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Haematological, blood biochemical constituents and histopathological responses of growing rabbits fed different levels of moringa leaves

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Abstract: This study was conducted to investigate the effect of different supplemental levels of *Moringa oleifera* leaves (ML) on haematological profile, blood biochemical constituents and histopathological changes of growing rabbits. Thirty six male New Zealand White (NZW) rabbits aged 4-5 weeks with initial average body weight of 566.5 g were randomly blocked by weight into four treatment groups (9 rabbits each). Rabbits of each group were individually housed and fed ad libitum on a basal diet supplemented with 0, 0.15, 0.30 and 0.45 % ML for a feeding period of 56 days. At the end of the experimental period three representative rabbits from each group were slaughtered for blood and histological examinations. The results showed that all haematological and blood plasma biochemical parameters were within the normal range, however significant ($P<0.05$) differences were recorded among treatment groups for red and white blood cells count and haemoglobin (Hb) concentration, where the highest values were recorded for rabbits fed 0.3 % ML. Differential leukocytes (%) showed comparable values among groups. Blood plasma total protein, albumin and globulin concentrations were significantly ($P<0.05$) improved with increasing ML supplementation level, meanwhile, urea, alanine lipoprotein transaminase (ALT) and creatinine recorded the highest values with 0.45 % ML diet. Blood glucose was ($P<0.05$) decreased with 0.15 and 0.30% ML, while it was significantly increased with increasing level of moringa to 0.45%. Thyroid hormones (T_3 and T_4) were higher ($P< 0.05$) with 0.15 and 0.30% ML treatments than control, but. Microscopic examination of the red blood corpuscles showed clear deformation and agglutination with 0.45% ML treatment. Liver and kidney tissues showed obvious harmful damages with 0.45% ML diet. It's safe to conclude that, *Moringa oleifera* dry leaves showed positive physiological effects on growing rabbits when fed at 0.15 to 0.30% of the diet.
Keywords: *Moringa oleifera* dry leaves, rabbits, haematological profile, blood biochemical constituents and histopathological examination.

Introduction:

Feed additives are important materials that can improve the efficiency of feed utilization and animal performance. Using medicinal herbs and plants (MH&P) with humans has been known since the old civilization. Inversely many synthesized chemicals such as antibiotics and hormones caused many hazards to animals, plants and human. The World Health Organization (WHO, 2002)[1] encourages using MH&P to substitute or minimize the use of chemicals through the global trend to go back to nature.

Moringa oleifera lam is commonly named as the miracle tree or Horseradish tree, it has an impressive range of medicinal uses with high nutritive value throughout the world. Several biological properties ascribed to different parts of this tree, the leaves have been reported to be a valuable source of β-carotene, vitamins (B-complex, C, D and K) beside some important macro and micro-elements as calcium, potassium, zinc, iron, copper and selenium. The previous studies indicated that moringa leaves are free from anti-nutritional factors, e.g. phenols, tannins, saponins[2].

Several studies pointed to that moringa leaves are effectively prevent morphological changes and oxidative damage in human and animals by enhancing the inhibiting generation of free radicals and promoting the immune system against pathogens[3]. Moreover, [4]found that moringa dry leaves fed at 150-330 mg/kg body weight of rabbits could enhance growth performance, improve carcass traits and promote energy and protein utilization for growing rabbits .However, some drawback effects were noticed on rats and rabbits fed high dosage of moringa exceeded 200-300 mg/kg wt. and with increasing level 3-4 times severe organ damage, mostly liver and kidneys could occur[3].

This study was conducted to investigate the effect of different supplemental levels of moringa dry leaves on haematological profile, blood biochemical constituents and histopathological changes for growing rabbits.

Materials and Methods

Thirty six male New Zealand White (NZW) rabbits aged 4-5 weeks with initial average live weight of 566.5 g were randomly blocked by weight into four treatment groups (9 rabbits each). Rabbits of each group were individually housed in galvanized wire cages for 56 days where they were fed *ad lib* amounts on a uniform diet supplemented with 0, 0.15, 0.30 and 0.45 % *Moringa oleifera* dry leaves (ML) for treatments D1, D 2, D 3 and D 4, respectively. Four batches of rabbits diets were formulated of 30% alfalfa hay, 25% ground yellow corn, 25% wheat bran, 14% soybean meal (44%), 3% cane-molasses, 1.5% lime stone, 1% sodium chloride and 0.5% vitamin& mineral premix. A Moringa powder dry leaf was added and thoroughly hand mixed with other ingredients. Experimental diets were pelleted at 0.3 cm diameter and packed in polyethylene bags until feeding as described by[5]. At the end of the experimental period three representative rabbits from each group were slaughtered for blood and histological examinations.

Haematological study:

Blood samples for experimental rabbits groups were individually collected. Blood plasma was separated after adding EDTA and centrifuged at 4000 rpm for 15 minutes, then stored at – 20 °C until analysis. Whole blood samples were subjected to blood picture investigation. Red blood cells (RBC), white blood cells (WBC) and WBC differential were estimated according to[6]. Packed cell volume (PCV) was estimated using standard micro-hematocrit tubes for 5 minutes. Haemoglobin (Hb) was estimated according to[7].

Blood biochemical analysis:

All the biochemical constituents of blood plasma for experimental rabbits groups were measured calorimetrically used the specific kits using the Chemistry Auto-Analyzer (Olympus AU400). Total protein was estimated according to[8]. Albumin was determined using method of[9]. Globulin was calculated by subtracted albumin from total protein. Albumin: globulin ratio was calculated by divided the albumin on globulin value. Urea was determined using method of[10]. Cholesterol was estimated according to[11]. Glucose was determined according to[12]. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined applying the method of[13]. Creatinine was estimated according to[14]. Triiodotherionine (T_3) and Thyroxine (T_4) were determined using specific kits.

Histological study:

Tissue samples of liver and kidney were taken from slaughtered rabbits. Samples were fixed in 10% formalin-saline solution, taken the histological sections (4-5 microns) using paraffin technique. The sections were stained with haemotoxylline and eosin (H&E) stains. The sections were examined using light microscope, and photographed using digital camera.

Statistical analysis:

Collected data of the present study were subjected to statistical analysis as one way analysis of variance using SPSS (2008)[15]. The statistical model was as follow: $Y_{ij} = \mu + T_i + e_{ij}$ where μ: observation, μ: the overall

mean, T_i: effect of treatment, and e_{ij}: the experimental error. The significant differences between means were separated applying Duncan's Multiple Range Test[16].

Results and Discussion

Results in Table (1) indicate that growth performance of growing rabbits was improved with moringa supplemented diets, however with increasing the supplementation level over than 0.30% was associated by lower average daily gain even than control.

Hematological study:

Results of the experiment showed that all haematological (Table 2) and blood plasma biochemical parameters (Table 3) were generally within the normal range of rabbits, however significant (P<0.05) differences were recorded among treatments for particularly, red blood cells (RBCs), white blood cells (WBCs) count and haemoglobin (Hb) concentration, where the highest value of RBCs and Hb were recorded for rabbits fed 0.3 %, while the highest value of WBCs were recorded for 0.45% DL diet. The adverse levels of blood RBCs and Hb with increasing moringa leaves to 0.45% could be regarded to the poisonous effect of some phytochemical substances presented in moringa leaves and there extracts[4,3]. In this concern, [17]reported that using the methanolic extract of moringa leaves at 250-750 mg/kg in mice has noted an increase in circulating antibody titre and immunoglobulins. [18]found that 10-100 mg/kg of *Moringa oleifera* water extract (leaves and pod), appear to cause an increase in hemoglobin in mice following a single oral dose. [19]found that WBCs, RBCs,

Table 1: Mean daily intake and growth performance of rabbits fed different levels of moringa leaves.

Item	Experimental diets				SEM
	D1 (0% ML)	D2 (0.15% ML)	D3 (0.30% ML)	D4 (0.45% ML)	
Initial weight, g	560	573	571	562	24.07
Final weight, g	2024^{b}	2192^{a}	2219^{a}	1997^{c}	62.01
Average daily gain, g	26.14^{b}	28.91^{a}	29.43^{a}	25.63^{b}	1.15
Dry matter intake, g/h/d	148	146	155	144	2.74
Daily intake of moringa, mg/kg body wt.	0.0	150^{c}	329^{b}	485^{a}	15.62

Cited from El-Badawi *et al.* (2015). a, b, and c: Means in the same row having different superscripts are significantly different at P<0.05.

Table 2: Haematological values (Mean ± SE) of rabbits fed diets supplemented with different levels of moringa leaves.

Parameters	Experimental treatments				Normal range*
	T1,Control (0% ML)	T2 (0.15% ML)	T3 (0.30% ML)	T4 (0.45% ML)	
RBCs $x10^6$/ mm^3	5.96^{c} ±0.11	6.21^{b}±0.09	6.74^{a}±0.08	5.82^{c}±1.04	3.7-7.5
WBCs $x10^3$/ mm^3	6.80^{c} ±0.31	7.40^{b}±0.64	8.20^{a}±0.15	8.70^{a}±0.23	5.2-16.5
Hb, g/ dl	11.92^{d}±0.83	13.01^{b}±0.87	14.32^{a}±1.61	12.43^{c}±0.72	8.9-15.5
PCV, %	38.15^{d}±2.15	41.50^{b}±1.64	43.00^{a}±1.52	42.71^{a}±1.16	26.7-47.2
MCV, mm^3	64.00^{b}±0.53	66.81^{b}±0.39	63.80^{b}±0.61	73.36^{a}±0.87	50 -75
MCH, pg/ cell	20.00^{b}±0.41	21.01^{a}±0.51	21.25^{a}±0.44	21.35^{a}±0.60	19.2-29.5
MCHC, %	31.25^{b}±0.93	31.35^{b}±0.74	33.32^{a}±0.61	29.10^{c}±0.54	31.1-37.0
Differential leukocytes, %					
Lymphocytes	60.10±2.36	60.25±2.71	65.00±3.49	67.20±5.14	43-80
Neutrophils	35.12±1.05	35.20±1.67	30.01±1.26	28.00±1.21	34 -70
Monocytes	2.98±0.27	2.53±0.01	2.61±0.03	3.00±0.61	0 - 4
Basophils	0.37^{b}±0.05	0.12^{c}±0.01	0.68^{a}±0.03	0.10^{c}±0.01	0 - 0.84
Eosinophils	1.42^{b}±0.03	1.90^{a}±0.14	1.68^{a}±0.05	1.75^{a}±0.12	0 - 2

a, b, c and d: Means in the same row having different superscripts are significantly different at P<0.05.

*: Hewitt *et al.*,1989[33].

WBCs, RBCs, Hb and platelets (PLT) values of Albino rats were significantly (P<0.05) increased with addition of *Moringa oleifera* leaves extract at 100, 200 and 300 mg/kg body weight compared with control, but with the high level (300 mg) these values were decreased compared with 200 mg. They added that with growing rabbits values of RBCs, PLT and Packed cell volume % (PCV) were (P<0.01) increased with feeding 2.5 g/kg of body weight fresh moringa leaves compared with control. [20]reported that PCV, RBC, Hb, WBC, mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets and leukocyte differential counts for growing rabbits were not significantly different with moringa leaves supplemented at 0, 5, 10 and 15% of the diet. [21]noted that feeding growing rabbits on diets supplemented with 0% (control), 5, 10 and 15% *Moringa oleifera* leaf meal did not associated with any significant difference among groups for PCV, RBC, MCV, MCH, MCHC, WBC, lymphocytes, neutrophils. However significant effect among diets was only observed on Hb concentration and basophils and eosinophils contents.

The microscopic examination of red blood corpuscles for experimental animals presented in Figures (1a, 1b, 1c and 1d). showed normal red blood corpuscles shape with control diet (Fig. 1a) normal and fewer small size red blood corpuscles with 0.15% moringa (Fig. 1b) and normal with few small size corpuscles of faint colour for 0.30% moringa (Fig. 1c). While with the highest supplementation level (0.45% ML) obvious changes on blood RBCs were observed characterized by faint colour, peripheral hemoglobin, deformation and clear agglutination (T 4, Fig. 1d). The present results pointed to that moringa leaves at 0.45% supplementation had negative effect on the biological function of blood cells which might lead to death.

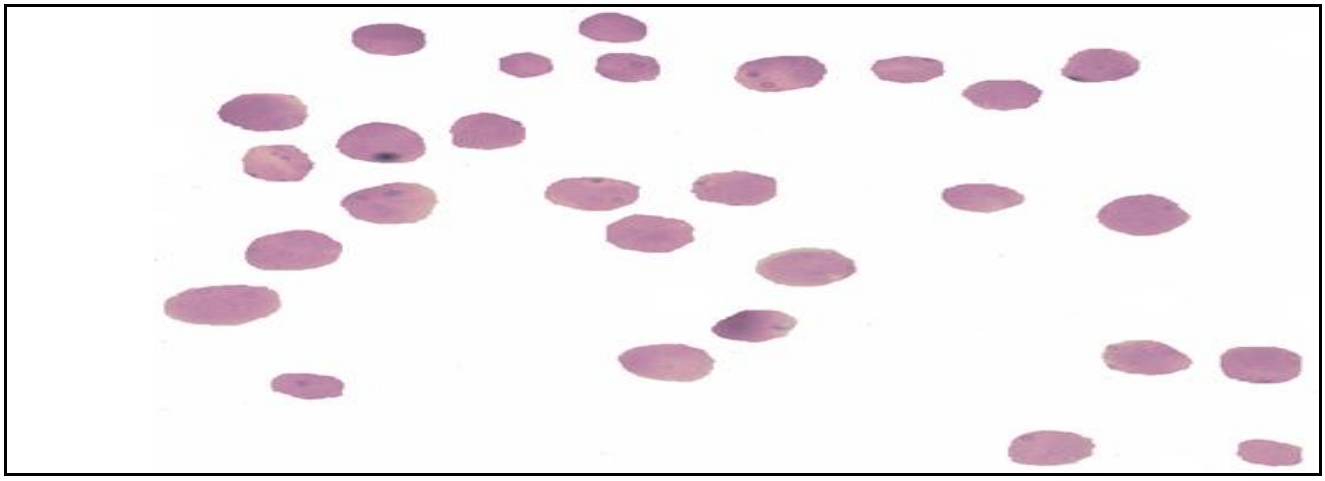

Fig. 1a: Control group (T1, 0%ML) showing red blood corpuscles with normal shape (Giemsa stain, X 1000).

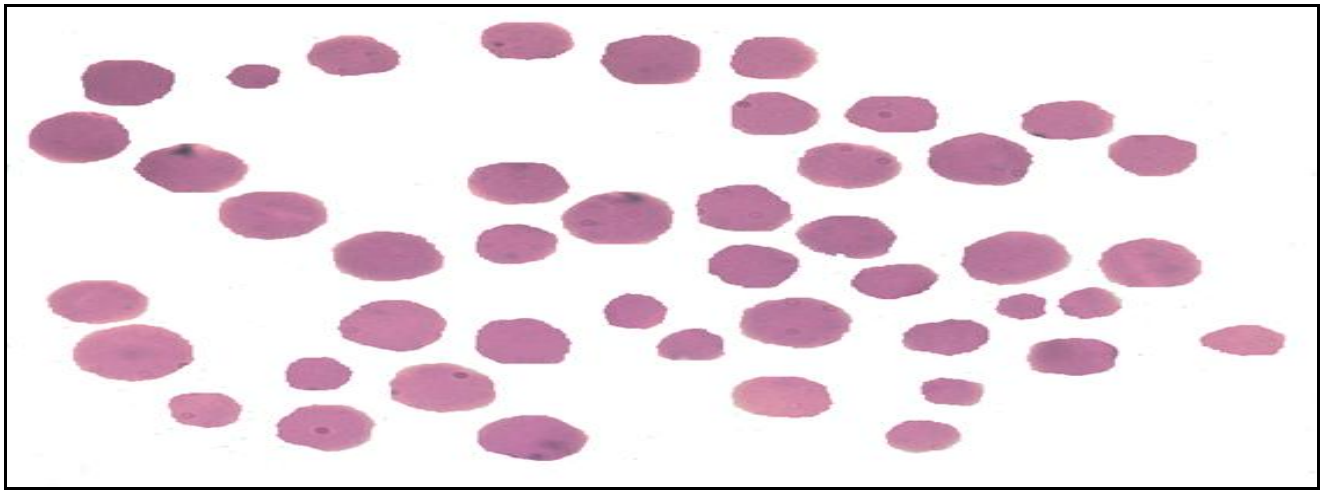

Fig. 1b: T2, 0.15%ML) showing normal and few small size red blood corpuscles (Giemsa stain, X 1000).

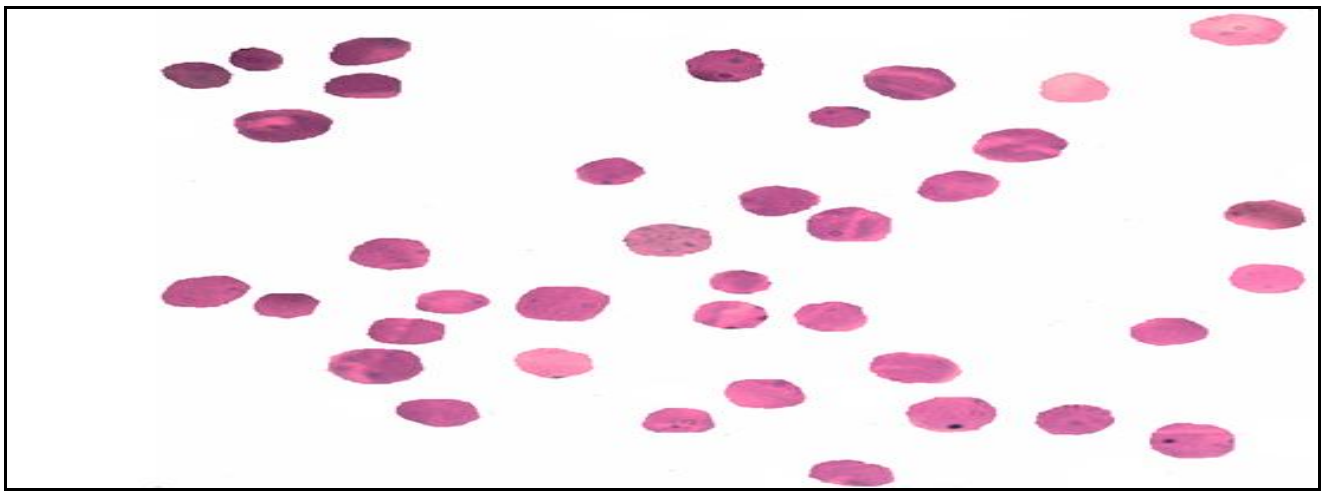

Fig. 1c: T3, 0.30%ML) showing normal and few small size with some faint colour red blood corpuscles (Giemsa stain, X 1000).

Fig. 1d: T4, 0.45%ML) showing faint colour, peripheral hemoglobin, deformation and clear agglutination of red blood corpuscles (Giemsa stain, X 1000).

Blood biochemical study:

Blood plasma total protein, albumin and globulin concentrations were significantly (P<0.05) improved with increasing ML supplementation level, meanwhile, urea, alanine lipoprotein transaminase (ALT) and creatinine concentrations recorded the highest values with 0.45 % ML. Glucose values were significantly (P<0.05) decreased with 0.15 and 0.30% ML, but it were significantly increased with increasing moringa to 0.45% compared with control (Table 3). The decreased glucose may be regarded to the increase of glucose utilization, while such trend was significantly decreased with 0.45% ML diet. It seems that moringa leaves used at low supplementation levels (0.15 to 0.30%) could enhance insulin secretion, but adverse effect could be expected with the higher level (0.45%). Similar conclusion had been given by[3]. Our results were also in agreement with those reported by[20]. Serum total protein, albumin, globulin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) values were not significantly different for growing rabbits fed diets supplemented with 5, 10 and 15% moringa leaf meal. They found that urea values were increased with 15% than other diets. [21]reported that there were no significant difference on serum biochemical parameters included total protein, albumin, globulin, glucose, urea, creatinine, cholesterol, AST, ALT and ALP for growing rabbits fed diets contained 5, 10 and 15% moringa leaf meal compared with control. While urea and creatinine values were insignificantly increased especially with high levels (10 and 15%) in comparison

with the lower level (5%). [22]found that 8 g of moringa leaf powder covered for 40 days was able to reduce total cholesterol by 14%, LDL-C by 29%, vLDL-C by 15% and triglycerides by 14% with no significant increase in HDL-c. [23]stated that moringa leaf extract appears to inhibit significantly cholesterol micelle formation (40% inhibition at 10 mg/ml) and can directly bind to bile acids (no influence on pancreatic cholesterol esterase), suggesting an inhibitory effect on cholesterol absorption. [24]reported that 200 mg/kg water extract of moringa leaves appears to have hypoglycemic properties in otherwise normal rats, reducing fasting blood glucose by 26.7% over the course of 8 hours after acute ingestion and reducing the spike in glucose from an oral glucose tolerance test by 29.9% relative to control. [23]reported that the leaf extract of moringa appears to inhibit intestinal sucrose with an IC_{50} of 780 +/ –210 µ g/ml, with weak inhibitory potential against pancreatic amylase. [24]found that 200 mg/kg of water extract of moringa leaves determined to be more effective than 100 or 300 mg when fed to diabetic rats over the course of 21 days, there appear to be time dependent decrease in fasting blood glucose reaching up to a 69.2% drop and a reduction in an oral glucose tolerance test after 21 days by 51.2%.

Table 3: Blood plasma biochemical constituents (Mean ± SE) of rabbits fed diets supplemented with different levels of moringa leaves.

Parameters	Experimental treatments				Normal range *
	T1,Cont (0% ML)	T2 (0.15% ML)	T3 (0.30% ML)	T4 (0.45% ML)	
Total protein, g/ dl	6.53^{b}±0.04	6.37^{b}±0.05	7.01^{a}±0.06	6.98^{a}±0.06	5.0 -7.5
Albumin,g/ dl	3.46^{b}±0.27	3.68^{b}±0.25	3.82^{a}±0.32	3.81^{a}±0.29	2.5- 4.0
Globulin,g/ dl	3.07^{b}±0.11	2.69^{c}±0.08	3.19^{a}±0.09	3.17^{a}±0.13	1.5-3.3
Urea, mg/ dl	33.42^{b}±2.52	31.70^{b}±1.97	30.15^{b}±1.40	41.38^{a}±2.68	17.5-54.3
Cholesterol, mg/ dl	68.70±3.06	72.15±2.87	65.82±1.85	66.78±1.17	10- 80
Glucose, mg/ dl	95.10^{b}±2.16	72.49^{c}±3.21	76.51^{c}±2.62	112.80^{a}±1.79	75-140
ALT, IU/ L	96.61^{b}±1.75	96.80^{b}±1.32	102.71^{b}±3.72	131.50^{a}±3.44	55-260
AST, IU/ L	45.00±1.65	43.72±0.94	45.23±1.73	44.06±1.02	10- 98
Creatinine, mg/ dl	1.29^{b}±0.02	1.15^{b}±0.02	1.33^{b}±0.01	2.11^{a}±0.04	0.5- 2.6
T_4, µg/ dl	6.20^{d}±0.15	6.91^{b}±0.34	7.24^{a}±0.11	6.68^{c}±0.20	6.4- 8.3
T_3, ng/ dl	76.71^{d}±4.01	94.95^{b}±3.98	101.22^{a}±4.67	86.51^{c}±5.16	89.4-115

a, b, c and d: Means in the same row having different superscripts are significantly different at P<0.05.

*: Hewitt *et al.*,1989[33].

[25]reported that a methanolic extract of moringa leaves at 200-400 mg/kg of rats had increased liver enzymes. [26]stated that oral administration of methanolic extract of moringa seeds at 1600 mg/kg was elevated liver enzymes of rats (400-800 mg/kg confirmed safe). [27]stated that 150-300 mg/kg of the aqueous-ethanol extract of moringa leaves appears to be protective against gentamicin-induced nephrotoxicity, this may be due to antioxidative effect of the extracts on protect kidney against oxidative toxins. [24]reported that 200 mg/kg water extract of moringa leaves appears to abolish all urinary proteins and sugars for diabetes rats, the antioxidant properties of moringa appear to reduced urinary proteins and glucose in diabetic animals, suggesting protective effects that may attenuate the rate of kidney failure diabetes.

Triiodotherionine (T_3) and Thyroxine (T_4) were significantly (P<0.05) higher with ML treatments than control and the highest values were recorded with 0.30 % ML, however obvious decrease was shown with 0.45 % ML treatment. The depression in T_3 and T_4 with increasing of moringa may be due to depression in thyroid gland secretion results from and effect in thyroid gland [(3)]. [28]stated that 10 days supplementation of 175 and 350 mg/kg of moringa leaf extract in rat's diet, female rats appeared to experience a decrease in circulating T_3 by 30% with an increase in T_4 by 15%, while male rats did not experience any change at either dosage.

Histological study:

Histopathological examination of liver and kidney for experimental treatments are presented in Figures 2a, 2b, 2c, and 2d and Figures 3a, 3b, 3c, and 3d, respectively. It is clear from Figures 2a, 2b, 2c, and 2d that liver sections showed moderate changes associated with increasing level of supplemental moringa. Liver structure of the control group (T1) showed normal appearance of hepatocytes, portal (central) vein and bile ducts; however, there are some few necrotic areas and some lymphocytic cells aggregation (Fig. 2a). A similar trend was also observed for liver sections from T2 (0.15%) and T3 (0.30%), except a marked increase in necrotic areas and disrupted hepatic cords with few infiltrated fluids (Fig. 2b). This case was more obvious in the liver sections from T3, indicative moderate changes in liver histology (Fig. 2c). The high level of moringa

(T_4) revealed many disruptions in the hepatic parenchyma including enlarged portal vein and changes in bile duct lining epithelium accompanied with an increase in the necrotic areas and the infiltrated fluids (Fig. 2d). These changes may reflect hyperactivity of liver tissues resulting from the higher metabolic processes of rabbits to satisfy their accelerated growth rates. It is possible also that moringa has some substances or compounds which may exert anti-nutritional, toxic or harmful effects on liver functions.

Concerning kidney histology sections (Fig. 3a, 3b, 3c, and 3d) revealed mild changes in the T3 (Fig. 3c) and some deleterious observation in the T4. Sections, especially the changes in the glomeruli size of both cortical and medullary areas (Fig. 3d). These observations indicate that the supplemental level of moringa should not exceed 0.30% to be used safely in rabbit's diet. However, from the nutritional and physiological point of view, further research is needed to examine both liver and kidney function tests in details; this may help in suggesting the appropriate and or safe supplemental level of moringa to rabbit diets, since in this study the adverse effect on liver and kidney tissues associated with feeding of 0.45% ML (485 mg moringa/kg body weight). These results were in agreement with the conclusion of [3] who stated that 150-200 mg/kg rats body weight of leaf water extract was nontoxic and appear to be safe from all tested toxicity, while a relatively small increase (3-4 times the recommended doses) cause genotoxic damage and may promote cancer formation whereas doses higher than that cause overt organ damage (mostly liver and kidneys). On the contrary, [29]found that the LD_{50} was 1585 mg/kg and when mice were injected with moringa leaf water extracts, and when orally administrated over 60 days had failed to cause toxicity. However, [30]reported that oral administration of moringa leaf water extract at 3000 mg/kg wt. daily resulted in genotoxic potential in PMBC immune cells of mice, meanwhile 1000 mg/kg confirmed safe with no damage to organs. Leaf ethanol extract had failed to exert any clinical signs of toxicity up to 6400 mg/kg acutely[31]. 50% ethanol extract appears to be clinically nontoxic up to 2000 mg/kg[32]. While, a methanolic extract of moringa leaves had elevated liver enzymes at 200-400 mg/kg of Wister rats[25]. [26]found that a moringa seed methanolic extract did not acutely toxic up to 3000 mg/kg in rats. They added that chronic ingestion noted no toxicity aside from elevated liver enzymes associated with 1600 mg/kg oral intake, but 400-800 mg/kg confirmed safe. It seems that oral administration of dry moringa leaves is more effective than either water or ethanolic extracts for animals and humans.

Conclusion

From results of the present study it could be concluded that the oral administration of dry moringa leaves between 150 to 329 mg/kg body weight is useful in improving blood biochemical constituents, haemological parameters and liver and kidney tissues of growing NZW rabbits. However, deleterious effects characterized by high liver enzymatic activity, low thyroid hormones level, deformation of red blood corpuscles and sever damage of liver and kidney tissues for rabbits fed 0.45% ML (485 mg/kg body weight).

Acknowledgment

The team work of this study like to express their deep thanks to Dr. Youssef Fawzy Ahmed Prof. of Animal Reproduction, Animal Reproduction Department, National Research Centre, Giza, Egypt for his help in examining formation of red blood corpuscles.

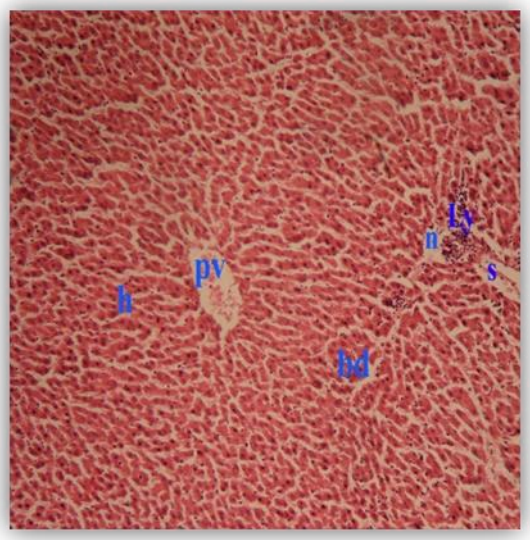

Fig. 2a: T.S. in the liver from the control group (T1, 0%ML) showing normal hepatocytes (h) and well-arranged hepatic cords with normal portal vein (pv) and bile duct (bd). There are also, some necrotic areas (n) and lymphocytic cells (ly) near the blood sinuses (s), but no histopathological changes could be seen (H&E x 100).

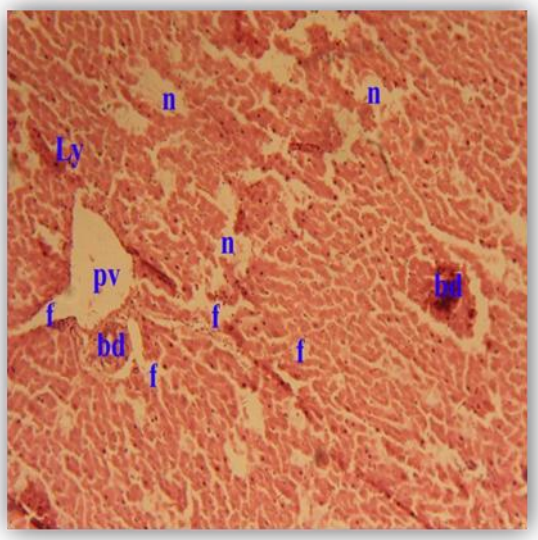

Fig. 2b: T.S. in the liver from T2 (0.15%ML) showing slightly dilated portal vein (pv) and bile duct (bd) with many necrotic areas (n) and in filtrable fluids (f) between the hepatic cords. Also there are marked disruptions in the arrangement of the hepatic cells (H&E x 100).

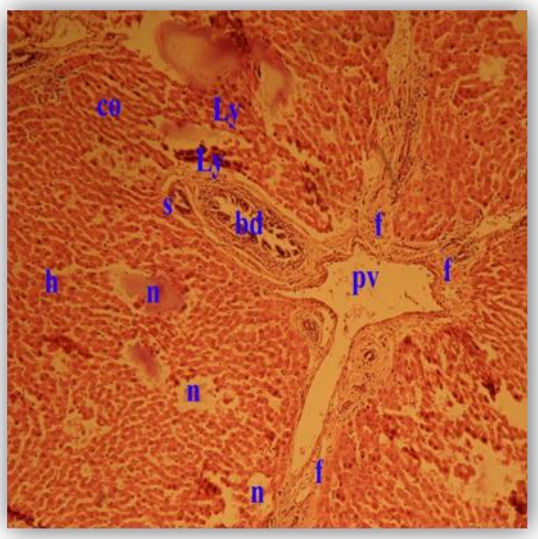

Fig. 2c: T.S. in the liver from T3 (0.30%ML) showing a squeezed portal vein (pv) and bile duct (bd) accompanied with some congested (co) areas and many infiltration fluids (f) between the hepatic cells. Also, there are many necrotic (n) areas surrounding the blood sinusoids (s) and large lymphocytes (ly) scattered on the surface of the section (H&E x 100).

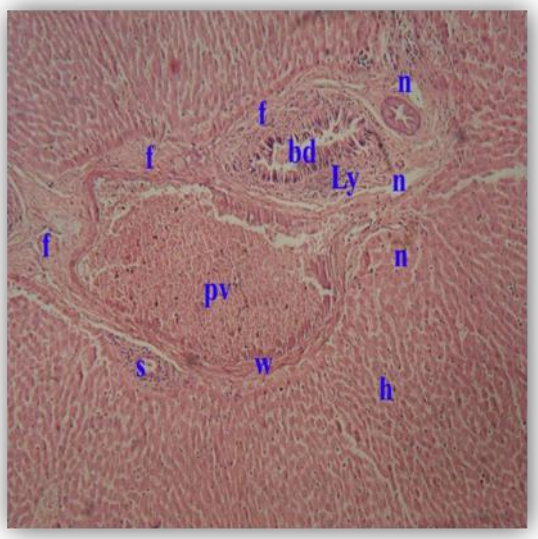

Fig. 2d:T.S. in the liver from T4 (0.45%ML) showing a greatly enlarged portal vein (pv) and a marked thickness of its membrane (w). Also, there are an obvious decrease in the liver cells (h) size with dilated and squeezed bile duct (bd) and ductile (arrow). Also, there are many fluids surrounding both the pv and (bd) with some granular degenerative (fibrotic) cell masses (arrow head) (H&E x 100).

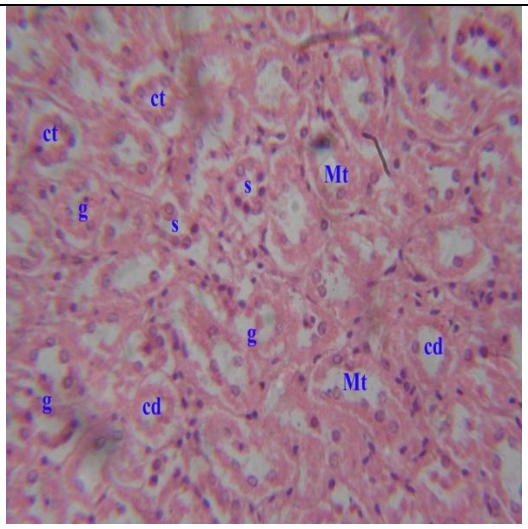

Fig. 3a: T.S. in the kidney from control group (T1, 0%ML) showing many regular medullary collecting tubules (Mt) and small segments of the fine collecting ducts (s) coming from the Henle's loop. Also, there are many cortical tubules (ct) and main collecting ducts in both the cortex and medullary regions. Also, many glomeruli (g) are present in both regions indicating the preseuse of glomerulus from cortical or medullary nephrons (H&E x 400).

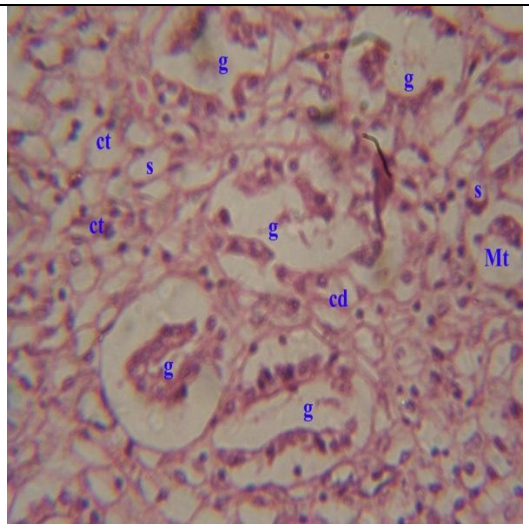

Fig. 3b: T.S. in the kidney from T2 (0.15%ML) showing greatly dilated cortical and medullary glomeruli (g) with many cortical tubules (ct) and medullary collecting tubules (Mt) (H&E x 400).

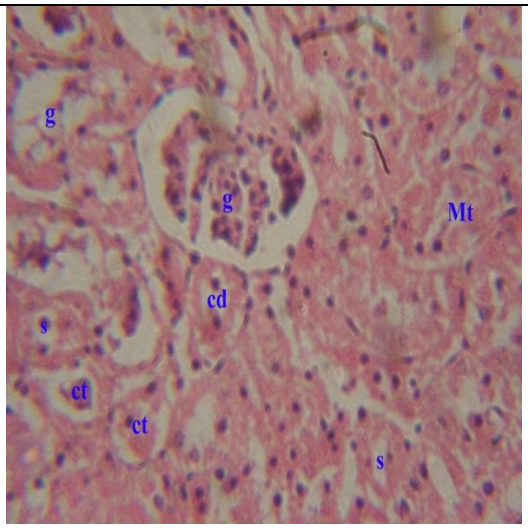

Fig. 3c: T.S. in the kidney from T3 (0.30%ML) showing a small cortical glomerulus (g) and a large dilated medullary one. Also there are small cortical tubules (ct) and enlarged medullary collecting tubules (Mt) (H&E x 400).

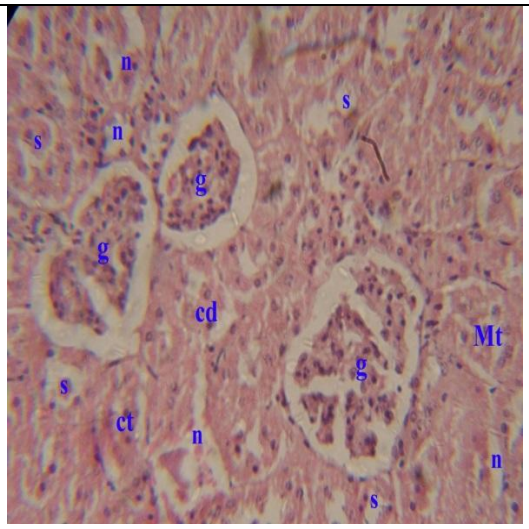

Fig. 3d: T.S. in the kidney from T4 (0.45%ML) showing greatly dilation of both cortical and medullary glomeruli (g) associated with irregular collecting tubules and some pre-necrotic areas (n) surrounded with large lymphocytes indicative of moderate dysfunction. (H&E x 400).

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