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A 9-week Feeding Study of *Cuminum cyminum* and *Hibiscus sabdariffa* in Bovans Chicks

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Abstract: The objective of the present study was to investigate the effects of low levels of dietary *Cuminum cyminum* seeds and *Hibiscus sabdariffa* calyces on the growth, organ pathology, haematological and serobiochemical parameters of Bovans chicks. *C. cyminum* seeds and *H. sabdariffa* calyces were fed to 7-day-old Bovans chicks at 2 and 10% of the diet for 9 weeks. The 10% *H. sabdariffa* calyx was toxic but not fatal to chicks and caused reduced body weight gain, inefficient feed utilization, enterohepatotoxicity, anaemia and alterations in serum aspartate aminotransferase and creatine kinase activities and cholesterol, total lipid and uric acid concentrations. These changes were also observed in the chicks fed *C. cyminum* seed at 10% of the diet and *H. sabdariffa* calyx at 2% of the diet but were less marked.

Key words: *Cuminum cyminum*, *Hibiscus sabdariffa*, Bovans chicks

INTRODUCTION

Cuminum cyminum L. (Umbelliferae), a popular aromatic herb, is locally known as *Kamoon* or *Cumin* and widely distributed in Sudan and other countries. It is used in traditional medicine by people in rural areas as an appetizer, antispasmodic and anthelmintic and as a remedy for flatulent colic, asthma, chronic cough and gonorrhoea (Haroun *et al*., 2002). Phytochemical analysis of the aerial parts of the plant demonstrated the presence of flavonoid glycosides, essential oil rich in cuminaldehyde, α-β pinene, γ-3-carene, 1, 8 lineole, terpenes and sesquiterpenes (Ageel *et al*., 1987; Banerjee *et al*., 2002).

Hibiscus sabdariffa (Malvaceae), locally known as karkadeh, is prevalent in various regions of Sudan and other African countries and is used in folk medicine as aphrodisiac, antispasmodic, diuretic and soft drink and for the treatment of gonorrhoea, hepatic disorders, hypertension, pneumonia and pyrexia (Ageel *et al*., 1987; Odigie *et al*., 2003). The calyces of this plant contain flavonal gycosides, alkaloids, amino acids, anthocyanin pigments, pectins, ascorbic, citric and malic acids, calcium and iron (Chen *et al*., 2003, Odigie *et al*., 2003). The calyces having a variety of pigments are utilized in the preparation of jellies and jams (Mclean, 1973).

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In spite of the efforts made in many countries to encourage the production, collection and manufacture of medical materials causalities have over the years been observed among villagers and animals as a result of over dosage because, in general, there is no standardized dosage system in traditional medical practice. Some medicinal plants used in folk medicine have such narrow therapeutic indices that their use is dangerous and should be carefully researched.

Because of the common use of *C. cyminum* and *H. sabdariffa* in the treatment of various disorders as well as the lack of information on their toxicity in birds, rodents and livestock, we investigated the effects of low levels of dietary *C. cyminum* seeds and *H. sabdariffa* calyces on the growth, organ pathology, haematological and serobiochemical parameters of Bovans chicks in pursuance of our investigations on the toxicity of traditionally used medicinal plants (Ibrahim *et al*., 1993, 2004; Bakheit and Adam, 1996).

MATERIALS AND METHODS

Plant Material

Cuminum cyminum seeds and *H. sabdariffa* calyces were purchased from a local market, ground separately to a fine powder and then mixed into a basal diet (Table 1).

Experimental Design

Sixty one day-old Bovans cockerels were obtained from Coral Company Ltd. Khartoum and housed within the premises of the Faculty of Veterinary Medicine, University of Khartoum, under light/dark cycle with diet and water provided *ad libitum*. This experiment was carried out in the year 2006. After 7 days, the chicks were randomly allotted to 5 groups of 12 chicks each. Chicks in group 1 were the controls and fed normal basic diet. Chicks of groups 2 and 3 were fed diets containing 2% (w/w) and 10% (w/w) of ground *C. cyminum* seeds, respectively. Chicks in groups 4 and 5 received diets containing 2% (w/w) and 10% (w/w) of ground *H. sabdariffa* calyces, respectively. All chicks were fed their designated experimental diets for 9 weeks.

Average body weights, body weight gains and feed conversion ratios (kg feed kg^{-1} gain) for each group were estimated weekly. Batches of 4 birds from each group were slaughtered at weeks 3, 6 and 9 for pathological examinations. Blood samples were collected from each of the killed birds for haematology and serum analysis.

Blood Analyses

Haemoglobin (Hb) Concentration, Packed Cell Volume (PCV), Red Blood Cell (RBC) Counts, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were estimated by standard methods (Schalm *et al*., 1975). Sera were analyzed for the activities of aspartate aminotransferase (AST), acid phosphatase (ACP) and Creatine Kinase (CK) and for concentrations of total lipid, cholesterol and uric acid using commercial kits (Stanbio Laboratory Inc., San Antonio, TX).

Table 1: Percent composition of basal diet fed

Ingredients	(%)
Sorghum	58
Soya bean	4
Sesame cake	14
Groundnut cake	12
Wheat bran	5
Marble dust	1
Dicalcium phosphate	1
Super concentrate	5
Total	100

Pathological Examinations

Necropsies were made on all birds to identify gross lesions and specimens of liver, kidneys, spleen, heart, proventriculus and intestines were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin and eosin (H and E) for histopathological examinations.

Statistical Analysis

The significance of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

RESULTS

Effect on Growth

The effects of treatment with diets containing 2 and 10% *C. cyminum* seeds or 2 and 10% *H. sabdariffa* calyces on body weight, body weight gain and feed efficiency of the chicks are presented in Table 2. The chicks fed a diet consisting of 10% *H. sabdariffa* calyces (group 5) for 3, 6 and 9 weeks had the lowest growth rate, but none of the birds died during the 9-week period.

Haematological Changes

In the chicks fed a diet containing 10% *H. sabdariffa* calyces (group 5) for 3 weeks, there were decreases in Hb, RBC, PCV and MCV without effect on MCHC Table 3. After 6 and 9 weeks, changes in erythrocytic series of this group persisted. In chicks fed a diet consisting of 10% *C. cyminum* seeds (group 3) for 6 or 9 weeks, the values of Hb, RBC, PCV and MCV were lower ($p<0.05$) than those in the control and other groups.

Serobiochemical Changes

In the chicks fed 10% *H. sabdariffa* (group 5) for 3, 6 and 9 weeks Table 4, there were increases in the activity of AST and CK and concentration of cholesterol, total lipid and uric acid. Serum enzyme activity and concentrations of cholesterol, total lipid and uric acid were also higher ($p<0.05$) in the

Table 2: Growth changes in Bovans chicks fed diets containing *C. cyminum* or *H. sabdariffa* for 9 weeks

Treatment	Body weight (g)	Weight gain (g)	Feed conversion ratio (kg feed kg^{-1} gain)
Three weeks			
Control (normal diet)	257±6^{a}	202±4^{a}	2.4±0.3^{b}
2% *C. cyminum*	263±11^{a}	203±5^{a}	2.8±0.2^{ab}
10% *C. cyminum*	232±7^{ab}	174±7^{ab}	2.8±0.2^{ab}
2% *H. sabdariffa*	243±8^{ab}	188±6^{ab}	3.4±0.2^{a}
10% *H. sabdariffa*	223±5^{a}	164±3^{b}	3.5±0.2^{a}
Six weeks			
Control (normal diet)	607±4.9^{a}	350±2.9^{a}	3.3±0.13^{b}
2% *C. cyminum*	581±6.3^{ab}	318±2^{ab}	3.9±0.3^{ab}
10% *C. cyminum*	511±6^{b}	279±5.1^{b}	3.6±0.4^{ab}
2% *H. sabdariffa*	521±9^{b}	278±2.9^{b}	4.1±0.3^{a}
10% *H. sabdariffa*	458±5^{c}	235±4^{c}	4.7±0.2^{a}
Nine weeks			
Control (normal diet)	934±3^{a}	327±5^{ab}	3.7±0.2^{b}
2% *C. cyminum*	876±10^{ab}	295±6^{b}	4.2±0.8^{ab}
10% *C. cyminum*	854±9^{b}	343±3^{a}	3.7±0.4^{b}
2% *H. sabdariffa*	857±7^{b}	336±4^{a}	4.4±0.3^{ab}
10% *H. sabdariffa*	727±5.4^{c}	269±5^{c}	5.5±0.3^{a}

Values are means±SE. Means within column with no common letters are significantly different ($p<0.05$)

Table 3: Haematological changes in chicks fed *C. cyminum* or *H. sabdariffa* for 9 weeks

	Diets				
Parameters	Control	*C. cyminum* (2%)	*C. cyminum* (10%)	*H. sabdariffa* (2%)	*H. sabdariffa* (10%)
Three weeks					
Hb (g dL^{-1})	10.5±0.4^{a}	10.1±0.4^{a}	9.9±1.2^{a}	8.9±0.2^{a}	7.1±0.3^{b}
RBC (10^{6} mm^{3})	2.3±0.1^{a}	2.4±0.1^{a}	2.3±0.1^{a}	2.1±0.2^{a}	1.9±0.2^{b}
PCV (%)	36.5±1.1^{a}	33.4±0.3^{a}	31.9±1.2^{a}	26.3±1.1^{ab}	23.5±1.2^{b}
MCV (m^{3})	158.7±5.4^{a}	149.1±4.1^{ab}	139.2±3.7^{ab}	125.2±3.2^{b}	123.7±2.1^{b}
MCH (pg)	45.7±4.2^{a}	42.1±3^{a}	43.0±2.2^{a}	42.4±3.0^{a}	37.4±1.9^{b}
MCHC (%)	28.8±1.1^{a}	30.2±0.9^{a}	31.0±0.8^{a}	33.8±1.2^{a}	30.2±0.9^{a}
Six weeks					
Hb (g dL^{-1})	10.4±0.3^{a}	9.7±0.9^{a}	7.2±0.4^{b}	8.1±0.9^{ab}	5.8±0.4^{c}
RBC (10^{6} mm^{3})	2.4±0.3^{a}	2.3±0.2^{a}	1.9±0.1^{ab}	2.0±0.1^{ab}	1.6±0.0.2^{b}
PCV (%)	35.2±0.9^{a}	34.3±0.7^{a}	25.0±0.3^{b}	29.0±0.9^{ab}	22.8±0.4^{b}
MCV (m^{3})	146.7±5.1^{a}	149.4±4.3^{a}	131.6±3.1^{b}	145.0±2.9^{a}	142.5±3.1^{a}
MCH (pg)	43.3±3.2^{a}	42.2±1.5^{a}	37.9±0.8^{a}	40.5±1.0^{a}	36.3±1.4^{a}
MCHC (%)	29.5±1.2^{a}	28.3±0.8^{a}	28±0.7^{a}	27.0.±1.2^{a}	25.4±0.3^{b}
Nine weeks					
Hb (g dL^{-1})	10.1±0.3^{a}	8.1±0.4^{a}	6.5±0.6^{b}	7.9±0.3^{ab}	5.5±0.2^{c}
RBC (10^{6} mm^{3})	2.2±0.1^{a}	2.1±0.1^{a}	1.8±0.2^{ab}	1.9±0.9^{ab}	1.6±0.2^{b}
PCV (%)	36.±0.8^{a}	32.0±0.5^{ab}	23.4±0.3^{ab}	26.7±0.6^{ab}	21.9±0.3^{b}
MCV (m^{3})	163.6±3.4^{a}	152.4±2.7^{ab}	130.0±3.1^{b}	140.5±2.3^{ab}	136.9±1.2^{ab}
MCH (pg)	45.9±4.1^{a}	38.6±3.1^{ab}	36.0±2.2^{ab}	41.6±0.9^{a}	34.4±1.2^{b}
MCHC (%)	28.1±0.9^{a}	25.3±0.7^{b}	27.8±0.9^{a}	29.6±0.7^{a}	25.1±0.4^{b}

Values are means±SE, Means within rows with no common letter (s) are significantly different ($p<0.05$)

Table 4: Serobiochemical changes in chicks fed *C. cyminum* or *H. sabdariffa* for 9 weeks

	Diets				
Parameters	Control	*C. cyminum* (2%)	*C. cyminum* (10%)	*H. sabdariffa* (2%)	*H. sabdariffa* (10%)
Three weeks					
AST (IU)	52.4±3.1^{b}	56.2±2.9^{b}	53.2±1.8^{b}	59.2±1.3^{b}	117.0±2.7^{a}
CK (IU)	129.6±1.7^{b}	135.7±1.8^{b}	134.6±2.1^{b}	130.0±2.1^{b}	199.0±2.2^{a}
ACP (IU)	11.3±1.4^{a}	9.6±1.3^{a}	10.2±1.3^{a}	10.4±1.4^{a}	10.0±1.3^{a}
Cholesterol (mg dL^{-1})	141.3±3.1^{b}	150.0±3.5^{b}	143.3±4.1^{b}	145.1±4.2^{b}	179.0±3.1^{a}
Total lipid (mg dL^{-1})	5.4±0.3^{b}	4.9±0.4^{b}	5.1±0.4^{b}	6.1±0.3^{ab}	7.7±0.3^{a}
Uric acid (mg dL^{-1})	4.1±0.3^{b}	4.2±0.2^{b}	4.0±0.3^{b}	4.5±0.3^{b}	6.9±0.2^{a}
Six weeks					
AST (IU)	55.6±3.2^{b}	49.0±3.1^{b}	133.0±3.1^{ab}	69.0±2.9^{b}	166.0±3.3^{a}
CK (IU)	128.5±2.1^{c}	132.±1.8^{c}	162.0±3.1^{b}	153.0±1.9^{b}	208.0±3.2^{a}
ACP (IU)	10.2±1.6^{a}	10.4±1,4^{a}	10.3±1.3^{a}	10.2±1.2^{a}	10.3±1.3^{a}
Cholesterol (mg dL^{-1})	142.6 ±3.2^{b}	145±3.6^{b}	189.0 ±3.1^{a}	174.5±3.2^{a}	184.0±3.2^{a}
Total lipid (mg dL^{-1})	5.6±0.4^{b}	5.6±0.3^{b}	6.9±0.3^{ab}	6.3±0.3^{b}	8.6±0.2^{a}
Uric acid (mg dL^{-1})	4.2±0.3^{b}	4.4±0.3^{b}	6.3±0.4^{a}	5.9±0.3^{a}	5.7±0.3^{a}
Nine weeks					
AST (IU)	48.0±4.1^{b}	69.8±3.1^{b}	163.3±3.5^{a}	135.0±3.3^{ab}	159.8±3.2^{a}
CK (IU)	134.0 ±2.1^{b}	159.0±3.2^{ab}	177.9±2.4^{a}	184.5±2.5^{a}	199.0±3.1^{a}
ACP (IU)	10.3±1.3^{a}	9.9±01.4^{a}	9.8±1.3^{a}	9.9±0.4^{a}	10.1±1.3^{a}
Cholesterol (mg dL^{-1})	149.5 ±4.2^{b}	150.6±3.9^{b}	192.0±3.1^{ab}	195.0±3.2^{ab}	231.0±3.2^{a}
Total lipid (mg dL^{-1})	5.5±0.4^{b}	5.4±0.3^{b}	6.3±0.3^{ab}	6.1±0.3^{ab}	7.7±0.2^{a}
Uric acid (mg dL^{-1})	4.1±0.3^{b}	4.6±0.3^{b}	6.5±0.2^{ab}	5.7±0.2^{ab}	8.6±0.3^{a}

Values are means±SE. Means within row with no common letter (s) are significantly different ($p<0.05$)

chicks fed a diet containing 10% *C. cyminum* (group 3) or 2% *H. sabdariffa* (group 4) for 6 or 9 weeks than those in the control and chicks on 2% *C. cyminum* diet (group 2) no significant changes in ACP activity were observed in the serum of test chicks.

Pathological Changes

After 3 weeks of treatment, no significant macroscopical or microscopical changes were observed in the vital organs of the chicks on the 2% *C. cyminum* (group 2), 10% *C. cyminum* (group 3) or 2%

H. sabdariffa diets (group 4). In the chicks fed a diet containing 10% *H. sabdariffa* (group 5), the microscopical examinations showed that the centrilobular hepatocytes and cells of renal proximal convoluted tubules contained fatty vacuoles. In this group, the lesions observed at weeks 6 and 9 of treatment included catarrhal enteritis, fatty cytoplasmic vacuolation or necrosis of the centrilobular hepatocytes and of the cells of renal tubules with accumulation of lymphocytes. These changes were also observed in the chicks of groups 3 and 4, but were less pronounced. No lesions were observed in the proventriculus, spleen or heart of the chicks of groups 3, 4 and 5 or in the vital organs of the chicks of group 2 or of the control group 1 throughout the 9-week feeding period.

DISCUSSION

In spite of the use of *C. cyminum* seed and *H. sabdariffa* calyx in Sudanese traditional medicine for the treatment of various ailments toxicological information is unavailable. The results of the present study indicated that feeding Bovans chicks with 2 and 10% *H. sabdariffa* or 10% *C. cyminum* of the normal diet is toxic as evidenced by growth impairment, of lesions in the vital organs and of haematological and serobiochemical alterations.

It is well known that susceptibility of animals to feeding plant material is dependent on the type of the active constituents and concentrations in the amount added to the diet as well as the rate of their metabolic conversion in the liver to metabolites and consequent excretion. For chicks, the dietary levels (2 and 10%) represented non-toxic concentrations of some plants and are exemplified by *Nigella sativa* (Al-Homidan *et al*., 2002). On the other hand, levels of 2% or more of dietary *Abrus precatorius*, *Rhazya stricta* and *Ammi visnaga* have been toxic to chickens and rodents (Omer *et al*., 1992; Adam 1999; Ibrahim *et al*., 2004).

No research has been done to investigate the safety of *C. cyminum* seed or *H. sabdariffa* calyx fed to chicks. The present study suggests the hazard associated with feeding of the calyx of *H. sabdariffa* may be related to the concentration and properties of the compounds in *H. sabdariffa* calyx as compared with those in *C. cyminum* seed. Phytochemical investigations of *H. sabdariffa* calyces have so far demonstrated the presence of flavone glycosides, alkaloids, anthocyanin pigments, amino acids, pectins, acids and probably other constituents (Chen *et al*., 2003; Odigie *et al*., 2003). *Cuminum cyminum* aerial parts contain flavonoid gycosides, essential oil rich in cuminaldehyde, α-β pinene, ä-3-carene, 1, 8 lineole, terpenes and sesquiterpenes (Ageel *et al*., 1987; Banerjee *et al*., 2002).

In the chicks fed diets consisting of 2 and 10% *H. sabdariffa* calyces or 10% *C. cyminum* seeds, the damage to the intestines, liver and kidneys could explain depression in growth. However, the mechanism whereby the plant constituents damage body tissues can not be derived from the present study, but the damage to these organs probably contributed to the increased serum AST and CK activity and cholesterol, total lipid and uric acid concentrations.

The anaemia is microcytic normochromic as indicated by the low MCV and normal MCHC values. These findings suggest that the plants constituent (s) may be involved in a derangement of the haemopoietic process.

Further investigations into the appropriate isolation, characterization and concentration of the active constituents in *H. sabdariffa* calyces and *C. cyminum* seeds are deemed necessary for elucidating their respective modes of action.

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