Journal of Applied Sciences

ISSN 1812-5654

OPEN ACCESS
Journal of Applied Sciences
ISSN 1812-5654
DOI: 10.3923/jas.2022.233.240

Research Article

Intake of *Moringa oleifera* Leaf Powder Positive Biochemical and Haematologic Effects in Albino Rabbits

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Abstract

Background and Objective: *Moringa oleifera* is a widely acclaimed 'miracle plant' whose medicinal and nutritional importance require documentation. The effect of *Moringa oleifera* leaf powder (MOLP) intake on haematologic, hepatic and renal function indices was studied using standard methods. **Materials and Methods:** Twenty-four Albino rabbits, weighing 0.9-1.8 kg, were distributed into group I animals fed the control diet and groups II-IV fed 500, 750 and 1000 mg kg^{-1} b.wt., of MOLP, respectively for 28 days. Feed intake and body weights of the animals were measured daily. **Results:** Groups fed 500 and 750 mg kg^{-1} of MOLP consumed more of the feed and showed a non-significant (p>0.05) but a more progressive increase in mean body weights. Intake of MOLP did not significantly (p>0.05) alter most of the biochemical and hematologic indices studied but serum total protein, albumin and haemoglobin concentrations increased significantly (p<0.05), while red blood cell indices increased non-significantly (p>0.05) with the increase in MOLP dosage. Generally, 500 and 750 mg kg^{-1} MOLP diets gave better positive indices than 1000 mg kg^{-1} MOLP. **Conclusion:** Apparently, MOLP intake has no harmful effects on the liver, kidney and blood parameters of animals but may enhance the building capacity, repair and maintenance of body tissues. However, the study calls for caution in the use of very high doses.

Key words: Drum stick, liver function, kidney function, haemoglobin, diet fortification

Citation: Igwe, C.U., C.C. Ezerioha, C.I. Iheme, D.I. Ukairo, I.O. Onyeocha and V.A. Onwuliri, 2022. Intake of *Moringa oleifera* leaf powder positive biochemical and haematologic effects in Albino rabbits. J. Appl. Sci., 22: 233-240.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Moringa oleifera, commonly known as drum stick, is the most widely cultivated species of the Moraceae family. It is indigenous to South Asia and was chiefly grown as an ornamental plant, on fence hedges and along roadsides on the coastal plains and lower foothills. It is a rapidly growing tree that is now widely cultivated in many locations in the tropics including Nigeria[1,2].

Moringa is a slender softwood that branches freely. It is an extremely fast-growing tree that reaches a height of over 10 m (33 ft) and a diameter of 20-40 cm at chest height. The stem is normally straight but occasionally poorly formed with extended branches that grow in an organized manner. The canopy is umbrella-shaped. The leaves are feathery with green to dark green elliptical leaflets that are 1-2 cm long[3]. The plant bears conspicuous, lightly fragrant flowers that are generally white to cream coloured, 2.5 cm in diameter and are borne in sprays. The flowers are produced profusely in auxiliary, drooping panicles 10-25 cm long[1]. The fruits of *M. oleifera* commonly known as pods are trilobed and capsular. The mature fruits of the plant are brownish while the immature ones are greenish but could have reddish colouration in some varieties. The pods when fully matured and dry usually release their seed content by splitting lengthwise[4]. While seed kernels with low viable seeds appear whitish, mature viable seeds are commonly dark and round with brown to the black coloured hull and can germinate within 14 days. On the hull of each seed are extensions of 3 white wing-like structures that run the entire length of the seed[5].

Moringa oleifera tree is grown mainly for human and animal food. It has high nutritional value and an impressive range of other uses. In addition to its compelling water purifying powers[6]. *Moringa oleifera* has been used for biomass, biogas, gum, pulp, dye and biopesticides production as well as a domestic cleaning agent and juice-clarifier[7]. Various parts of the plant have been reported to act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, anti-diabetic, antibacterial and antifungal activities[8] as well as anti-sickling[9] and anti-poison[10] effects.

M. oleifera plant is generally known in the developing world as a vegetable, a medicinal plant and a source of vegetable oil[1,11]. It is commonly described as a miracle plant and thus traditionally recommended and widely consumed by many families, especially among the rural and semi-urban dwellers. Therefore, the present study was designed to investigate the effect of intake of *M. oleifera* leaf powder supplemented diet on the haematologic indices, liver and kidney organ function parameters of albino rabbits.

MATERIALS AND METHODS

Plant sample collection and processing: Fresh leaves of *Moringa oleifera* were sourced in January, 2020 from a farm in Umuawulu town, Awka South Local Government Area of the State of Anambra in Nigeria. A plant taxonomist, Prof. J.C. Obiefuna of the School of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Nigeria authenticated the leaf samples. Leaves plucked from the stem stalks were washed in clean running water and air-dried at room temperature to a constant weight. The dried leaves were milled into powder form with a mechanical blender. The *M. oleifera* leaf powder (MOLP) was preserved in an airtight container, labelled and stored in a refrigerator at 4°C until required.

Experimental animals: Twenty-four healthy male Albino rabbits aged 3-6 months and weighing 0.9-1.8 kg were obtained from laboratory animal breeder, Emil Veterinary Services located in Owerri, Nigeria. They were housed in stainless steel cages and allowed an acclimatization period of 2 weeks under standard laboratory conditions. The animals were fed freely with feed pellets sourced from Guinea Feed Nigeria Ltd.® and water.

At the end of the acclimatization period, the rabbits were weighed and then distributed into 4 groups. Each group consisted of six rabbits with all the groups having comparably equal weight ranges. Group I - IV animals were every morning fed 0, 500, 750 and 1000 mg of MOLP per kg body weight per day, respectively for 28 days. After each day's treatment period, all the animals per group were allowed free access to 100 g of the control diet and water *ad libitum*. The feed intake of the animals was measured daily, while their body weights were taken at 4 days intervals.

Federal University of Technology Owerri ethical committee approved the study protocol before the commencement of the study. The animals were humanely handled and treated in line with the Principles of Laboratory Animal Care (NIH publication, 1985 to 1993, revised, 1985).

Collection of blood sample: On the 28th day of the experimental period, feed and water were withdrawn overnight from the animal groups. Then, each animal was humanely exposed to diethyl ether vapour for aesthesis and approximately 10 mL of blood sample was immediately collected from the animals via cardiac puncture. Five millilitres of the blood samples were each gently dispensed into heparin and EDTA bottles. The blood samples were thoroughly but gently mixed. The heparin and EDTA anticoagulated blood were each centrifuged at 3000 rpm for 5 min and the plasma was separated into fresh labelled bottles for determination of the biochemical and haematological parameters, respectively.

Liver function profile: The activities of plasma aspartate and alanine aminotransferases (AST and ALT) were assayed with the aid of AST and ALT kits (Randox Laboratories Ltd., UK)[12]. Assay of alkaline phosphatase (ALP) activity and determination of total protein, albumin and total bilirubin concentrations was carried out by colourimetric methods[13] with the aid of commercially available reagent kits from Teco Diagnostics, Ca, the USA and Randox Laboratories Ltd., UK, respectively.

Kidney function profile: Blood urea nitrogen and creatinine concentrations were, respectively determined using colourimetric methods based on Berthelot urease and Jaffe reactions[13] with the aid of Randox urea and creatinine reagent kits (Randox Laboratories Ltd., UK). Blood serum potassium, sodium and chloride concentrations were also determined using colourimetric methods[13] with the aid of proprietary reagent kits (Teco Diagnostics Ca, USA).

Haematological analysis: The haematological parameters assessed include haemoglobin concentration and packed cell volume as well as the blood cells counts (red cell count, white cell count and platelet count) and the red blood cell indices involving mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and mean corpuscular volume. These were analysed with aid of a haematology autoanalyzer (Mindray BC 2300, USA). The autoanalyzer is based on non-cyanide haemoglobin and direct current detection methods for measurement of haemoglobin concentration and blood cell count, respectively. The red blood cell indices are automatically computed by the instrument from the values of haemoglobin concentration, packed cell volume and red blood cell count.

Statistical analysis: The data obtained were expressed as Mean±Standard deviation. One-way Analysis of Variance (ANOVA) ran on statistical software (GraphPad Prism version 5.3, USA) was used to analyze the data, while the comparison of the means was achieved with the aid of Turkey *post hoc* test of the same software. The limit of statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Moringa oleifera, popularly known as drum stick tree, possesses high pharmacologic and therapeutic values. It has been suggested that its consumption in regular diets could be used to reduce the risk of many degenerative diseases[14,15]. Thus, the plant has been widely investigated for the treatment/management of many human and animal ailments. Therefore, the present study investigated the possible toxic or beneficial effects of regular intake of the plant's leaf on haematologic, hepatic and renal function indices of animals.

Data obtained from this investigation presented in Fig. 1 showed that animals fed 500 mg kg^{-1} body weight of MOLP progressively had the highest mean fed intake (86.67±10.31 g) followed by the animals on 750 mg kg^{-1} of MOLP (79.33±21.31 g) from baseline feed intake values of 50.50±11.12 g and 53.13±8.48 g, respectively when compared with the other animal groups. The animals fed 1000 mg kg^{-1} MOLP and the control group had the lowest mean feed intake values at 70.25±5.65 and 72.00±33.21 g, respectively, while their baseline values were 54.55±3.55 and 51.80±27.33 g. This implies a higher consumption rate of the feed by the animals fed 500 and 750 mg kg^{-1} MOLP within the treatment days. This observation indicates a possible appetite enhancing potential of the plant leaf which could be attributable to the plant's vitamins and mineral contents. However, the observation was not consistent with the intake of the highest dose (1000 mg kg^{-1}) of MOLP which showed an initial rise and then a progressive decrease in the mean fed intake within the treatment days. This could be due to the possible negative effect of the MOLP diet at high doses. *M. oleifera* like most plants contain tannins, oxalates, phytates and other anti-nutrient secondary metabolites which at high doses may directly influence diet intake and nutrient utilization. Anti-nutrients are naturally occurring substances that could bind to mineral nutrients forming indigestible complexes. High levels of oxalates decrease copper availability to the body by binding with copper to form insoluble salts and also block the absorption of soluble calcium salts, while high levels of tannins cause decreased animal consumption and utilization of feed by forming indigestible protein complexes[16].

Progressive positive increases in body weights of all the *M. oleifera* fed groups were observed which peaked at 15.3, 10.7 and 7.0 g for 500, 750 and 1000 mg kg^{-1} MOLP groups, respectively in Fig. 2. The body weight changes of the 1000 mg kg^{-1} MOLP group were comparable to those of the control group, which peaked at 6.9 g. The observed higher increases in body weights of the 500 and 750 mg kg^{-1} MOLP fed animal groups could be attributed to their higher feed

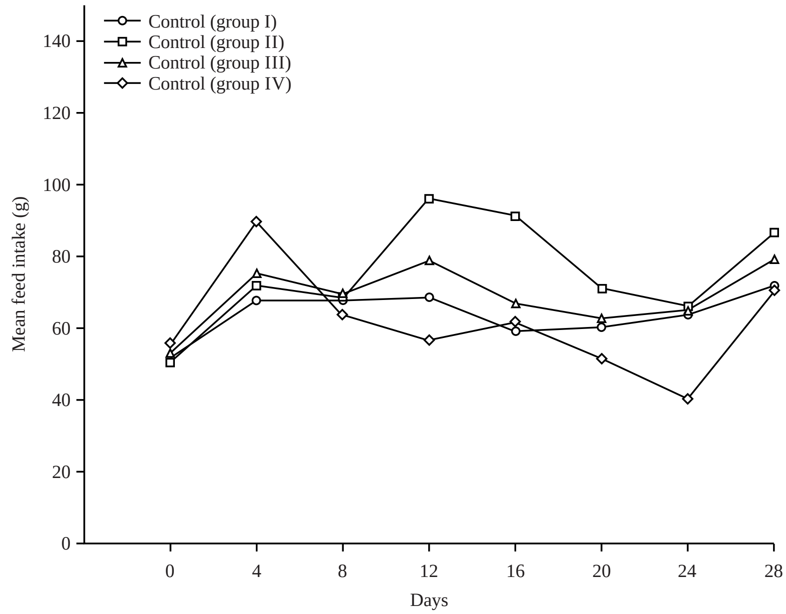

Fig. 1: Mean feed intake (g) of Albino rabbits fed 500 mg kg^{-1} (group II), 750 mg kg^{-1} (group III) and 1000 mg kg^{-1} (group IV) of *Moringa oleifera* leaf powder

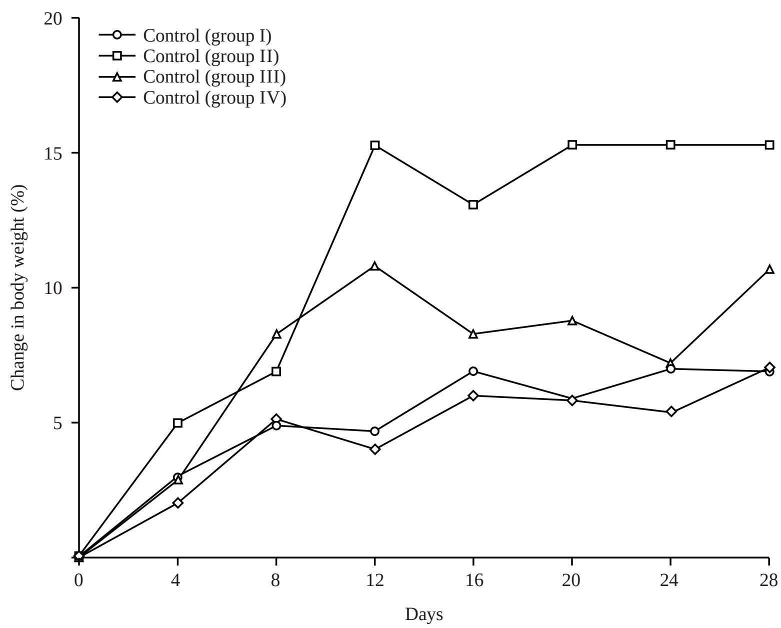

Fig. 2: Percentage change in mean body weights of Albino rabbits fed 500 mg kg^{-1} (group II), 750 mg kg^{-1} (group III) and 1000 mg kg^{-1} (group IV) of *Moringa oleifera* leaf powder

intake and in extension to the reported high nutritious value of the plant[10,17]. Furthermore, it has also been reported that *M. oleifera* contains large amounts of proteins, fibre, lipids, carbohydrates and fatty acids which must have contributed to the observed increase in body weights of these animals[18]. As noted earlier, the group fed 1000 mg kg^{-1} of MOLP showed a lower percentage increase in body weight. This could be as a result of their reduced consumption of feed, which could in turn be due to environmental and physiological factors associated with the animals or most probably due to the negative effect of the MOLP diet at high doses. As stated earlier, *M. oleifera* like most plants contains naturally occurring anti-nutrient secondary metabolites which at high doses may directly influence diet intake and nutrient utilization, slow growth rates and decrease palatability of feed[18].

Table 1 shows the effect of MOLP intake on liver function parameters of the animal groups. MOLP intake at 500 and 750 mg kg^{-1} body weight did not significantly (p>0.05) affect ALT (35.33±5.84 and 44.67±4.78 IU L^{-1}) AST (25.00±8.29 and 34.00±5.02 IU L^{-1}) and ALP (70.86±5.45 and 74.32±7.13 IU L^{-1}) activities when compared with those of the control animals (37.00±5.22, 32.33±9.74 and 72.54±6.08 IU L^{-1}), respectively. However, MOLP intake at 1000 mg kg^{-1} body weight elicited a significant (p<0.05) increase in only ALT activity (56.00±6.00 IU L^{-1}). ALT, AST and ALP are liver function biomarker enzymes that are commonly used to assess liver organ damage because they are naturally localized within the hepatocytes and/or liver canaliculi. Thus, significant increases in their activities in the blood indicate a disruption in liver function, damage to liver cells and/or liver cholestasis[10]. The observed significant increase in ALT activity of the 1000 mg kg^{-1} MOLP animal group may be attributed to possible damage to hepatocytes as a consequence of the high Moringa leaf intake. The significant increase in ALT activity in the face of normal activities of AST and ALP further confirms the higher sensitivity of ALT to liver tissue damage compared to AST and ALP. On the other hand, the non-significant changes observed with both AST and ALP activities, portray the isolated increase in the result of ALT as possibly inconsequential[19], but calls for caution in high dose intake of Moringa leaf extract.

Results of the present study also showed a non-significant (p>0.05) increase in the total bilirubin concentrations (7.10±2.72, 7.00±1.72 and 9.75±1.85 mmol L^{-1}, respectively) of the MOLP fed animal groups with an increase in MOLP dose in comparison with the control group (6.67±2.06 mmol L^{-1}). Similarly, the administration of the MOLP feeds elicited increases in total protein and albumin concentrations of the animals, with the only significant (p<0.05) value of total protein observed being in the 750 mg kg^{-1} MOLP fed group (84.33±6.94 mmol L^{-1}). Serum bilirubin, total protein and albumin concentrations are measured for supplementary information towards an assessment of liver damage as markers of liver excretory and synthetic functions. Thus, marrying the observed results of bilirubin, total protein and albumin with those of the earlier discussed liver enzymes, goes to confirm that intake of *M. oleifera* leaf-based diet may not be deleterious but rather may enhance the excretory and synthetic functional capacity of the liver cells[20].

Table 2 showed that the urea and creatinine concentrations of the animals fed 500 mg kg^{-1} (7.27±1.78 and 88.00±7.15 mmol L^{-1}) and 750 mg kg^{-1} (6.95±0.50 and 90.00±7.00 mmol L^{-1}) of MOLP generally decreased, although non-significantly (p>0.05) in comparison with those of the control animals (8.20±0.88 and 98.33±5.25 mmol L^{-1}) and the 1000 mg kg^{-1} MOLP fed group (11.53±1.69 and 119.00 ±12.68 mmol L^{-1}). Urea and creatinine are excretory products of the kidney. An increase in their blood concentrations is indicative of kidney dysfunction which points to an inability of the organ to clear them from the blood[21]. Meanwhile, the urea and creatinine concentrations of the animal group fed 1000 mg kg^{-1} MOLP were higher than those of the other groups. This observation, in conjunction with the earlier discussed effect of 1000 mg kg^{-1} MOLP on the animals' feed intake, body weight changes and ALT activity, further suggest a potential negative effect of intake of high doses of the plant's leaves. However, the observed general

Table 1: Liver function profile of Albino rabbits fed *Moringa oleifera* leaf powder

Parameters	Group I (Control)	Group II (500 mg kg^{-1})	Group III (750 mg kg^{-1})	Group IV (1000 mg kg^{-1})
ALT (IU L^{-1})	37.00±5.22^{a}	35.33±5.84^{a}	44.67±4.78^{ab}	56.00±6.00^{b}
AST (IU L^{-1})	32.33±9.74^{a}	25.00±8.29^{a}	34.00±5.02^{a}	34.01±3.98^{a}
ALP (IU L^{-1})	72.54±6.08^{a}	70.86±5.45^{a}	74.32±7.13^{a}	75.91±6.12^{a}
TB (mmol L^{-1})	6.67±2.06^{a}	7.10±2.72^{a}	7.00±1.72^{a}	9.75±1.85^{a}
TP (mmol L^{-1})	66.67±2.56^{a}	64.21±4.75^{a}	84.33±6.94^{b}	70.50±3.56^{ab}
ALB (mmol L^{-1})	49.00±2.03^{a}	50.33±1.41^{a}	56.00±4.55^{a}	49.50±3.35^{a}

Values are Mean±Standard deviation, values with different superscripts per row are statistically significant (p<0.05), ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, TB: Total bilirubin, TP: Total protein and ALB: Albumin

Table 2: Kidney function profile of Albino rabbits fed *Moringa oleifera* leaf powder

Parameters (mmol L^{-1})	Group I (Control)	Group II (500 mg kg^{-1})	Group III (750 mg kg^{-1})	Group IV (1000 mg kg^{-1})
Urea	8.20±0.88^{ab}	7.27±1.78^{ab}	6.95±0.50^{b}	11.53±1.69^{a}
Creatinine	98.33±5.25^{ab}	88.00±7.15^{a}	90.00±7.00^{a}	119.00±12.68^{b}
Chloride	111.33±3.86^{a}	108.33±236^{a}	111.00±3.00^{a}	114.6±1.69^{a}
Potassium	8.30±1.34^{a}	6.33±0.61^{a}	7.15±0.45^{a}	8.07±0.65^{a}
Sodium	140.67±6.13^{a}	145.33±4.78^{a}	145.50±2.50^{a}	148.67±5.44^{a}

Values are Mean±Standard deviation, values with different superscripts per row are statistically significant (p<0.05)

Table 3: Haematologic profile of Albino rabbits fed *Moringa oleifera* leaf powder

Parameters	Group I (Control)	Group II (500 mg kg^{-1})	Group III (750 mg kg^{-1})	Group IV (1000 mg kg^{-1})
PCV (%)	26.30±1.30[a]	40.80±3.04[b]	36.50±3.65[b]	26.80±1.20[a]
HGB (g dL^{-1})	8.27±2.01[a]	13.35±0.74[b]	11.93±1.53[b]	9.33±0.45[a]
PLT (×10^3 μL^{-1})	466.33±213.06[ab]	279.00±56.00[b]	483.00±20.02[ab]	709.33±117.12[a]
RBC (×10^3 μL^{-1})	4.34±2.15[a]	6.14±0.06[a]	5.48±1.05[a]	4.52±0.25[a]
WBC (×10^3 μ L^{-1})	8.40±5.29[a]	10.70±1.66[a]	11.10±5.96[a]	10.00±0.40[a]
MCV (fl)	66.93±7.64[a]	66.77±5.64[a]	67.80±6.48[a]	66.60±2.50[a]
MCH (pg)	19.13±1.27[a]	21.67±1.75[a]	20.97±1.93[a]	19.75±0.95[a]
MCHC (g dL^{-1})	28.87±2.75[a]	32.5±1.04[a]	39.93±1.31[a]	30.75±0.15[a]

Values are Mean±Standard deviation, values with different superscripts per row are statistically significant (p<0.05), PCV: Packed cell volume, HGB: Haemoglobin concentration, PLT: Platelet count, RBC: Red blood cell count, WBC: White blood cell count, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration and MCV: Mean corpuscular volume

positive effect of the plant leaves on kidney function substantiates the reported diuretic potential of the plant in ethnomedical practice[15,22].

Table 2 also showed that there were non-significant (p>0.05) changes in plasma chloride, sodium and potassium concentrations of the MOLP fed animal groups in comparison with the control group. These observations are in tandem with a previously reported no significant decrease in serum electrolytes after intake of aqueous extract of *M. oleifera* leaves[23]. Alterations in serum chloride and sodium ion concentrations have been associated with dietary changes. Chloride is excreted together with sodium ions in urine and sweat. Conservation of serum sodium depends on its content in the extracellular fluid (ECF) and blood volume. Changes in serum sodium concentration are attributable to increased intake, decreased sodium loss, water imbalance and excretion. Similarly, changes in serum potassium may occur due to increased intake, gastrointestinal or urinary loss, increased cellular uptake in stool or intake of large doses of diuretics[24]. The animals fed 500 and 750 mg kg^{-1} of MOLP showed significantly (p<0.05) higher packed cell volume (PCV, 40.80±3.04 and 36.50±3.65%) and haemoglobin concentration (HGB, 13.35±0.74 and 11.93±1.53 g dL^{-1}) than the control (26.30±1.30% and 8.27±2.01 g dL^{-1}) and the 1000 mg kg^{-1} MOLP fed (26.80±1.20% and 9.33±0.45 g dL^{-1}) groups, respectively in Table 3. HGB is the oxygen carrier macromolecule of the blood, while low blood PCV value is indicative of anaemia, haemorrhage or bone marrow failure. *Moringa oleifera* contains many minerals and vitamins especially zinc, iron and vitamin C[17]. Zinc and vitamin C assist in protecting and boosting the haematologic indices of the blood as well as the immune system[25], while the iron is required for HGB formation. Thus, the observed significant increases in PCV and HGB could be attributed to the reported high contents of minerals, vitamins and amino acids in *M. oleifera*[9,26].

The platelet count of the MOLP fed animals increased progressively (p<0.05) with the increase in MOLP doses from 279.00±56.00 through 483.00±20.02 to 709.33±117.12×10^3 μL^{-1} for the 500, 750 and 1000 mg kg^{-1} MOLP fed groups, respectively in Table 3. *Moringa oleifera* leaves contain phytosterols and phenolic compounds[15], of which have been associated with the modulation of platelet count[27]. The presence of these molecules could have elicited the observed increase in platelet count which was significantly (p<0.05) highest in the animal group fed 1000 mg kg^{-1} of MOLP. Platelets, also called thrombocytes are the components of blood involved in blood clot formation with the clotting factors. Excessive elevation in blood platelet count could be detrimental to the normal liquidity and flow of blood[22]. The later comment further buttresses the need for caution in the intake of high doses of *M. oleifera* based diets.

Table 3 also showed non-significant (p>0.05) increases in the Red Blood Cell (RBC) and White Blood Cell (WBC) counts as well as the RBC indices [Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)] of the animal groups. RBCs are unique cells of the blood that circulate HGB for oxygenation of all body cells, while the red cell indices are used to mathematically define the concentration and distribution of HGB in individual RBCs. On the other hand, WBCs are components of the immune system. They are involved in protecting the body against both infectious diseases and foreign antigens. The observed positive outcome in the RBC and WBC results of the *M. oleifera* fed groups could be due to the reported high content of zinc, iron and vitamin C[17], as well as the earlier discussed increase in HGB concentration of the animals.

CONCLUSION

The study has shown that the intake of *Moringa oleifera* leaves does not have a potentially harmful effect on the tissue cells of the liver, kidney and blood but may enhance the building capacity, repair and maintenance of body tissues. Thus, the study confirms the folkloric recommendation of the plant as a nutritious vegetable for humans and their livestock. However, high dose intake of the plant leaves could have an inhibitory effect on feed intake and potential on nutrient utilization, which may translate to a reduction in body weight gain of animals and possibly blood haemoglobin lowering effect.

SIGNIFICANCE STATEMENT

This study discovered that intake of *Moringa oleifera* leaves does not have a potentially harmful effect on the tissue cells of the liver, kidney and the blood, rather it could enhance the building capacity, repair and maintenance of body tissues. The findings can be beneficial to people with reduced access to nutritious food to enhance their food quality. The study will help nutritionists in the exploration and development of policies for possible fortification of low-quality foods and thus help the growing population of the world achieve food security.

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