Antagonistic Effect of An Aqueous Root Extract of *Eryngium creticum* on the Hyperglycemic Response Caused by *Cerastes cerastes* Envenomation in Rats.

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Abstract ❑ **The possible antagonistic effects of *Eryngium creticum* were tested on *Cerastes cerastes* envenomation using rats. The results indicated a hypoglycemic effect as the most significant antagonizing action of an aqueous root extract of this plant. Other hematological changes have also been recorded.**

Keyphrases ❑ ***Eryngium creticum, Cerastes cerastes,* Envenomation.**

Snakes and scorpions are the deadliest of natural enemies of the inhabitants of the rural areas. In Jordan, five species of venomous snakes have been reported (1). Four of them, namely ***Cerastes cerastes, Vipera palaestinae, Echis coloratus*** and ***Pseudocerastes persicus fieldi*** belong to the family Viperidae, while the fifth, ***Walterinnesia aegyptia,*** belongs to the family Elapidae.

Since ancient times, plants are considered the first choice to protect from ailments or cure diseases. One of the local plants used against scorpion stings in Jordan is ***Eryngium creticum*** (2,3). Three species of the genus Eryngium are found in Jordan, namely ***E. creticum***, ***E. falcatum*** and ***E. glomeratum,*** family Umbelliferae, subfamily Saniculoidae (4). ***E. creticum*** (Syrian Eryngium) is commonly found in cultivated and fallow fields, waste places and road sides in the Mediterranean ecozone of Jordan (5).

The aqueous extracts of different Eryngium species are used in the folk medicine for their diuretic, antidiabetics and antispasmodic properties as well as for treatment of liver diseases, low-back pain, asthma, whooping cough and in certain poisoning conditions (6-9). In Palestine, the crushed leaves and roots of ***E. creticum*** are applied to wounds and also used orally and topically to overcome the toxic effects of snake and scorpion venom (10). In Cyprus, the fleshy roots of ***E. creticum*** are used against snake bites in humans and animals and, in some parts of the country, the shepherds feed it to their goats and sheep when bitten by ***Vipera lebetina*** (11). ***E. creticum*** root extract was reported to prolong the life of guinea pigs significantly from 20 minutes to 8 hours when combined with ***Leiurus quinquestriatus*** scorpion venom (2). The aqueous extract of ***E. creticum*** roots caused a dose dependent inhibition of the spontaneous movements of isolated jejunal segments of rabbits and guinea pigs (3). It also inhibited the tonic contraction of jejunal segments caused by ***L. quinquestriatus*** scorpion venom. Based on these observations, this study was designed to test the antagonistic effect of the aqueous extract of ***E. creticum*** root on the hypoglycemic response and hematological alterations caused by ***C. cerastes*** snake venom ***in vivo***.

Experimental:

Materials and Methods:

Collection of the snakes and preparation of the venom: Snakes were collected from Wadi Araba. The venom, obtained by milking the snakes, was stored immediately in deep freeze, lyophilized thereafter and kept at 4°C. For determination of one and two LD_{50} concentrations, the lyophilized crude venom was dissolved in 0.2M phosphate buffered saline (pH = 7.4). For each experiment, fresh solutions were prepared.

Collection and preparation of the plant material: E. creticum plants were collected from Al-Salt and Zei area, Jordan, in March/April and June 1990. The collected plant material was authenticated by Prof. D. Al-Eisawi, Department of Biological Sciences, Faculty of Science, University of Jordan. A voucher specimen has been deposited at the Department of Pharmacy, University of Jordan. ***E. creticum*** roots were air-dried and then powdered mechanically. An aqueous extract was prepared by refluxing powdered root (10 g) with dist. H_2O (100 mL) for 45 min., and then filtering twice through filter paper (Whatman No. 4). The filtered solution was brought up to 100 mL with dist. H_2O so that 1 mL extract was equivalent to 100 mg of starting material.

Experimental animals: Locally bred male Albino rats (Rattus rattus) weighing 150-400 g were used. They were maintained on a normal diet.

Determination of LD_{50} of C. cerastes snake venom: Adult male rats of the same weight were grouped into 8 main groups, each composed of 5 animals. The animals were injected intraperitoneally (i.p) with various concentrations of the venom, calculated to their body weight. The number of dead rats was scored after 24 h. LD_{50} value was evaluated according to the method of Alawi and Jeryes (12) and was found to be 1.86 mg/kg body weight.

Hematological Examinations: The animals were fasted overnight before the experiments. The rats were grouped into 6 main groups and, on the day of the experiment, the animals were treated as follows: ***Group 1*** (the control group; 4 animals); was injected i.p. by only physiological saline. ***Group 2*** (12 animals); received i.p. injection of LD_{50} of ***C. cerastes*** snake venom. The dose of the venom has been matched with their body weight. ***Group 3*** (12 animals); received i.p. injection of 2 LD_{50} of snake venom (3.75 mg/kg body weight). ***Group 4*** (12 animals); received i.p injection of 1 LD_{50} ***C. cerastes*** snake venom and then let to drink ***E. creticum*** aqueous root extract. The calculated amount of plant extract (10 mL/100g body weight) was administered to each animal. ***Group 5*** (12 animals); received i.p. injection of 2 LD_{50} of ***C. cerastes*** snake venom and let to drink ***E. creticum*** aqueous root extract. The amount of plant extract administered to each animal was related to its body weight (10mL/100g body weight). ***Group 6*** (12 animals) were allowed to drink the plants root extract ***ad libitum*** without receiving venom injection.

Experimental Protocol:

After one hour of the previous treatment, the control group and four animals from each group were anesthetized with ether. Then 0.5-1 mL of blood samples were collected directly from the heart into EDTA-anticoagulant tubes and the following hematological tests were performed: Haematocrit (PCV), WBC (s) and RBC (s) counts were done by the usual routine manual methods. Erythrocyte sedimentation rate (E.S.R) was determined by the macro method according to Westergren. Also differential WBC(s) count was made.

The same procedure was repeated after two and three hours on the other groups of 4 rats.

Biochemical Analysis: Plasma glucose level was measured in the collected samples at one, two and three hours intervals by using a spectrophotometric method (13).

Statistical Analysis Data of the hemotological and biochemical tests were presented as means ± S.D. and compared to the control means by using Student's t-test of α-level of 0.05.

RESULTS and DISCUSSION

Table I shows the comparative differential blood count of the control group and the treated animals. ***Table II*** gives the mean values of the hematocrit, red blood cell count, white blood cells count, erythrocyte sedimentation rate and blood glucose concentration of the control group and the treated groups of animals.

Effect of E. creticum extract (Table II): Animals which received ***E. creticum*** extract showed a significant drop in glucose level ($P<0.05$).

Effect of 1LD_{50} (Table II): PCV was increased within the first hour, remaining unchanged in the second hour and returned to the control values in the third hour. Also, WBC count was increased reaching a maximum of 17.8 x 1000/mm^3 in the third hour. The differential count of WBC showed an increase in N% and M% and a decrease in L%.

Effect of LD_{50} and E. creticum extract (Table II): The group that received ***E. creticum*** in addition to LD_{50} dose showed a drop in PCV values already within the first hour, which continued further in the second hour when compared with group of the animals that received only 1 LD_{50} dose. In first hour, after administration of the ***E. creticum*** extract to the 1 LD_{50} treated animals, the value of the WBC was decreased to the value of the control group animals. In the second and third hour, ***E. creticum*** extract did not influence the high WBC count of the 1 LD_{50} treated animals. A significant increase in % of N's numbers and significant decrease in % of L's numbers throughout the experiment were observed when compared with control animals.

Effect of 2 LD_{50} (Table II): In the first hour, WBC and % of N count were increased and % of L, M, and E count were decreased, while other parameters were not changed. An increase of the PCV value was observed in the second hour which returned to the control value in the third hour. WBC count was increased significantly in the second and third hour. Elevation of the serum glucose level started in the second hour to reach a significant difference in the third hour. A drop of the RBC's was also observed in the third hour.

Effect of 2 LD_{50} and E. creticum extract (Table II): In the first and second hour, PCV count was in-

Table I: **Comparative Differential Count of the Control Group and Treated Animals (N: Neutrophils; L: Lymphocytes; (± S.D).**

	N%			L%		
	1h	2h	3h	1h	2h	3h
Control group	36.7 ± 18.7			64 ± 16.9		
1 LD *(C. cerastes venom)*	64.2[a] ± 5.9	61.8 ± 8.5	60 ± 8.5	35 ± 5	42 ± 4.6	37 ± 6.8
2 LD50 *(C. cerastes venom)*	58.7[a] ± 2.8	62.5 ± 5.9	67.4 ± 8	40.3 ± 1.3	37.6 ± 5.9	32.5 ± 7.9
***E. creticum* extract alone**	36 ± 3.1	55 ± 3.3	46.2 ± 2.4	63.8 ± 3	45 ± 3.3	53.8 ± 2.4
1 LD50 + *E. creticum* extract	58.3[a]±11.2	57.8[a]±10.9	64.3[a]±12.2	43.8[a]±11.3	44.3[a]±8.9	41.5[a] ± 8.9
2 LD50 + *E. creticum* extract	58.7 ± 1	68.8 ± 1.3	58.4 ± 2.1	41.3 ± 0.9	31.2 ± 1.3	41.6 ± 2.1

Table I **(Contd): Comparative Differential Count of the Control Group and Treated Animals (M: Monocytes; E: Eosinophils; B: Basophils) (± S.D).**

	M%			E%			B%		
	1h	2h	3h	1h	2h	3h	1h	2h	3h
Control group	0.3 ± 0.5			0.5 ± 0.8					
1 LD *(C. cerastes venom)*	0.6 ± 0.9	0.9 ± 1.3	1.4 ± 2.1	0.4 ± 0.9	0.4 ± 0.7	0.2 ± 0.4	0.4 ± 0.8	0.4 ± 0.9	0.5 ± 0.9
2 LD50 *(C. cerastes venom)*	0.1 ± 0.4		0.1 ± 0.3	0.1 ± 0.4	0.2 ± 0.6	0.1 ± 0.3			
***E. creticum* extract alone**	0.2 ± 0.4	1 ± 1.3	0.8 ± 1	0.2 ± 0.4	0.2 ± 0.6	0.2 ± 0.8		0.2 ± 0.6	0.1 ± 0.3
1 LD50 + *E. creticum* extract	0.6 ± 1	0.4 ± 0.5	1.5 ± 1.1	0.6 ± 1	0.1 ± 0.3	0.5 ± 0.8	0.3 ± 0.5	0.1 ± 0.3	0.1 ± 0.3
2 LD50 + *E. creticum* extract	0.1 ± 0.4	0.8 ± 1.2	0.5 ± 1.1	0.1 ± 0.4	0.2 ± 0.4	0.1 ± 0.3		0.1 ± 0.3	

[a]Indicates statistically significant difference to the control ($P<0.05$). [b]Indicates statistically significant difference within the treated animals ($P<0.05$).

creased while other parameters were not changed when compared with 2 LD_{50} data. In the third hour, a decrease in the WBC and % of N count as well an increase of % of L and M count were observed after administering the ***E. creticum*** extract. A significant drop of serum glucose level was reached in the third hour when compared with the 2 LD_{50} treated animals.

Comparison of 1 LD_{50} and 2 LD_{50}: The most significant difference between 1 LD_{50} and 2 LD_{50} has been observed in serum glucose levels. Animals treated with 2 LD_{50} showed higher serum glucose levels after 3 hours from injection. Animals treated with 1 LD_{50} and 2 LD_{50} doses showed significant increase in WBC's count and in % of N ($P< 0.05$) ***(Tables I, II).*** Similar observations of leucocytosis with increased % of N were reported to 24 hours after i.p. injection of sublethal doses of ***C. vipera*** venom to rabbits (14). In our experiments, this leucocytosis may be influenced by the route of injection. However, a reducing effect of ***C. cerastes*** venom on circulating WBC's was observed by Abdalla, ***et al.*** (15) after i.v. injection of 0.12 mg/kg venom to guinea pigs and by Mohammed, ***et al.*** (14) after i.p injection of venom to rabbits. Such observations indicated that fluctuations in differential count and species variability are experienced in working with ***Cerastes*** venom. 1 LD_{50} and 2 LD_{50} -treated animals showed after receiving ***E. creticum*** extract a decrease of the WBC's which may be due to counteraction of this plant extract to the effect of the venom ***(Table II).***

The increase of PCV observed after 1 LD_{50} and 2 LD_{50} doses and the decrease of these values after treatment with ***E. creticum*** extract are insignificant. 1 LD_{50} and 2 LD_{50} doses decreased the RBC count after 3 hours from injection. This decrease in RBC's count three hours after ***C. cerastes*** venom injection resembled the reported one (14). However, no effect of ***C. cerastes*** venom on RBC's count was observed in guinea pigs (15). 1 LD_{50} and 2 LD_{50}- treated animals did not show any significant changes in RBC's following ***E. creticum*** administration ***(Table II).*** ESR did not change

Table II: Hematological Findings in Control Group and in Treated Animals (±SD).

	ESR (mm)a			PCV (%)		
	1h	2h	3h	1h	2h	3h
Control group	0.8 ± 0.3			45.5 ± 6.2		
1 LD *(C. cerastes venom)*	0.9 ± 0.4	1 ± 0.6	1.1 ± 0.5	49.8 ± 2.2	49.8 ± 3.9	45 ± 4.1
2 LD_{50} *(C. cerastes venom)*	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	45.1 ± 2.1	50.6 ± 3.8	46.1 ± 3.4
***E. creticum* extract alone**	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.4	48.1 ± 6.9	50 ± 2.9	48.7 ± 4.5
1 LD_{50} + *E. creticum* extract	0.5 ± 0.1	0.6 ± 0.4	0.7 ± 0.3	47.8 ± 8.8	44 ± 5.3	46.8 ± 3.6
2 LD_{50} + *E. creticum* extract	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.3	50.8 ± 2.9	50.2 ± 2.6	45.6 ± 3.2

Table II: (Contd.) Hematological Findings in Control Group and in Treated Animals (±SD).

	WBC ($x10^3/mm^3$)			RBC ($x\ 10^6/mm^3$)			GL		g/dl)
	1h	2h	3h	1h	2h	3h	1h	2'	3h
Control group	6.4 ± 1.5			5.2 ± 0.6			82 ± 15.8		
1 LD *(C. cerastes venom)*	11.1 ± 1.4	13.5 ± 4.0	17.8[a]±0.5	5.3 ± 0.4	5.2 ± 0.7	4.7 ± 0.7	65.3±35.7	80.4±19.9	68.6±15.7
2 LD_{50} *(C. cerastes venom)*	10.6 ± 2.5	14.9[a]± 4.4	24.1[a]±3.8	5.5 ± 0.5	5.3 ± 0.9	4.4 ± 0.2	84.6 ±24.2	115 ± 52.7	248.3[ab]±170.5
***E. creticum* extract alone**	8.3 ± 2.5	9.2 ± 2.1	6.8 ± 1.8	6.6 ± 1.9	6.5 ± 1.6	4.4 ± 1.7	48.2[a]±26.1	43[a] ± 8.8	42.8[a]±1.8
1 LD_{50} + *E. creticum* extract	6.5 ± 1.4	13.1 ± 4.9	15.4 ± 3.0	5.3 ± 0.5	4.8 ± 0.9	5.2 ± 1.0	62 ± 25.4	122.6±52.4	107.6±52.5
2 LD_{50} + *E. creticum* extract	9.5 ± 0.5	17.4 ± 0.6	13.4±10.5	4.9 ± 0.2	5.2 ± 0.3	4.0 ± 0.4	83.3 ± 33.4	72 ± 24.2	48.8[b]±7.5

[a]Indicates statistically significant difference to the control ($P<0.05$). [b]Indicates statistically significant difference within the treated animals ($P<0.05$).

with time for 1 LD_{50} and 2 LD_{50} doses although it has been observed that an ***in vitro*** inhibition of ESR of normal blood took place by low concentration of ***Vipera xanthina palestine*** venom (16).

Animals treated with 1 LD_{50} dose showed a decrease in blood glucose levels already after one hour which remained low during the three hours of the experiment as reported for ***C. cerastes*** (17). A significant hyperglycemia was observed with 2 LD_{50} doses ***(Table II).*** Hyperglycemia resulting from snake venoms was described by several authors (18-22) and was reported to be due to mobilization of glycogen in the liver and muscle, possibly by a direct inhibition of glucokinase or indirectly by stimulating the release of adrenaline (22). In a study of the hyperglycemia produced by ***C. viper*** venom in mice, glycogen content in the brain, liver and kidney was found to decrease with an increased glucose 6-phosphate in the liver and kidney (23). Depressed succinic dehydrogenase in all organs suggested depressed metabolic activity and indicated that the decreased glycogen content is due to diminished glucose uptake. Such depressed glucose uptake might be attributed to decreased insulin like activity and seemed to be the results of stimulated release of catecholamines. Catecholamines may be responsible for insulin insufficiency at the level of pancreas and in other tissues (23). Animals treated only with ***E. creticum*** extract showed already in the first hour a significant decrease in blood glucose level ***(Table II)*** as reported (9). When 2 LD_{50}-treated hyperglycemic animals received ***E. creticum*** extract, a significant drop of the blood glucose level was observed in the third hour. This hypoglycemic effect of ***E. creticum*** extract was not recorded when 1 LD_{50} dose given animals received plant extract.

Although the mechanism of action is not clear, it seems that the 1 LD_{50} dose blocks the hypoglycemic action of ***E. creticum in vivo.***

In conclusion, the obtained data indicated a certain counteraction of the ***E. creticum*** on the ***C. cerastes*** venom. The hypoglycemic effect can be considered as the most significant antagonizing ac-

tion of the plant extract. Further investigations are needed to determine the mechanism of action of the antagonizing effect as well as the nature of the active principles of ***E. creticum*** responsible for this activity.

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Differential Inhibition of Prostaglandin H Synthase Isozymes by Non-Steroidal Anti-inflammatory Drugs: Use of Ibuprofen Isomers

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Abstract ❑ The activity of series of nonsteroidal anti-inflammatory agents (naproxen, ibuprofen isomers and racemate, and indomethacin) were examined for their inhibition of bovine prostaglandin endoperoxide H synthase-1 (PGHS-1 or cyclooxygenase 1, COX-I), and PGHS-2 (COX-2) isozymes, and inhibition of arachidonic acid (AA)-induced platelet activation. IC50 values and selectivity ratios were de-