Subacute Toxicity Studies of *Moringa oleifera* Leaf

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Abstract: Twenty five cockerels (5 weeks old) were used for the study and were divided into 5 groups (A to E) of five chicks each, while group A served as the control group and received normal saline, groups B, C, D and E were administered daily with an oral dose of 100, 200, 300 and 400mgkg^{-1} respectively for 28 days. Parameters such as body weight gain, hematological, gross and histopathological changes were obtained during this Period on weekly basis. The same parameters were also taken 1 week post treatment (day 35) to determine the effect of the withdrawal of this extract on the chicks. Despite the wide margin of safety of the extract, prolong exposure of birds to the extract resulted to lesions at histological levels in the tissues and organs suggesting that the ethyl acetate fraction of the crude aqueous extract of *Moringa oleifera* leaf should be used with caution. The tissue lesions and hemathological parameters returned to normal by day 7 after the withdrawal of the extract treatment. Also further studies should be carried out before the commencement of its use clinically.
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Introduction

The therapeutic index of drugs which is the relative benefit to toxicity of various doses when determined will help in defining the dosage of a drug that will most benefit the end users. The ethyl acetate fraction of *Moringa oleifera* leaf has been shown to possess some antibacterial activity at *in-vitro* level (Kwaghe and Ambali, 2009) and the acute toxicity study has revealed its potential as a herbal product with a wide safety margin (Kwaghe and Ambali, 2011), the long-term effects of administration of the extracts is yet to be determined. It is widely acknowledged that, if a drug/product is toxic to the end users following its long administration, such products may not be passed for use as a drug (Clarke and Clarke, 1977). It is on the basis of this fact that the sub-acute toxicity studies of the ethyl acetate extract of *Moringa oleifera* leaf is to be determined.

Therefore, this study was carried out to determine the prolonged effects of the ethyl acetate fractionated portion of the extract on the various tissues and organs of chickens so as to find the necessary measures to take in case of its toxicity.

Materials and Methods

Sample collection and identification

Fresh sample of *Moringa oleifera* leaves was collected from Shagari Lowcost, Maiduguri. The plant was identified and authenticated by a taxonomist with the Department of Biological Sciences, University of Maiduguri.

Preparation of the extract

Fresh leaves of the plant *Moringa oleifera* was collected and air dried in the laboratory, grounded into fine powder and stored in a glass container at 4^{0}C. The powdered sample was exhaustively extracted with distilled water using reflux method. The crude aqueous extract was concentrated *in-vacuo,* properly labeled and stored in the refrigerator at 4^{0}C (Trease and Evans, 1989).

Fractionation of the aqueous extract

The crude aqueous extract obtained was suspended in cool distilled water and then filtered. The filtrate was subsequently fractionated using chloroform, ethyl acetate, normal butanol successively. The organic solvents used were of different polarities and the fractionation for each organic solvent was done until the organic layers were visibly clear in order to obtain chloroform, ethyl acetate and n-butanol soluble fractions and residual aqueous fraction in sequence as described by *Cho et al.* (2003) and Motahashi *et al.* (2004). The organic solvents were evaporated and recovered by distillation living the extract in the round bottom flask which was

poured into a beaker and placed in an oven to evaporate to dryness.

Experimental birds

Twenty five day old cockerels were purchased from Tanya Agro-vet. Consult Ltd., Maiduguri and were kept under intensive management for 5weeks in the Animal House Department of Veterinary Medicine, University of Maiduguri. Throughout the period of the study, feed and water were given *ad libitum*. The feed given was from a commercial source (Vital Feed International, Jos, Nigeria).

Sub-acute toxicity study

Twenty five chicks (5weeks old) were used for this study which were randomly divided into five groups of 5 chicks each (Groups A, B, C, D and E). The body weights of the birds were obtained prior to the commencement of the treatment and subsequently on weekly basis during the period of extract administration and a week after the end of the administration of the extract. Group A served as the negative control group and normal saline was administered orally daily for a period of 28 days. The chicks in Groups B, C, D and E were treated daily with 100, 200, 300 and 400mgkg^{-1} respectively with the ethyl acetate fraction of the extract for 28 days. The effects of this prolonged administration of the extract was then determined. Blood samples was obtained from the wing vein from all the birds in each group on weekly basis and processed to determine the hematological parameters such as RBC, Hb, PCV, WBC (total and deferential) and platelet counts. Blood was also taken a week after the stoppage of the administration of the extract and processed to obtain similar parameters. Tissue samples such as the liver, kidney, intestine, lungs, spleen and heart were collected and processed for histopathological analysis to assess tissue damage.

Determination of body weight

The body weights of the birds were determined every week using Harvard Trip Balance (manufactured by OHAUS, Florham Park N. J. 07932, U.S.A).This was used to determine the effects of the extracts on weight gain of the birds.

Statistical analysis

Data collected were summarized as mean ± standard deviations (S.D). One way ANOVA was used to analyze the differences between the means and P value ≤0.05 was considered significant (Armitage, 1980). Graphpad Instant (1998), Computer Statistical software package was used for the analysis.

Haematological studies

Hematological parameters that were determined in the treated birds including the controls were red blood cells (RBC) count, Hb (hemoglobin concentration), packed cell volume (PCV), white blood cell (WBC)count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) using standard procedures as described by (Coles 1974;1977 and Schalm *et al.* (1975).

Gross lesions

Birds were sacrificed and examined for gross lesions.

Histopathology

Tissue samples of the liver, lungs, kidney, intestine, spleen and heart were taken and fixed in 10% formalin. The tissues were dehydrated through graded concentration of ethanol (70%, 95% and absolute), cleared in xylene and embedded in paraffin wax. The embedded tissues were stained with hematxylene and eosin (H&E) for light microscope examination. Lesions observed were photographed using Vanox T Olympus photograghing microscope (Drury and Wallington, 1979).

Results

Clinical signs

The clinical signs observed in all the Groups (Groups B, C, D, and E) following the administration of the extract were mainly gasping and sneezing. Group A being the negative control birds remained normal with no clinical signs throughout the period of the experiment.

Body weight

Treatment with the ethyl acetate soluble fraction of aqueous extract of *Moringa oleifera* leaves did not indicate any significant difference in body weight when compared with the control group (Table 1). The body weights of birds before treatment were 339.14 $\pm$ 35.86, 321.78 $\pm$ 57.25, 344.40 $\pm$ 28.84 and 333.16 $\pm$ 17.8g for birds in Groups B, C, D and E respectively. On the 28th day of treatment with the extract the birds attained the weights of 878.80 $\pm$ 88.53, 764.20 $\pm$ 120.85, 791.80 $\pm$ 41.32 and 775.00 $\pm$ 109.26g for Groups B, C, D, and E respectively.

Haematological parameters

There was a highly significant increase in the red blood cell values (Table 2), from day 14 to day 28 of the treatment in all the groups (Groups B to E) when compared with the control group, (group A) with a P

value of less than 0.001 (P<0.001). Even at one week post cessation of treatment the P value was highly significant though there was a decrease in the RBC values when compared to the same group at day 28 (Table 2).

The haemoglobin concentration (Table 3), of the birds at day 7 of the treatment was significantly higher in group E (400mgkg^{-1}) with P<0.05 when compared to the control group. By day 21 there was highly significant difference in the P value (P<0.001) in groups C, D, and E (200, 300, and 400 mgkg^{-1}). By day 7 post treatment, there was decrease in the Hb value in all the groups (100, 200, 300, and 400 mgkg^{-1}) though the P value in birds treated with 100mgkg^{-1} (group B) and 400mgkg^{-1} (group E) was moderately significant (P<0.01) when compared to the control group.

The result of WBC evaluation is shown in Table 4. There was significant increase in the values of the WBC count with P<0.001 from day 7 to day 35 of treatment in all the treatment groups (groups B to E) as compared with the control.

The packed cell volume (PCV) value increased significantly as compared with the control by day 28 of treatment in all the groups (Table 5). By day 7 post treatment, the PCV value dropped to almost normal and there was no significant difference between the treatment groups and that of the control group.

Looking at the platelet values (Table 6), there was significant increase in the platelet value in groups C, D, and E birds at day 7. By day 14, all the group platelet values were highly significant as compared with the control group. This highly significant difference (P<0.001) was maintained up to the end of the study. There was a significant decline in the platelet value at day 7 post treatment.

Table 1: The effects of treatment of cockerels with varying doses of *M. oleifera* ethyl acetate leaf extract on its mean[a] body weight (g).

Dose	Treatment in days					
(mg/kg)	0	7	14	21	28	35 PT
Control (Grp A)	328.54 ± 6.56	427.84 ± 26.44	565.62 ± 24.56	684.00 ± 89.35	870.80 ± 46.96	862.40 ± 63.53
100 (Grp B)	339.14 ± 35.86	431.34 ± 44.25	545.42 ± 47.99	762.80 ± 84.16	878.80 ± 88.53	884.80 ± 81.31
200 (Grp C)	321.78 ± 57.25	395.66 ± 84.81	530.90 ± 125.96	682.12 ± 113.29	764.20 ± 120.85	773.40 ± 125.19
300 (Grp D)	344.40 ± 28.84	406.20 ± 36.99	555.20 ± 38.66	717.00 ± 42.06	791.80 ± 41.32	780.80 ± 48.32
400 (Grp E)	333.16 ± 17.86	410.07 ± 25.21	532.64 ± 37.54	766.82 ± 41.41	775.00 ± 109.26	780.20 ± 112.38

a---mean ± standard deviation based on 5 observations

Table 2: The effects of prolong treatment of cockerels with varying doses of *M. oleifera* (ethyl acetate leaf extract) on its mean[a] erythrocyte counts (×10^6/mm^3).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	3.25±0.12	3.39±0.17	3.57±0.06	3.52±0.15	3.35±0.08
100 (B)	3.23±0.15	4.19±0.06*	5.69±0.10*	5.82±0.05*	4.29±0.05*
200 (C)	2.90±0.19	4.67±0.12*	5.83±0.08*	5.88±0.04*	4.70±0.05*
300 (D)	3.11±0.25	5.26±0.33*	5.92±0.05*	6.06±0.04*	4.78±0.08*
400 (E)	3.13±0.13	5.68±0.09*	6.09±0.04*	6.19±0.08*	4.90±0.03*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 3: The effects of prolong treatment of cockerels with *M. oleifera* (ethyl acetate leaf extract) on its mean[a] haemoglobin concentration (g/dl).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	5.34±0.39	9.44±1.76	6.60±0.00	6.26±0.32	6.42±0.16
100 (B)	6.40±0.31	7.06±1.91	7.36±0.39*	7.42±0.63*	6.06±0.22*
200 (C)	5.80±0.69	8.46±1.36	7.90±0.40*	7.54±0.15*	6.60±0.00
300 (D)	5.88±0.53	9.70±0.92	8.08±0.47*	7.88±0.43*	6.42±0.16
400 (E)	7.34±2.34*	7.64±1.41	8.54±0.29*	7.72±0.77*	5.98±0.18*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 4: The effects of prolong treatment of cockerels with varying doses of *M. oleifera* (ethyl acetate leaf) extract on its mean[a] White blood cells count ($X10^3/mm^3$).

Dose (mg/kg)	Duration of Treatment (days)				
	7	14	21	28	35 PT
Control	3140.0 ± 54.8	4010.0±41.8	4060.0±54.8	4110.0±54.8	3430.0±27.39
100 (A) (B)	4050.0±158.1*	4350.0±215.1*	4440.0±74.2*	4530.0±57.0*	4160.0±102.47*
200 (C)	4250.0±70.7*	4740.0±89.4*	4780.0±83.7*	4990.0±114.0*	4300.0±50.00*
300 (D)	4600.0±476.9*	4990.0±108.4*	5030.0±481.7*	5150.0±70.7*	4460.0±54.77*
400 (E)	5200.0±122.5*	5040.0±74.2*	5180.0±44.7*	5270.0±57.0*	4520.0±4472*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 5: The effects of prolong treatment of cockerels with *M. oleifera* (ethyl acetate leaf extract) on its mean[a] packed cell volume (%).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	30.60±4.51	30.80±1.30	34.00±1.00	31.80±1.30	29.60±0.55
100 (B)	32.60±5.23	31.20±2.17	32.60±1.14	34.80±0.84*	29.6±0.55
200 (C)	22.80±5.63	33.00±2.74	33.00±1.41	34.80±0.45*	30.00±1.00
300 (D)	27.20±6.38	31.20±2.78	33.60±0.89	36.20±1.48*	30.00±0.71
400 (E)	30.40±3.05	32.20±3.03	32.80±2.17	36.20±0.84*	30.80±0.84

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 6: The effects of prolong treatment of cockerels with *M. oleifera* (ethyl acetate leaf extract) on its mean[a] platelet value ($X10^3/mm^3$).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Countrol (A)	102,000± 2738.6	102,000± 4472.1	107,000± 2738.6	111,000± 4183.3	98,000± 2738.6*
100 (B)	99,000± 4183.3	152,000± 1565.2*	172,000± 4472.1*	177,000± 2738.6*	165,000± 3535.5*
200 (C)	118,000± 8366.6*	191,000± 4183.3*	180,000± 5000.0*	183,000± 2738.6*	173,000± 2738.6*
300 (D)	130,000± 3535.5*	200,000± 5000.0*	191,000± 8215.8*	195,000± 6123.7*	169,000± 4183.3*
400 (E)	134,000± 4183.3*	205,000± 7905.7*	231,000± 4183.3*	239,000± 6519.2*	170,000± 5000.0*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

There was constant increase of percentage neutrophils from day 7 to day 28 of treatment (Table 7). In all the treatment groups, the increase was significantly higher than the control group.

The percentage monocytes (Table 8) was highly significant (P value <0.001) in all the treatment groups throughout the period of the experiment.

The percentage of eosinophils increased from day 7 of extract administration in all the groups up to day 14 (P<0.01) (Table 9). By day 21 there was drop in the percentage monocytes in all the groups with a P value that is not significant (P>0.05). By day 28, there was a sharp increase in the percentage of monocytes in all the groups with a P value of 0.001 which is highly significant (Table 9).

As shown in Table 10, the percentage of basophils in birds of the control group was zero percent and remained so throughout the study period. There was increase in the basophilic percentage in all the other groups at various periods of the treatment, the difference was however not significant throughout (P>0.05).

The percentage of lymphocytes (Table 11) dropped significantly in all the groups by day 7 of treatment when compared with the control values. This decrease in values continued in all the groups throughout the period of the treatment (28 days). By one week post treatment, there was increase in the percentage of lymphocytes which was found to be highly significant (P<0.001).

Table 7: The effects of treatment of cockerels with ethyl acetate leaf extract of *M. oleifera* on its mean[a] differential leucocytes counts of neutrophils (%).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	31.20±1.30	30.80±0.84	31.40±1.14	31.20±1.10	31.40±1.67
100 (B)	34.40±0.55*	36.00±0.71*	37.40±0.89*	37.60±0.55*	34.60±1.14*
200 (C)	35.60±0.55*	36.80±0.84*	37.80±0.84*	37.80±0.84*	34.40±1.14*
300 (D)	34.20±0.84*	36.60±1.14*	37.40±1.14*	37.40±1.14*	35.60±0.89*
400 (E)	35.60±0.89*	36.60±1.14*	37.80±0.84*	37.80±1.30*	34.80±0.84*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 8: The effects of treatment of cockerels with *M. oleifera* ethyl acetate leaf extract on its mean[a] differential leucocytes counts of neutrophils (%).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	31.20±1.30	30.80±0.84	31.40±1.14	31.20±1.10	31.40±1.67
100 (B)	34.40±0.55*	36.00±0.71*	37.40±0.89*	37.60±0.55*	34.60±1.14*
200 (C)	35.60±0.55*	36.80±0.84*	37.80±0.84*	37.80±0.84*	34.40±1.14*
300 (D)	34.20±0.84*	36.60±1.14*	37.40±1.14*	37.40±1.14*	35.60±0.89*
400 (E)	35.60±0.89*	36.60±1.14*	37.80±0.84*	37.80±1.30*	34.80±0.84*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 9: The effects of treatment of cockerels treated with *M. oleifera* ethyl acetate leaf extract on its mean[a] differential leucocytes counts of eosinophils (%).

Dose (mg/kg)	Duration of Treatment (days)				
	7	14	21	28	35 PT
Control (A)	4.00±0.71	5.00±0.71	5.40±0.55	6.20±1.30	4.60±0.55
100 (B)	5.40±0.55*	7.00±0.71*	5.80±0.84	9.40±1.14*	7.00±0.71*
200 (C)	4.60±0.55	6.60±0.55*	6.20±0.84	9.60±0.55*	6.00±0.71*
300 (D)	6.00±1.00*	7.20±1.30*	6.20±0.84	10.00±0.71*	7.40±0.55*
400 (E)	6.20±0.84*	7.00±1.23*	6.00±0.71	11.20±0.84*	6.80±1.10*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 10: The effects of treatment of cockerels treated with *M. oleifera* ethyl acetate leaf extract on its mean[a] differential leucocytes counts of basophils (%).

Dose (mg/kg)	Duration of Treatment (days)				
	7	14	21	28	35 PT
Control (A)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.000	0.00±0.00
100 (B)	0.00±0.00	0.20±0.45	0.00±0.00	0.40±0.55	0.00±0.00
200 (C)	0.20±0.45	0.40±0.55	0.40±0.55	0.20±0.45	0.20±0.45
300 (D)	0.40±0.55	0.20±0.45	0.20±0.45	0.20±0.45	0.20±0.45
400 (E)	0.40±0.55	0.20±0.45	0.20±0.45	0.20±0.45	0.00±0.00

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

Table 11: The effects of treatment of cockerels treated with *M. oleifera* ethyl acetate leaf extract on its mean[a] differential leucocytes counts of lymphocytes (%).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	59.40±1.52	58.80±1.92	57.20±1.48	55.60±3.36	58.20±1.48
100 (B)	52.20±1.48*	48.60±1.82*	49.00±1.87*	41.00±1.58*	50.40±1.52*
200 (C)	51.40±1.14*	48.00±1.23*	49.20±1.30*	40.40±1.52*	52.00±1.87*
300 (D)	51.60±2.07*	47.80±2.59*	49.00±2.00*	40.00±1.14*	49.60±0.89*
400 (E)	49.20±1.64*	47.60±1.52*	48.00±1.00*	38.40±1.95*	51.40±1.14*

*P<0.05 significant compared to control
a---mean ± standard deviation based on 5 observations

The effect of treatment on the Mean Corpuscular Haemoglobin is shown in Table 12. Significant decrease in the values was observed from day 14 of treatment up the last day of the study in all the treatment groups compared with the control. By 1 week post treatment, there was a slight increase in these values in all the groups which suggests that withdrawal of the extract administration resulted in the increase of the Mean Corpuscular Haemoglobin concentration though there was still significant difference between these values when compared with those in the control group.

Determination of the Mean Corpuscular Volume (Table 13) indicates decrease in the values in all the treatment groups (Groups B to E) which was significant from day 14 to the end of the study.

The effect of treatment on Mean Corpuscular Haemoglobin Concentration (MCHC) is shown in Table 14. Birds in group B (100mgkg^{-1}) showed a significant increase at day 21 of the extract administration (22.60 $\pm$ 1.52) compared to the control (19.43 $\pm$ 0.57) and this was followed at 1 week post treatment with a significant drop in the value when compared to the control group (20.47 $\pm$ 0.05). Those in Group C showed significant increase in MCHC value (26.43 $\pm$ 5.55) only at day 7 of the treatment (Table 14). In Group D birds there was a significant increase in the MCHC (Table 14) only at day 21 (24.21 $\pm$ 1.67) of treatment as compared to the control group (19.43 $\pm$ 0.57). In Group E, the increase in the MCHC value was significant at day 21 (26.21 $\pm$ 1.80) when compared to the control group (19.43 $\pm$ 0.57) and one week post treatment (19.42 $\pm$ 0.84), with significant decrease when compared to the control (21.69 $\pm$ 0.55).

Table 12: The effects of treatment of cockerels treated with *M. oleifera* ethyl acetate leaf extract on Mean Corpuscular Haemoglobin (Pg)

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	16.43±1.31	28.53±6.74	18.05±0.31	17.84±1.38	19.16±0.08
100 (B)	19.82±0.95	16.84±4.40*	12.94±0.70*	12.75±1.00*	14.13±0.05*
200 (C)	20.20±1.96	18.08±2.73*	13.57±0.52*	12.82±0.29*	14.04±0.05*
300 (D)	19.07±1.55	18.42±1.00*	13.66±0.87*	13.02±0.76*	13.43±0.08*
400 (E)	22.99±6.62*	13.45±2.57*	14.03±0.54*	12.48±1.25*	12.20±0.03*

Mean ± S. D, n =5
*P<0.05 significant compared to control

Table 13: The effects of treatment of cockerels treated with *M. oleifera* ethyl acetate leaf extract on Mean Corpuscular Volume (Fl).

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	9.39±1.07	9.10±0.36	9.53±0.25	9.05±0.30	8.84±0.16
100 (B)	10.09±1.63	7.45±0.52*	5.73±0.24*	5.98±0.19*	6.90±0.22*
200 (C)	7.81±1.50	7.20±0.62*	5.66±0.19*	5.91±0.05*	6.38±0.10*
300 (D)	8.73±1.46	5.97±0.86*	5.68±0.18*	5.98±0.25*	6.28±0.17*
400 (E)	9.71±1.76	5.66±0.48*	5.39±0.35*	5.85±0.17*	6.29±0.18*

Mean ± S. D, n =5
*P<0.05 significant compared to control

Table 14: The effect of treatment of cockerels with *M. oleifera* ethyl acetate leaf extract on Mean Corpuscular Haemoglobin Concentration (%)

Dose (mg/kg)	Treatment in days				
	7	14	21	28	35 PT
Control (A)	17.64±1.90	31.18±6.34	19.43±0.57	19.71±1.27	21.69±0.55
100 (B)	20.16±2.73	22.72±6.53	22.60±1.52*	22.01±2.08	20.47±0.05*
200 (C)	26.43±5.55*	25.84±5.13	23.95±0.98*	21.67±0.48	22.00±1.01
300 (D)	22.50±5.31	31.39±4.99	24.21±1.67*	21.79±1.24	21.40±0.71
400 (E)	24.03±5.41	24.80±5.26	26.12±1.80*	21.56±2.11	19.42±0.84*

Mean ± S. D, n =5
*P<0.05 significant compared to control

Gross lesions

No gross lesions was observed in all the birds throughout the period of treatment.

Histopathological changes

All the changes observed in the various tissues and organs at histopathological level are dose dependent. The kidneys revealved multifocal haemorrhages, congestion, focal and diffused mononuclear cell infiltration of the intestitium, hyaline degeneration, tubular degeneration and necrosis and protein cast in the tubules. One week post treatment, the kidneys showed extensive haemorrhage, severe congestion, renal tubular epithelial necrosis, infiltration of mononuclear cells and a lot of haemosiderin (Fig. 1).

Changes observed in the heart were focal and diffuse mononuclear cell infiltration, congestion of blood vessels, degeration of myofibres of the heart mostly hydropic change, myocarditis and areas of myocardial necrosis. A week post treatment still showed diffuse and focal mononuclear cell infiltration, congestion, hydropic changes and necrotic myofibres (Fig. 2).

The lesions observed in the lungs are severe congestion, extensive haemorrhagic areas, interstitial infiltration of mononuclear cells in the intestitium leading to oblitration of the alveoli (Fig. 3). After one week post treatment the changes observed are resolution of mononuclear cell infiltration of the alveoli and interstitium and massive macrophages. In the bronchiole, there was edema, subepithelial mononuclear cell infiltration, necrosis of the epithelium and desquamation (Fig. 4).

In the spleen, changes are subcapsular haemorrhages, hypoplasia of splenic follicles, slight hypoplasia of the cortical capsule of the spleen, and a few active follicles. A week post treatment indicated wrinkling of the capsule of the spleen, crowding of the splenic arterioles, red pulp hyperplasia, marked congestion of the spleen and regeneration of some germinal centres (Fig. 5).

In the intestine, there was villus atrophy, matting of the villus, sloughing of villus epithelium, necrosis and desquation of villus epithelium, subepithelial and submucosal mononuclear cell infiltration. One week post treatment also indicated the following changes in the intestine, villi atrophy, subepithelial cell infilteration and epithelial necrosis (Fig. 6).

In the liver, changes such as congestion of blood vessels, hyperplastic liver, bile duct hyperplasia, vacuolar degeneration especially in the periportal area, hydropic change, focal infiltration of mononucleocytes and multifocal areas of necrosis were observed. A week post treatment there was mild congestion, hydropic changes, periportal accumulation of infiltrating mononuclear cells and periportal necrosis (Fig. 7).

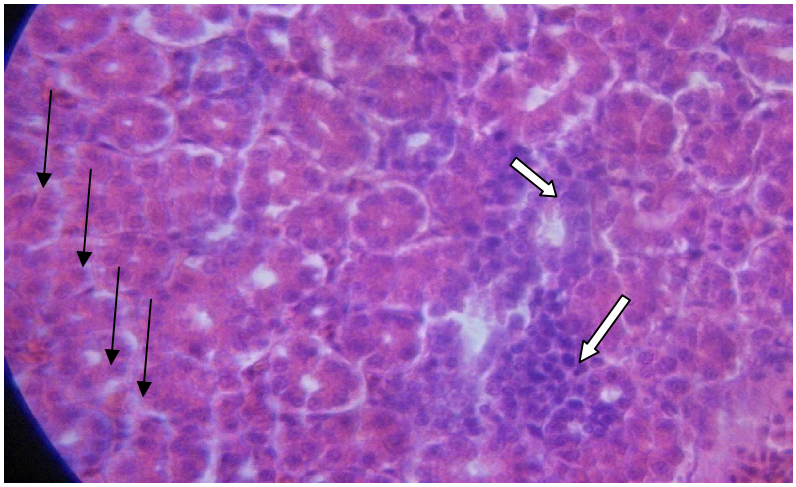

Figure 1: Kidney of a 9 week old chick fed with 300mg/kg of ethyl acetate fraction of aqueous Extract Of *Moringa oleifera* leaves one week post treatment showing renal tubular necrosis (thin black arrows) and interstitial mononuclear cell infiltrations (thick white arrows). H and E x 400.

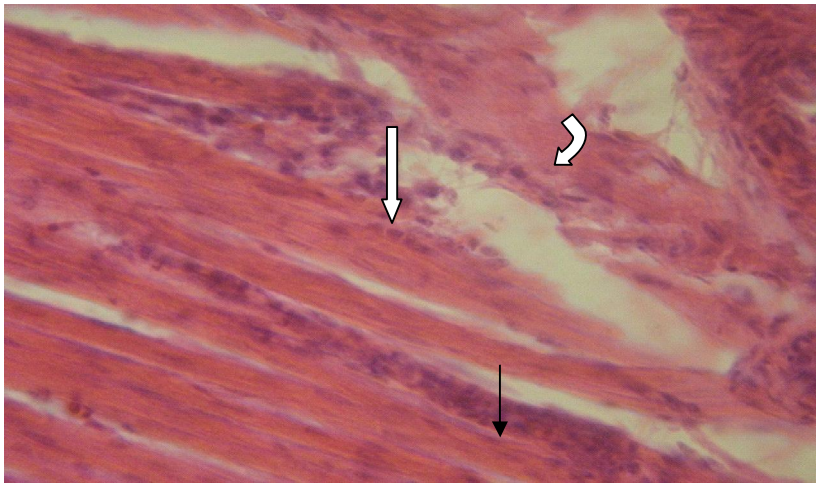

Figure 2: Myocardium of an 8 week old chick fed with ethyl acetate fraction of aqueous extract of *Moringa oleifera* leaves showing mononuclear cell infiltrations (thick white arrow), congestion (thin black arrow) and degeneration and necrosis of myocytes (thick bent white arrow). H and E x 400.

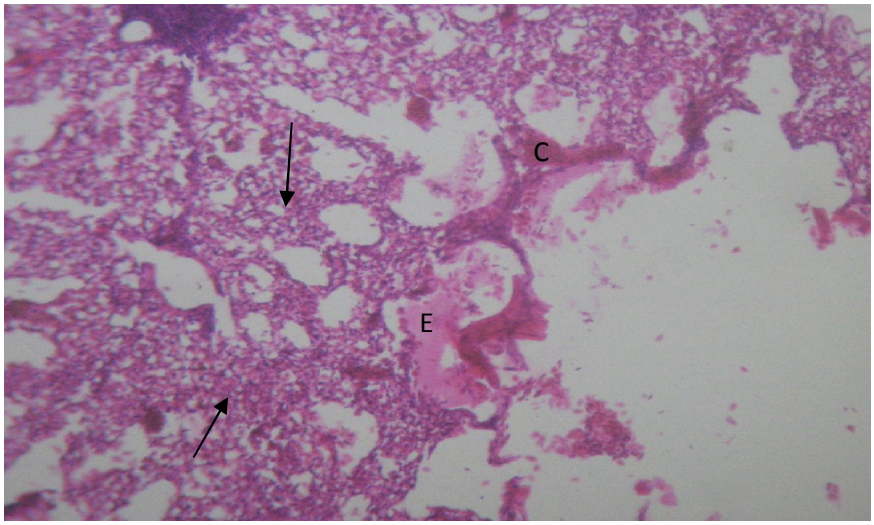

Figure 3: Lung of an 8 week old chick fed with 400mg/kg of ethyl acetate fraction of aqueous extract of *Moringa oleifera* leaves showing congestion (c), interstitial and mononuclear cell infiltrations (thin arrows) and edema (E). H and E x 200.

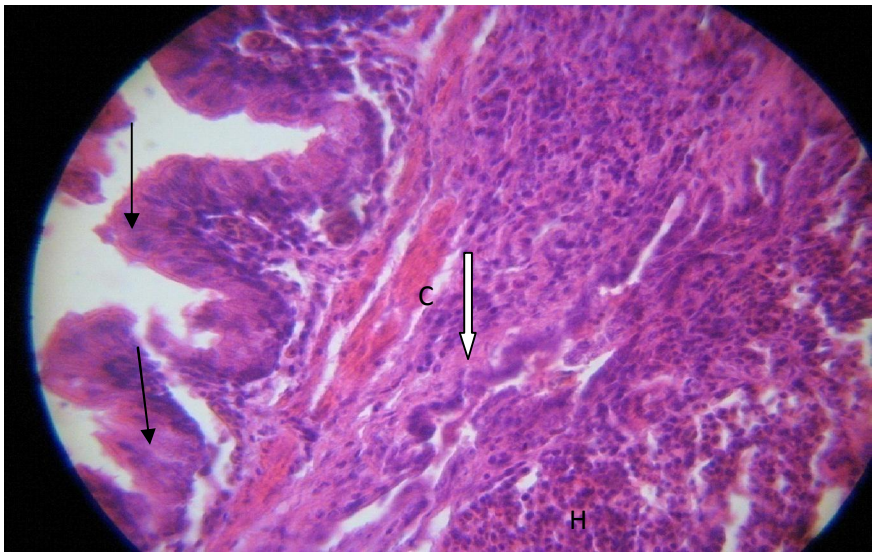

Figure 4: Brochiole of a 9 week old chick fed with 100mg/kg of ethyl acetate fraction of aqueous extract of *Moringa oleifera* leaves one week post treatment showing congestion (C), subepithelial mononuclear cell infiltrations (thick white arrows), necrosis of the epithelium (thin black arrows) and hemorrhages (H). H and E x 400.

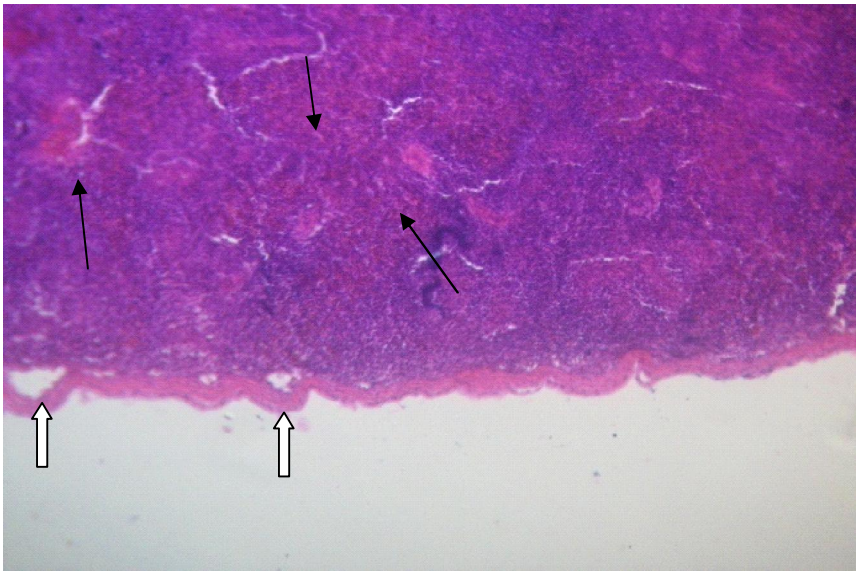

Figure 5: The spleen of a 9 week old chick fed with 400mg/kg of ether acetate fraction of aqueous extract of *Moringa oleifera* leaves showing, folding of the splenic capsule (black thin arrows) red pulp hyperplasia(thick white arrows) H and E x 200.

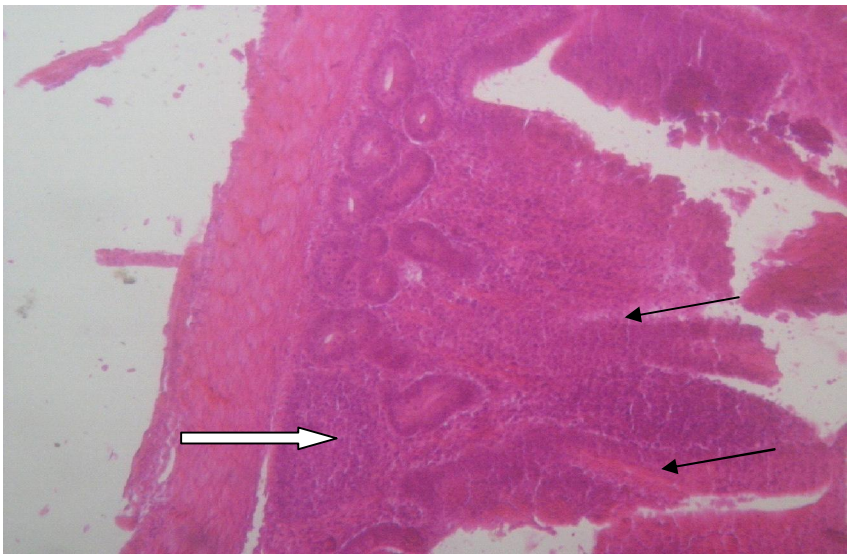

Figure 6: The Intestine of a 9 week old chick fed with 300mg/kg of ether acetate fraction of aqueous extract of *Moringa oleifera* leaves showing villus matting (thin black arrows), submucosal mononuclear cellular infiltration (thick white arrow). H and E x 200

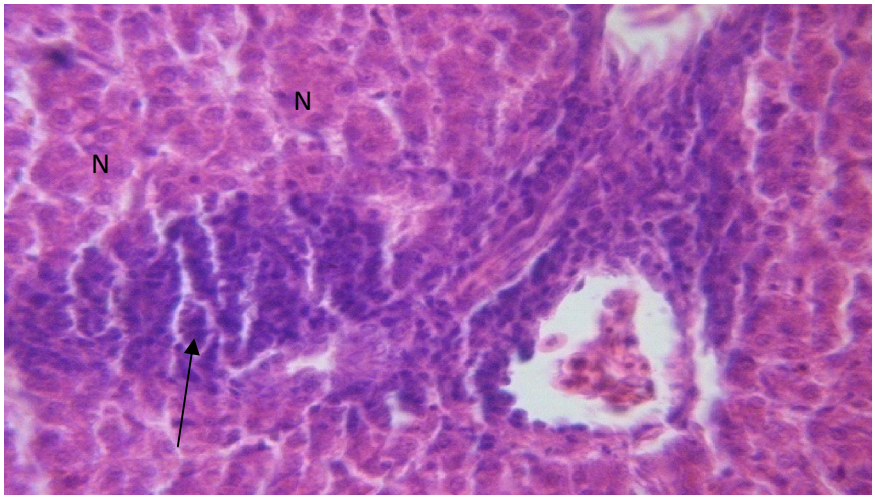

Figure 7: The liver of a 9 week old chick fed with 400mg/kg of ether acetate fraction of aqueous extract of *Moringa oleifera* leaves showing periportal necrosis(N) and mononuclear cell infiltration (thin arrow). H and E x 400.

Discussion

The result of the study is a further evidence of the safety of the extracts of *Moringa oleifera* leaves in the birds tested based on the fact that the extract did not affect the weight gain of the treated birds when compared with the control group. This is indicative that the extract does not have significant effect on feed conversion of the bird despite the intestinal lesions observed in the treated birds. This may imply the lesions are not severe enough to cause serious mal-absorption in the host.

The significant increase in the RBC, hemoglobin concentration and packed cell volume is likely to be as a result of contraction of the spleen which was observed histopathologically as wrinkling of the spleen capsule. This agrees with the findings of Stockham and Michael, (2002) who suggested that the possible causes of erythrocytosis could be due to either hemoconcentration (dehydration or endotoxic

shock), increased total RBC mass or by physiologica contraction of the spleen. In transient erythrocytosis, PCV returns to within reference interval after the stimulus is removed (Stockham and Michael, 2002). In addition Schalm *et al.*, (1975) reported that polycythemia might occur in excitable animals as a result of the injection of a mass of concentrated erythrocyte into circulation upon contraction of the spleen. Relative polycythemia reverts to normal as dehydration is relieved or when circumstances leading to contraction of the spleen no longer exist (Schalm *et al.* (1975). In the present study, this phenomenon also occurred suggesting that it was a case of relative polycythemia since all the parameters returned to normal upon withdrawal of the extract.

The increase in WBC count observed in this study may be as a result of response to antigen (extract) or to inflammation and necrosis of the various tissues and organs caused by the extract. The later is an indication of the attempt by the body mechanism to effect repair of the damaged tissues. While the former could be an immunological reaction to the administered extract. Both possibilities agreed with the reports of Kashinath, (1990) and Schalm *et al.* (1975). They reported that many biological compounds have been reported to stimulate immune functions and the persistence of antigenic load in the body results in the development of leukocytosis (increase number of WBC) at the site of injury improves wound healing.

The thrombocytosis observed in this study could be a reactive thrombocytosis similar to the one reported by Schalm *et al.* (1975) as a result of either acute hemorrhage, trauma, fractures, infections, malignancies and/ or iron deficiency. Platelet produced by splenic hematopoietic may contribute to the circulating platelet mass in health and disease (Stockham and Michael, 2002). Platelets are required for the formation of primary hemostatic plugs for the repair of small vascular defects, and this is achieved by magnifying minute stimuli into explosive production of fibrin to form secondary hemostatic plugs (Stockham and Michael, 2002).

Eosinophilia as observed in this study isp an indication of damage to tissues and organs that contain a high concentration of mast cells such as the skin, lungs, gastrointestinal tract and female genital organs. Such tissue injury leads to the degranulation of mast cells resulting in histamine release which ultimately is said to attract eosinophils from the bone marrow into circulation (Schalm *et al.*, 1975). Increased lymphocytes and monocytes in circulation in the present study could be due to tissue damage as well. Monocytosis in acute or chronic diseases is as a result of increased production in the bone marrow since there is no large storage pool of monocytes (Schalm *et al.*, 1975) in the body. Lymphopenia could be as a result of acute inflammation caused by bacterial, viral, endotoxemia or stress (Stockham and Michael, 2002). This study demonstrated that the extract caused inflammation as shown by lesions in various organs of the birds and as such could be responsible for the lymphopenia observed

Low MCV and high MCHC values may be produced when erythrocytes are in hypoosmolar plasma. Hyponatremia and hypochloremia decreases cytoplasmic osmolarity thereby resulting in hypoosmolal environment to which the erythrocytes have to adjust to *in-vivo*. When put in a diluent prior to counting, osmosis results in escape of water from the erythrocytes thus decreasing the volume of erythrocytes which eventually led to decrease in MCV and increase in MCH as obtained in the studies which also agrees with the earlier report of (Stockham and Michael, 2002). The hydropic changes observed in the organs is an indication of pre-necrotic changes. Also, the focal necrosis with mononuclear cell infiltration observed in the lungs, liver, heart, kidneys and intestine is indicative of cellular death and the body's attempt to get rid of the necrotic debris at these points. This well defined mononuclear cell infiltration is part of the healing or regenerative process. There was contraction of the spleen which was observed histopathologically by the wrinkling of the capsule of the spleen and crowding of the arterioles. In the liver, observed lesions might have been the product of metabolism of the active or toxic principle of the extract since the liver is the primary organ of biotransformation (Kaplan *et al.*, 1988). Although this portion of the extract contains flavonoids which are said to have hepatoprotective activity (Narayana *et al.*, 2001), lesions were observed in the liver which was contrary to what was reported by Pari and Kumar (2003), who stated that when liver damage was induced in rats using antitubercular drugs, the results of the study showed that treatment with *Moringa oleifera* extract enhances the recovery from hepatic damage. The extract had adverse effect on the kidney. There was tubular degeneration and necrosis, cellular infilteration and the formation of protein cast. This suggests that the active principle may be excreted through the kidneys and it is toxic to the tubular epithelial cells. The concentration of mononuclear cells in the parenchyma means that the parenchyma cells of the kidney have been destroyed possibly as a result of the extract. The presence of the protein cast indicates that the glomerulus and Bowman's capsule have been affected. There was no death recorded during the sub-acute toxicity study despite the lesions in the organs tested in the present study. The lesions

were more pronounced with increase dose of the extract.

In general, lesions were found in the various tissues and organs suggesting that the ethyl acetate fraction of the extract of *Moringa oleifera* leaf have some toxic potential, nevertheless no death was recorded and the extract had no effect on weight gain. Thus, it should be administered with caution when in use.

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