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Histopathological Evaluation of Spleen, Liver and Kidneys from Pigs Fed on *Moringa oleifera* Leaf Meal Diets

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Abstract: *Moringa oleifera* leaves and seeds have for a long time been used as food and medicine. Despite increased usage, little information is available on its application as a nutraceutical agent as well as toxicity. The objective of this study was to determine the histopathological consequences of prolonged inclusion at varying levels of *Moringa oleifera* leaf meal (MOLM) diets on the spleen, liver and kidneys of growing pigs. Twenty four growing pigs were selected and assigned to four treatment diets (T) containing; 0% (T1), 3% (T2), 6% (T3) and 12% (T4) MOLM concentrations. Each treatment had two replicates of 3 pigs and the experimental period lasted 7 weeks after which four pigs from each treatment were selected, sacrificed and the spleen, liver and kidney samples collected for histopathological analysis. The results revealed that increased MOLM in the diet (>3%) led to the enlargement of splenic follicles (white pulp) as well as capsular and parenchymal fibroses. In the liver, increased MOLM in the diet led to loss of lobular architecture with damaged cellular outlines, dilation of sinusoidal spaces, vascular congestion and occasional nuclear changes in hepatocytes leading to hepatocytic necrosis and distortion of the portal triad. In the kidneys, higher levels of MOLM led to glomerulonephritis essentially presenting as glomerular oedema leading to reduced Bowman's space. In the renal tubules, there appeared to be protein casts in the tubular lumen. In conclusion, prolonged inclusion of MOLM (>3%) in the diet negatively affected the histoarchitecture of the spleen, liver and the kidneys and may, in extreme circumstances, result in reduced animal performance.

Key words: Hepatotoxic · Flavonoids · Renal Toxicity · Saponins · Spleen

INTRODUCTION

Increasing feed costs in livestock industry has led to the adoption of diverse feed ingredients by farmers in order to improve the animal feed conversion efficiencies as well as cutting down the costs of production [1]. *Moringa oleifera* (MO) is a plant in the family Moringaceae and, due to its adaptation to a wide variety of soils and climatic conditions and its rich nutritional value; it has been used for numerous purposes, for example, human food, animal feeds and medicinal purposes [2-6]. The plant parts such as leaves, seeds, roots and fruits have been extensively used as feed ingredients to enhance growth performance or to substitute, to some extent, some protein sources such as soybean meal, sunflower and cotton seed cake [7-9].

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Furthermore, in spite of the recorded higher growth rates in MO based diets in majority of the animal nutrition studies, depressed feed intake and reduced growth rate associated with high levels of MO in the diet have similarly been documented [8]. The decreased performance in animals under high moringa levels in diet may be due to high levels of Phytochemicals such as alkaloids, flavonoids, saponins and tannins [10]. This implies therefore that MO has phytochemicals that influence either positively or negatively the physiological functioning of the body systems through modes of action that have not been conclusively established.

Histopathology can be used to assess the physiological performance of animals especially those under test diets. The main organs involved in detoxification are the liver and kidneys and their tissue structure when subjected to test diets may be affected. Spleen, on the other hand, is involved in haemopoietic and immune functions and would also give an indication on how the diet affects the two important body functions. Aqueous extract of *Moringa oleifera* seed, if consumed at higher quantities (Greater that 800mg/kg), negatively affect the normal histological appearance of the spleen causing mild and moderate expansion of white pulp leading to a decreases in WBC count and platelet concentration in rats [11].

At moderate doses, MO leaf extract ingestion has been found safe for the renal tissues and have shown nephroprotective effects in cases of drug administration [12-15]. However, other studies have established that moringa leaf consumption at higher doses or chronic use could predispose animals to hepatic and kidney damage and may result in renal failure [16, 17].

In spite the increased use of MO plant in animal feeds and research, there is no consensus on the optimal levels of inclusion in animal diets and, even so, their effects on the spleen, liver and kidney morphology remain conflicting. This study was therefore designed to determine the effects of prolonged inclusion of different levels of MO plant leaves in the pig's diet on the spleen, liver and kidney structures in growing pigs.

MATERIALS AND METHODS

Study Site: This study was conducted at the University of Nairobi, College of Agriculture and Veterinary Sciences, Nairobi County. The area receives an average of 869mm annual rainfall with average daily temperature of 19°C.

Animals and Experimental Design: This study was conducted in accordance with the University of Nairobi Faculty of Veterinary Medicine Biosafety, Research Animal Use and Ethics guidelines. Twenty four (24) large white growing pigs (2.5 months old) were selected and assigned to four dietary treatments (T): 0% (T1), 3% (T2), 6% (T3), and 12% (T4) MOLM, each with 2 replicates of 3 pigs in a concrete floor housing system according to the guidelines of Reese *et al.* [18].

Treatment Diets: Growing pig diets were formulated using the NRC guidelines [19] using maize meal, wheat pollard and vegetable oil as energy sources while MOLM, cotton seed meal, sun flower meal, fish meal and soybean meal as protein sources. Vitamin mineral premix, Di calcium phosphate and limestone were also included as vitamin and mineral sources.

The feeds raw materials were sourced from a reliable local feed manufacturer and preliminary laboratory tests done to confirm their proximate nutritional composition.

Moringa oleifera leaves: *Moringa oleifera* leaves were obtained from the University of Nairobi field station, Kibwezi which is an arid land. MO is a plant in the family Moringaceae, Genus Moringa and species *Moringa oleifera* Lam.

Feed Intake and Weight Gains: Feed were weighed each morning and fed in 3 portions to minimize feed wastage. At the end of the day, feed left in the troughs were weighed for the calculation of average daily feed intakes [20]. Water was provided *ad libitum*.

Housing and Management: Pigs were housed in groups of three each with a space of $6m^2$ concrete floor system. These housing were well lit and ventilated and no environmental modifications such as ambient temperature regulations were used. Concrete floor feed and water troughs were used with high levels of hygiene standards maintained. Pig houses were cleaned twice daily, in the morning and evening, together with the feed troughs to ensure that fresh feed were available to the pigs on each day.

All the mixed feeds and the raw materials were stored in a dry, well ventilated, slatted raised floor system storage facility within the pig experimental premises.

General Body Examination and Weighing of the Animals: At the start of the experiment, each pig was examined and weighed as recommended by Whittington *et al*. [19] followed by weekly weighing for a total of 7 weeks. Furthermore, each pig was monitored closely throughout the experiment period so that in the unlikely event of injuries and disease, there was a timely response in terms of treatment and management of the health conditions.

Sample Collection and Histopathological Analysis: At the end of the experiment period, feed was withdrawn for 12 hours; pigs were weighed and physically examined for signs of disease or toxicity. Four pigs per treatment were randomly selected and slaughtered as per the recommended guidelines [21]. After manual evisceration, visceral organs (Spleen, liver and kidneys) from the selected pigs were weighed and thereafter, 50 grams tissue from each organ was picked and processed for histopathological analysis. In brief, small pieces of each organ were Fixed by immersion in phosphate buffered formalin (10%) and routinely processed (Ethanol dehydration) to serial paraffin sections (7 µm thick) which were then stained with haematoxylin and eosin. The stained sections were examined and photographed using a Leica ® DM 500 light microscope. Photographs of the tissue sections from the treatment groups were analyzed for any changes by comparing to the control group. Analysis of tissues was done using qualitative methods with emphasis laid on histomorphology and general histoarchitecture.

RESULTS

The general pig body condition across the treatments was good and no animal mortality was observed during this study. The starting pig weights averaged 26kg while the final weights were 67.3kg (T1), 66.3kg (T2), 65.1kg (T3) and 64.9 (T4). With the advancement in the experiment time, there appeared to be a reduction in the rates of weight gains in pigs with highest levels of *Moringa oleifera* in the diet. However, final weights for the different treatment groups did not differ significantly (P>0.05). The liver, spleen and kidney weights for T1, T2, T3 and T4 groups did not differ significantly (P>0.05) Table (1).

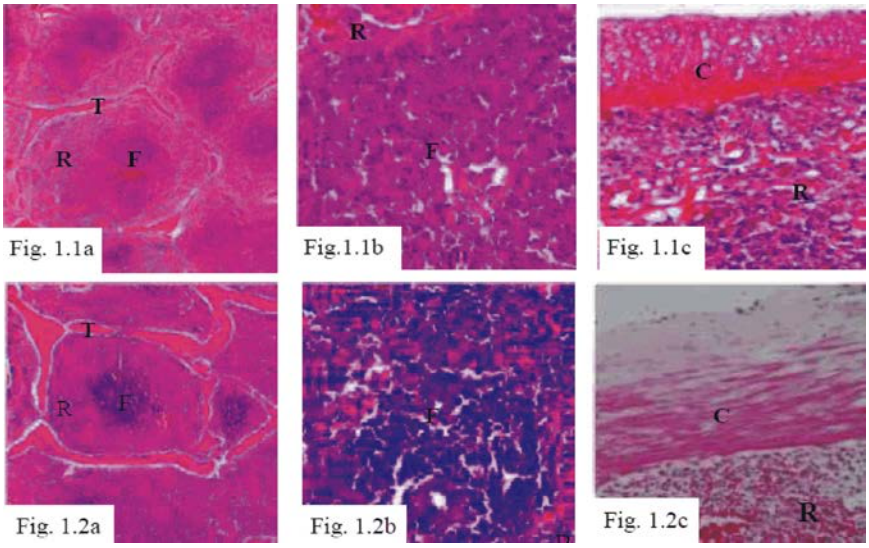
Effects of MOLM on the Spleen: Spleen from control T1 and T2 experimental group had normal histoarchitecture The white pulp contained lymphoid follicles embedded within the red pulp and trabecular extensions of the capsule extended into the tissues Figs (1.1a and 1.2a). The follicles appeared as nodular aggregations of lymphocytes surrounded by the red pulp Figs (1.1b and 1.2b). The splenic parenchyma was enveloped by an extensive capsule Figs (1.1c and 1.2c). Spleen from T3 group, on the other hand, showed red pulp atrophy characterized by reduced cellularity Fig (1.3a). This atrophy was further aggravated in T4 Fig (1.4a), leaving prominent white pulp in both cases. Red pulp atrophy was accompanied by white pulp fibrosis in T3 Fig (1.3b) culminating into mild focal hyperplasia in T4 Fig (1.4b). The degeneration of parenchyma was further accompanied by parenchyma and white pulp fibroses Figs (1.3c and 1.4c).

Effects of MOLM on the liver: The liver in controls (T1) showed normal lobular architecture conspicuously outlined by the surrounding interlobular septae Fig (2.1a). In T2, the liver also appeared normal with normal lobular architecture and cellular outlines Fig (2.2a) though the sinusoids showed mild dilatation around the central veins. In the T3 and T4 groups, the liver maintained normal lobular outline as in T2 but with apparent loss of lobular architecture Figs (2.3a and 2.4a) characterized by hepatic degeneration presenting as damaged cellular outlines, nuclear pyknosis in hepatocytes and leukocytic infiltration Figs (2.3b and 2.4b). In addition, there was sinusoidal congestion. These changes were comparatively more amplified in T4 group leading to hepatic necrosis. In both cases, there was vascular congestion around central veins Figs (2.3c and 2.4c).

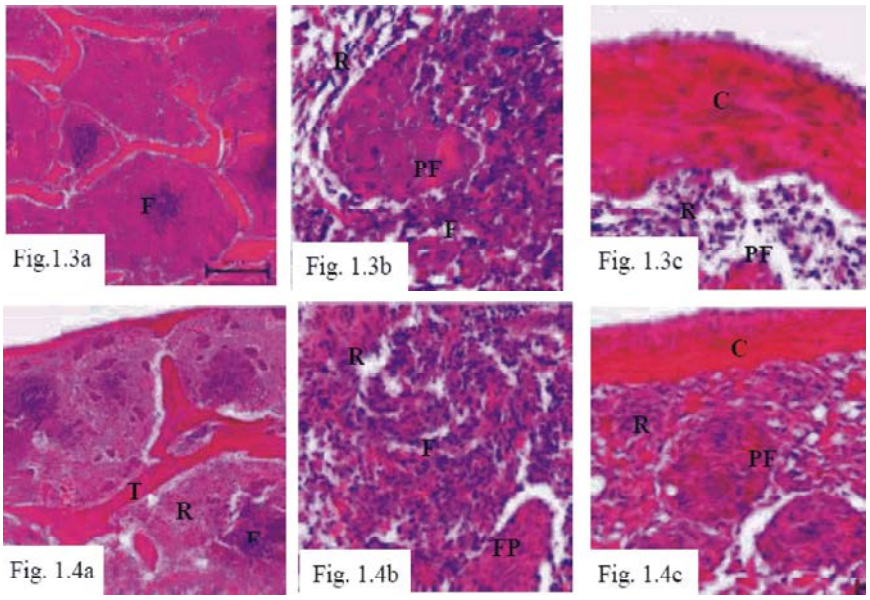
Table 1: Average liver, spleen and kidney weights (grams) from pigs under MOLM diets (±Standard deviation) (n=16)

	Dietary treatments (#MOLM inclusion levels, %)				
Organ	T1 (0%MOLM)	T2 (3%MOLM)	T3 (6%MOLM)	T4 (12%MOLM)	P Value
Liver (grams)	1294.2±212.3	1589.0±127.9	1627.5±101.1	1653.8±98.7	0.52
Spleen (grams)	107.0±25.1	164.7±17.0	133.3±16.7	108.5±19.1	0.31
Kidney (grams)	165.2±51.7	229.1±41.9	187.7±42.2	299.3±42.2	0.28

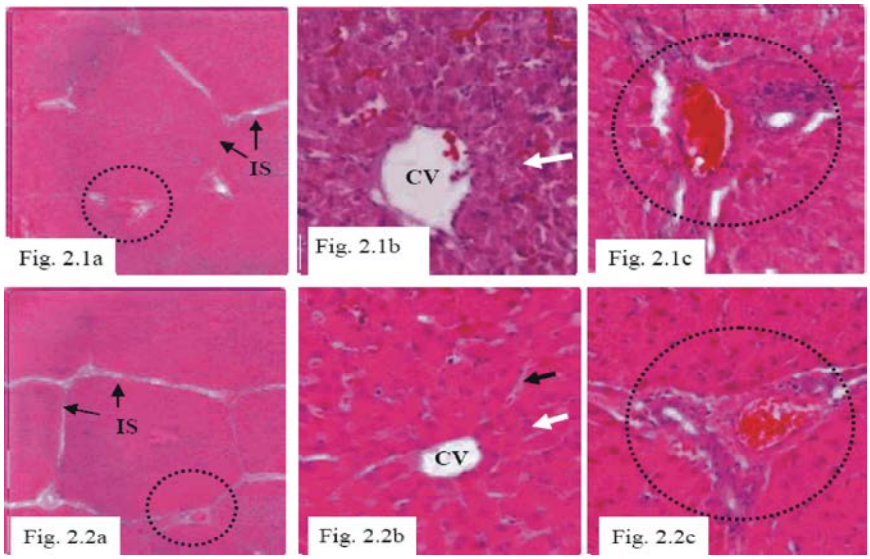
#MOLM: *Moringa oleifera* leaf meal, T=treatment



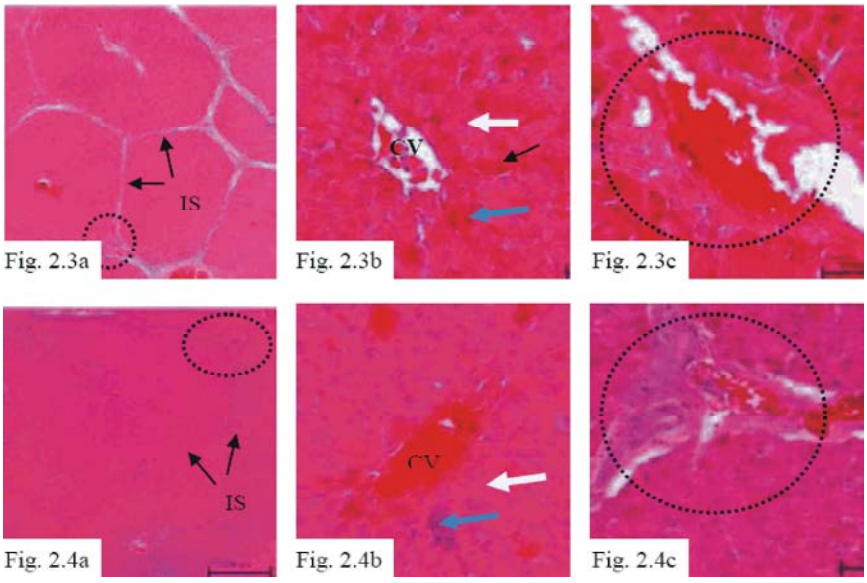
Figs. 1.1a-c: and 1.2a-c: Show sections of the spleen parenchyma in control group (T1) and T2 respectively. In both cases the red pulp (R), white pulp (F), trabecular (T) extensions of the capsule into the tissue appear normal (Figs. 1.1a and 1.2a). The white pulp (F) appears as nodular aggregations of lymphocytes surrounded by red pulp (R) (Figs. 1.1b, 1.2b). Underneath, the capsule (C) lays normal red pulp (R) tissue (Figs. 1.1 c, 1.2c). Bars- (Figs. 1.1a and 1.2a) =200µm, (Figs. 1.1b-c and 1.2b-c) = 20=µm



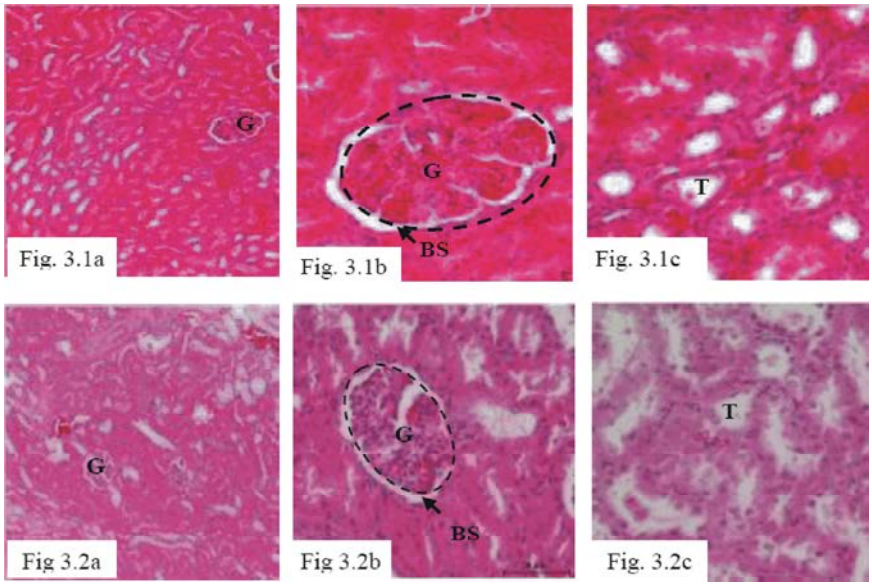
Figs. 1.3a-c and 1.4a-c: Show sections through splenic parenchyma in T3 and T4 treatment groups respectively exhibiting various degrees of structural alterations. Fig. 1.3a demonstrate red pulp atrophy (R) characterized by reduced cellularity (Fig. 1.3a). This was further aggravated in T4 (Fig. 1.4a) and in both cases, this atrophy left prominent white pulp (F). Trabeculae (T) extended into the parenchyma from the capsule and appear expanded. Accompanying the red pulp atrophy were the white pulp (F) atrophy in T3 (Fig. 1.3b) culminating as white pulp hyperplasia in T4 (Fig. 1.4b). In both cases, capsular (C) and parenchyma fibrosis (PF) were common features (Figs. 1.3b-c and Figs. 1.4b-c). Bars- (Figs. 1.1a and 1.2a) =200µm, (Figs. 1.1b-c and 1.2b-c) = 20µm.



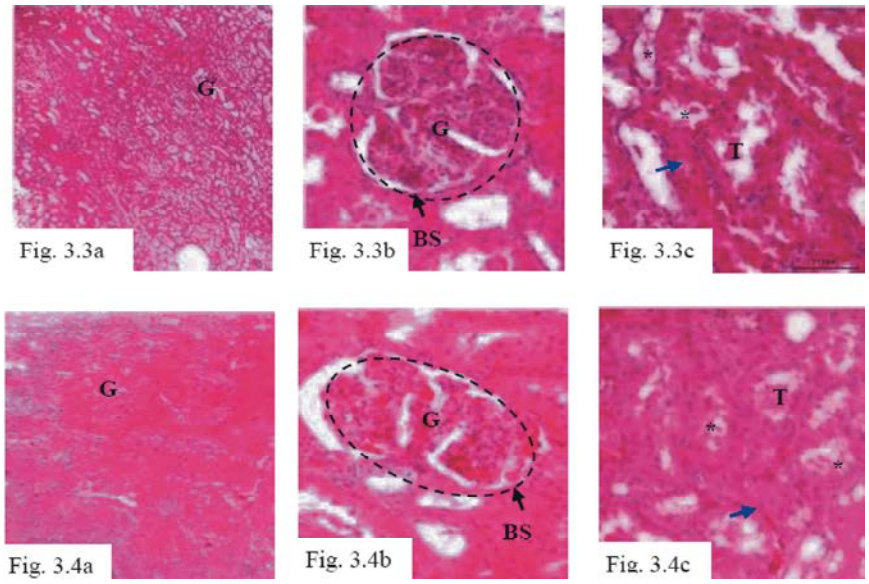
Figs. 2.1a-c and 2.2a-c: Are sections through liver parenchyma of control (T1) and T2 treated groups respectively. The lobules show normal architecture and are conspicuously demarcated by the interlobular septae (IS) (Figs. 2.1a and 2.2a). Hepatic triads are clearly demonstrated (hatched circle) and hepatocytes have normal cellular outlines (white block arrows) but the sinusoids appear mildly dilated (black block arrows) around the central veins (CV) (Figs. 2.2b). In both cases, vascular congestion was evident hatched circle (Figs. 2.1c and 2.2c). Bars- (Figs. 2.1a and 2.2a) =200µm, (Figs. 2.1b-c and 2.2b-c) =20µm.



Figs. 2.3a-c and 2.4a-c: Show sections through the liver parenchyma of pigs in groups T3 and T4 respectively. The liver lobules in T3 (Figs. 2.3a) and T4 (Figs. 2.4a) show normal outline but with apparent loss of lobular architecture. There was also apparent hepatocytic degeneration characterized by nuclear pyknosis (white block arrow) and leukocyte infiltration (blue block arrow) (Figs. 2.3b and 2.4b). These changes were more aggravated in T4 group compared to T3. Accompanying these changes was sinusoidal congestion (black slender arrow). Vascular congestion around the hepatictriad (hatched circles) was also evident (Figs. 2.3c and 2.4c). IS=interlobular septae, CV=central vein, Bars- (Fig. 2.3a and 2.4a) =200µm, (Figs. 2.3b-c, 2.4b-c) =20µm.



Figs. 3.1a-c and 3.2a-c: Demonstrate sections through kidney parenchyma of groups T1 and T2 pigs respectively, showing normal architecture with glomeruli (G) delineated by Bowman's capsule circumscribing the normal Bowman's space (BS) (Figs. 3.1b and 3.2b). The tubules (T) appeared normal (Figs. 3.1c and 3.2c). G=Glomerulus, BS=Bowman's space. Bars- (Figs. 3.1a and 3.2a) =200µm, (Figs. 3.1 b-c and 3.2b-c) = 20µm



Figs. 3.3a-c and 3.4a-c: Sections through the kidneys of T3 and T4 showing extra capillary proliferative glomerulonephritis (hatched circle) presented as oedema and congestion of glomeruli (G) with mononuclear infiltration (Figs. 3.3b and 3.4b). In T4, there was progression of the inflammatory reaction into extr-capillary necrotizing glomerulonephritis (hatched circle) appearing as sharply demarcated sub capsular necrosis, oedema and severe hydropic glomerular degeneration (Fig. 3.4b). Tubular degeneration accompanied by cystic dilation (T) proteinaceous material filling the lumen (asterisk), perivascular oedema and congestion (blue arrow) were evident (Figs. 3.3c and 3.4c). G=glomerulus, BS=Bowman's space. Bars- (Figs. 3.3a and 3.4a) =200µm, (Figs. 3.3b-c and 3.4b-c) = 20µm

Effects of MOLM on the kidney: The kidneys of controls (T1) and T2 groups displayed normal architecture Figs. (3.1a-c and 3.2a-c). The glomerulus appeared normal; clearly delineated by the Bowman's capsule as well as the parietal squamous cell epithelium, circumscribed by a normal Bowman's space and the glomerular tuft appearing lobular. In T3, the kidney showed extra capillary proliferative glomerulonephritis presented in the form of oedema and congestion of the glomerulus with mononuclear cell infiltration Fig. (3.3 b). Kidneys of T4 group showed extra capillary necrotizing glomerulonephritis Fig. (3.4b). There were sharply demarcated sub-capsular necrotic areas with inflammatory cells surrounded by oedema and severe glomerular degeneration. Renal tubules had cystic dilatations and the tubular lumina were completely obliterated and filled with fluid. Protein casts were also observed in the lumina of tubules and the tubules seemed to be surrounded by cell debris Figs. (3.3c and 3.4c).

DISCUSSION

Throughout the experimental period, no pig mortalities were recorded and the overall final pig weights did not vary significantly. The spleen, liver and kidney weights also did not vary significantly with the increase in the MOLM concentration in the diet.

Spleen contains hematopoietic and lymphoid elements; hence it is a primary site for extramedullary haematopoiesis. Spleen also removes degenerate, aged red blood cells, particulate materials and circulating bacteria from the blood circulation [22]. Results from this study show that MOLM in higher concentrations resulted in alterations in splenic histoarchitecture characterized by parenchymal and capsular fibrosis, reduced cellularity, white pulp hyperplasia and degenerated red pulp. Parenchymal fibrosis has been reported in previous studies, mainly in association with inflammatory, toxic, or neoplastic lesions of the spleen [22]. In this case parenchyma fibroses may have resulted from toxicity arising from the phytochemicals such as tannins and flavonoids present in MO leaves [23, 10]. It is therefore plausible to suggest that higher concentrations of MOLM in the diet led to accumulation of tannins, flavonoids and other phenolic compounds in the animal's body systems and may have resulted in the inflammatory reactions in the spleen. This is supported by the findings of Dike and Luteino [11] who reported that, sections of the rat's spleen treated with 800mg/kg and 1600mg/kg aqueous extract of *Moringa oleifera*, showed mild and moderate expansion of white pulp. Consequently, it is logical to suggest therefore that MOLM in the pig diets should be included at lower levels to avoid negative effects on the spleen which could ultimately impair its performance.

Liver on the other hand is an accessory organ of the digestive system and plays a key role in blood detoxification. This means therefore that if the diet had some toxic elements, the first organ to be affected is the liver. In controls (T1) and the lower levels of MOLM (T2) the normal histological structures of the liver were retained. At higher concentrations of MOLM in the diet (T3 and T4), it appeared MOLM had some mild toxic effects since the normal hepatic outline appeared lost and central veins appeared congested. Hepatic necrosis was also noted as a sign of hepatitis. Hepatic reactions at increased levels of MOLM may have been due to the accumulation of tannins, flavonoids and other phenolic compounds in the liver. These results confirm previous reports which suggested that, lower levels of MO had no negative effects in the rat liver [17]. However, chronic use could predispose animals to hepatic and kidney damage [16]. Indeed, this latter suggestion seems to have vindicated the result of high level use of MOLM in this study since high levels of tannins, flavonoids and phenolic compounds are known to have serious effects on the liver structure and function.

Kidneys play an important role in excretion mainly through ultra-filtration and if affected negatively by any dietary compound, the detoxification process may be compromised and subsequently, the normal functioning of the body may as well be affected [12]. In this study, higher levels of MOLM in the diet negatively affected the glomeruli, renal tubules and renal cells. These results seems to contradict those of Ezejindu *et al.* [15] where it was reported that *Moringa oleifera* extracts did not have a negative effect on the renal histoarchitecture. This may be attributed to the lower dosages and short duration of administration of *Moringa oleifera* extracts in their study as opposed to this study where the duration was relatively longer. Oedema noted in the glomeruli may have resulted from the obstruction of ultra-filtration process caused by the phenolic compounds found in MOLM. Furthermore, these compounds in MOLM diets may also have had a negative effect on the renal cells hence the observed marked nephritis in the highest concentration of MOLM in the diet.

CONCLUSIONS

In conclusion, MOLM can be included in the diet of pigs up to a level of 3% after which it will exert toxicological effects on the spleen, liver and the kidneys.

However, further studies need to be conducted to ascertain the exact nature of phytochemicals in the MO leaves associated with the histopathological changes observed in these organs.

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REFERENCES

1. FAO, 2002. Protein sources for The animal feed industry. Expert Consultation and Workshop. 29 April-3 May 2002. Bangkok.
2. Choudhury, S. and M.P. Sinha, 2015. Comparative Studies of *Moringa oleifera* and *Murraya koenigii* Leaf Extracts as a Nutraceutical and a Potent Antibacterial Agent. Advances in Biological Research, 9(2): 103-108.
3. Richter, N., P. Siddhuraju and K. Becker, 2003. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (Oreochromis niloticus L.). Aquaculture, 217(1): 599-611.
4. Sanchez, S., Ledin and I. Ledin, 2006. Biomass production and chemical composition of *Moringa oleifera* under different management regimes in Nicaragua. Agroforestry Systems, 66(3): 231-242.
5. Popoola, J.O. and O.O. Obembe, 2013. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. Journal of Ethnopharmacology, 150(2): 682-691.
6. Iqbal, M.A., A. Iqbal, N. Akbar, H.Z. Khan and R.N. Abbas, 2015. A Study on Feed Stuffs Role in Enhancing the Productivity of Milch Animals in Pakistan- Existing Scenario and Future Prospect. Global Veterinaria, 14(1): 23-33.
7. Nuhu, F., 2010. Effect of Moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. Masters thesis. Kwame Nkurumah University of Science and Technology, Ghana.
8. Gakuya, D.W., P.N. Mbugua, B. Kavoi and S.G. Kiama, 2014. Effect of Supplementation of *Moringa oleifera* Leaf Meal in Broiler Chicken Feed. International Journal of Poultry Science, 13(4): 208-213.
9. Mukumbo, F.E., V. Maphosa, A. Hugo, T.T Nkukwana, T.P. Mabusela and V. Muchenje, 2014. Effect of *Moringa oleifera* leaf meal on finisher pig growth performance, meat quality, shelf life and fatty acid composition of pork. South African Journal of Animal Science, 44(4): 388-400.
10. Okiki, P.A., I.A. Osibote, O. Balogun, B.E. Oyinloye, O. Idris, A. Olufunke, S.O. Asoso and P.T. Olagbemide, 2015. Evaluation of Proximate, Minerals, Vitamins and Phytochemical Composition of *Moringa oleifera* Lam. Cultivated in Ado Ekiti, Nigeria. Advances in Biological Research, 9(6): 436-443.
11. Dike, E.C. and L.H. Luteino, 2015. Effect of aqueous extract of *Moringa oleifera* seed on haematological parameters and the spleen in male albino rats. IOSR Journal of Dental and Medical Sciences, 14(4): 35-41.
12. Paliwal, R., V. Sharma, S.S. Pracheta, S. Yadav and S.H. Sharma, 2011. Anti-nephrotoxic effect of administration of *Moringa oleifera* Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice, Biology and Medicine, 3(2): 27-35.
13. Awodele, O., I.A. Oreagba., S. Odoma., J.A.T. da Silva and V.O. Osunkalu, 2012. Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). Journal of Ethnopharmacology, 139(2): 330-336.
14. Ouédraogo, M., A. Lamien-Sanou, N. Ramdé, A.S. Ouédraogo, M. Ouédraogo, S.P. Zongo, O. Goumbri., P. Duez and P.I. Guissou, 2013. Protective effect of *Moringa oleifera* leaves against gentamicin-induced nephrotoxicity in rabbits. Experimental and Toxicologic Pathology, 65(3): 335-339.
15. Ezejindu, D.N., O.O. Udemezue and A.J. Akingboye, 2014. Protective effects of *Moringa oleifera* leaf extract on mercury induced renotoxicity in adult Wistar rats. International Journals of Scientific Research Publications, 4(3): 1-4.
16. Oyagbemi, A.A., T.O. Omobowale, I.O. Azeez, J.O. Abiola, R.A.M. Adedokun and H.O. Nottidge, 2013. Toxicological evaluations of methanolic extract of *Moringa oleifera* leaves in liver and kidney of male Wistar rats. Journal of Basic and Clinical Physiology and Pharmacology, 24(4): 307-312.
17. Owolabi, J.O. and P.O. Ogunnaike, 2014. Histological evaluation of the effects of Moringa leaf extract treatment on vital organs of murine models. Merit Research Journal of Medicine and Medical Sciences, 2(10): 245-257.

18. Reese, D.E., K.M. Eskridge and W.W. Stroupe, 2010. How to conduct on-farm pig experiments. Lincoln, EC270. http:// extensionpublications.unl.edu/ assets/pdf/ec270.pdf.
19. NRC, 2012. Nutrient Requirements of Swine. The National Academies Press. 11th ed. Washington D.C.
20. Whittington, D.L., C.M. Nyachoti, J.F. Patience, H.W. Gonyou, R. Zijlstra and S.P. Lemay, 2003. Feed intake - a checklist of nutritional, environmental and management strategies to achieve success. The Pig Site, Manitoba. http:// www.thepigsite.com/ articles/ 915/feed-intake-a-checklist-of-nutritional-environmental-and-management-strategies-to-achieve-success/
21. Ray, F.K, 2004. Pork Carcass evaluation and procedures. Division of Agricultural Sciences and Natural Resources. Oklahoma State University. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-2044/ANSI-3725web.pdf.
22. Suttie, A.W, 2006. Histopathology of the spleen. Toxicol. Pathol, 34(5): 466-503.
23. El Hilaly, J., Z.H. Israili and B. Lyoussi, 2004. Acute and chronic toxicological studies of Ajuga iva in experimental animals. Journal of Ethnopharmacology, 91(1): 43-50.