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The Effect of Low Levels of Dietary *Peganum harmala* L. and *Ballota undulata* or Their Mixture on Chicks

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Abstract: The effects of diet containing 10% of *Peganum harmala* L. or 10% of *Ballota* undulata leaves or their 1:1 mixture (5 + 5%) on Chicks treated for 2 weeks were investigated. A depression in growth and hepatotoxicity characterized treatment with *P. harmala* and *B. undulata* given alone. Hepatotoxicity, widespread congestion and hemorrhage in Chicks fed *Peganum harmala* L. 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: *Peganum harmala* L., *Ballota undulata*, toxicity, dietary, Chicks, plants

INTRODUCTION

The *Peganum harmala* L. (Syrian rue) is a wild-growing flowering plant that belongs to the Zygophylaceae family where the active principle of this plant seeds is recognized to be the harmaline (Movafeghi *et al*., 2009). It is frequently found in the Middle East and North Africa regions. Since ancient times, it has been claimed that this plant has important medicinal effects. Extracted plant's seeds are known to possess hypothermic and hallucinogenic properties (Hamden *et al*., 2008). In addition, within the Middle East and North Africa, it was popular for its traditional usage as an emmenagogue and an abortifacient agent. There are several reports in the literature that indicate its diversified pharmacological effects such as anti-bacterial, antifungal and Monoamine Oxidase (MAO) inhibition. With emphasis on treatment of some dermatological conditions this plant was also considered in treatment of some hypothermic conditions and cancer (Arshad *et al*., 2008). This plant was also known to interact with α_2-Adrenoceptor subtypes inducing hallucination. Other reports from literature indicate diversified unwonted effects of this plant when taken in high dosage such as visual disturbances, loss of coordination agitation and in same cases paralysis.

A great number of plant species have been tested for their effects on fertility regulation beginning about 50 years ago and were subsequently fortified by national and international agencies. The role of diversified plant products in inducing male and female infertility in experimental animals has drawn the attention of researchers over the turn of the century (El Dwairi, 2007).

Ballota undulata (Lamiaceae) is a Mediterranean plant but has a more continuous distribution in a wider range of relatively moist microhabitats, up to 800 m above the sea level; it has <15 leaves, usually erects and undulates, with white to dark pink flowers (Citoglu *et al*., 2004). Its distribution is affected positively by elevation. *Ballota undulata* prefers low-pH soils, share soil microhabitats with high clay and silt and organic matter. These habitats have low sand content, low pH and relatively high soil moisture (Zaghloul, 2003; Bader *et al*., 2003). The most important constituents of *Ballota undulata* are monoterpenes and sesquiterpenes (Al-Bakri and Afifi, 2007).

This plant was suggested to exert anti-allergic, antispasmodic, antimicrobial and anti-inflammatory properties (Al-Bakri and Afifi, 2007).

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*., 2000).

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 *P. harmala* and *B. undulata* 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.

MATERIALS AND METHODS

Plant material: *Peganum harmala* L. and *B. undulata* leaves were purchased from a local market, separately ground and then mixed in a basal diet (Table 1).

Experimental design: One day-old chicks were purchased from a local market. 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^{-1}) of *P. harmala* leaves and 10% (w w^{-1}) of *B. undulata* leaves, respectively. Group 4 received a diet containing a mixture of 5% (w w^{-1}) 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 (Scham *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 praffin wax, sectioned at 5 urn 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
Soyabean	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% *Peganum harmala* L. (group 2), 10% *B. undulata* (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 2 plants (group 4) had the lowest growth rate over the 2 weeks 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 2-4, 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% *Peganum harmala* L. 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 variolation 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 chicks fed *P. harmala*, B. undulata or their mixture for 2 weeks

Treatments	Body weight (g)	Body weight gain (g)
One week		
Control (normal diet)	98±2.66[a]	35±1.4[a]
10% *P. harmala* L.	102±3.8[a]	42±1.5[a]
10% *B. undulata*	100±3.4[a]	31±0.6[b]
5% mixture of two plants	97±4.1[a]	33±1.9[b]
Two weeks		
Control (normal diet)	146±5.3[a]	52±2.2[a]
10% *P. harmala*	116±2.4[b]	20±0.7[b]
10% *B. undulata*	111±3.7[c]	13±0.6[c]
5% mixture of 2 plants	107±1.6[c]	14±0.8[c]

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

Table 3: Hematological changes in chicks fed diets containing *P. harmala*, *B. undulata* or their mixture for 2 weeks

	Diets			
Parameters	Control	*P. harmala* (10%)	*B. undulate* (10%)	Mixture of 2 plants (5%)
Hb (g dL^{-1})	7.2±0.5[a]	6.3±0.3[b]	7.1±0.3[a]	7.3±0.6[a]
PCV (%)	19.7±1.3[a]	18.1±0.5[b]	19.9±0.6[a]	21.1±0.6[a]
RBC ($I0^6$ mm3)	3.1±0.5[b]	2.9±0.7[b]	3.4±0.5[a]	3.3±0.2[a]
MCV (m3)	80.3±3.2[b]	71.0±2.6[a]	71.2±2.4[c]	80.7±3.1[b]
MCH (pg)	27.3±1.5[a]	26.9±0.7[a]	22.9±0.6[b]	24.8±1.5[b]
MCHC (%)	35.7±1.3[a]	33.8±1.2[a]	34.3±l.2[a]	33.8±1.6[a]

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

Table 4: Serobiochemical changes in chicks fed diet containing *P. harmala*, *B. undulata* or their mixture for 2 weeks

	Diets			
Parameters	Control	*P. harmala* (10%)	*B. undulate* (10%)	Mixture of 2 plants (5%)
AST (IU)	19.1±2.1[a]	21.5±2.3[a]	19.1±l.4[a]	19.3±1.7[a]
ALT (IU)	15.9±1.6[a]	16.1±0.7[a]	14.1±0.3[a]	16.3±0.7[a]
Total protein (g dL^{-1})	2.8±0.3[a]	1.1±0.5[c]	1.6±0.7[b]	1.8±0.4[b]
Albumin (g dL^{-1})	1.8±0.7[a]	0.8±0.3[c]	1.9±0.5[b]	1.4±0.1[b]
Globulin (g dL^{-1})	1.4±0.05[a]	0.7±0.05[c]	0.8±0.07[b]	0.8±0.04[b]
Cholesterol (mg dL^{-1})	162.9±3.6[a]	121.7±4.7[b]	152.4±2.3[a]	157.3±4.7[a]
Uric acid (mg dL^{-1})	4.2±0.5[a]	4.5±0.7[a]	2.7±0.7[b]	1.6±0.4[c]

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

DISCUSSION

Although the *P. harmala* and *B. undulata* leaves are commonly used in Jordan 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% *P. harmala* and 10% *B. undulata* or 5% mixture of two plants in the diet were toxic but not lethal to chicks when fed for 2 weeks. Inappétence as well as the damage to the liver could explain the depression in growth.

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 *P. harmala* leaves. The vascular changes in the chicks fed 10% *B. undulate* leaves or 5% mixtures of two plants were less intense. The hypoproteinemia detected in chicks fed 10% *P. harmala* leaves, 10% *B. undulata* leaves, or 5% mixture of two plants might have been due to hepatotoxicity. This as well as the occurrence of hypocholesterolernia particularly in chicks fed *P. harmala* is farther evidence of liver damage. The absence of change in the activity of serum AST or ALT in the chicks fed *P. harmala* and *B. undulata* leaves singly or combined might have been due to enzyme excretion.

In the present study, serum uric acid concentration did not show significant changes; this finding might indicate the absence of significant nephrotoxicity in chicks. In chicks fed *P. harmala* 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 chicks which had been fed 10% *Cassia italica* or normocyte 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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