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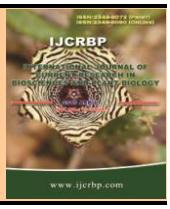

Original Research Article

Effects of Dietary *Grewia tenax* (Goddaim) Fruit and its Ethanolic Extract Given by Different Routes of Administration on Bovans-Type Chicks

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A b s t r a c t

Grewia tenax (Goddaim) fruits at 2%, 10% of basic diet ethanolic extract were administered in drinking water (500 mg/Kg/day), via intraperitoneal route (50 mg /kg/day), and via intramuscular route (10/mg/kg/day) for varying periods were fed to 7-day old Bovans-type chicks. After feeding period of 4 weeks and recovering period of 3 weeks, 4 chicks were slaughtered at weeks 2, 4 and 7. Blood samples were collected for hematological and serum analysis. Chicks were examined for gross lesions and specimens of liver, kidneys, heart, spleen and intestines were processed for histopathology. Mean body weights, weight gains, feed conversion ratios were drastically affected throughout the 7-weeks study period in chicks receiving *Grewia* ethanolic extract *via* oral, intraperitoneal, and intramuscular routes comparing those fed on 2% *Grewia* with those fed on 10% *Grewia*, which exhibited an intense yellow discoloration at the combs and shanks. Enterohepatonephropathy is a characteristic feature of plant toxicities in chicks particularly induced by feeding at 10% of the basic diet or administering ethanolic extract by the oral route (500mg/kg/day), i.p route (50mg/kg/day), and i.m. route (10mg/kg/day). Lesions were correlated with changes in serum aspartate aminotransferase activity and concentrations of total protein, albumin, globulin, total bilirubin, cholesterol, uric acid, and calcium as well as with alterations in hemoglobin, packed cell volume, red blood cells, mean corpuscular volume and mean corpuscular hemoglobin concentration values.

K e y w o r d s

Dietary *Grewia tenax*
Goddaim fruit
Ethanolic extract
Bovans-type chicks

Introduction

Since the time man first discovered the plant kingdom as a rich and convenient source of his food, he returned to this kingdom repeatedly and found remedies for illness too (Murthy et al, 2011). *Grewia tenax* roots, leaves, juice and fruit decoctions have been used in Africa and Southeast Asiatic countries for a variety of medical purposes (Khemiss et al., 2006). Guddaim is the local name of *Grewia tenax*, is one of the valuable plant species in Sudan. It is largely spread in arid area such as sand and near mountains, especially in the Savanna plantation area of the Northern and Middle of Sudan (Aboagarib et al., 2014).

All parts of the plant are a rich source of medicinally useful components. Leaves are easily available in abundant amounts and have been intensively used in traditional medicine for the treatment of venous insufficiency, hemorrhoids, hypoglycemia, diarrhea and fungal or microbial infections (Hotez et al., 2008; Syeda et al., 2014).

There is commercial potential in using the fruits in beverages, ice cream, yogurt, and baby food. In Sudan, beverages are prepared by soaking the fruits in water for 3–4 h followed by hand pressing, sifting, and sweetening. The juice is regarded as a good thirst-quencher, especially during the hot season. Because of its high iron, the fruits are used by tribal members as an iron supplement for anemic children (Aboagarib et al., 2014) and the sweetened porridge is given to pregnant and lactating women to improve their health and milk production (Gebauer et al., 2007).

Materials and methods

This study was carried out according to the guidelines of the Animal and Human Ethical Committee of Omdurman Islamic University (Sudan).

Successive extractions of plant materials

The plant (Fig. 1) was identified, classified and authenticated by botanists in Medicinal and Aromatic Plants, Research Institute (MAPRI) The National Research Center (NRC), Khartoum, Sudan.

The fruits of *Grewia tenax* were collected from western Sudan, cleaned and then coarsely ground in a mortar. The ground materials were extracted successively with petroleum ether (60-80%) and ethanol (95%) using a Soxhelt extraction method (Soxhelt, 1879). The ethanolic extract was evaporated to dryness under vacuum to give a residue which was subjected to toxicity evaluation in chicks.

Fig. 1: *Grewia tenax* plant.

Experimental plan

Seventy two of 7days-old chicks of both sexes were housed in pens at the faculty of Pharmacy, Khartoum University. The chicks were divided randomly into 6 groups each of 12 chicks. Group 1 chicks were (control group) and fed with starter diet. Fruits of *Grewia tenax* were added, to starter mash and fed at 2% (group 2) and 10% (group 3) of the basal diet. The ethanolic extract of the fruit was provided daily to chick in drinking water at 500 mg kg⁄day (group 4) or injected at 50mg ⁄kg day via intraperitoneal route (group 5), or at 10mg ⁄kg⁄day via intramuscular route (group 6). At the end of 4th week the administration of alcoholic extract was stopped and the experimental diets were withdrawn and replaced by control ration for a 3-week period.

Batches of 4 chicks/ group were slaughtered at week 2, 4 and 7 and blood samples were collected. Growth rates, clinical signs, mortality, pathology and alteration in hematology and clinical chemistry were investigated.

Methods used for determination of serum constituents

Blood samples were obtained from chicks by cutting the cervical blood vessels during slaughter according to Muslim practice into clean dry bottles and allowed to clot overnight at room temperature. The collected blood was centrifuged at 3000 rpm for 10 min and sera were stored at –20ºC until analyzed.

Serum AST (aspartate transaminase), albumin, globulin, bilirubin, uric acid, total cholesterol, total proteins, and calcium were measured by the specific

enzymatic colorimetric methods. Hematological parameters like hemoglobin (Hb) concentration, mean corpuscular volume (MCV), packed cell volume (PCV), red blood cell (RBC) count, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated.

Histopathological methods

The specimens of tissues were collected immediately after slaughter of birds or death and fixed in 10% Normal saline, embedded in paraffin wax, sectioned at 5µm and stained routinely with haematoxylin and eosin (H&E) using Harris's haematoxylin formula.

Statistical analysis

The differences between mean values of the parameters were analyzed by the unpaired student's t-test (Snedecor and Cochran, 1967).

Results

There were no significant differences ($p>0.05$) in mean body weights between chicks on *Grewia* food at 2% (group 2) or 10% (group 3) and the control (group 1) throughout the 7 weeks of the experiment. On the other hand, the average body weight of chicks given *Grewia* ethanolic extracts at 500 mg/kg in drinking water (group 4), 50mg/kg via i.p route (group 5) and 10mg/kg via i.m route (group 6) was markedly decreased ($p<0.05$) at week 2, 3 and 4 of the toxicity period and throughout the 3-week recovery interval when compared with the controls in group 1 and chicks on Grewia food at 2% (group 2) or 10% (group 3). The lowest body weight gain was shown by chicks given the ethanolic extract (group 4, 5 and 6) during the toxicity period. The chicks in the latter groups were in appetent and weak and their food conversion ratios were drastically affected when compared to other groups (Table 1).

Table 1. Change in average food conversion ratio in chicks given *Grewia tenax* extract by different routes of administration.

Groups	Toxicity period	Recovery Period
1 (control)	3.3 ± 1.5	2.5 ± 0.2
4 (500 mg/kg in water)	4.5 ± 0.14^{NS}	4.5 ± 0.14^{NS}
5 (50mg/kg i.p route)	$7.7 \pm 2.9^{*}$	12.8 ± 2.8^{NS}
6 (10mg/kg i.m route)	5.03 ± 0.28	$8.2 \pm 1.5^{**}$
NS = Not significant; * = $p<0.05$; ** = $p<0.01$.		

None of the chicks fed *Grewia* fruits (groups 2 and 3) or given their ethanolic extract by different routes of administration (group 4, 5 and 6) died during the experiment. There was an intense yellow discoloration at the shanks and combs particularly in chicks on 10% *Grewia* food. The yellow discoloration observed in groups 2 and 3 was not seen at the end of the recovery period.

At weeks 2 and 4 the yellow discoloration was also observed on the reflected skin and the enlarged liver with linear hemorrhage on the thigh of chicks in groups 2 and 3. The intensity of this color was found to decrease towards the end of the recovery period. The liver showed fatty change with scattered areas of congested sinusoids and the renal glomeruli revealed varying degrees of lymphocytic aggregates but the epithelial cells of the convoluted tubules were not significantly affected either during the feeding period or after withdrawal from the test diets. Other tissues of chicks in group 2 and 3 and of the controls (group 1) did not show significant lesions. In group 4 chicks given the ethanolic extract Grewia fruit at 500mg/kg/day in water, the lesions were similar to those described for groups 2 and 3. Enteritis was not seen although detachment of the intestinal epithelium of a few chicks was seen between weeks 2 and 4. No yellow discoloration was observed at post-mortem examination, at the end of the recovery period. In group 5 chicks given the ethanolic extract of *Grewia tenax* fruit at 50mg/kg/day *via* the i.p. route, there was fatty change and hepatocellular necrosis between weeks 2 and 4 and slight desquamation of the intestinal epithelium. The renal lesions were not different from those observed in previous groups. In group 6 (10mg/kg/day *via* i.m. route) slight hemorrhage was seen on the thigh without yellow discoloration in the tissues. The renal, hepatic and intestinal damage was moderate and similar in nature to that seen in groups 4 and 5.

Changes in serum constituents

Changes in serum constituents of chicks in *Grewia* diet at 2% (group 2) or 10% (group 3) and of the control group (group 1) are presented in Table 2. The cholesterol concentration was higher (p<0.01) in group 2 and 3 than group 1. Total protein and uric acid concentrations were higher (p<0.02) in group 2 than group 3 and control group. Serum AST activity was significantly increased (p< 0.01) in group 3 than groups 2 and 1. There were no significant changes in the concentration of calcium, bilirubin, albumin and globulin in the serum of the control chicks (group 1) or the test chicks in groups 2 and 3. During the recovery period the concentration of total protein was significantly elevated (p<0.05, 0.01) in both groups 2 and 3 and was due to tendency of increase in globulin concentrations. The concentration of total cholesterol was still higher (p<0.05, 0.01) in groups 2 and 3 than that of the control group and AST activity returned to normal. Changes in Serum Constituents of chicks in Grewia ethanolic extract by different routes of administration are summarized in Table 3. There was a significant increase (p<0.001) in serum protein concentration in group 4 (500mg/kg/day in water), group 5 (50mg/kg/ via i.p. route) and group 6 (10mg/kg/day via i.m. route). Serum cholesterol and uric acid concentrations increased (p<0.05, 0.001) in groups 4 and 6. Bilirubin, calcium, and AST activity did not change in the serum of the test chicks (group 4 – 6) or the control (group 1). However, AST activity increased (p<0.02) in the test groups during the recovery period and uric acid concentration was higher (p<0.05) in groups 4 and 6 than other groups.

Hematological findings

Hematological changes in chicks fed with *Grewia tenax* fruit at 2 % (group 2) or 10% (group 3) are shown in Table 4. There was no significant change in the values of Hb or PCV in the test chicks (groups 2 and 3) when compared with those of the controls (group 1). MCHC significantly increased (p< 0.02) in groups 2 and 3 but MCV increased (p<0.05) in group 2 only. During the recovery period, no significant changes were observed in Hb, RBC or MCHC but significant increases (p<0.01, 0.001) in PCV and MCV of the test chicks were recorded. There was no significant change in the values of Hb, PCV, or MCHC (Table 5) in chicks given Grewia ethanolic extract at 500 or 10 mg/kg/day via i.m. route (group 6). MCV was higher (p<0.02 - 0.001) in the test chicks (groups 4 - 6) than the control (group1). During the recovery period PCV and MCV were higher (p<0.05, 0.001) in the test groups than the controls.

Table 2. Change in serum constituents of chicks fed *Grewia tenax*.

Groups	AST (IU)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)
Toxicity period (4 weeks)								
1(Control)	93.67 ± 4.11	3.20 ± 0.16	2.17± 0.17	1.03 ± 0.1	0.36 ± 0.05	115.67 ± 9.74	4.07 ± 1.11	12.00 ± 1.63
2 (2% GTE)	101.33 ± 42.91 ^{NS}	3.57 ± 0.17†	2.00 ± 0.14^{NS}	1.57 ± 0.08^{NS}	0.50 ± 0.00^{NS}	161.67 ± 24.58***	5.70 ± 0.42***	12.67 ± 4.99^{NS}
3(10% GTE)	135.33 ± 10.87***	3.33 ± 0.9^{NS}	2.10 ± 0.2^{NS}	1.25 ± 0.05^{NS}	0.50 ± 0.00^{NS}	161.67 ± 24.58***	3.10 ± 0.22^{NS}	12.00 ± 3.27^{NS}
Recovery period (3 weeks)								
1 (Control)	92.33 ± 2.87	3.33 ± 0 .34	2.4 ± 0.29	0.93 ± 0.80	1.08 ± 0.01	102.67 ± 18.62	3.63 ± 0.12	11.33 ± 2.49
2 (2% GTE)	56.00 ± 46.33^{NS}	3.93 ± 0.66*	2.20 ± 0.28^{NS}	1.73 ± 0.10^{NS}	1.60 ± 1.56^{NS}	168.17 ± 22.88***	6.40 ± 0.57†	13.33 ± 4.25^{NS}
3 (10% GTE)	58.00 ± 43.91^{NS}	4.93 ± 0.75***	3.00 ± 0.62^{NS}	1.93 ± 0.03^{NS}	0.50 ± 0.00 ^{NS}	150.67± 38.52***	4.37 ± 2.31^{NS}	12.00 ± 4.23^{NS}

GTE- *Grewia tenax* extract; NS = Not significant; * = p<0.05; **= p<0.02; *** = p<0.01; † = p<0.001.

Table 3. Change in serum constituents of chicks given *Grewia tenax* ethanolic extract by different routes of administration.

Groups	AST (IU)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Bilirubin (mg/dl)	Cholesterol (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)
Toxicity period (4 weeks)								
1 (control)	33.67 ± 4.11	3.20 ± 0.16	2.17 ± 0.17	1.03 ± 0.17	0.36 ± 0.45	115.67 ± 9.74	4.07 ± 1.11	11.00 ± 1.63
4 (GTE in water 500mg/kg/d)	20.00 ± 11.31^{NS}	3.93 ± 0.66*	2.20 ± 0.28†	1.73 ± 0.18**	0.62 ± 0.35^{NS}	168.67 ± 22.88†	6.40 ± 0.57***	7.67 ± 0.94
5 (GTE i.p. 50mg/kg/d)	22.00 ± 74^{NS}	4.93 ± 0.75†	3.90 ± 0.62†	1.03 ± 0.03^{NS}	0.50 ± 0.00^{NS}	137.33 ± 22.88***	4.77 ± 2.64^{NS}	10.00 ± 4.32
6 (GTE i.m. 10mg/kg/d)	32.67 ± 44.19^{NS}	4.20 ± 0.16†	2.97 ± 0.48***	1.23 ± 0.02^{NS}	0.40 ± 0.28^{NS}	186.67 ± 18.86†	6.03 ± 1.79*	8.00 ± 1.63^{NS}
Recovery period (3 weeks)								
1 (control)	23.37 ± 2.87	3.33 ± 0.34	2.40 ± 0.29	0.93 ± 0.01	0.80 ± 0.01	102.00 ± 18.62	3.63 ± 0.12	11.33 ± 2.49
4 (GTE in water 500mg/kg/d)	102.33 ± 2.36†	3.97± 0.09***	2.33 ± 0.63^{NS}	1.54 ± 0.01^{NS}	0.42 ± 0.11^{NS}	115.33 ± 22.07^{NS}	4.30 ± 1.27^{NS}	10.67 ± 5.25^{NS}
5 (GTE i.p. 50mg/kg/d)	99.33 ± 3.68**	3.35 ± 0.25^{NS}	2.27 ± 0.25†	1.08 ± 0.3^{NS}	0.62 ± 0.35^{NS}	96.67 ± 12.36^{NS}	4.33 ± 0.61*	12.00 ± 1.63^{NS}
6 (GTE i.m. 10mg/kg/d)	103.33 ± 0.94†	3.93 ± 0.34*	1.97 ± 0.76^{NS}	1.97 ± 0.06^{NS}	0.90 ± 0.00^{NS}	152.33 ± 35.72**	4.33 ± 0.61*	6.67 ± 2.49^{NS}

GTE- *Grewia tenax* extract; NS = Not significant; * = p<0.05; **= p<0.02; *** = p<0.01; † = p<0.001.

Table 4. Hematological changes in chicks fed with *Grewia tenax*.

Groups	Hb (g/dl)	PCV (%)	RBCs' count (x10^6 mm^3)	MCV (m^3)	MCHC (%)
Toxicity period (4 weeks)					
1(Control)	8.9 ± 0.36	35± .82	2.67 ± 0.9	131.50 ± 7.29	25.44± 1.10
2 (2% GTE)	9.2 ± 0.22^{NS}	32.33 ± 2.05^{NS}	2.32 ± 0.14^{NS}	139.34 ± 0.48*	28.53±1.33**
3(10% GTE)	9.07 ± 0.05^{NS}	32 ± 0.82^{NS}	2.43 ± 0.05^{NS}	131.61 ± 5.64^{NS}	28.36±.85**
Recovery period (3 weeks)					
1 (Control)	9.37 ± 0.31	31± 1.63	2.7±0.8	115.01±8.18	30.35 ± 2.58
2 (2% GTE)	9.1 ± 0.08^{NS}	35 ± .82***	1.71 ± 0.0^{NS}	204.94± 5.43†	26.01 ± 0.54^{NS}
3 (10% GTE)	9.43 ± 0.21^{NS}	35.67±1.25***	1.8± .08^{NS}	198.34± 6.67†	26.47 ± 0.75^{NS}

GTE- *Grewia tenax* extract; NS = Not significant; * = p<0.05; **= p<0.02; *** = p<0.01; † = p<0.001.

Table 5. Hematological changes in chicks given *Grewia tenax* ethanolic extract by different routes of administration.

Groups	Hb (g/dl)	PCV (%)	RBCs' count (x10^6 mm^3)	MCV (m^3)	MCHC (%)
Toxicity period (4 weeks)					
1 (control)	8.9 ± 0.36	35 ± 0.82	2.67 ± 0.7	131.5 ± 7.29	25.44 ± 1.10
4 (GTE in water 500mg/kg/d)	8.63 ± 0.41^{NS}	36.33 ± 1.25^{NS}	1.15 ± 0.4^{NS}	315.90 ± 30.89†	23.75 ± 0.44**
5 (GTE i.p. 50mg/kg/d)	8.47 ± 0.29^{NS}	35.83 ± 1.25^{NS}	2.25 ± 0.18^{NS}	157.95 ±12.66**	23.89 ± 0.77^{NS}
6 (GTE i.m. 10mg/kg/d)	9± 0.36^{NS}	34 ± 1.63^{NS}	2.3 ± 0.19^{NS}	148.26 ±5.55***	26.52 ± 1.5^{NS}
Recovery period (3 weeks)					
1 (control)	9.37 ± 0.31	31 ± 1.63	2.7 ± 0.8	115.01 ± 8.18	30.35 ± 2.58
4 (GTE in water 500mg/kg/d)	9.4 ± 0.08^{NS}	32.67 ±2.62^{NS}	2.32 ± 0.24^{NS}	143.55 ± 24.63*	28.97 ± 2.44^{NS}
5 (GTE i.p. 50mg/kg/d)	9.10 ± 0.08^{NS}	36 ± 2.16***	2.27 ± 0.12^{NS}	159.85 ± 18.78**	25.38 ± 1.68^{NS}
6 (GTE i.m. 10mg/kg/d)	9.10 ± 0.08^{NS}	35.33 ± 1.70†	2.57 ± 0.19^{NS}	138.71 ± 14.92**	25.81 ± 1.11^{NS}

GTE- *Grewia tenax* extract; NS = Not significant; * = p<0.05; **= p<0.02; *** = p<0.01; † = p<0.001.

Discussion

As expected there were no differences among the chicks in terms of body weights for the 7-weeks pretrial duration. This is explained by the feeding of identical well-balanced ration to the chicks for a sound randomized allotment for testing.

Incorporation of Grewia tenax fruits into the basal diet at 2 and 10% were chosen for a variety of reasons. Firstly, these low dietary levels represent non-toxic concentrations for chicks of the number of plants such as *Cassia occidentalis* (Mercer et al., 1967), *Cassia obtusifolia* (Herbert and Flory, 1983) and *Cucurbita maxima* (Mohamed, 1992). However, 2 or 5% of dietary *Abrus precatorius* and *Ricinus communis* seeds have been found to be toxic for chicks as reported by Omer et al. (1992) and El Badwi et al. (1992) respectively.

Secondly, moderate dietary concentration at 20% of the defatted and debittered *Azadirachta indica* kernel meal decrease food consumption and body weight gain in rats (Sankaram et al., 1987). Thirdly, considerable variations in the toxicity to poultry and livestock of different plant constituents are well documented (Bakhiet et al., 2006). Therefore, it appears that susceptibility of chicks to feeding with plants materials is, at least, dependent on the type of active constituents' and their concentration in the specified amount added to the diet as well as the rate of their metabolic conversion in the liver to metabolites and consequent excretion (Bakhiet et al., 2006).

No studies had been done to delineate toxic effects of *Grewia tenax* on chicks. Dietary *Grewia* fruit was not accompanied by prominent growth changes while the ethanolic extract of this plant fruit given by different routes of administration caused growth changes without signs of morbidity or mortality. The development of yellow discoloration at the shanks and combs of chicks particularly on 10% *Grewia* food is suggestive of hepatic excretory dysfunction. The result might, however, indicate that Grewia fruit is a rich source of pigments or flavonoids that had accumulated in the blood circulation as a consequence of hepatic malfunction. A similar observation of yellow discoloration at the shanks and combs of brown Hisex chicks fed dietary *Azadirachta indica* leaves for 4 weeks was reported by Ibrahim et al. (1992) who found changes in the values of erythrocyte count, hemoglobin concentration, packed cell volume, mean corpuscular volume and mean corpuscular hemoglobin and associated with yellow discoloration on the legs and combs and hepatonephropathy. Tissue recovery was incomplete 2 weeks after removal from the experimental *Azadirachta indica* diets.

Lesions in the liver of chicks fed dietary Grewia or given the ethanolic extract of the plant products by different route of administration comprised congestion, lymphocytic nodules, fatty change and/ or individual cell necrosis. Degenerated renal tubules and inflammatory cell response were present in the cortex. These findings indicate that renal tubular toxicity is a sequela of plant toxicities in chicks. The increase in activity of AST and in the concentration of uric acid and decreases in the concentration of total protein in serum as well as the histopathological changes were indicative of damage to the liver and kidneys. Significant increases in serum uric acid concentrations corroborate the findings of Omer et al (1992) and confirm changes in glomerular filtration. Analysis of the parameters suggested that uric acid concentration would be useful diagnostically because of uniform changes along with pathological features of renal toxicity. Post-mortem lesions along with microscopic observations in renal tubules and glomeruli indicated nephrotoxicity of the constituents contained in the plants investigated. Since AST is not a liver specific enzyme, we believe that damages to other organs such as those occurring in the kidneys and gastrointestinal tract could be a contributing factor to the changes in the activity of serum AST.

Khemiss et al. (2006) reported the effect of aqueous extract of *Grewia tenax* fruit (AEGTF) on the variation of iron absorption in male Wistar rats. Addition of AEGTF at different concentrations favors significantly this iron transfer from the mucous side toward the serous one. The maximum of iron absorption was carried out in the presence of AEGTF at 10 mg/ml and 5 min of incubation time in stomach, duodenum and jejunum. AEGTF used at high doses (20 and 30 mg/ml) reduced significantly iron uptake suggesting a probable toxic effect of this extract. Histological studies confirmed the presence of cytotoxic signs as multinucleated giant cells and the disappearance of enterocyte border brush. In the present study, lesions of major importance were found in the intestines and heart. Congestions and hemorrhage particularly at the

site of i.m. injection with the ethanolic extract at 10 mg/kg/day were attributable to irritant and/or endotheliotoxic effects resembling those observed in chicks which had been injected by the i.m. route with *Balanites aegyptiaca* kernel saponin (Nakhala et al., 1992).

Degenerative changes in liver and kidneys as well as congestion and accumulation of lymphocytes between the cardiac muscle fibers of some of the chicks receiving the plant materials points to an inflammatory cell response. Such findings are similar to some recent studies that described the Toxic Effects of ochratoxin A in chicks (Stoev, 2010; Lautert et al., 2014; Xiaozhe et al., 2014).

Conclusion

Enterohepatonephropathy is a characteristic feature of *Grewia tenax* toxicities in chicks particularly induced by feeding at 10% of the basic diet or administering ethanolic extract by the oral route (500mg/kg/day), i.p. route (50mg/kg/day), and i.m. route (10mg/kg/day). Lesions were correlated with changes in serum aspartate aminotransferase (AST) activity and concentrations of total protein, albumin, globulin, total bilirubin, cholesterol, uric acid, and calcium as well as with alterations in Hb, PCV, RBC, MCV and MCHC values.

Recommendations

More studies are needed to elucidate the mechanism responsible for the development of lesions in the liver, kidneys and intestines by plant constituents. The isolation, characterization and concentration of the active principles of the *Grewia tenax* fruits had to be done. Long term experiments to examine the carcinogenicity of this plant and/or their isolated active principles should be undertaken.

References

Aboagarib, E.A.A., Yang, R., Hua, X., Siddeeg, A., 2014. Chemical compositions, nutritional properties and volatile compounds of Guddaim (*Grewia tenax* Forssk) Fiori fruits. J. Food Nutr. Res. 2(4), 187-192.

Bakhiet, AlO., Ibrahim, E.A., 2006. Response of Bovans chicks to dietary *Commiphora myrrha*, *Glycyrrhiza glabra* or their mixture. J. Biol. Sci. 6, 950-953.

El-Badwi, S.M., Adam, S.E.I., Hapke, H.J., 1992. Experimental *Ricinus communis* poisoning in chicks. Phytother. Res. 6, 205-208.

Gebauer, J., El-Siddig, K., El Tahir, B. A., Salih, A. A., Ebert, G., Hammer, K., 2007. Exploiting the potential of indigenous fruit trees: *Grewia tenax* (Forssk.) Fiori in Sudan. Genetic Res. Crop Evol. 54(8), 1701-1708.

Herbert, C.D., Flory, W., 1983. Determination of the oral toxicity of *Cassia obtusifolia* seeds in chickens. Vet. Hum. Toxicol. 25(3), 164-166.

Ibrahim, I.A., Khalid, S.A., Omer, S.A., Adam, S.E., 1992. On the toxicology of *Azadirachta indica* leaves. J. Ethnopharmacol. 35(3), 267-273.

Khemiss, F., Ghoul-Mazgar, S., Moshtaghie, A.A., Saidane, D., 2006. Study of the effect of aqueous extract of *Grewia tenax* fruit on iron absorption by everted gut sac. J. Ethnopharmacol. 103, 90–98.

Lautert, C., Ferreiro, L., Wolkmer, P., Paim, F.C., da Silva, C.B., Jaques, J.A., Lopes, S.T., Santurio, J.M., 2014. Individual in vitro effects of ochratoxin A, deoxynivalenol and zearalenone on oxidative stress and acetylcholinesterase in lymphocytes of broiler chickens. Springerplus 3, 506. doi: 10.1186/2193-1801-3-506.

Mercer, H.D., Neal, F.C., Himes, J.A., Edds, G.T., 1967. *Cassia occidentalis* toxicosis in cattle. J. Am. Vet. Med. Assoc. 151(6), 735-741.

Mohamed, A.B., 1992. Effect of various levels of dietary *Lupinus termis* and *Cucurbita maxima* on chicks. M.Sc., Veterinary Sciences dissertation, University of Khartoum, Sudan.

Murthy, P.K., Joseph, S.K., Murthy, P.S., 2011. Plant products in the treatment and control of filariasis and other helminth infections and assay systems for antifilarial/anthelmintic activity. Planta Med. 77(6), 647-661.

Nakhla, H.B., Mohamed, O.S.A., Abu El Futuh, I.M., Adam, S.E.I., 1992. Effect on chicks of *Balanites aegyptiaca* kernel saponin given by different routes of administration. Vet. Hum. Toxicol. 33, 224.

Omer, S.A., Ibrahim, F.H., Khalid, S.A., Adam, S.E.I., 1992. Toxicological interactions of *Abrus precatorius* and *Cassia senna* in the diet of Lohmann broiler chicks. Vet. Hum. Toxicol. 34(4), 310–313.

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., Jacobson, J., 2008. Helminth

infections: the great neglected tropical diseases. J. Clin. Invest. 118(4), 1311–1321.

Sankaram, A.V.B., Murthy, M.M., Bhaskaraiah, K., Subramanyam, M., Sultana, N., Sharma, H.C., Leuschner, K., Ramaprasad, G., Sitaramaiah, S., Rukmini, C., Rao, P.U., 1987. Chemistry, biological activity and utilization of some promising neem extracts. In: Schmutterer and Ascher, 1987. pp. 127-148.

Snedecor, G.W., Cochran, W.G., 1967. Statistical Methods. The Iowa State University Press, Ames, Iowa.

Soxhlet, F., 1879. "Die gewichtsanalytische Bestimmung des Milchfettes". Dingler's Polytechnisch. J. (in German) 232, 461–465.

Steov, S.D., 2010. Studies on carcinogenic and toxic effects of Ochratoxin A in chicks. Toxins (Basel). 2(4), 649–664.

Syeda, J. B. T., Subba Reddy, G.V., Jayaveera, K.N., 2014. Anthelmintic activity of crude extract of *Grewia tenax*. World J. Pharm. Pharmaceut. Sci. 3(8), 1710-1715.

Xiaozhe Qi, Xuan Yang, Siyuan Chen, et al., 2014. Ochratoxin A induced early hepatotoxicity: new mechanistic insights from microRNA, mRNA and proteomic profiling studies. Scient. Rep. 4, 5163.