ISSN: 0145-6296

Veterinary and Human TOXICOLOGY

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An Estimation of Citrullus Colocynthis Toxicity for Chicks

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ABSTRACT. Citrullus colocynthis seed was fed at 2% and 10% of the basal diet to 7-d-old Bovans-type chicks for 6 w. Average body weights and efficiency of feed utilization were markedly depressed in the chicks on 10% Citrullus feed, and the serum activities of LDH, AST and CK and concentrations of total lipid and zinc were significantly increased. The concentration of serum total iron binding capacity was particularly reduced in chicks on 2% Citrullus feed. The concentrations of other serum and blood constituents and of hepatic copper, manganese and zinc were not significantly changed. Lesions seen in the intestines, livers, kidneys and other tissues were fully reversed 4 w after removal from the experimental diet.

Citrullus colocynthis (Cucurbitaceae), locally known as handal, is found throughout the plains of western and central Sudan where it is claimed to possess several medicinal properties. C colocynthis fruits and seeds are used in traditional medicines as purgatives, anthelmintics and molluscicidal agents (1,2). The main constituents in the plant are cucurbitacins A, B, C and D and α-elaterin (3). C colocynthis alcoholic extract, colocynithin and hydrated colocynithin were efficient insecticidal substances against American cockroaches, adult honey bee, house fly and red bug (4).

The comparative toxicity of various oral dosages of the plant fruit in calves, sheep and goats has been communicated previously (5). It is common practice for livestock and poultry producers to buy sorghum or wheat grain for use in animal and chicken diets, but these may be contaminated with weed seeds and other parts of plants which grow in sorghum or wheat fields. When contaminants are found the obvious concern is the establishment of contamination rates and evaluation for safe use in livestock and poultry diets.

Information on the toxicological effects of C colocynthis in poultry is lacking. We report investigations of feeding small concentrations of C colocynthis seeds to Bovans-type chicks.

MATERIALS AND METHODS

Experimental Design

Thirty-six 1-d-old Bovans cockerels were purchased from Coral Co, Khartoum, and housed within the premises of the Faculty of Veterinary Science, University of Khartoum, with light at night and early morning and with feed and water provided ad libitum. At 7-d of age, the chicks were assigned to 1 of 3 equal groups. Group 1 chicks were controls and fed a basal starter diet. C colocynthis seeds were bought from Omdurman Central Market, ground and fed at 2% of the basal diet to chicks in Group 2 and at 10% of the diet to Group 3. Feeding was continued for 6 w, when the test diets were replaced by the basal feed for 4 w (recovery period).

Four chicks/group were slaughtered at 3, 6 and 10 w for pathological examinations. Blood samples were collected at each slaughter for hematology and serum chemistry analysis. All chicks were examined for gross lesions. The liver, kidneys, heart, proventriculus, intestine and spleen were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

Evaluations

Mean body weight and feed conversion ratios (kg feed consumed/kg weight gain) were recorded for each group weekly. Blood samples were examined for hemoglobin concentrations (Hb), packed cell volumes (PCV), red blood cell (RBC) counts, mean corpuscular volumes (MCV) and mean corpuscular hemoglobin concentrations (MCHC) according to the methods described by Schalm (6). Serum samples were analyzed for the activities of lactic dehydrogenase (LDH), alkaline phosphatase (ALP),

aspartate transaminase (AST), gamma glutamyl transferase (GGT) and creatine kinase (CK), and for concentrations of uric acid, total protein, cholesterol, total lipid, phosphorus, calcium, magnesium, total iron and total iron binding capacity (TIBC) by commercial kits (Stanbio Laboratory Inc, San Antonio, TX; Bio-Analytics, Palm City, FL; King Diagnostic Inc, Indianapolis, IN). Serum zinc and manganese and hepatic zinc, copper and manganese were determined by atomic absorption spectrophotometry (Shimadzh Model AA-670, Germany).

Statistical Analysis

Statistical significance was assessed by Student's "t"-test (7).

RESULTS

Effect on Growth

Changes in average body weight gains and feed conversion ratios in the chicks fed C colocynthis for 6 w are presented in Table 1. Group 2 fed 2% Citrullus feed had significantly increased ($p<0.02$–0.01) growth rate during the 6-w feeding period compared to the other 2 groups. However, the average body weight and efficiency of feed utilization of the chicks on 10% Citrullus feed (Group 3) were lower ($p<0.02$–0.001) than those of the chicks in Group 2 and for the controls (Group 1) at 3 and 6 w. Four weeks after removal from the test diets, the average body weight gain and efficiency of feed utilization of the chicks in Group 3 were significantly lower ($p<0.02$–0.01) than those of the chicks in Groups 2 and 1.

Pathological Findings

Chicks fed 10% Citrullus feed (Group 3) had catarrhal enteritis with lymphocytic infiltration and erosions of the intestinal epithelium, cytoplasmic fatty vacuolation and focal necrosis of the centrilobular hepatocytes, congestion of the hepatic blood vessels and of the renal cortex, degeneration of the epithelial cells of the convoluted tubules and lymphocytic accumulation between the cardiac muscle fibers. Chicks on the 2% Citrullus feed (Group 2) had lesions that were less severe than in the chicks fed 10% Citrullus feed (Group 3). No lesions were seen in the proventriculus or spleens of the chicks fed the test diets. There were no such changes in the control chicks (Group 1).

Table 1. Growth parameters in chicks fed C colocynthis

Group	w 3	w 6	w 10
	Average body weight (g)		
1	160.8 ± 1.17	342.0 ± 11.30	635.0 ± 49.4
2	183.0 ± 6.36*	403.3 ± 4.70**	629.16 ± 41.2^{NS}
3	147.36 ± 0.50**	325.6 ± 7.95**	527.5 ± 3.9**
	Average weight gain (g)		
1	52.1 ± 0.23	181.2 ± 10	293.0 ± 13.9
2	79.08 ± 0.12***	220.3 ± 0.6**	226.0 ± 2.3**
3	55.8 ± 0.14*	178.24 ± 0.7^{NS}	201.9 ± 10.6**
	Feed conversion ratio (kg feed / kg gain)		
1	2.40 ± 0.56	2.02 ± 0.3	3.47 ± 0.56
2	3.14 ± 0.19**	2.40 ± 0.14*	3.74 ± 0.65^{NS}
3	3.74 ± 0.56***	6.62 ± 0.16****	6.63 ± 0.14***

Groups : 1 = Control; 2 = 2% Citrullus; 3 = 10% Citrullus.

NS = Not significant; * = $p < 0.05$; ** = $p < 0.02$; *** = $p < 0.01$; **** = $p < 0.001$

Table 2. Serum chemistry determination in chicks fed C colocynthis

Group	LDH (iu)	CK (iu)	AST (iu)	Total lipid (mg/100ml)	Zinc (mg/100ml)	TIBC (ug / dl)
	Parameters					
1	65.2 ± 21.5	129.6 ± 11.9	54.0 ± 13.6	5.0 ± 0.56	0.35 ± 2.8	134.9 ± 37.3
2	143.9 ± 14.4	414.9 ± 206.6*	67.5 ± 14.8	5.8 ± 0.47	0.30 ± 2.9	48.8 ± 8.6*
3	176.09 ± 0.77***	638.27 ± 379.5*	148.66 ± 9.26*	6.09 ± 0.60**	0.74 ± 0.34*	241.4 ± 98
	Recovery period					
1	93.3 ± 42.4	135.8 ± 28.1	50.8 ± 20.4	4.2 ± 0.9	0.40 ± 0.01	142.0 ± 0.4
2	102.9 ± 50.9^{NS}	481.7 ± 52.9***	51.0 ± 21.7^{NS}	6.8 ± 0.72**	0.29 ± 0.08****	124.4 ± 54^{NS}
3	71.1 ± 20.1^{NS}	959.1 ± 45.8***	51.54 ± 19^{NS}	6.23 ± 0.73*	0.35 ± 0.04^{NS}	169.7 ± 29.6^{NS}

Groups: 1 = Control; 2 = 2% Citrullus; 3 = 10% Citrullus
NS = Not significant; * = $p < 0.05$; ** = $p < 0.02$; *** = $p < 0.01$; **** = $p < 0.001$

Serum Chemistry Assays

There were significant differences ($p<0.05$–0.01) in the activities of CK, LDH and AST and in the concentrations of total lipid and zinc between chicks fed 10% Citrullus diet and the control chicks (Group 1) and chicks in Group 2 fed 2% Citrullus diet (Table 2). In Group 2 chicks, serum CK activity was higher ($p<0.05$) than in the control chicks (Group 1), and TIBC was lower ($p<0.05$) than in the chicks from Groups 3 and 1. Citrullus feeding had no effect on serum calcium, phosphorus, magnesium, total protein, bilirubin, cholesterol, uric acid, iron, manganese, copper and ALP. At the end of the recovery period the concentrations of AST, LDH and TIBC returned to normal, but those of CK and total lipid were still higher ($p<0.001$) than in the control chicks. Zinc level was lower ($p<0.001$) in Group 2 than in Groups 3 and 1.

Hepatic Copper, Zinc and Manganese Levels

Table 3 shows the significant differences ($p<0.05$) in hepatic zinc and copper concentrations between the test and control groups at the end of 10 w. The hepatic manganese level did not change during Citrullus feeding or after withdrawal from the test diets. No significant differences ($p<0.05$) were observed in Hb, PCV, RBC, MCV or MCHC between the test and control groups.

DISCUSSION

There is complete lack of information on the response of chicks to various levels of dietary C colocynthis. In the present study, the effects produced by feeding C colocynthis to the Bovans chicks were considered highly important because of the common use of the plant seed or fruit in traditional medicine as purgatives in rural areas. The fact that

Table 3. Hepatic copper, zinc and manganese contents of chicks fed C colocynthis

Group	Cu (mg/100ml)	Zn (mg/100ml)	Mn (mg/100ml)	Cu (mg/100ml)	Zn (mg/100ml)	Mn (mg/100ml)
	Feeding period			Recovery period		
1	1.04 ± 0.04	0.2 ± 0.03	0.21 ± 0.09	1.2 ± 0.02	0.11 ± 0.01	0.26 ± 0.01
2	0.84 ± 0.02^{NS}	0.18 ± 0.04^{NS}	0.26 ± 0.01^{NS}	0.99 ± 0.02^{NS}	0.2 ± 0.08*	0.18 ± 0.09^{NS}
3	1.02 ± 0.01^{NS}	0.25 ± 0.02^{NS}	0.3 ± 0.05^{NS}	0.95 ± 0.01*	0.2 ± 0.003*	0.28 ± 0.04^{NS}

Groups : 1 = Control; 2 = 2% Citrullus; 3 = 10% Citrullus
NS = Not significant; * = $p < 0.05$

weight gains were depressed in the chicks on 10% Citrullus feed suggests that the plant seed contained 1 or more toxic substances that impaired weight gain. Cucurbitacins A, B, C and D and α-elaterin are the active constituents found in C colocynthis (3). Unfortunately, exact data pertaining to chemical analysis of the different parts of the plant are not available. However, the possibility of a dietary or nutritional deficiency being responsible for the poor rate of gain of the 10% group should not be excluded.

In this study, enterohepatonephrotoxicity was the main pathological process observed in the chicks fed C colocynthis. Splenic hemosiderosis and extensive hemorrhage, which had been previously noticed in small ruminants (5), were not documented. This might be due to variability in the concentration of an endotheliotoxic substance in the different parts of the plant.

Necropsy findings and serum chemistry indicate that C colocynthis caused hepatic damage in chicks. Significant elevations in serum AST, LDH, total lipid and zinc, but not in ALP or GGT, were observed in chicks fed C colocynthis. It is necessary, however, to consider the possibility of injury to other organs, such as heart, kidneys and intestines, as contributing to the changes in the activities of LDH and AST in the serum of the experimental chicks since these enzymes are not liver specific. The absence of changes in serum ALP, GGT, bilirubin and cholesterol suggests that interference with the excretory ability of the liver was not a major feature of the Citrullus-induced hepatotoxicity. Elevated serum CK activity pointed to muscular involvement; unfortunately, the musculature of the test chicks was not examined. The absence of decreased total protein concentrations in the serum of the C colocynthis-fed chicks was not surprising as this has been previously noted in chicks fed 2% Cassia senna (8) and in calves fed Senecio jacobea (9). On the other hand, the decrease in serum total protein was noticed in chicks fed Ricinus communis or Abrus precatorius (10,11). Elevated total serum protein concentrations in chicks fed Cucurbita maxima has been interpreted the result of dehydration (12).

The present study showed no change in the concentration of uric acid in the serum of Citrullus-fed chicks. This might be due to relatively mild renal damage, as compared to that previously caused in chicks by Azadirachta indica (13), A precatorius (11) or ochratoxin A (14).

The significance of the microelement status and their interactions in livestock, chickens and laboratory animals has been previously described (15). In the present study, copper, iron and manganese were not found to change whether in the serum or liver of chicks fed C colocynthis. However, Citrullus feeding was associated with decreased TIBC and elevations in serum zinc concentrations. At the end of the recovery period, significant increases in the liver concentrations of zinc and decreases in liver copper were detected. Work is needed on the chemical composition and metabolism of the isolated compounds from different parts of C colocynthis.

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