferric chloride (FeCl<sub>3</sub>) (1 ml). For each, the experiment was done in triplicate. After that, the absorbance values were measured using a spectrophotometer at 700 nm, and lC<sub>50</sub> was determined.

*Hydrogen peroxide scavenging activity (HPSA):* The ability of plant extracts to scavenge hydrogen peroxide was determined by following the method of Ruch, 1989. Accordingly, a solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). Three different concentrations of extract (25, 50 and 75  $\mu$ g/ml) prepared in distilled water were added to a 0.6 ml hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging capacity was calculated by

$$[H_2O_2] = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance of the control, AS is the absorbance of the sample.

Experimental design of in-vivo studies: *Experimental groups*: Forty-eight (48) male Wistar albino rats were divided into eight groups of six rats each. Anaemia was induced in rats except the normal control group (NOR) by intraperitoneal administration of 40 mg/kg of PHZ for three days. The rats were divided into groups I to VIII and treated for 28 days as follows:

NOR: Normal rats fed with normal chow; NEG (Negative control): Anemic rats fed with normal chow; POS (Positive control): Anemic rats fed with normal chow, plus 25 mg/kg hydroxyurea; MOB: Anaemic rats fed with rat chow blended with 10 % *Moringa oleifera* bark; MOF: Anaemic rats fed with

rat chow blended with 10 % *Moringa oleifera* flower; MOL: Anaemic rats fed with rat chow blended with 10 % *Moringa oleifera* leaves; MOR: Anaemic rats fed with rat chow blended with 10 % *Moringa oleifera* root; MOS: Anaemic rats fed with rat chow blended with 10 % *Moringa oleifera* seed

*Induction of anaemia:* In the experimental rats, anaemia was induced through intraperitoneal injection of phenylhydrazine (40 mg/kg body weight) into the rats for three consecutive days (Sheth *et al.*, 2021). The packed cell volume (PCV) and haemoglobin (Hb) were determined, and rats with PCV less than 36% and Hb less than 12 g/dl were considered anaemic and included in this study.

*Collection of blood sample:* The blood sample was collected via cardiac puncture after dislocation at the cervical region. It was dispensed into commercially prepared concentrations of ethylene diamine tetra acetic acid containers gently mixed and allowed to settle, after which it was centrifuged for 10 minutes at 4000rpm to obtain plasma. Samples were then stored in a refrigerator until it was needed.

Determination of haematological parameters: The full blood count was determined using an automatic counter (Sysmex kx-21N, Tokyo, Japan), following the procedure outlined by Dacie *et al.*, (2001).

#### **RESULTS AND DISCUSSION**

Phytochemical screening of ethanol extract of various parts of *Moringa oleifera*: Chemical groups in the ethanol extracts of the various parts of *Moringa oleifera* are presented in Table 1. The leaf contained alkaloids, flavonoids, tannins, saponins and glycosides, while the bark, with minor components, contained only saponin, steroids and glycosides.

 Table 1: Phytochemical Screening of the various parts of M.

Group	Moring	ga oleifera			
	Bark	Flower	Leaf	Root	Seed
Alkaloids	-	-	+	-	+
Flavonoids	+	+	+	+	+
Saponins	+	+	+	+	+
Steroids	+	+	-	+	+
Tannins	-	+	+	-	-
Terpenoids	-	-	-	-	+
Phobatannin	-	-	-	-	+
Glycoside	+	+	+	-	-
Carbohydrate	-	-	+	+	+
Amino acids proteins	-	-	+	-	+

DPPH radical scavenging activities: Table 2 shows the result of DPPH radical scavenging activities of Moringa oleifera parts. Moringa oleifera parts exhibited profound *in-vitro* DPPH radical scavenging potentials in a concentration-dependent manner. The standard (gallic acid) showed remarkably higher DPPH radical scavenging activities than the extracts. The concentration of *Moringa oleifera* parts needed to scavenge 50% of the DPPH radicals (IC<sub>50</sub>) was also evaluated in this study. The IC<sub>50</sub> values for *Moringa oleifera* parts were MOL 24.26  $\pm$  2.92 µg/ml, MOF 43.69  $\pm$  2.68 µg/ml, and gallic acid 16.71  $\pm$  0.63 µg /ml. The IC<sub>50</sub> value of the standard was lower than that of the extracts.

*Ferric reducing antioxidant power assay*: Like DPPH, the ferric-reducing antioxidant potential of the *Moringa oleifera* extracts showed concentrationdependent increases in absorbance values at a wavelength of 700 nm (Table 2). The standard (gallic acid) at all tested concentrations had higher absorbance values than the extract. The  $IC_{50}$  was determined in this study. The  $IC_{50}$  for gallic acid (0.89 µg/ml) was higher than that of MOF, MOS, and MOL extract (0.39, 0.39, 0.67 µg/ml, respectively) but lower than MOB and MOR (0.93, 1.29 µg/ml respectively).

*Hydrogen peroxide scavenging activity*: Hydrogen peroxide scavenging activities (HPSA) of *Moringa oleifera* parts showed that the various parts had profound hydrogen peroxide scavenging potentials in a concentration-dependent manner (Table 2). The standard (gallic acid) exhibited remarkably higher hydrogen peroxide scavenging activities than the extract. The concentration of *Moringa oleifera* parts needed to scavenge 50% of the hydrogen peroxide (IC<sub>50</sub>) was also estimated in this study. The IC<sub>50</sub> for gallic acid was lower than all the extracts.

 Table 2. antioxidants activities for DPPH, FRP and HPSA assays.

Plant extract	DPPH	FRP	HPSA			
	(µg/mL)	(µg/mL)	(µg/mL)			
MOB	$83.67 \pm 2.27*$	$0.93\pm0.02$	$0.13\pm0.03$			
MOF	$43.69 \pm 2.68*$	0.39±0.01*	0.29±0.01*			
MOL	$24.26 \pm 2.92*$	$0.67 \pm 0.06$	0.19±0.01*			
MOR	$387.36 \pm 8.02*$	1.29±0.37*	0.15±0.01			
MOS	ND	0.39±0.02*	$0.14 \pm 0.01$			
GA	16.71 ±0.63	0.89±0.10	0.12±0.01			
ND - not detected; *Significance when compared to Gallic acid						
across the column						

The presence of alkaloids, flavonoids, tannins, saponins, and glycosides in the various parts of *M. oleifera*, from the phytochemical screening, suggested their involvement in the bioactivities of the plants. Such activities have been associated with various parts of diverse plants without excluding *M. oleifera* (Vergara-Jimenez *et al.*, 2017; Nimyel and Ilori, 2022). While Vergara-Jimenez *et al.* (2017) used only leaf extract, Nimyel and Ilori (2022) used root bark extracts with different solvents and argued that ethanol

extract had more phytochemicals. The in-vitro antioxidant assay was conducted to determine whether the Moringa oleifera bioactive phytochemicals could prevent DPPH, FRP, and HPSA oxidation. The observed antioxidant activities in leaf and flower extracts are similar to the gallic acid used regarding their DPPH and FRP IC<sub>50</sub>, indicating that they are rich in phytochemicals with antioxidant activities. The activities were also dose-dependent, like that of pure gallic acid used as the study's standard, suggesting that the antioxidant properties of Moringa oleifera allow it to mop up free radicals in the assay, stabilize them, and then stop the free radicals chain reactions. Furthermore, various Moringa oleifera extracts, mainly the bark, seed, root and leaves, showed notable in vitro hydrogen scavenging capacity (HPSA), with the seed and leaves giving the best results

Effect of Moringa oleifera parts on haematological parameters of the PHZ-induced anaemic rats: Table 3 shows the effect of Moringa oleifera parts on the haematological parameters of PHZ-induced anaemic. The result revealed a significant (p < 0.05) decrease in RBC, HCT, HB and LYM in the anaemic group compared to the non-anaemic group. Interestingly, treatment with different parts of Moringa oleifera significantly (p < 0.05) improved RBC, HCT, HB and LYM levels relative to the untreated group. There was a significant increase in WBC, MCH, MCHC, and NEU in anaemic rats  $(14.07 \pm 0.64 \times 10^3, 22.6 \pm 0.44)$ pg,  $32.5 \pm 0.85$  g/dl,  $33 \pm 1.00$  %, respectively) compared to the normal control  $(9.98 \pm 0.92 \text{ x } 10^3,$  $18.98 \pm 0.42$  pg,  $30.45 \pm 0.5$  g/dl,  $29.05 \pm 1.80$  %, respectively) but lowered significantly with the treatment of different parts of Moringa oleifera.

Table 3: Effect of Moringa oleifera parts on haematological parameters of the PHZ-induced anaemic rats.

Parameter	NOR	NEG	POS (HU)	MOB	MOF	MOL	MOR	MOS
P-LCR (%)	$14.50 \pm 0.70$	14.57 ± 0.93	17.38 ± 1.13 <sup>ab</sup>	17.20±1.31*	17.78 ± 0.55 <sup>ab</sup>	16.38 ± 1.42 <sup>ab</sup>	16.78 ± 0.61 <sup>ab</sup>	16.38 ± 0.73 <sup>ab</sup>
MPV (f l)	$8.40 \pm 0.40$	$8.20 \pm 0.17$	8.65 ± 0.86	8.63 ± 0.42	8.68 ± 0.26	8.53 ± 0.33	8.70 ± 0.43	$8.68 \pm 0.46$
PDW (f l)	10.93 ±1.2	$10.50 \pm 0.26$	$11.08 \pm 1.93$	$10.93 \pm 1.10$	$11.58 \pm 1.90$	$10.90 \pm 1.13$	$10.85 \pm 0.75$	$10.83 \pm 1.68$
WBC (103)	9.98 ± 0.92 <sup>∞</sup>	14.07 ± 0.64 <sup>sb</sup>	8.60 ± 0.70 <sup>ab</sup>	8.30 ± 0.26 <sup>abc</sup>	7.03 ± 0.64 <sup>sbc</sup>	10.30 ± 0.29 <sup>be</sup>	8.53 ± 0.57**	7.90 ± 0.57**
RBC (10°) UI	6.73 ± 0.59 <sup>b</sup>	5.58 ± 0.63*	5.95 ± 0.65	5.86 ± 0.13*	5.28 ± 0.47*	$5.98 \pm 0.54$	6.10 ± 0.41 <sup>ab</sup>	5.85 ± 0.67*
HB g/dl	$12.78 \pm 0.48$	$12.60 \pm 0.2$	$13.43 \pm 1.07$	$13.33 \pm 0.31$	$12.51 \pm 0.91$	14.08 ± 0.53 💑	14.10 ± 0.99 💑	$12.75 \pm 0.83$
HCT %	42.13 ± 0.92 <sup>b</sup>	38.80 ± 0.85°	44.33 ± 0.56 <sup>to</sup>	44.80 ± 0.44°	39.53 ± 0.94 <sup>ab</sup>	45.65 ± 0.48 <sup>ab</sup>	46.50 ± 0.88 <sup>ab</sup>	41.58 ± 0.93 <sup>b</sup>
MCV (fl)	62.25 ± 0.55 <sup>∞</sup>	69.57 ± 0.90∞	74.18 ± 0.67 <sup>ab</sup>	76.40 ± 0.56 <sup>abc</sup>	74.67 ± 0.70 <sup>ab</sup>	76.28 ± 0.94 <sup>abc</sup>	76.25 ± 0.81 <sup>abc</sup>	70.88 ± 1.07 <sup>abc</sup>
MCH (pg)	18.98 ± 0.42 <sup>bc</sup>	22.60 ± 0.44°	22.53 ± 1.08°	22.73 ± 0.51°	23.68 ± 1.07°	23.58 ± 0.60°	23.18 ± 1.85°	21.83 ± 1.53°
MCHC(g/dl)	30.45 ± 0.5 <sup>b</sup>	32.50 ± 0.85 <sup>∞</sup>	30.33 ± 0.95°	29.83 ± 1.68 <sup>b</sup>	31.75 ± 1.22	30.88 ± 0.51	30.35 ± 1.50°	30.70 ± 0.96°
PLTX10 <sup>3</sup> /ul	927.50 ± 1.29 <sup>₅</sup>	885.33 ± 2.08∞	481.75 ± 1.71 <sup>sbc</sup>	486.00 ± 1.00 <sup>abc</sup>	507.75 ± 2.22***	529.75 ± 1.71 <sup>abe</sup>	558.25 ± 1.29**	563 ± 2.16 <sup>abc</sup>
LYM%	71.20 ± 1.65°	67.00 ± 1.14°	67.93 ± 0.72°	80.10 ± 1.48 <sup>ab</sup>	78.30 ± 1.04 <sup>ab</sup>	78.93 ± 1.12 <sup>ab</sup>	74.65 ± 1.06 <sup>ab</sup>	83.40 ± 0.74 <sup>ab</sup>
NEU %	29.05 ± 1.80 <sup>6€</sup>	33.00 ± 1.00 <sup>a</sup>	32.15 ± 0.60°	19.90 ± 1.50 <sup>abc</sup>	21.70 ± 1.37 tbc	$21.03 \pm 1.14^{\text{abc}}$	25.98 ± 0.87 <sup>abc</sup>	$16.60 \pm 0.84^{\text{abc}}$
RDW-SD (fl)	42.10 ± 1.34 <sup>bc</sup>	53.03 ± 1.34∞	46.43 ± 1.65 <sup>ab</sup>	52.93 ± 1.36**	52.20 ± 1.83™	54.38 ± 0.88∞	55.2 ± 1.67 <sup>™</sup>	37.58 ± 1.02 <sup>abc</sup>
RDW-CV%	18.60 ± 1.15 <sup>ab</sup>	21.80 ± 1.34 <sup>c</sup>	16.90 ± 1.42°	19.50 ± 1.34 <sup>bc</sup>	19.63 ± 1.59 <sup>bc</sup>	20.00 ± 1.84 <sup>c</sup>	19.85 ± 1.00 <sup>c</sup>	13.55 ± 0.59 <sup>abc</sup>

Results are expressed as mean ± SD, n=6. A means significant when compared to NOR, b is significant compared to anaemic control, and c is significant compared to the hydroxyurea group. NOR: Normal control; NEG: Anaemic control, POS: (HU) hydroxyurea treated group, MOB: Moringa oleifera bark, MOF: Moringa oleifera flower, MOL: Moringa oleifera leaf, MOR: Moringa oleifera root, MOS: Moringa oleifera seed. HCT: haematocrit; HGB: haemoglobin; LYMP: lymphocyte; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; MPV: mean platelet volume; NEU: neutrophil; PDW: plate volume distribution width; PLT: platelet; RBC: red blood cell; RDWCV: red blood cell volume distribution width-CV; RDWSD: red blood cell volume distribution width-SD; WBC: white blood cell. Phenylhydrazine is a renowned hemolytic drug that distorts the erythrocyte membranes via lipid peroxidation resulting from massive intravascular hemolysis. The membrane distortion caused haemoglobin destruction as the globin portion is denatured and precipitated. This counted for the reduced RBC recorded in this study.

Anaemic condition is also associated with declining Hb, RBC, and HCT values. Both human and rat studies have shown a significant correlation between RBC, Hb, PCV, and red cell indices, that is, MCV, MCH, and MCHC (Aloke et al., 2021). In the present study, the Hb and HCT of the anaemic rats dropped compared to the normal rats compared to the untreated anaemic group. Still, the values significantly improved after treatment with HU, MOB, MOL, MOR, and MOS, supporting the findings of the previous studies that certain plants contain phytochemicals that can enhance red cell indices (Gheith and El-Mahmoudy, 2018). The body's response to low HCT levels, which culminated in the synthesis of blood cells to prevent oxygen deprivation in the circulation, may be responsible for the increase in HCT levels. By reversing the effects of the toxicant - PHZ, the antioxidants present in plant materials may be responsible for this anti-anaemic activity. Extensive intravascular hemolysis is linked to the alteration of MCHC values (decreases or increases); reticulocytosis often increases MCV and MCH (Prasad et al., 2018). According to the haematological index data in the study, the anaemic rats altered the indices, while HU

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and *M. oleifera* improved most of them, with the best improvement displayed by MOL. Thus, the *Moringa oleifera* leaf is a choice plant material for mitigating anaemic conditions.

Growing data have suggested that anaemic condition arises when phenylhydrazine damages red blood cells through the formation of ROS (Banerjee et al., 2020), supporting the decrease in the RBC numbers of PHZinduced anaemic rats in this study. However, treatments with hydroxyurea and various parts of Moringa oleifera improved the value dramatically. This can be attributed to abundant antioxidant bioactive components in the plant materials, which counteracted phenylhydrazine's harmful effects. It was thus postulated that the Moringa oleifera components might have stimulated stem cells to create new blood cells through erythropoiesis (Suzanne et al., 2020). These findings align with research on Moringa oleifera leaf extract (Rabeh et al., 2021) which increased Hb concentration and RBC after anaemia induction with PHZ. The anaemic rats fed with various Moringa oleifera parts had a considerable increase in Hb content, proving that the plant effectively boosts

blood production. The rise in white blood cells (WBC) experienced in anaemic rats could result from the rat's immune system responding by producing more white blood cells to fight any infections that might be the source of the anaemia (Quasaaid *et al.*, 2022); this was also curtain by the *M. oleifera* various parts. Although WBC was suppressed by treatment with *Moringa oleifera* parts, the value was brought to normal with *Moringa oleifera* leaves (MOL).

Figure 1 shows the histology sections of selected bone marrows of the various groups in the study. The typical architecture of the bone marrow in the normal control rats was seen in the bone marrow film (Fig. 1A). In contrast, moderate myeloblastic and lymphoblastic cellular traits with some diffused cells were seen in the anaemic untreated rats (Fig. 1B). However, in the groups treated with the standard drug (Fig. 1C) and *Moringa oleifera* parts Fig. 1 D, E, F, G and H), the result showed varying frequencies of mild and moderate myeloblastic and lymphoblastic cellular traits with some diffused cells across groups indicating improvement in the bone marrow.



Fig. 1 Photomicrographs of rat bone marrow at x400 magnification, H&E staining. Observable across groups A-H are bone marrow films with varying frequencies of mild to moderate myeloblastic and lymphoblastic cellular traits (red arrow) with some diffused cells across groups

Due to inefficient erythropoiesis in the bone marrow and the short red blood cell survival in circulation, it has been demonstrated that oxidative stress plays a critical role in haemolytic anaemia (Kale *et al.*, 2019). The histopathology of the bone marrow showed a typical bone marrow architecture for healthy rats and mild lymphoblastic and myeloblastic cellular characteristics in anaemic rats. These agreed with the elevated white blood cells in PHZ rats in the study on haematological parameters. Lymphoblastic leukaemia is associated with high WBC production in response to injuries induced by toxic compounds (Ahmed *et al.*, 2023). The treatment groups exhibit some diffused cells across groups and minor myeloblastic and lymphoblastic cellular characteristics, which is a sign that *Moringa oleifera* root, bark, seed, leaves and flower had an ameliorative effect on the bone marrow. Anaemia condition has been connected with an increase in tissue hypoxia, which, in-turns resulted in elevated serum erythropoietin, an essential molecule in the expansion and differentiation of bone marrow and spleen (Wang *et al.*, 2021). This present study

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reported that *Moringa oleifera's* bark, flower, leaves, root, and seeds have significant antioxidants and anaemic-fighting potential against PHZ-induced hemolytic anaemia, supporting their folklore use in alternative medicine. The various positive effects of *Moringa oleifera* parts adduced to the bioactive phytochemicals observed in them, immune response elicitation suggested that some phytochemicals regulate some genes.

### REFERENCES

- Ahmed, IA; Senan, EM; Shatnawi, HAS; Alkhraisha, ZM; Al-Azzam, MMA (2023). Hybrid Techniques for Diagnosing Acute Lymphoblastic Leukemia Based on Fusion of CNN Features. *Diagnostics*. 13(6): 1026.
- Aloke, C; Uche, EC; Ajuka, ON; Nkemjika, OP; Oswald, EC; Godwin, UC; Ekakitie, O; Adewale, IA; Okoro, CC; Peter, OB; Ginikachukwu, AG; Charles, UB (2021). HPLC profiling and studies on Copaifera salikounda methanol leaf extract on phenylhydrazine-induced hematotoxicity and oxidative stress in rats. *Arab. J. Chem.* 14(12): 103428.
- Banerjee, A; Dey, T; Ghosh, AK; Mishra, S; Bandyopadhyay, D; Chattopadhyay, A (2020). Insights into the ameliorative effect of oleic acid in rejuvenating phenylhydrazine induced oxidative stress mediated morpho-functionally dismantled erythrocytes. *Toxicol. Rep.* 7: 1551–1563.
- Ezeonu, CS; Ejikeme, CM (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New J. Sci.* 1-9.
- Forman, HJ; Zhang, H (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nat. Rev. Drug Discov.* 20(9): 689-709.
- Gheith, I; El-Mahmoudy, A (2018). Laboratory evidence for the hematopoietic potential of Beta vulgaris leaf and stalk extract in a phenylhydrazine model of anemia. *Braz. J. Med. Biol. Res.* 51(11).
- Giordano, P; Urbano, F; Lassandro, G; Faienza, MF (2021). Mechanisms of Bone Impairment in Sickle Bone Disease. *Int. J. Environ. Res.* 18(4).
- Gopalakrishnan, L; Doriya, K; Kumar, DS (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Sci. Hum. Wellness.* 5(2): 49-56.

- Kale, OE; Awodele, O; Akindele, AJ (2019). Acridocarpus Smeathmannii (DC.) Guill. & Perr. Root enhanced reproductive behavior and sexual function in male Wistar rats: Biochemical and pharmacological mechanisms. J. Ethnopharmacol. 230: 95–108.
- Leonard, A; Bonifacino, A; Dominical, VM; Luo, M; Haro-Mora, JJ; Demirci, S; Uchida, N; Pierciey, FJ Tisdale, JF Bone marrow Jr: (2019). characterization in sickle cell disease: inflammation and stress erythropoiesis lead to recovery. suboptimal CD34 Br. J. Haematol. 186(2): 286-299.
- Nimyel, CD; Lori, JA (2022). Phytochemistry, Proximate Analysis And Acute Toxicity Study Of Ethanolic Moringa Oleifera Root Bark Extract. *Niger. J. Chem. Res.* 27:1.
- Ousaaid, D; Ghouizi, AE; Laaroussi, H; Bakour, M; Mechchate, H; Es-safi, I; Kamaly, OA (2022). Anti-Anemic Effect of Antioxidant-Rich Apple Vinegar against Phenylhydrazine-Induced Hemolytic Anemia in Rats. *Life* 12(2): 239.
- Oyaizu, M (1986). Studies on Products of Browning Reactions: Antioxidative Activities of Product of Browning Reaction Prepared from Glucosamine. *Jpn. J. Nutr.* 44: 307-315
- Prasad, YPS; Hari, P; Shajina, M; Mirshad, PV; Rahiman, OMF (2018). Hematinic and antioxidant potential of aqueous extract of Sesamum indicum seeds against phenylhydrazine-induced hemolytic anemia in albino rats. *Natl. J. Physiol. Pharm. Pharmacol.* 8(8): 1092-1096.
- Rabeh, NM; El. Kady, KA; Elmasry, HG; Abdelhafez, BI (2021). Effect of Feeding Moringa Oleifera (Moringaceae) Leaves Extract On Rats With Induced Iron Deficiency Anemia. *Nat. Volatiles. Essent.* 8(5): 13276-13287.
- Ruch, RJ; Crist, KA; Klaunig, JE (1989). Effects of culture duration on hydrogen peroxide-induced hepatocyte toxicity. *Toxicol. Appl. Pharmacol.* 100(3): 451–464.
- Sheth, PA; Pawar, AT; Mote, CS; More, C (2021). Antianemic activity of polyherbal formulation, Raktavardhak Kadha, against phenylhydrazineinduced anemia in rats. J. Ayu. Med. Sci. 12(2): 340–345.

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- Suzanne, BB; Adeline, FY; Theodora, KK; Dairou, H; Pradel, KL; Aristide, KM; Mathieu, N; Gabriel, AA; Anne, NNR; Clerge, T (2020). Hemopoietic effects of some herbal extracts used in treatment of infantile anemia in Cameroon. *World J. Pharm. Res.* 6(1): 147-155.
- Tanabe, P; Spratling, R.; Smith, D; Grissom, P; Hulihan, M (2019). Understanding the Complications of Sickle Cell Disease. Am. J. Nurs. 119(6): 26.
- Vats, S; Gupta, T (2017). Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of Moringa oleifera Lam. from Rajasthan, India. *Physiol Mol Biol Plants*. 23(1): 239–248.

- Vergara-Jimenez, M; Almatrafi, MM; Fernandez, ML (2017). Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. *Antioxidants*. 6(4).
- Wang, H; Liu, D; Song, P (2021). Exposure to hypoxia causes stress erythropoiesis and downregulates immune response genes in spleen of mice. *BMC Genom* 22: 413.
- Watanabe, S; Okoshi, H; Yamabe, S; Shimada, M (2021). Moringa oleifera Lam. In Diabetes Mellitus: A Systematic Review and Meta-Analysis. *Molecules*. 26(12).