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Effect of Young and Old *Moringa oleifera* Leaf Extract on Haematological, Renal and Liver Indices in *Rattus novergicus*

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Authors' contributions

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

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ABSTRACT

Aim: The use of *Moringa oleifera* leaves has gained worldwide acceptance in use by animals and humans. The study aimed to determine the effects of ethanolic leaf extract of young and old *Moringa oleifera* on haematological, renal, and liver indices in Wistar rats.
Design: A completely randomized design was used for the study.
Place and duration of study: The study was carried out in the Animal Science Department of Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development and lasted for six weeks.

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Materials and methods: A total of twenty male Wistar rats of age eight weeks old were used for the study. The Wistar rats were kept in aluminum cages under a 12-hours light and 12-hours dark cycle. The rats were divided into four treatments; Treatment (T1) received 1 mL/bw/day of normal saline, Treatments 2, 3, and 4 received 100 mg/kg/bw of iron (III) hydroxide polymaltose, 100 mg/kg/bw of young *Moringa oleifera* extract (YMoE) and 100mg/kg/bw of old *Moringa oleifera* extract (OMoE) as treatments respectively for six weeks. Phytochemical screening of young and old *Moringa oleifera* was done separately using standard extraction procedures.
Results: Phytochemical screening revealed the presence of triterpenoid, glycosides, flavonoid, and saponins in both young and old *Moringa oleifera* leaves, however, alkaloids and tannins were found only in young leaves of *Moringa oleifera*. Both extract (YMoE and OMoE) significantly ($P<0.05$) influenced rat's feed intake and body weight. An insignificant ($P>0.05$) effect of the treatment on haematological parameters was observed. However, there was a significant ($P<0.05$) effect of YMoE and OMoE treatments on haemoglobin which mirrored the effect of iron (III) hydroxide polymaltose. The study found no significant effect ($P>0.05$) of YMoE and OMoE treatments on liver enzymes, and Blood urea nitrogen. Creatines levels showed elevation in the group that received iron (III) hydroxide polymaltose, while those administered with Moringa extract had similar effect to the normal saline treatment. Histopathological examinations showed normal kidney and liver architecture in normal saline and *Moringa oleifera* treatments. Mild renal epithelium degeneration was observed in the iron (III) hydroxide poly-maltose treatment.
Conclusion: The findings from this study suggest that both young and old *Moringa oleifera* leaves may effectively manage anaemia without causing kidney or liver damage.

Keywords: Moringa Oleifera; phytochemicals; hematological indices; liver enzymes; creatinine levels.

1. INTRODUCTION

Blood is a vital homeostatic fluid in the human body, it is responsible for transporting nutrients and elimination of waste products from cells. It accounts for 10% of body weight of humans [1]. A reduction in blood haemoglobin concentration results in a condition termed as anaemia.

Anemia is a global public health issue, primarily caused by nutritional iron deficiency. It is prevalent among pregnant women and children under age five. The effects of anemia in children under age five and pregnant women include disrupted physical and mental development, low intellectual ability, and weakened immunity, and increasing labor complications [2], [3].

Efforts made at curbing the effects of anaemia have not reached the point of satiation in developing countries. The high cost of conventional prognosis and treatment, and the risk of blood transfusion-transmitted infections (TTIs) has made the destitute population in developing countries to depend on herbal medicinal plants for the treatment and management of anemia [4]. Given these reasons, it has become imperative to explore traditional medicinal plants which have been assumed to have potential anti-anemic effects.

Moringa oleifera, a medicinal plant that has been used since antiquity for the treatment and management of a wide range of diseases has been reported to possess haematinic efficacy [5]. Research since the 1970s has revealed *Moringa oleifera* plant's valuable medicinal properties and essential nutrients. Standardized extraction procedures have revolutionized the process, allowing for the extraction of bioactive compounds without altering the original composition and structure of the plant [5]. However, there is limited literature regarding the leaf type (young or old) of *Moringa oleifera* leaves which possesses the haematinic effect. To this regard the present study sought to investigate the phytochemical diversity in young and old *Moringa oleifera* leaves, the effect on body weight, feed intake, biochemical and haematological indices.

2. MATERIALS AND METHODS

2.1 Study Location and Study Period

The study was carried out at the Department of Animal Science Education of the Faculty of Agriculture Education at Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (AAMUSTED), Mampong-Ashanti. The research was carried for six weeks. Figure 1 shows the study site.

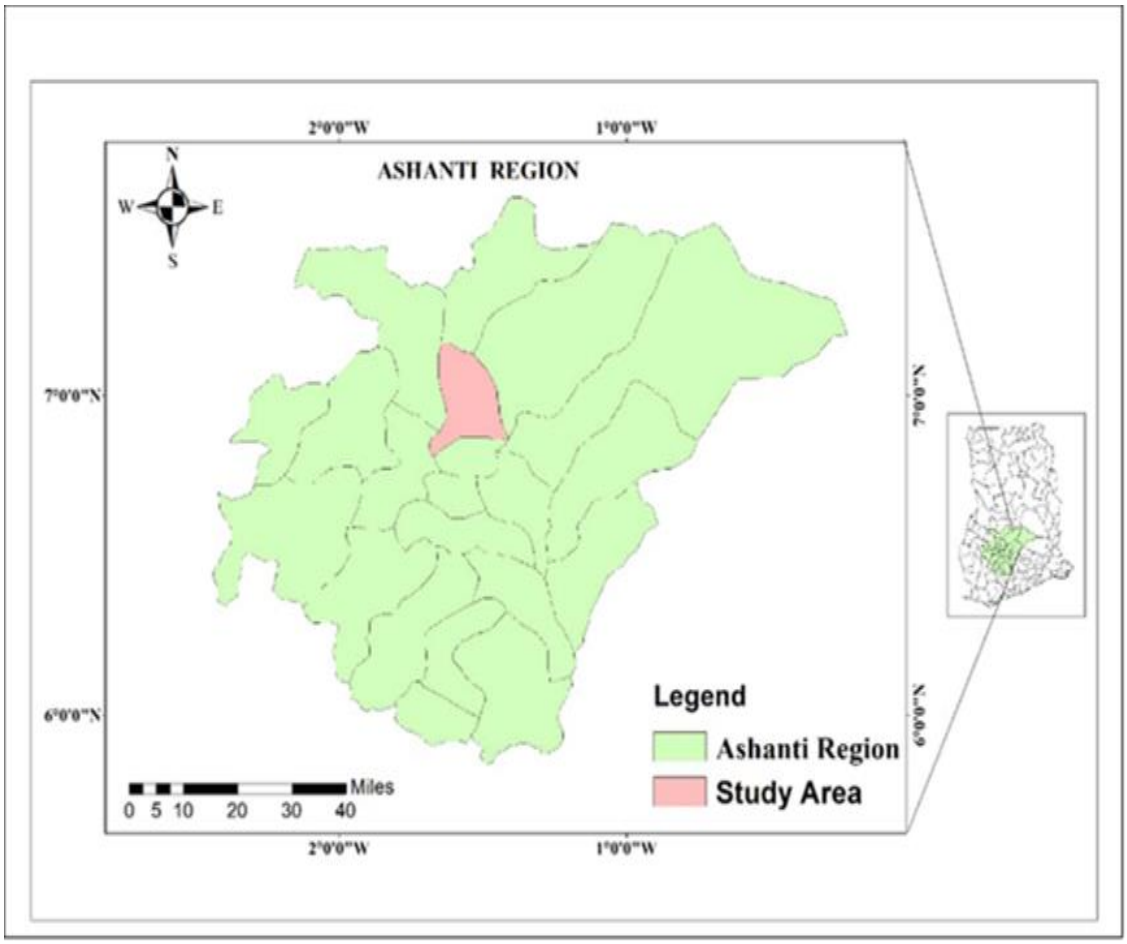

Figure 1. Map of the study area. Designed by using ArcMap vision 10.7

2.2 Experimental Design and Experimental Animals

The study utilized a completely randomized design (CRD). A total of twenty (20) sexually matured male Wister Rats (*Rattus novergicus*) weighing between 130 to 150g were purchased from Noguchi Memorial Research Institute for the study. The animals were transported to the Animal Farm located at the Department of Animal Science Education of the faculty of Agriculture Education, AAMUSTED where the experiment was conducted. The animals were given a period of 14 days to acclimate before being randomly allotted into four groups, each consisting of five replicates. Treatments were administered via the oral route using sterile oral gavage for a period of twenty-eight days.

2.3 Treatment and Dose Administered

Table 1 shows the treatment and dose administered during the experimental period.

Table 1. Treatment and dose administered

Group	Treatment	Dose Administered
1	9% Normal physiological Saline	1 mL /bw/day
2	Iron (III) Hydroxide Polymaltose	100 mg /bw /day
3	Ethanolic leaf extract of young *Moringa oleifera*	100 mg /bw/ day
4	Ethanolic leaf extract of old *Moringa oleifera*	100 mg /bw/ day

2.4 Animal Maintenance

2.4.1 Housing

The experimental animals were housed in galvanized metal cages with a 2 mm wire mesh coving. The cages were divided into four (4) compartments with the following dimensions 70cm x 60cm x 40cm. Softwood shreds were used as beddings for the experimental animals, the shreds were changed periodically (every

three days). The animals were kept under a controlled environmental condition at a temperature of 27 ± 3 °C under a 12-hour day-night cycle.

2.4.2 Feeding and watering

Each treatment group was provided with two earthenware bowls as feeders and waterers. The feed was offered daily. Clean water was provided *ad libitum*. The experimental animals were fed in the morning between 6:00 and 08.30 hours GMT each day.

2.4.3 Feed for the experimental animals

The experimental animals were fed with commercially formulated feed (basal diet) purchased from Galdus Ghana Limited. The feed was composed of the following ingredients: Maize, soya bean meal, wheat peas, corn gluten, rapeseed meal, nutricell, wheat, grits lecithin, feed-phosphate, lysine, sulfate, fishmeal, Limefine, sunflower oil, mould inhibitor, salt-NaCl, Vitamin E, premix, Choline chloride liquid, xylamase, phytase, sunflower hippo, and oat (Source: Label, Koudjis Animal Nutrition)

2.5 Plant Collection

Respective leaves of *Moringa oleifera* were identified and collected at Nyamebekyere a suburb in Mampong municipality. In this study, leaves of *Moringa oleifera* were considered young when their appearance was whitish-green and measured < 0.9 to 1.8 cm in length by 0.5 to 0.96 cm in width. Also, *Moringa oleifera* leaves were considered old when their appearance showed a dark green color and measured ≥ 0.9 to 2.54 cm in length by ≥ 0.5 to 1.5 cm in width **[6], [7]**

2.5.1 Plant Extraction

The leaflets were separated from the stem and cleaned with distilled water to remove any debris. They were allowed to air dry for two weeks at room temperature (26±1°C) at AMMUSTED Science laboratory. The leaves were then pulverized to a fine powder using the Retsch Milling solution at 2000 rpm for five minutes.

Fifty (50g) of the coarse form of the sample (leaves) was macerated for 3 days in 500 mL of 90% ethanol in sterile plastic containers with intermittent shaking at 26 °C to ensure adequate dilution and extraction. The extract was then filtered with Whatman filter paper (1.5 Sigma Aldrich, USA) and then concentrated to semi-solid form at 42 °C (to avoid denaturation of the active ingredients) using a Clifton water bath.

16 g and 45 g of YMoE and OMoE crude extracts, respectively, were dissolved in 100 ml of sterile distilled water to make a stock solution with a concentration of 0.16 g/mL and 0.4568 g/mL, respectively. The preparation of sub-stocks in microlitres was done by diluting the stock solution with sterile distilled water to the concentration of interest (100 mg/bw/day). The stock solution was kept refrigerated (- 4^0 C) until the time of treatment administration.

2.5.1 Procedure for phytochemical screening

The respective leaves of young and old *Moringa oleifera* were screened separately for the following phytochemicals: anthraquinones, steroids, triterpenoids, alkaloids; test for cyanogenic glycosides; test for phenolic compounds; saponins, tannins, steroids, flavonoids; and terpenes using standard phytochemical reagents and procedures. The procedures employed in the phytochemical screening process have been described by **[8], [9]**.

2.6 Daily Feed Intake

Feed intake was all measured in grams (g/day) and recorded for each treatment group. Feed intake was taken daily and calculated as the difference between the feed given and the feed left over.

2.7 Body Weight

The bodyweight (g) of the experimental rats was recorded (control, group 2, group 3, group 4, and group 5). The body weight was determined using a Camry top-loading sensitive scale with a reading or sensitivity of 0.1 g, produced in China by the Jadever Company Limited.

2.8 Haematological Parameters

The blood sample was taken via cardiac puncture on day 28, using a sterile syringe and needle manufactured by Jiangsu Shenli Medical Production Company Limited, China, and distributed by Letap Pharmaceuticals Limited, Ghana, and transferred into ethylenediaminetetraacetic acid (EDTA) tubes for

haematological assays. Markers such as white blood cell (WBC), red blood cell (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), haemoglobin (HGB), and platelet (PLT) were determined using a haematology auto-analyzer (Automatic Haematology Analyzer, with a model number Rayto RT-7600s made in Guangzhou, China).

2.9 Data Analysis

Data from the study was expressed in terms of mean ± standard error of the mean (SEM), graphs, and tables using Minitab statistical software (Version 20.0). Parameters in the groups were compared by one-way ANOVA, means were separated using Tukey HSD. All data were analyzed at a 95% confidence interval, and values were considered statistically significant at $p < 0.05$, the statistical model used was defined as:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \varepsilon$$

Where:

Y represents the response variable being measured (feed intake, body weight, haematological indices, renal function, and liver function).
β_0 is the intercept term, representing the baseline or average response when all the explanatory variables are zero.
β_1, β_2, β_3, and β_4 the coefficients associated with the explanatory variables X_1, X_2, X_3, and X_4 respectively.
X_1 represents the first treatment, ethanolic leaf extract of old *Moringa oleifera*.
X_2 represents the second treatment, ethanolic leaf extract of young *Moringa oleifera*.
X_3 represents the third treatment, Iron (III) hydroxide polymaltose.
X_4 represents the control treatment, normal physiological saline.
ε represents the random error term.

3. RESULTS

3.1 Phytochemical Screening

Results of the phytochemical analyses of young and old *Moringa oleifera* Leaves (YMoL and OMoL) revealed that there is diversity in the phytochemical constituents of the two leaves. Triterpenoids, glycosides, flavonoids, and saponins were found in both leaves, however, alkaloids, and tannins were only found in the young leaves of *Moringa oleifera*. Anthraquinones, steroids, and terpenoids were not detected in either of the leaf types. Table 2. Provides summary of the phytochemical constituents in YMoL and OmoL.

3.2 Feed Intake

There was a significant increase in the weekly feed-intake of the experimental models, except for week 3. The group that was administered with OMoE had the highest mean feed-intake when compared with the groups administered with normal saline, iron (III) hydroxide poly-maltose, and YMoE.

Table 2. Phytochemical constituents in YMoL and OmoL

Phytochemical	YMoL	OMoL
Anthraquinones	- -	- -
Steroids	- -	- -
Triterpenoids	+ +	+ +
Alkaloids	+ +	- -
Glycosides	+ +	+ +
Terpenoid	- -	- -
Flavonoids	+ +	+ +
Tannins	+ +	- -
Saponins	+ +	+ +

Detected = ++, Not detected = - -, YMoL = Young Moringa oleifera leaves, OMoL = Moringa oleifera leaves

The group that received YMoE also had a significant increase in the weekly feed intake during the period during weeks 1,2, and 3 when compared with the normal saline and Iron (III) hydroxide poly-maltose, however, in week three the YMoE group recorded the highest average feed-intake. The normal saline and Iron (III) hydroxide poly-maltose groups showed a steady increase in weekly feed intake, but there was a significant difference in mean feed intake levels when compared to YMoE and OMoE groups.

3.3 Body Weight

The body weight of the rats was not significantly influenced by the treatment on days 7 and 14.But on day 21, a statistically significant ($p < 0.015$) influence of the treatments was observed. YMoE and OMoE was similar as compared to the normal saline and iron (III) hydroxide polymaltose which had similar values. On day 28 OMoE was statistically similar to YMoE but different from the

normal saline and iron(III) hydroxide polymaltose, the YMoE treatment was statistically similar with OMoE, iron (III) hydroxide Polymaltose but statistically different from the normal saline group.

3.4 Haematological Indices

The treatment effect on red blood cells (RBCs), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean Platelet volume (MPV), white blood cells (WBCs), and platelets were not statistically significant ($p > 0.05$). However, there was a variation in WBCs and MCV. Contrarily, the treatment effect on Haemoglobin (Hbg), was statistically significant ($p < 0.05$), where rats treated with OMoE had an increase (16.733 g/dL**)** haemoglobin level when compared with rats treated with normal saline having the lowest mean (11.77 g/dL). **(**Table 5).

3.5 Liver Function Test

Results from the liver function test showed that treatment had statistically insignificant effect on the parameters accessed (Table 6).

Table 3. Weekly feed consumption rate by the experimental animals during the 28 days of treatment administration

Week	Normal Saline	Iron (III) Hydroxide Polymaltose	YMoE	OMoE	p-value
1	59.7±1.29^{c}	58.57±4.09^{c}	76.71±2.18^{b}	96.29±4.81^{a}	0.000
2	61.71±6.68^{b}	66.29±2.09^{b}	83.00±1.72^{a}	83.14±2.67^{a}	0.001
3	64.57±1.85^{b}	69.71±1.41^{b}	80.14±1.92^{a}	78.14±1.65^{a}	0.000
4	69.57±4.59	77.57±4.08	79.57±2.92	80.29±2.47	0.165

^{abc} *Means with different superscripts in the same row are significantly different (p< 0.05). Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. Means were separated using the Tukey HSD method at a 95% confidence interval.*

Table 4. Effect of the treatments on body weight of the experimental *Rattus novergicus*

Week	Normal Saline	Iron (III) Hydroxide Polymaltose	YMoE	OMoE	p- value
0	189.20±6.62	190.2±14.2	194.0±18.1	203.60±9.69	0.854
7	181.00±6.89	191.2±11.9	215.4±13.7	221.0±10.7	0.062
14	194.00±8.39	198.4±12.2	228.0±13.5	232.2±11.8	0.068
21	203.00±9.96^{b}	202.6±10.1^{b}	242.6±14.0^{a}	248.0±10.9^{a}	0.015
	207.0±13.7^{c}	215.2±13.6^{bc}	250.4±15.6^{ab}	257.0±11.7^{a}	0.047

$^{abc:}$ *Means with different superscripts in the same row are significantly different (p < 0.05). Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. Means were separated using the Tukey HSD method at a 95% confidence interval.*

Table 5. Effect of treatment on haematological indices

Parameters	Normal Saline	Iron (III) Hydroxide polymaltose	YMoE	OMoE	P-value
WBC (10^9/L)	4.61±1.71	4.54±0.894	4.96±0.936	4.85±0.618	0.992
RBCs(10^12/L)	6.050±0.686	6.970±0.214	6.810±0.344	7.353±0.160	0.215
Hbg (g/dL)	11.77±1.60^{b}	15.300±0.721^{a}	14.36±0.219^{ab}	16.733±0.273^{a}	0.025
HCT (%)	32.27±6.34	39.267±0.817	37.83±1.39	39.33±2.10	0.462
MCV (fL)	56.23±2.96	53.43±1.24	55.63±1.10	58.13±1.03	0.312
MCH (pg)	20.33±0.296	21.67±0.867	21.667±0.751	21.600±0.709	0.405
MCHC (g/dL)	37.200±0.666	38.933±0.869	38.17±1.34	39.27±1.96	0.697
MPV (fL)	6.56±0.176	6.733±0.120	6.700±0.100	6.700±0.208	0.819
PLATELETS (10^9/L)	396.3±28.3	419.7±28.6	401.7±22.3	423.7±28.4	0.863

^{abc} *Means with different superscripts in the same row are significantly different (p < 0.05). Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA. Means were separated using the Tukey HSD method at a 95% confidence interval. White Blood cells (WBC), Red Blood Cells (RBC), Haemoglobin (Hbg), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin Concentration (MCH), Mean Platelets volume (MPV).*

Table 6. Effect of the treatments on liver function tests (ALT, AST, ALP, GGT, Total protein, Total Bilirubin)

Parameters	Normal Saline	Iron (III) Hydroxide polymaltose	YMoE	OMoE	P-value
ALT (U/L)	17.333±87.6	4.33±2.85	4.667±0.667	18.7±10.7	0.411
AST (U/L)	259.0±37.2	200.7±21.3	201.67±9.21	158.7±50.3	0.281
ALP (U/L)	258.3±68.8	298.7±21.1	277.0±99.6	288.3±61.2	0.971
GGT (U/L)	15.7±14.2	9.00±1.15	7.33±1.76	5.33±1.86	0.767
T. Protein (g/L)	70.80±2.32	74.00±2.60	69.63±3.13	68.87±4.87	0.726
T. Bilirubin(µmol/L)	24.33±8.55	18.47±3.41	12.27±1.36	19.73±4.96	0.493

Each column represents the mean ± SEM. Group comparison was done by one-way Anova. ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), ALP (alkaline phosphate), GGT (Gamma-glutamyl transferase), T. Protein (Total protein), T. Bilirubin (Total Bilirubin).

Table 7. Renal function test after four weeks of treatments administration

Parameter	Normal saline	Iron (III) hydroxide polymaltose	YMoE	OMoE	P-Value
Urea(mmol/L)	6.49 ± 0.549	6.64 ± 0.701	5.243± 0.075	5.246 ± 0.043	0.101
Creatinine (µmol/L)	63.70 ± 5.99	64.97 ± 6.73	49.73 ± 1.93	47.13 ± 2.75	0.06

Each column represents the mean ± SEM. Group comparison was done by one-way ANOVA.

3.6 Renal Function Test

Table 7 shows the findings on the results of renal function after four weeks of treatment administration. Urea and creatine levels did not show any statistically significant ($p > 0.05$) differences between all the treatments.

3.7 Kidney Histology

The observation of the histopathological examination of the kidney (Fig. 1) showed a normal histological structure for the normal saline treatment (A), young and old *Moringa oleifera* treatments (C and D respectively). Diffuse degeneration of renal epithelium was observed in the iron (III) hydroxide polymaltose treatment(B). No tubular congestion was observed in any of the groups. H&E. 100X. Arrows point to (G) glomeruli, (BC) Bowman's Capsules, (BS) Bowman's Space, (PCT) Proximal Convoluted Tubules, (DCT) Distal Convoluted Tubules, (RT) Renal Tubules.

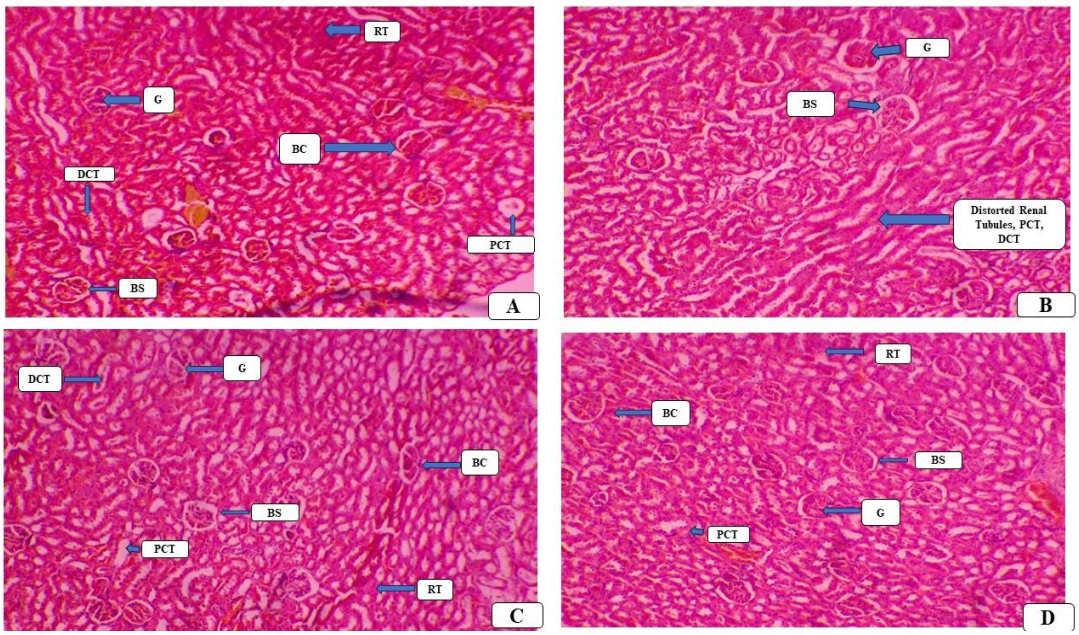

Fig. 2. Kidney histology following the administration of treatments

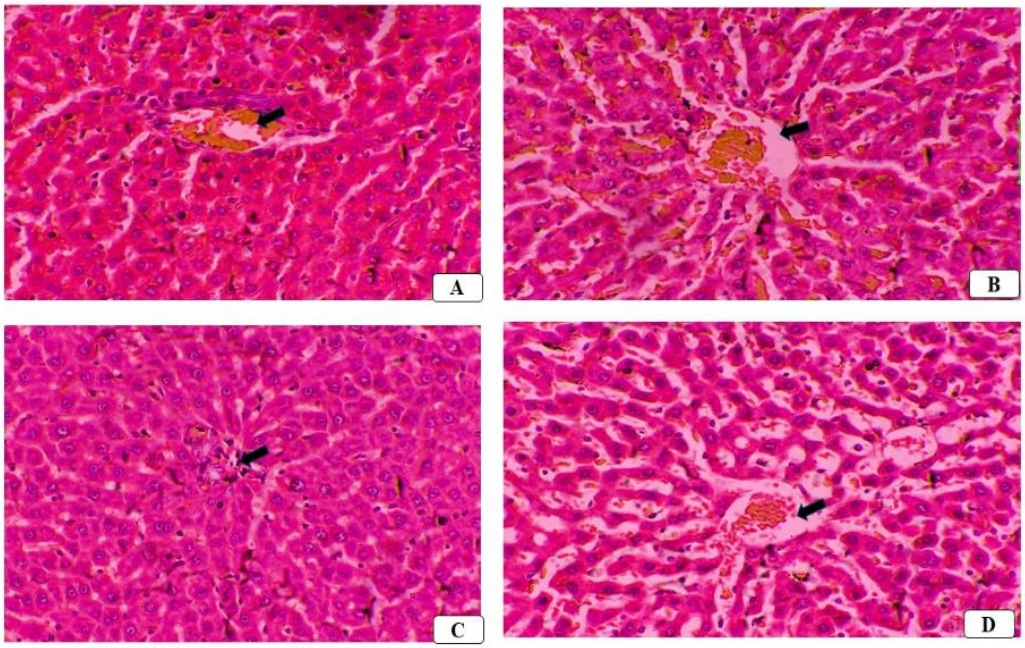

Fig. 3. Liver histology following the administration of treatments

3.8 Liver Histology

Fig. 2 shows the histopathological examination of the liver following four weeks of treatment administration. Micrographs from the liver (Fig.2) showed a normal histological structure for the Normal saline (A), Iron (III) hydroxide polymaltose (B), young *Moringa oleifera* (C), *Moringa oleifera* (D) treatments. The central vein indicated by an arrow, hepatic cords, and sinusoids were undeformed. No fatty infiltration of hepatic cells and vacuolar degeneration were seen.

4. DISCUSSION

4.1 Phytochemicals

Phytochemicals, also known as bioactive compounds, are non-nutritive plant chemicals with the capacity to exert physiological effects. Phytochemical constituents identified in the ethanolic extracts of both young and old *Moringa oleifera* leaves in the current study agrees with a study by [10]. An intriguing finding of this study was the presence of alkaloids and tannins found in the young leaves of *Moringa oleifera* which was absent in the old *Moringa oleifera* extract this is in accordance with earlier studies by [11].

The findings of the qualitative phytochemical screening support a study which hypothesis that the phytochemical content and antioxidant activity of fresh *Moringa oleifera* leaves are influenced by the age of the leaves and the extraction solvent used, as well as their interaction [12]. In their study, they reported that old (aged 45 days) *Moringa oleifera* leaves are best suited to produce extracts with the most potent antioxidant activity, this assertion contradicts the findings of this study as the young *Moringa oleifera* leaves had more phytochemicals. The disparity between the finding of this present study and that of [12] could be attributed to the type of menstruum used in the extraction process. The presence of these Phytochemicals could account for the much-touted medicinal properties of these leaves in various disease conditions management.

4.2 Feed Intake

The highest feed intake recorded under OMoE and YMoE treatments in the present study corroborates with a study by [13] who reported that mice treated with 900mg/kg of the *moringa* extract showed an increase in body weight as a result of high feed intake when compared with the controls, the authors attributed this significant increase to the fact that *Moringa oleifera* has the potency to increase feed intake even though, their work did not indicate the type of *Moringa oleifera* leaves that was used in the extract preparation [13], [14].

The observed significant feed intake in the rats could give an indication that the taste of *Moringa oleifera* leaves extract was not objectionable to the experimental rats [14]. This could be the causal factor of the observed high feed intake levels from improved palatability. The extent of feed digestibility is linked to low fibre content, *Moringa oleifera* leaves have a relatively low fibre content [15]. The observed low fiber content could also be the reason for the observed relative increase in feed intake among the treatment that received old and young *Moringa oleifera* extract when compared with those administered with normal saline and Iron (III) hydroxide polymaltose. The finding on the feed intake also suggests that both old and young leaves of *Moringa oleifera* have similar effects on daily feed intake.

The observed reduction in feed intake in week four among the treatments could be attributed to reduced lusciousness that may have resulted from prolonged administration of OMoE and YMoE. Similar results were obtained in an experiment by [16] who reported that high (75% and 100% of Moringa foliage) and prolonged administration of Moringa in diet may result in reduced feed intake and weight as Moringa is thought to comprise various anti-nutritional compounds such as phytase, tannins, cyanide, and oxalates, which may alter the digestibility, metabolism, and absorption of nutrients [16]. It is interesting to note that the observed insignificant feed intake in week four could imply that prolonged intake of *Moringa oleifera* extract might be effective in weight management, as reduced feed intake could invariably result in reduced weight gain.

4.3 Body Weight

The present study revealed that the effect of the administration 100 mg/kg/bw of ethanolic leaf extract of young and old *Moringa oleifera*, Iron (III) hydroxide polymaltose on body weight had a significant effect on days 21 and 28 when compared with the normal saline treatment. The observed increase in body weight could be ascribed to increased feed intake that was observed among the moringa-treated groups. This finding also follows a similar report by [17]. The observed improvement in body weight gain following administration of young and old *Moringa oleifera* may be attributed to the rich content of nutrients in the old leaves of moringa which was efficiently metabolized for growth.

Previous studies have reported on the weight gain effect of *Moringa oleifera.* Interestingly, none of these studies have indicated the leaf type, which was used, however, the finding from these studies have revealed that both young and old leaves of *Moringa oleifera* possesses similar effect of body weight [18]–[21].

A study by [22] and [23] have reported a contrast result to that of the findings of this study. Their study found no significant increase in body weight following the administration *Moringa oleifera* extracts regardless of the dosage, when compared to the control group [23], [22]. The observation suggests that the administered extract did not induce significant changes in the metabolic processes of the animals under study, which may have potentially influenced their hormonal regulation and body weight. Moreso, the findings of this investigation exhibit notable disparity when compared to the outcomes reported by [24]. The results of their study showed a decrease in body weight among female rats who were administered *Moringa oleifera* extract while being fed a high-fat diet. The reduction in body weight seen in their study was attributed to the suppression of cholesterol deposition in body tissues or the inhibition of 3-Hydroxy-3-Methyl-Glutaryl-CoenzymeA (HMG CoA) reductase activity. HMG CoA reductase is a crucial regulatory enzyme in the biosynthetic pathway of cholesterol [25]. The relevance of this finding suggests that administration of Ethanolic leaf extract of young and old *Moringa oleifera* 100 mg /bw/ day may be a viable recommendation for individuals seeking to achieve weight loss goals. Nevertheless, the researchers also noted that the administration of *Moringa oleifera* extract did not yield a statistically significant impact on the body weight of male rats. This observation may suggest that the extract possesses potential utility in the context of weight management [26].

4.4 Haematological Indices

The results of this study are in line with that of [22], who found that irrespective of the dose administered, aqueous extract of *Moringa oleifera* did not have significant effect on Red Blood Cells, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, as well White Blood Cells, Haemoglobin and Platelet of the test animals compared with the control.

The observed significant increase in haemoglobin concentration by the old and young *Moringa oleifera* treatments suggests that *Moringa oleifera* leaves may contain an appreciable amount of iron and also facilitate its absorption as an adequate amount of this element is necessary for Haemoglobin synthesis and for animal tissues such as the kidneys and bones to take part in the production of RBCs this is similar to an earlier report by [27].

Improved haemoglobin levels can enhance oxygen-carrying capacity, which can possibly benefit individuals with conditions such as iron-deficiency anaemia. This finding agrees with earlier studies [28].

Again, the reported presence of phytochemical constituents in the extract and also the presence of minerals and vitamins may have contributed to the significant increase in haemoglobin concentration in the rats that were administered with young and old *Moringa oleifera* extracts.

The bone marrow's ability to produce blood is directly influenced by these constituents, which are known as haemopoietic factors [29]. Studies have also indicated that *Moringa oleifera* also contains various micronutrients such as iron, zinc, copper, calcium, manganese, magnesium, potassium, sodium [30] sulfur, vitamin E, beta carotene, thiamine, riboflavin, niacin, pyroxide, biotin, ascorbic acid, cholecalciferol, selenium, tocopherol and vitamin K [31], [15], [32]. There is a potential for ethanol used as an extraction solvent in the study to have extracted most of these chemicals, hence potentially influencing the observed effects on the haematological parameters.

These compounds have played an essential part in the formation and maturity of the body's immune systems, particularly in relation to the cellular components involved in hemopoiesis. The essentiality of micronutrients found in *Moringa oleifera* leaves lies in their role in facilitating the growth, differentiation, and proliferation of cells within the immune system.

The amino acids in *Moringa oleifera* are significant in the process of globin production, which plays a crucial role in the synthesis of haemoglobin [33] Iron, a trace metal found in *Moringa oleifera*, plays a crucial role in the synthesis of haemoglobin, a protein found in red blood cells as reported by [34].

4.5 Liver Function Test

The observed insignificant levels of the liver biomarkers suggest that both young and old *Moringa oleifera* extract did not cause any hepatocellular damage this is in agreement with several studies [35]–[37] that reported that *Moringa oleifera* extract has demonstrated a potential protective effect against liver damage caused by hepatotoxic substances such as carbon tetrachloride and acetaminophen. Its administration has been associated with a reduction in the concentrations of liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase, which serve as indicators of hepatic injury. More so, the findings from this study also suggest that administration of *Moringa oleifera* at low dose 100 mg/bw/kg is safe and it did not cause any significant change in the liver enzymes. This is in concordance with a study by [38].

The observed effect of both young and old *Moringa oleifera* extract on liver enzymes could be as result of phytochemicals such as triterpenoids, alkaloids, glycoside, flavonoids, and saponins detected in the leaves through the qualitative analysis. The collected data is consistent with [39].

4.6 Kidney Function Test

In this study, results show that administration of 100 mg/bw/kg of old and young *Moringa oleifera* extract did not have significant effect on serum urea levels when compared with rats that received a similar dose of iron (III) hydroxide polymaltose and normal saline this finding corroborates with the findings of [40]. Urea is a significant nitrogenous compound that serves as the primary result of the degradation of proteins and amino acids. Elevated blood urea nitrogen (BUN) levels have been observed in conjunction with renal diseases, obstruction of the urinary system caused by a renal calculus, congestive heart failure, desiccation, pyrexia, shock, and gastrointestinal haemorrhage [41]. In comparison with the findings indicated by, a similar finding was observed as the administration of old and young *Moringa oleifera* extract did not have any detrimental effect on the renal function of the experimental rats. This finding is in line with a study who have reported that *Moringa oleifera* has been shown to be non-toxic and has been shown to promote nephron-hepatic function [42].

The observed decrease in Creatinine levels in the YMoE and OMoE groups, as compared to the Iron (III) hydroxide polymaltose group, suggests the possibility of moringa having protective effects on renal function. Reduced levels of creatinine are frequently correlated with enhanced renal function, hence indicating possible therapeutic significance. This observation may imply a beneficial influence of *Moringa oleifera* extracts on renal health. This is in agreement with a study by [42].

4.7 Kidney and Liver Histology

The young and old *Moringa oleifera* treatments (plates C and D) displayed a kidney section that showed a histological structure indicative of normalcy. This finding implies that the administration of these extracts at 100 mg/bw/kg did not result in any structural abnormalities or detrimental effects on the kidney tissues, suggesting the possible protective effects of young and old *Moringa oleifera* extracts on kidney function. This conclusion is very similar to the findings reported by [43], who suggested that *Moringa oleifera* has the potential to have beneficial effects on the liver and kidneys by repairing the damage that Monosodium glutamate causes to these organs, as demonstrated by observations made in rats. However, it was observed that the iron (III) hydroxide polymaltose treatment had a widespread deterioration of the renal epithelium. This finding elicits concerns over the possible detrimental impacts of prolonged usage of synthetic Iron (III) hydroxide polymaltose on the integrity of renal tissue [43].

The liver structures were undeformed, this is indicative that both old and young *Moringa oleifera* exhibited a similar effect when compared with the normal saline and, iron (III) hydroxide polymaltose treatments. This affirms the already established claim that *Moringa oleifera* is nontoxic and has hepatoprotective effect. This finding correlates with a report by a study which stated that consumption of *Moringa oleifera* daily as an herb and spice should be encouraged as it has no detrimental effect on liver function and histology [44]. A study has reported that in as much as *Moringa oleifera* may have a beneficial effect on liver function and histology, they have cautioned that high doses (1000 and 2000 mg/kg) of moringa extract caused a deleterious effect on the liver, kidney, and the brain on Wistar rats [45]. Therefore, moderate to low doses of *Moringa oleifera* given via oral administration may be safe and may not have cytotoxic effects on the brain, liver, or kidneys.

5. CONCLUSIONS

These findings collectively suggest that both young and old *Moringa oleifera* leaf extracts have relatively mild effects on the parameters studied in this experimental model, with some similarities to the effects of iron (III) hydroxide polymaltose. The findings from this study are indicative that both young and old leaves of moringa have similar hematopoietic effects, with no detrimental effect on kidney, liver functions as well as liver and kidney histology. Additional research should aim to isolate, identify, and quantify the exact phytochemical constituents present in both young and old leaves of *Moringa oleifera*, to elucidate their respective impacts on body weight, blood parameters, renal and liver histology.

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ETHICAL CONSIDERATION

This research followed The Guide for the Care and Use of Laboratory Animals by the National Institutes of Health in the United States (NIH Publication No. 85-23). Written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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