Combined effect of *Ambrosia maritima* L. shoots and *Citrullus colocynthis* L. Schrad. seeds in chicks

Amal O. Bakhiet and Adam S.E.I. *
Department of Veterinary Medicine, Pharmacology and Toxicology, University of Khartoum, P.O. Box 32, Khartoum-North, Sudan

ABSTRACT

The effect of diet containing 2% of *Ambrosia maritima* L. Shoots or 2% of *Citrullus colocynthis* L. Schrad. seeds or their 1: 1 mixture on Bovans chicks treated for 6 weeks was examined. Although *A. maritima* L. shoot or *C. colocynthis* L. Schrad. seed alone increased mean body weights of chicks, mild enterohepatonephropathy accompanied by alterations in serum lactic dehydrogenase (LDH), gamma glutamyl transferase (GGT) and creatine kinase (CK) activities and concentrations of total iron, total iron-binding capacity (TIBC), cholesterol, total lipid, and uric acid and red blood cells was observed. Feeding the mixture of the two plants for 6 weeks caused more marked effects with prominent impairment of growth and incomplete disappearance of organ lesions 3 weeks after removal from the test diet.

INTRODUCTION :

Ambrosia maritima L. (Asteraceae) is commonly found in Sudan and used in local folk medicine practices for the treatment of gastrointestinal disorders and as a molluscicide (1). The plant contains sesquiterpene lactones and terpenoid compounds and probably other substances (2, 3).

Citrullus colocynthis L. Schrad (Cucurbitaceae) is also commonly found in the country and is used as a purgative, anthelmintic, molluscicide and insecticide (1). The main constituents in the plant are cucurbitacins A, B, C and D and α –elaterin (4). Our previous studies have shown that feeding the mixture of *C. colocynthis* and

* Author to whom correspondence should be addressed at Department of Veterinary Medicine, King Saud University, P.O. Box 1482, Buraidah, Al – Qassim, Saudia Arabia.

Cassia senna fruits or *C. colo-cynthis* fruits and *Nerium olea-nder* leaves caused more mark-ed effects than individual plants in rats (5, 6). Since interactions among plant drugs may modify toxicity and/or bioactivity (7) and no information is available on the interaction of *C. colocynthis* L. Schrad. seeds and *A. maritima* L. shoots in poultry and animals, the present study was planned to investigate the health effects in chicks fed low dietary levels of the plants singly or combined through hematological, serobiochemical, pathological and growth parameters.

MATERIALS AND METHODS :

Plant material :

Ambrosia maritima L. shoots and *Citrullus colocynthis* L. Schrad. seeds were purchased in a local market of Omdurman City, Sudan, as dry plant materials and authenticated by Professor E. M. Abdel Bari, Department of Botany, Faculty of Science, University of Khartoum, Sudan, were used in this study. The plant tissues were separately ground to a fine powder with a mortar and pestle and then mixed in a commercial starter diet (Table 1).

Chicks :

Fourty eight 1 day old Bovans cockerels, purchased from Coral Company, Khartoum, were used. The chicks were reared in a poultry house at the Faculty of Veterinary Sciences, University of Khartoum, artificially illuminated at night and early morning with fluorescent lights for 16h/day, and allowed free access to feed and drinking water.

Toxicity study :

At 7 days, the chicks were randomly allotted to 4 groups of 12 chicks each. Group 1 continued to be fed the normal diet and served as control; group 2 received a diet containing 2% (w/w) of *A. maritima* L. Shoots, group 3 received a diet containing 2% (w/w) of *C. colocynthis* L. Schrad. seeds and group 4 received a diet containing a mixture of 1% *A. maritima* L. shoots and 1% *C. Colocynthis* L. Schrad seeds. Feeding on the control and test diets continued for 6 weeks (feeding period) at which time the test diets were replaced by the control diet for 3 weeks (recovery period).

The average body weight, body weight gain and feed efficiency (kg feed/kg gain) were determined weekly for each group. From each group, 4 randomly selected chicks were slaughtered at 3 and 6 weeks after the beginning of the test diet and at 3 weeks after replacing the test diets by the control diet for pathological examination. Blood samples were collected from each bird at slaughter for hematology and serum chemistry analyses. Necropsies were done on all chicks to identify gross lesions. Specimens of proventriculus, intestine, liver, spleen, kidneys and heart were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm and stained with hematoxylin and eosin (H&E) for histopathological examinations.

Chemical and hematological analyses :

Sera were analysed for the activities of gamma glutamyl transferase (GGT), lactic dehydrogenase (LDH) and creatine kinase (CK) and for concentrations of total lipid, cholesterol, total iron and total iron-binding capacity (TIBC) using commercial kits (Stanbio Laboratory Inc., San Antonio, Texas, USA).

Red blood cell (RBC) counts, packed cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were estimated by standard methods (8).

Statistical analysis :

Data were analysed for statistical significance using one way ANOVA followed by Meuman-Keuls multiple mean comparison test (9).

RESULTS :

Growth changes :

Changes in mean body weights and feed efficiency of the chicks fed 2% *A. maritima* L. shoots (group 2), 2% *C. Colocynthis* L. Schrad. seeds (group 3) or their mixture at 1% each (group 4) for 3 and 6 weeks are presented in Table 2. After 3 weeks of treatment, the chicks in groups 2 and 3 had higher ($p< 0.05$) body weights than control (group 1) while those in group 4 had the lowest ($p<$

0.05 - 0.001) body weights and feed efficiency at 6 weeks. The birds in group 3 had the highe-st ($p< 0.01 - 0.001$) body weig-ht and feed efficiency and tho-se in group 4 had the lowest ($p< 0.01 - 0.001$) growth para-meters at the end of the 3 week recovery period.

Pathologic changes :

The chicks on 2% *A. maritima* L. diet (group 2) and those on 2% *C. colocynthis* L. Schrad diet (group 3) showed mild focal catarrhal enteritis, fatty cytoplasmic vacuolation of the centrilobular hepatocytes and degeneration of the renal tubular cells with slight lymphocytic infiltration in the renal cortex, hepatic parenchyma, intestinal lamina propria and between some of the cardiac muscle fibers at 3 and 6 weeks. In the chicks fed the combination of the two plants at 1% each, (group 4), lesions were more intense and included catarrhal enteritis, congestion of the intestinal blood vessels, hemorrhagic foci and degeneration or necrosis of the centrilobular hepatocytes (Figure 1) and of the cells of the renal tubules. In this group, the organ damage was reduced but neither the centrilobular hepatocytes nor the cells of the renal proximal tubules had completely reverted to normal at the end of the recovery period. No lesions were seen in the control (group 1) or in the spleen and proventriculus of the test chicks.

Changes in serum constituents :

These are summarized in Table 3. In the chicks on 2% *A. maritima* L. diet (group 2), 2% *C. colocynthis* L. Schrad. diet (group 3) or mixture of the two plants at 1% each (group 4), the activities of LDH and CK and concentrations of total iron were higher ($P<0.05 - 0.001$) than control (group 1) at 3 and 6 weeks and at the end of recovery period. The activity of serum GGT was higher ($P< 0.05 - 0.001$) and the concentration of cholesterol was lower ($P< 0.01 - 0.001$) in groups 2 and 4 than the control at 3 and 6 weeks. The GGT activity was higher ($P< 0.05$) in group 4 than control at the end of the recovery period but that in group 2 returned to normal. In group 3, GGT activity did not change during feeding but was higher ($P<0.05$) than control at the end of the recovery period. The concentration of cholester-

ol in group 3 did not change either during feeding or recovery period and that of total lipid was only lower ($P<0.05$) in group 2 than controls during feeding. TIBC was lower ($P< 0.05 - 0.01$) in groups 2 and 3 and higher ($P< 0.01 - 0.001$) in group 4 than controls at 3 and 6 weeks. At the end of recovery period, TIBC was lower ($P<0.05 - 0.01$) in groups 2, 3 and 4 than controls.

Hematologic changes :

These data are presented in Table 4. The values of RBC, PCV, Hb, MCH, MCHC or MCV did not change in chicks on 2% *C. colocynthis* L. Schrad. diet (group 3). The values of RBC and PCV were lower ($p<0.05$) and those of MCH and MCHC were higher ($P<0.05$) in chicks on 2% *A. caritima* L. diet (group 2) than controls at 3 and 6 weeks. The values of MCV was only higher ($p<0.01$) in chicks fed the combination of the two plants at 1% each (group 4) for 3 weeks and lower ($p<0.05 - 0.01$) in group 2 at 6 weeks and also in groups 2 and 4 than control at the end of the recovery period.

Table (1). Percent (%) composition of starter diet

Ingredients	%
Sorghum	58
Soybean	4
Sesame cake	14
Groundnut cake	12
Wheat bran	5
Marble dust	1
Dicalcium phosphate	1
Superconcentrate	5
Total	100

Table (2) . Change in weight and feed efficieny of chicks fed *A. Maritima L. Shoots, C. colocynthis L. Schard . Seeds or their mixture .*

Groups	Feeding period (weeks)		Recovery period (weeks)
	0 – 3	4 - 6	7 - 9
Mean body weight (g)			
1. Control . (standard diet)	160 ± 4.2	342 ± 3.3	635 ± 4.9
2. 2% A.maritima	183 ± 6.4*	403 ± 4.7***	629 ± 4.1^{NS}
3. 2% C. Colocynthis	180 ± 2.5*	382 ± 6.8**	668 ± 3.5**
4. 1% A. Maritima + 1% C. colocynthis	171 ± 2.1^{NS}	328 ± 4.8*	532 ± 5.3***
Mean feed efficiency (kg feed / kg gain)			
1. Control . (standard diet)	2.4 ± 0.6	3.40 ± 0.3	3.5 ± 0.6
2. 2% A.maritima	3.1 ± 0.2*	2.02 ± 0.1**	3.7 ±0.7^{NS}
3. 2% C. Colocynthis	2.2 ± 0.2^{NS}	3.50 ± 0.4^{NS}	2.6 ± 0.8**
4. 1% A. Maritima + 1% C. colocynthis	3.1 ± 0.1*	6.30 ± 0.2***	4.7 ± 1.2**

Value are mean ± SE; NS= Not significant ; * P< 0.05 ; ** P< 0.001

Table (3) . Change in serum constituents of chicks fed *A. Maritima* L. Shoots, *C. colocynthis* L. Schrad. Seeds or their mixture .

Parameters	Feeding period (Weeks)								Recovery period (Weeks)			
	0 – 3 Groups				4 – 6 Groups				7 – 9 Groups			
	1	2	3	4	1	2	3	4	1	2	3	4
Fe(µg/dl)	118 ±4.3	234 ±3.4***	191 ±9***	236 ±5.1***	120 ±3.8	232 ±5.1***	189 ±10***	241 ±5***	119 ±4	184 ±0.9***	259 ±13***	352 ±4.5***
TIBC (µg/dl)	135 ±7	102 ±5.2*	103 ±6*	205 ±6**	137 ±8	100 ±6.5**	107 ±5*	203 ±8***	142 ±5	94 ±1.2**	124 ±5*	125 ±0.5*
Cholest.(mg/dl)	205 ±4.1	178 ±7**	202 ±6^{NS}	167 ±9***	204 ±5.1	176 ±8**	200 ±6^{NS}	165 ±8***	218 ±8.3	167 ±6***	217 ±9^{NS}	142 ±6***
T.lipid(mg/dl)	5 ±0.2	3.6 ±0.2*	5.8 ±0.4^{NS}	4.9 ±0.3^{NS}	4.9 ±0.1	3.2 ±0.1*	5.7 ±0.5^{NS}	4.7 ±0.4^{NS}	5.2 ±0.6	4.9 ±0.5^{NS}	6.8 ±0.7^{NS}	4.1 ±0.9^{NS}
GGT(iu)	42 ±3	60.4 ±2**	46 ±5^{NS}	82 ±3***	42 ±4	58 ±2*	44.2 ±2.8^{NS}	81 ±6***	45 ±3	44 ±1.8^{NS}	58 ±2*	58 ±1*
LDH(iu)	64.3 ±2	187 ±4***	145 ±5***	180 ±9.3***	62.1 ±6	185 ±6***	143 ±9***	179 ±9***	68 ±5	95 ±4.3*	102 ±8**	158 ±5***
CK (iu)	130 ±3	170 ±4**	146 ±5*	297 ±6***	129 ±9	168 ±4***	141 ±7*	298 ±5***	136 ±4	159 ±7*	181 ±5***	198 ±4***

Groups: 1= Control; 2= 2%*A. maritima*; 3= 2%*C. colocynthis*; 4= 1%*A. maritima*+1% C. colocynthis
Value are mean ± SE; ^{NS}= Not significant ; *P<0.05 ; ** P< 0.01 ; *** P< 0.001

DISCUSSION :

In the present study, the 7day pretrial period was aimed at achieving body weight uniformity of chicks at the start of feeding test diets, acclimatizing birds to poultry house environment at the Faculty of Veterinary Sciences and to allow unhealthy ones to be eliminated before the feeding trial commenced. It is well known that susceptibility of chicks or rodents to feeding with plant materials is dependent at least on the type of active constituents and concentration of the amount added to the diet as well as the rate of their metabolic conversion in the liver to metabolites and subsequent excretion (10, 11). The incorporation of *A. maritima* L. shoot and *C. colocynthis* L. Schrad. seed into the diet at 2% was chosen because this level represents a non-toxic concentration for chicks of a number of plants such as *Cassia italica* (10) and *Cucurbita maxima* (12). On the other hand, a level of 2% dietary *Abrus precatorius* and *Ricinus communis* seeds has been found toxic for chicks (13, 14).

The results of the present study indicate that the incorporation of *A. maritima* L. shoots and *C. colocynthis* L. Schrad seeds at 2% in the basal diet increased mean body weight of chicks. This increase in body weight may be due to the presence of relatively mild tissue damage and high protein content in these plants but neither growth promoter effect nor an appropriate proximal analysis of the plant tissues is available. The damage to the intestine, liver and kidneys of birds fed *A. maritima* L. shoot or *C. colocynthis* L. Schrad seed singly was mild but accompanied by elevated serum LDH, GGT and CK activities and decreased cholesterol concentration. The mechanism by which the plant constituents injured body tissues has yet to be defined.

There were increases in serum total iron and decrease in TIBC in the chicks fed *A. maritima* L. or *C. colocynthis* L. Schrad. alone. This finding as well as the differences in the values of RBC, PCV, MCH, MCHC and MCV particularly in the birds fed *A. maritima* L.

singly may indicate interference of the plant constituents with iron metabolism. Work would be required in this area to verify the role of iron and other trace elements in hematopoiesis. It has been found that similar results except for increased MCV occur with malabsorption of folic acid and vitamin B12 (15).

When the mixture of the two plants at 1% each was fed to chicks for 6 weeks, the toxic effect was enhanced as indicated by decrease in body weights and feed efficiency and occurrence of intense enterohepatorenal lesions. The vital organs of the chicks in this group had not completely reverted to normal at the end of the recovery period.

In conclusion, this study demonstrates that *A. maritima* L. shoot or *C. colocynthis* L. Schrad. seed alone at the dietary concentration used is growth promoter in chicks inspite of the occurrence of mild injury to the intestine, liver and kidneys. Combined toxicity is evidenced by growth impairment, severe tissue damage, hematol-ogical and serobiochemical alterations with incomplete reversion of vital organs to normal at the end of the 3 week recovery period. Further studies of the nutritive value, mode of actions and interactions of the active constituents of *A. maritima* L. shoots and *C. colocynthis* L. Schrad seeds had to be done.

REFERENCES :

Bakhiet, A. O. (1995). Comparative effects of some indigenous plants. PhD thesis, University of Khartoum, Khartoum.

El Sawy, M. F.; J. Duncan, S. ; Amir; H.; El Ruweini, and N. Brown (1989). The molluscicidal properties of *Ambrosia maritima* L. (Compositae). Temporal and Spatial distribution of *Biomphalaria alexandrina* in Egyptian village irrigation systems with reference to schistosomiasis transmission control. *Trop. Med. Parasitol.* 40, 103 – 106.

Abdalla, O.M.; Ali A. A. and Itokawa , H. (1991). Cytotoxic activity of sesquiterpene lactones isolated from *Ambrosia maritima*. *Pharm.* 46, 472 – 476.

Ageel, A. M.; Mossa, J.S. Tariq, M; Al-Yahya,M.A. and Al-Said , M.S. (1987). *Plants used in Saudi folk medicine.* King Saud University Press, Riyadh.

Al-Yahya, M. A., Al-F-arhan A. H. and Adam, S. E. I. (2000). Preliminary toxicity study on the individual and combined effects of *Citrullus colocynthis* and *Nerium oleander* in rats. Fitoterapia 71, 385 – 391.

Adam, S. E. I., Al-Yahya M. A. and Al-Farhan A. H. (2001). Combined toxicity of *Cassia senna* and *Citrullus colocynthis* in rats. Vet. Hum. Toxicol. 43, 70 – 72.

Hodgson, E. and Levi P. E. (1997). Modern toxicology, 2nd ed. Appleton and Lange, Stamford, C. T.

Schalm, O. W., Jain N. C. and Carroll G. H. (1975). Veterinary hematology, 3rd. edn. Lea and Febiger, Philadelphia.

Mendenhall, W. S. (1971). Introduction to probability and statistics, 3rd edn. Wadsworth Publishing Co. Inc., Belmont, CA.

Bakhiet, A. O. and Adam S.E.I (1996). Toxicity to Bovans chicks of *Cassia italica* seeds. *Phytother. Res.* 10, 156 – 160.

Adam, S.E.I.. (1999). Effects of various levels of dietary *Lepidium sativum* L. seeds in rats. *Am. J. Chin. Med.* 27, 397 – 405.

Mohamed, A. B. (1992) Effects of various levels of dietary *Lupinus termis* and *Cucurbita maxima* on chicks. MVSc. tesis, University of Khartoum, Khartoum.

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

El Badwi, S. M. A., Adam S. E. I. and Hapke H. J (1992). Experimental *Ricinus communis* poisoning in chicks. *Phytother. Res.* 6, 205 – 209.

Eichner, E. R. (1984). Macrocytic anemia. In: J. L. Spivak, ed. Fundamentals of clinical hematology. Harper and Row, philadelphia.

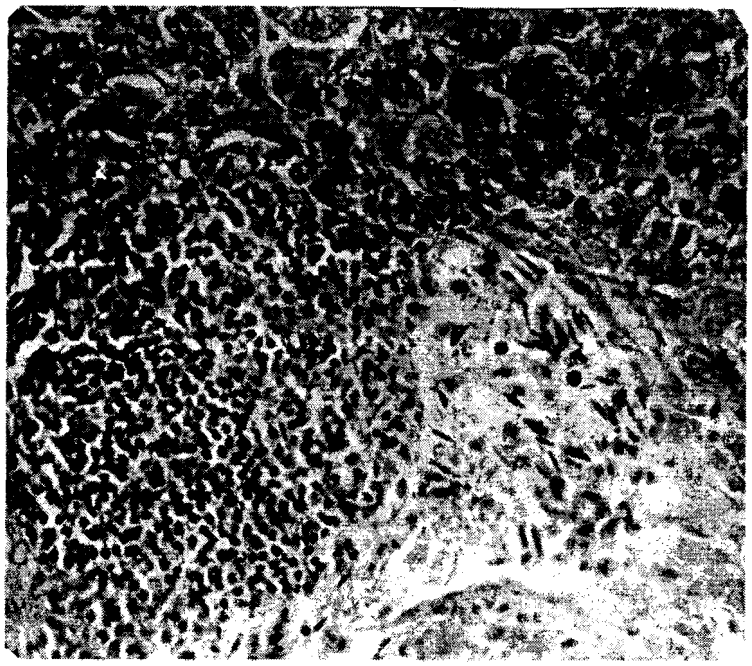

Figure 1. Hepatocellular necrosis in a chick fed a mixture of 1% *A. maritima* L. shoots and 1% *C colocynthis* L. Schrad. seeds for 6 weeks.H&E x90.

الملخص العربى

التأثير المشترك لنباتي امبروزيا ماريتيما والحنظل في الكتاكيت

امل عمر بخيت وصلاح الدين ابراهيم آدم

أجريت الدراسة لمعرفة تأثير عليقة تحتوى على ٢% من المجموع الخضرى لنبات الأمبروزيا أو ٢% من بذور الحنظل أو خليط منهما بنسبة ١:١ على كتاكيت بوفان. على الرغم من حدوث زيادة في أوزان الكتاكيت التي تمت تغذيتها على ٢% أمبروزيا أو ٢% حنظل إلا أنه قد لوحظت إصابة طفيفة في الأمعاء والكبد والكليتين مصحوبة بتغيرات في نشاط إنزيمات لاكتيك ديهايدروجنيز وجاما جلوتاميل ترانسفريز وكرياتين كاينيز وفي تركيز كل من الحديد الكلي والقدرة الكلية لارتباط الحديد والكولسترول والدهون وحامض البوليك في المصل وفي كريات الدم الحمراء. تغذية الكتاكيت بخليط من النباتين لمدة ٦ أسابيع أحدثت تأثيرات بالغة مع قصور النمو الواضح. لوحظ عدم الاختفاء الكامل للآفات المرضية في الأعضاء بعد توقف إعطاء هذا الخليط لمدة ٣ أسابيع.