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## The Effect of Low Levels of Dietary *Ruta graveolens* and *Solenostemma argel* or Their Mixture on Bovans Chicks

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**Abstract:** The effects of diet containing 10% of *Ruta graveolens* or 10% of *Solenostemma argel* leaves or their 1:1 mixture (5+5%) on Bovans chicks treated for 2 weeks were investigated. A depression in growth and hepatotoxicity characterized treatment with *R. graveolens* and *S. argel* given alone. Hepatotoxicity, widespread congestion and hemorrhage in chicks fed *R. graveolens* leaves alone were marked and accompanied by anemia and alterations in serum concentrations of total protein, albumin, globulin, cholesterol, and other serum constituents. Feeding the mixture of the two plants caused more marked depression in growth but no death among the chicks occurred.

**Key words:** *Ruta graveolens*, *Solenostemma argel*, toxicity

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### Introduction

*Ruta graveolens*, a member of the family Rutaceae and locally known as *Sadab*, is widely distributed in Sudan and other Afro-Asian countries, is used in traditional medicine as an aphrodisiac, emmenagogue and analgesic and for treatment of a variety of ailments from cramp to hysteria, helmenthoses, skin conditions and diseases of the womb (El Agraa *et al.*, 2002). Phytochemical investigations of the aerial parts of this plant have demonstrated the presence of rutin, furocoumarin and lemonin as its major active constituents (Srivastava *et al.*, 1998).

*Solenostemma argel* belongs to the Asclepiadaceae family and is locally known as *Hargel*. The plant leaves are used in folk medicine as antispasmodic, carminative, antiseptic and diuretic and for the treatment of gastrointestinal disturbances, hepatic injury, renal calculi and dysentery due to the presence of flavonoids, tannins, sterols, monoterpenes, triterpenes and pregnane glycosides (El Kamali, 1991; Kamel *et al.*, 2000).

It is well known that a plant or drug may interact with another plant or drug and as a consequence modifications in activity and/or toxicity can be observed. For example, simultaneous feeding of *Citrullus colocynthis* and *Cassia senna* resulted in an increased toxic effect on rats (Adam *et al.*, 2001). On the other hand, paracetamol-induced hepatotoxicity in rats was reduced by feeding the seeds of Kulthi, *Dolichos biflorus* (Laskar *et al.*, 1998).

Because of the paucity on the effect of *R. graveolens* and *S. argel* on rodents and poultry, the present study was designed to evaluate the toxicity to chicks fed the plants singly or combined through clinical, serobiochemical, hematological and pathological parameters.

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## Materials and Methods

### Plant Material

*Ruta graveolens* and *S. argel* leaves were purchased from a local market, separately ground and then mixed in a basal diet (Table 1).

### Experimental Design

One day-old Bovans chicks were obtained from Coral Company Ltd., Khartoum, and reared in pens within the premises of the College of Veterinary Medicine and Animal Production, Sudan University of Science and Technology, Khartoum North. The chicks were allowed free access to drinking water and feed. The pens were illuminated at night and early morning throughout the experimental period. After 2 weeks, the chicks were allotted at random to 4 groups, each of 10 chicks. Group 1 was the control and fed normal basal diet. Groups 2 and 3 were fed diets containing 10% (w/w) of *R. graveolens* leaves and 10% (w/w) of *S. argel* leaves, respectively. Group 4 received a diet containing a mixture of 5% (w/w) of each plant. All chicks were fed the designated experimental diets for 2 weeks.

Average body weight and body weight gain were measured weekly for each group. The chicks in each group were slaughtered after 2 weeks of treatment. Blood samples were collected from each of the killed chicks for hematology and serum analysis.

### Blood Analyses

Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), Red Blood Cell (RBC) counts, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were estimated by standard methods (Schalm *et al.*, 1975). Sera were analysed for the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and for concentrations of total protein, albumin, cholesterol and uric acid using commercial kits (Linear Chemicals, Barcelona, Spain).

### Pathological Examinations

Necropsies were performed on all chicks immediately after slaughter to identify gross lesions and specimens of liver, kidneys, spleen, intestines and heart were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin (H and E).

### 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).

Table 1: Percent composition of basal diet fed

Ingredients	(%)
Sorghum	58
Soya bean	4
Sesame cake	14
Ground nut cake	12
Wheat bran	5
Marble dust	1
Dicalcium phosphate	1
Superconcentrates	5
Total	100

## Results

### Effect on Growth

The effects of diet containing 10% *R. graveolens* (group 2), 10% *S. argel* (group 3) or 5% mixture of two plants (group 4) on body weight and body weight gain of the chicks are shown in Table 2. The chicks fed diets containing 5% mixture of two plants (group 4) had the lowest growth rate over the 2 week period but none of the chicks died during the course of the experiment.

### Hematological Changes

Hematological data are summarized in Table 3. In group 3, there were decreases in MCV and MCH while in group 2, the values of Hb, PCV and RBC decreased but those of MCV increased with no adverse effects on MCH or MCHC values. In group 4, the values of Hb, RBC, PCV and MCHC did not change but those of MCH decreased.

### Serobiochemical Changes

In groups 2, 3 and 4, there were decreases in the concentration of total protein, albumin and globulin. The concentration of serum cholesterol decreased in the chicks fed a diet containing 10% *R. graveolens* leaves (group 2). Uric acid concentration decreased in groups 2 and 4. Neither AST nor ALT activity was found to increase in any of the chicks in groups 2-4 (Table 4).

### Pathological Changes

There was mild fatty change in the liver of the chicks in groups 3 and 4. Hepatic fatty change was marked in group 2 with congestion of the blood vessels or hemorrhage especially in the heart. On microscopy, fatty cytoplasmic vacuolation and individual-cell necrosis of the centrilobular hepatocytes were observed but degeneration of the epithelial cells of the renal proximal convoluted tubules was mild. Other organs of the test chicks did not show significant lesions. There were no changes in the control (group 1).

Table 2: Growth changes in Bovans chicks fed *R. graveolens*, *S. argel* or their mixture for 2 weeks

Treatments	Body weight (g)	Body weight gain (g)
One week		
Control (normal diet)	94±6 <sup>a</sup>	31±1.2 <sup>ab</sup>
10% <i>R. graveolens</i>	98±8 <sup>a</sup>	38±1.3 <sup>a</sup>
10% <i>S. argel</i>	96±4 <sup>a</sup>	27±0.9 <sup>b</sup>
5% mixture of two plants	93±6 <sup>a</sup>	29±9.9 <sup>ab</sup>
Two weeks		
Control (normal diet)	142±7.8 <sup>a</sup>	48±4.3 <sup>a</sup>
10% <i>R. graveolens</i>	112±3.2 <sup>b</sup>	16±0.6 <sup>b</sup>
10% <i>S. argel</i>	107±1.3 <sup>c</sup>	9±0.9 <sup>c</sup>
5% mixture of two plants	103±1.4 <sup>c</sup>	10±0.6 <sup>c</sup>

Values are means±SE; means within columns with no common letters (a-c) differ significantly (p<0.05)

Table 3: Hematological changes in Bovans chicks fed diets containing *R. graveolens*, *S. argel* or their mixture for 2 weeks

Parameters	Diets			
	Control	<i>R. graveolens</i> (10%)	<i>S. argel</i> (10%)	Mixture of two plants (5%)
Hb (g dL <sup>-1</sup> )	6.7±0.5 <sup>a</sup>	5.8±0.4 <sup>b</sup>	6.5±0.3 <sup>a</sup>	6.8±0.6 <sup>a</sup>
PCV (%)	19.2±1.3 <sup>a</sup>	17.6±0.4 <sup>b</sup>	19.4±0.5 <sup>a</sup>	20.6±0.7 <sup>a</sup>
RBC (10 <sup>6</sup> mm <sup>3</sup> )	2.5±0.5 <sup>ab</sup>	2.2±0.2 <sup>b</sup>	2.9±0.7 <sup>a</sup>	2.8±0.4 <sup>a</sup>
MCV (m <sup>3</sup> )	76.8±3.2 <sup>b</sup>	88.0±4.5 <sup>a</sup>	67.0±2.9 <sup>c</sup>	76.3±5.2 <sup>b</sup>
MCH (pg)	26.8±1.5 <sup>a</sup>	26.4±1.4 <sup>a</sup>	22.4±0.9 <sup>b</sup>	24.3±1.5 <sup>b</sup>
MCHC (%)	34.9±1.7 <sup>a</sup>	33.1±1.9 <sup>a</sup>	33.5±1.6 <sup>a</sup>	33.0±1.8 <sup>a</sup>

Values are means±SE; means within rows with no common letters (a-c) differ significantly (p<0.05)

Table 4: Serbiochemical changes in Bovans chicks fed diet containing *R. graveolens*, *S. argel* or their mixture for 2 weeks

Parameters	Treatment groups			
	Control	<i>R. graveolens</i> (10%)	<i>S. argel</i> (10%)	Mixture (5+5%)
AST (IU)	18.2±2.3 <sup>a</sup>	20.6±2.1 <sup>a</sup>	18.2±1.9 <sup>a</sup>	18.4±1.8 <sup>a</sup>
ALT (IU)	15.0±0.9 <sup>a</sup>	15.2±2.2 <sup>a</sup>	13.2±1.1 <sup>a</sup>	15.4±1.5 <sup>a</sup>
Total protein (g dL <sup>-1</sup> )	2.5±0.5 <sup>a</sup>	0.8±0.2 <sup>c</sup>	1.3±0.2 <sup>b</sup>	1.5±0.4 <sup>b</sup>
Albumin (g dL <sup>-1</sup> )	1.5±0.2 <sup>a</sup>	0.5±0.1 <sup>c</sup>	0.9±0.04 <sup>b</sup>	1.1±0 <sup>b</sup>
Globulin (g dL <sup>-1</sup> )	1.0±0.03 <sup>a</sup>	0.3±0.01 <sup>c</sup>	0.4±0.01 <sup>b</sup>	0.4±0.02 <sup>b</sup>
Cholesterol (mg dL <sup>-1</sup> )	156.5±7 <sup>a</sup>	115.0±5 <sup>b</sup>	146.5±8 <sup>a</sup>	151.0±4 <sup>a</sup>
Uric acid (mg dL <sup>-1</sup> )	4.6±0.8 <sup>a</sup>	4.9±0.7 <sup>a</sup>	3.1±0.3 <sup>b</sup>	2.0±0.2 <sup>c</sup>

Values are means±SE; means within rows with no common letters (a-c) are significantly different (p<0.05)

## Discussion

Although the *R. graveolens* and *S. argel* leaves are commonly used in Sudan and other countries for the treatment of various ailments, toxicological information on chicks or rodents is unavailable. The results of the present study indicated that 10% *R. graveolens* and 10% *S. argel* or 5% mixture of two plants in the diet were toxic but not lethal to Bovans chicks when fed for 2 weeks. Inappetence as well as the damage to the liver could explain the depression in growth.

Phytochemical studies on the aerial parts of *R. graveolens* have shown that rutin, furocoumarin and lemonin are the major active constituents (Srivastava *et al.*, 1998). Volatile oils, flavonoids, tannins, sterols, monoterpenes, triterpenes, glycosides and saponins are the main constituents in *S. argel* (Kamel *et al.*, 2000). It has been suggested that the susceptibility of chicks, rodents and livestock to plant material is at least dependent on the type of active constituents and concentration in the amount added as well as the rate of their metabolic conversion to metabolites and consequent excretion (Barri *et al.*, 1983; Bakhiet and Adam, 1996a; Adam *et al.*, 2000).

The widespread congestion and hemorrhage could be due to altered permeability of the capillaries by the active constituents in *R. graveolens* leaves. The vascular changes in the chicks fed 10% *S. argel* leaves or 5% mixture of two plants were less intense. The hypoproteinemia detected in chicks fed 10% *R. graveolens* leaves, 10% *S. argel* leaves, or 5% mixture of two plants might have been due to hepatotoxicity. This as well as the occurrence of hypocholesterolemia particularly in chicks fed *R. graveolens* is further evidence of liver damage. The absence of change in the activity of serum AST or ALT in the chicks fed *R. graveolens* and *S. argel* leaves singly or combined might have been due to enzyme excretion.

In *R. graveolens*-fed Nubian goats, there were decreases in the concentration of serum cholesterol and total protein and increases in AST activity and urea concentration indicating hepatorenal malfunction (El Agraa *et al.*, 2002). In the present study, serum uric acid concentration did not show significant changes; this finding might indicate the absence of significant nephrotoxicity in Bovans chicks. In chicks fed *R. graveolens* leaves, the decrease in Hb, RBC, PCV and the increase in MCV without significant effect on MCHC indicate macrocytic normochromic anemia. Previous studies showed macrocytic anemia in Bovans chicks which had been fed 10% *Cassia italica* or normocytic normochromic anemia in chicks which had been fed *Ambrosia maritima* (Bakhiet and Adam, 1996a, b).

Investigations into the isolation and characterization of the active constituents in the plants are necessary for elucidating their modes of actions and interactions.

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