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The ameliorative effect of Alkanna orientalis extract against Cerastes cerastes venom renal , lipogram and cholinergic toxicity

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ABSTRACT **: The present study designed to evaluate the toxicity of Cerastes cerastes (Cc) venom on male albino rats and the possible ameliorative role of Alkanna orientalis (Ao) extract against venom toxicity. Forty-eight adult male albino rats (180 ± 200 g) divided into 6 groups. Group I injected intraperitonial with physiological saline (100µl). Group II injected intraperitonial with Ao extract (250 mg/kg/BW). Group III was injected with 1/10 LD50 of Cc venom (0.435 mg/kg/BW). Group IV injected intraperitonial with Cc venom (0.435 mg/kg/BW) then immediately injected with Ao extract (250 mg/kg/BW). Group V was given Ao extract orally (250 mg/kg/b.wt) then after 2 hours injected with Cc venom (0.435 mg/kg/BW) Group VI was injected with Cc venom (0.435 mg/kg/BW) then was injected immediately with immunoglobulin (300µl). Group III showed a significant increase in serum creatinin, urea, uric acid as well as a significant decrease in serum total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, glucose level, calcium and cholinesterase when compared with control group. As well as, Ao extract when given intraperitonial immediately or orally 2 hours before Cc venom as antidote, it minimizes the changes of previous biochemical parameters. groups IV and V when compared with group VI, it showed that Ao extract considered more effective antidote than antivenom immunoglobulin for Cc venom renal, lipogram and cholinergic toxicity. In conclusion, Ao extract showed a new therapeutic and prophylaxis agents against Cc venom renal, lipogram and cholinergic toxicity.**

KEYWORDS: Cerastes cerastes; Alkanna orientalis; family Boraginaceae; venom toxicity; haematological effect.

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I. INRODUCTION

Snake's envenomation is a critical public medical health problem **(Chippaux et al 1999)** resulting in high mortality and morbidity rate **(Theakston et al., 2003)**. Venomous snakes documented as the most dangerous poisoning animals all over the world and the fatality of its bites depending on the injected venom amount, location of the bite, the size of the victim, the snake species and the time between the bite and the injection of anti-venom **(Frobert et al., 1997)**. The toxic effects of the venoms result from both protein and non-protein components which have distinct actions **(Syed et al., 2008)**.

Cerastes cerastes venom was reported to cause local tissue damage, that lead to the extra cellular matrix disruption as well as, the basement membrane disruption that covering the body vessels and blood capillaries **(Anai et al., 2002)**.

Kebir and Laraba (2017) reported that, the cell membrane permeability changes due to the venom toxicity that resulting in the internal cell content discharge, oxidative stress and it cause an inflammatory response. Therefore, anti-

inflammatory and antioxidant drugs may be important in the treatment of snake bite. In addition, the neurological toxic effect of snakebites such as neuromuscular paralysis was documented by **(Ibrahim et al., 2017)**.

The usage of anti-venom can neutralize the venom as well as, it can prevent further damage but it cannot restore the damage that was already occurred **(Leon et al., 2000)**. Therefore, it is seriously to find out new natural or artificial venom inhibitors, which can compete the anti-venom therapy or complement the anti-venom action especially, in the local tissue damage restoring **(Girish and Kemparaju, 2006)**. Glycosaminoglycans, polysaccharides, flavonoids and alkaloids was documented as venom spreading factors inhibitors **(Girish and Kemparaju, 2007)**. Herbs and Plants was recorded as pharmacological ingredients source that represent about 25% of the recently used drugs **(De Smet, 1997)**. Because of the low-cost traditional medicinal plants, it is commercially used for diseases treatment especially in low-income population's countries **(Hasani et al., 2009)**.

Boraginaceae family includes several plants that widely distributed in mild and tropical regions and it was classified into 200 genus and 2000 species, most of these family members have important medicinal usage. Some plants of Boraginaceae family documented as snakebite antidote such as, Argusia argentea extract is effective in Trimeresurus flavoviridis bites **(Aung et al., 2010)**. Whole the plant of Carmona retusa reported as antidote paste for Dabois russelii bites **(Sekhar et al., 2011)**. Rosmarinic acid from cordial verbenacea extract is effective against Bothrops jararacussu bites **(Gomes et al., 2010)**.

Alkanna orientalis and Echium humil, belong to Boraginaceae family rich with stearidonic acid (SDA), alpha-linolenic acid (ALA) and gamma-linolenic acid (GLA) in addition to rosmarinic acid (RA). All previous ingradient was reported to be a powerful anti-inflammatory and antioxidant effect so; they can restore the Cerastes cerastes venom toxicity (**Abbaszadeh et al., 2011** and **Katrin K et al., 2012**).

Rosmarinic was reported as active ingredient of Alkanna orientalis that showed antioxidant, anti-inflammatory and antibacterial effect **(Petersen and Simmonds, 2003)**.As well as, rosmarinic acid able to inhibit the snake venom phospholipases A2 and metalloproteinase from Cordia verbenacea and B. jararaca **(Ticli et al., 2005)**.

On the same pattern, **(El Sohly et al., 1997)** reported flavonols fractions from aerial parts of Alkanna orientalis. Flavanoids are one of the foremost plant components that work against PLA2 and lipooxygenase, they showed anti-allergic, anti-inflammatory and enzyme inhibiting properties. Flavanoid weakly inhibits the group I PLA2 from Naja naja and strongly inhibits the group II PLA2 from Vipera **(Gopi et al., 2014)**.

II. MATERIAL AND METHODS

Experimental animals

Eighty eight male albino rats, weight range 180–200 gm, obtained from faculty of science animal house, Zagazig University. These rats quarantined 15 days before the experiments carried, settled in plastic cages, fed a standard diet and water, exposed to a 12 h light/dark cycle, and maintained at temperature range 22±2ºC. All Institutional and National Guidelines for the care and use of animals were followed.

***Cerastes cerastes* venom**

25 *Cerastes cerastes* vipers obtained from El Kharga (New Valley), settled in cages at in desert research center physiology lab. Vipers were milked by using a rubber into a small beaker, and then the venom was rapidly frozen by using liquid nitrogen until the study start.

***Cerastes cerastes* venom LD_{50}**

LD_{50} of *Cerastes cerastes* venom was calculated according to the method that described by ***(Ramakrishnan, 2016)***. 40 adult male albino rats were divided into 5 groups (gp I-V), injected intraperitonial (i.p.) with *Cerastes cerastes* venom with doses (2.0, 3.0, 4.0, 5.0, 6.0) mg/kg/BW for each group, respectively. 24 hours post injection the dead and alive rats in each group were recorded. The calculated LD50 was 4.35 mg / kg/BW.

Alkanna orientalis extraction

Alkanna orientalis extract was prepared according to ***(Sukhdev et al., 2008)*** method for intraperitonial administration. The plant obtained from Saint Katherine, South Sinai, Egypt, where it grow, and then it was left in air for drying away from direct sun heat. Then, we grinded the plant then immersed in 70% ethyl alcohol for 4 days. The extract filtered and concentrated at room temperature. The extract stored at 4ºC until using in the study.

Antivenom

We purchased Anti-venom immunoglobulin from VACSERA CO. Agouza .Giza, Egypt.

Animal treatment schedule

Forty eight male albino rats were divided into six groups (n=8/group), as the following:

Group I (control group):

Rats were injected (i.p.) with 100µl of 0.09% physiological saline

Group II (*Alkanna orientalis* treated group):

Rats were injected (i.p.) with *Alkanna orientalis* extract (250 mg/kg/BW) dissolved in 100µl distilled water.

Group III (*Cerastes cerastes* venom treated group):

Rats injected (i.p.) with *Cerastes cerastes* venom (0.435 mg/kg BW) that represent 1/10 LD 50, dissolved in 100µl of 0.09% physiological saline.

Group IV (*Cerastes cerastes* venom + *Alkanna orientalis* extract) group:

rats injected (i.p.) with 1/10 LD 50 *Cerastes cerastes* venom (0.435mg/kg/BW) dissolved in 100µl of 0.09% physiological saline then, immediately injected (i.p.) with *Alkanna orientalis* extract (250 mg/kg/BW) dissolved in 100 µl distilled water.

Group V (a prophylaxis group):

Rats were given *Alkanna orientalis* extract orally (250 mg/kg/BW) then after 2 hours were injected (i.p.) with 1/10 LD_{50} *Cerastes cerastes* venom (0.435 mg/kg BW) dissolved in 100µl of 0.09% physiological saline.

Group VI (*Cerastes cerastes* venom + antivenom):

Rats were injected (i.p.) with 1/10LD_{50} of *Cerastes cerastes* venom (0.435 mg / kg/BW) dissolved in 100 µl of 0.09 % physiological saline, then were immediately injected (i.p.) with 300 µl of antivenom immunoglobulin.

Blood sampling

In this study rats were sacrificed 2 hours after *Cerastes cerastes* venom injection and in group I & II, rats were sacrificed 2 hours after saline and *Alkanna orientalis* extract injection respectively, and blood samples were collected in sterile tubes without anticoagulant. The serum separated from clotted blood and used for the biochemical parameters determination.

Evaluation of some biochemical parameters

Biochemical parameters carried out by spectrophotometric analyzer (BT-260). Creatinin test measured according to method described by ***(Henry, 1974)***. Serum urea, measured according to ***(Patton and Crouch, 1977),*** Serum uric according to ***(Trinder, 1969b).*** Serum calcium according to ***(Tietz et al 1994)***, Cholinesterase level according to ***(Szasz, 1968)***, glucose according to method described by ***(Trinder, 1969a),*** Serum cholesterol According to method described by ***(Richmond, 1973),*** serum triglycerides according to method described by ***(Fossati and Prencipe ., 1990)*** and Serum LDL and HDL-cholesterol was determined according to ***(Stein 1986)***.

Statistical analysis

The obtained data were analyzed by the statistical analysis software (SAS–2013 program), for obtaining Mean values ±standard error. Subsequent multiple comparisons between the different groups were analyzed by Duncan's multiple comparison tests ***(Duncan, 1955)***, values at (P<0.05) were considered significant ***(Armitage and Berry, 1987)***.

III. RESULTS

Kidney function parameters and cholinesterase

Table (1) illustrate the effect of *Cerastes cerastes* venom and the ameliorative role of *Alkanna orientalis* extract and antivenom on some kidney function parameters, calcium and cholinesterase in the different groups. Rats that injected with *Cerastes cerastes* venom (group III), showed a significant increase ($P < 0.001$) in level of serum creatinin, urea, uric acid and a significant decrease ($P < 0.001$) in serum calcium and cholinesterase level as compared with control group (group I). While, *Alkanna orientalis* extract (group II) did not show any significant effect in all previous parameters when compared with control group.

On the other hand, when *Alkanna orientalis* was injected (i.p.) immediately after *Cerastes cerastes* venom or given orally 2 hours before *Cerastes cerastes* venom as a prophylaxis dose, (groups IV and V, respectively), it was able to ameliorate the renal toxic effect of the venom as well as cholinergic toxicity. In addition, it showed a significant decrease ($P < 0.001$) in serum level of creatinin, urea, uric acid and a significant increase ($P < 0.001$) in serum calcium and cholinesterase as compared with venom treated group. On the same pattern, antivenom immunoglobulin showed a significant ameliorative effect against the venom when given (i.p.) immediately after Cerastes cerastes crude venom injection.

Lipogram parameters and glucose level

Concerning to the effect of *Cerastes cerastes* venom and the ameliorative role of *Alkanna orientalis* extract and antivenom immunoglobulin on lipogram parameters and glucose level. Table (2) showed that, group III has a significant decrease ($P < 0.001$) in total cholesterol, triglyceride, HDL and LDL cholesterol as well as, serum glucose level in comparison with group I. While, *Alkanna orientalis* extract (group II) showed a significant decrease in triglyceride level and did not give any significant effect in the other parameters after 2 hours from (i.p.) injection of it as compared with control group as shown in Table (2). As well as, in groups IV and V *Alkanna orientalis* was able to ameliorate the toxic effect of the venom. It showed a significant increase ($P < 0.001$) in all previous parameters when compared with the venom treated group (group III). On the same pattern, antivenom immunoglobulin showed a significant ameliorative effect against

the venom when given (i.p.) immediately after *Cerastes cerastes* crude venom injection. It showed a significant increase ($P < 0.001$) in all previous parameters when compared with the venom treated group (group III), as shown in Table (2).

These results showed that *Alkanna orientalis* extract when given as antidote for *Cerastes cerastes* venom renal, cholinergic and lipogram toxicity was effective hand by hand such as synthesized antibody (anti-venom immunoglobulin) and these results clearly appeared by comparing the findings of groups IV and V with group VI.

IV. Discussion

Concerning to the effect on some kidney function parameter, serum calcium and serum cholinesterase. Our results showed that *Alkanna orientalis* extract did not cause any significant change in all previous determined parameters when given as a single intraperitonial dose to albino rats as compared with control group. While, 2 hours after *Cerastes cerastes* venom injection lead to a significant increasing in serum creatinin, urea and uric acid also, a significant decrease in serum cholinesterase and calcium as compared with control group.

On the same pattern, anti-venom and *Alkanna orientalis* extract when given as antidote for *Cerastes cerastes* venom toxicity according to the program of treatment, succeed to ameliorate the toxicity of *Cerastes cerastes* venom on kidney function parameters.

The renal toxicity occurred after *Cerastes cerastes* venom injection agree with ***Tilbury et al., (1987),*** as they documented acute renal failure with vascular lesions and tubular necrosis in renal cortex after different snake bites. In the present study, the elevation in serum uric acid, urea and creatinine levels indicate renal impairment. The increasing of urea and creatinine levels that observed in this study, reflect impairment of renal function ***(Mora, et al 2003 and Yousef et al, 2006)***. Also, a similar observation was reported in rats after administration of various viper venoms were reported ***(Schneemann et al., 2004)***.

The decreasing in serum cholinesterase levels in rats following *Cerastes cerastes* venom injection is in agreement with ***(Soares AM, Giglio, 2003),*** as they documented that Phospholipases A_2 is the major components of *Cerastes cerastes* venom and it exhibit a neurotoxicity. Also, ***(Kini, 2003)*** reported that PLA_2 exhibit a presynaptic neurotoxicity.

According to the present study results, the significant decreasing in calcium level after *Cerastes cerastes* venom injection is in agreement with ***(Fahmi et al ,. 2014)***, as they illustrated the effect of sub lethal doses of *Cerastes cerastes* and *Macrovipera mauritanica* venom after 1, 3, 6 and 24 h of envenomation, and the results showed that the venom caused a significant decreasing in calcium level in rabbit.

The protective action of *Alkanna orientalis* treatment is explicable in terms of their capacity for trapping free radicals and their stabilizing effect on the cytoplasmic membranes, they promotes protein sysnthesis, helps in regenerating kidney tissues ***(Naglaa et al ., 2012)*** . As well as, *Alkanna orientalis* considered a powerfull anti-oxidant as it rich with Flavanoids that considered as one of the foremost plant components that work against PLA2, lipooxygenase. The flavonoid was reported as strongly inhibits the group II PLA2 from *Vipera* ***(Gopi et al., 2014).*** As well as , *Alkanna orientalis* rich source of rosminaric acid, alpha - linolenic acid (ALA) and gamma- linolenic acid (GLA) that acts as powerful anti-inflammatory and anti-oxidant against *Cerastes cerastes* venom renal toxicity ***(Katrin et al., 2012)*** and ***(Ticli et al., 2005)***.

The protective effect of *Alkanna orientalis* extract against the significant decreasing in cholinesterase and the cholinergic effect of *cerastes cerastes* venom is in full agreement with ***(Diab et al., 2013)*** as they described the protective effect of *Echium humile* extract (family: *Boraginacea*) against malathion neurotoxicity and against the significant decreasing in cholinesterase as a result of malathion cholinergic toxicity .

As well as, *Alkanna orientalis* rich with flavonoids that considered as one of the most plant ingredients that work against PLA2, as flavonoid is strongly inhibits the group II PLA2 from Vipera that responsible for the major *Cerastes cerastes* venom toxicity so, it able to antagonize the hazardous neuotoxic effect of the crude venom as well as *Cerastes cerastes* effect on serum calcium level ***(Gopi et al., 2014).***

Concerning to the effect on glucose and lipogram parameters (Total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol) the obtained results revealed that, *Alkanna orientalis* extract when given as a single intraperitonial dose did not cause any significant change in all determined lipogram parameters and glucose with the exception of triglyceride showed a significant decreasing as compared with control group. While, *Cerastes cerastes* venom according to the same program of treatment caused a significant decreasing in all lipogram parameters, Total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol as well as, glucose level when compared with control group.

On the other hand , *Alkanna orientalis* extract and antivenom when given as antidote to *Cerastes cerastes* venom according to the program of treatment, succeed to modulate the *Cerastes cerastes* venom toxic effect and returned the lipogram and glucose parameter to about the normal value.

The significant hypoglycemic effect that observed after 2 hours from injection of the venom is in agreement with ***(Abu-Sinna et al., 1993, Al-Jammaz., 2002 and Al-Sadoon et al., 2013)***, as the reduction of blood glucose level reflects a disturbance in carbohydrate metabolism, which could be referred to insulin-releasing effect of some venom components. As well as, the decreasing in serum cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels in rats following *Cerastes cerastes* venom injection, these findings are in agreement with the other investigators ***(Abdel-Nabi, et al 1997 and Al-Jammaz, 2002).*** They reported that, a significant decreasing in serum cholesterol and triglyceride levels noted in laboratory animals that injected with snake venoms, suggested that the snake venom able to mobilize lipids from adipose tissues then splitting by lipolytic enzymes, that lead to free fatty acids liberation.

We suggested that *Cerastes cerastes* venom toxicity responsible for production of oxidative stress, which is the key contributor in hepatic injury and it known to produce reactive oxygen species (ROS) that is responsible for significant change in lipid profile and hepatic dysfunction.

The ameliorative effect of *Alkanna orientalis* that observed in this study against the hypoglycemic and hypolipemic effect of *Cerastes cerastes* venom explained by that, *Alkanna orientalis* rich with alpha-linolenic acid (ALA), gamma- linolenic acid (GLA) and rosminaric acid that acts as antioxidant and anti inflammatory against *Cerastes cerastes* venom toxicity ***(Katrin et al ., 2012*** and ***Ticli et al., 2005).***

ALA and GLA, act as anti-inflammatory and lipid lowering potential, it also cause vasodilation. In addition, GLA are important constituents of membrane phospholipids, including the mitochondrial membrane, where they enhance the integrity and the fluidity of the membrane ***(Horrobin, 1992).*** As well as, dietary supplementation with GLA alone has yielded variable results on circulating lipid levels ***(Graham et al., 1994 and Von Schacky., 2000).***

As well as, *Alkanna orientalis* considered a powerfull antioxidant as it rich with Flavanoids that considered as one of the foremost plant components that work against PLA2, lipooxygenase. They possess anti-inflammatory, anti-hypertensive, hypocholesterolemic, antiallergic, and enzyme inhibiting properties. The flavonoid strongly inhibits the group II PLA2 from *Vipera* that responsible for the major toxicity of *Cerastes cerastes* venom so, it able to antagonize the hazardous toxic effect of the crude venom ***(Gopi et al., 2014).***

V. CONCLUSION

The natural medical herbs are showing a new area for development of better therapeutic and prophylaxis agents against expected envenomation by Cerastes cerastes venom toxicity. The alcoholic extract of Alkanna orientalis has many benefits, as it is cheap, stable at room temperature and able to antagonize the hazardous toxic effect of Cerastes cerastes venom. Therefore, we recommend the usage of Alkanna orientalis extract as initial assist in Cerastes cerastes bitted victims therapy to minimize mortality and morbidity. As well as, this extract can be used as a prophylaxis for researchers who work in the fields where Cerastes cerastes are abundant.

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Table (1): Effect each of *Cerastes cerastes* venom, *Alkanna orientalis* and their combination on some kidney functions, calcium and cholinesterase in male albino rats (mean ± SE) (n = 8).

groups	mean ± SE of some kidney function parameters, calcim and cholinesterase (n = 8)				
	Creatinin mg\dl	**Urea** mg\dl	**Uric acid** mg\dl	**CHE** U\L	**Ca** mg\dl
1. Control group	0.78 ± 0.09^{b}	43.75± 2.2 ^{d}	4.26 ± 0.23 ^{d}	3901 ± 139 ^{ab}	10.48 ± 0.4^{a}
2. *Ao* extract group	0.74 ± 0.08^{bc}	45.75± 3.6 ^{d}	4.53 ± 0.22 ^{dc}	4004 ± 111 ^{a}	10.45 ± 0.2^{a}
3. *Cc*.venom group	1.41 ± 0.05^{a}	85.5± 4.1 ^{a}	8.80 ± 0.15 ^{a}	2145 ± 123 ^{d}	7.1 ± 0.3^{c}
4. *Cc*.venom $_{i.p}$ + *Ao* extract $_{i.p}$ group	0.79 ± 0.08^{b}	71.37± 5.3 ^{b}	4.41 ± 0.23 ^{cd}	3545 ± 118 ^{b}	9.56 ± 0.5^{b}
5. *Ao* extract $_{oral}$ then 2h + *Cc*.venom $_{i.p}$ group	0.71 ± 0.07^{dc}	43.25± 2.4 ^{d}	3.87 ± 0.14 ^{d}	3568 ± 133 ^{b}	10.56 ± 0.6^{a}
6. *Cc*.venom $_{i.p}$+Antivenom $_{i.p}$ group	0.79 ± 0.04^{b}	63.62± 2.3 ^{c}	5.73 ± 0.29 ^{b}	2592 ± 98 ^{c}	10.57 ± 0.5^{a}

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05).

Where : *Cc*.venom ====== Cerastes cerastes venom , *Ao* extract ====== *Alkanna orientalis* extract
i.p ========== intraperitonial

Table (2): Effect each of *Cerastes cerastes* venom, *Alkanna orientalis* and their combination on lipogram and glucose parameters in male albino rats (mean ± SE) (n = 8).

groups	mean ± SE of lipogram and glucose parameters (n = 8)				
	cholesterol mg\dl	**Triglyceride** mg\dl	**HDL cholestero** mg\dl	**LDL cholestero** mg\dl	**RBS** mg\dl
1. Control group	99.0 ± 2.5^{a}	80.1 ± 5.2 ^{a}	30.2 ± 1.1 ^{b}	56.3 ± 2.1 ^{c}	75.8± 2.3 ^{a}
2. *Ao* extract group	98.2 ±3.1^{a}	63.1 ± 4.3 ^{cd}	30.1 ± 0.9 ^{b}	55.3 ± 1.6 ^{c}	76.6± 3.1 ^{a}
3. *Cc*.venom group	80.5 ± 4.2^{c}	66.5 ± 3.2 ^{c}	23.1 ± 1.3 ^{c}	50.0 ± 1.4 ^{d}	49.8± 1.8 ^{b}
4. *Cc*.venom $_{i.p}$ + *Ao* extract $_{i.p}$ group	90.8 ± 5.1^{b}	58.0 ± 4.5 ^{d}	33.7 ± 1.4 ^{b}	67.2 ± 1.7 ^{a}	75.7± 3.5 ^{a}
5. *Ao* extract $_{oral}$ then 2h + *Cc*.venom $_{i.p}$ group	89.6± 2.1 ^{b}	75.5 ± 3.6 ^{ab}	32.5 ± 1.2 ^{b}	59.0 ± 1.8 ^{bc}	77.6± 0.9 ^{a}
6. *Cc*.venom $_{i.p}$+Antivenom $_{i.p}$ group	77.8 ± 3.6^{c}	77.0 ± 4.6 ^{a}	39.5 ± 1.4 ^{a}	64.5 ± 1.8 ^{ab}	42.2± 1.6 ^{c}

Means within the same column in each category carrying different litters are significant at (P ≤ 0.05).

Where:
Cc.venom ====== Cerastes cerastes venom , *Ao* extract ====== *Alkanna orientalis* extract
i.p ========== intraperitonial