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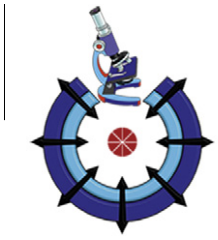

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ARTICLE

# Role of hyaluronidase inhibitors in the neutralization of toxicity of Egyptian horned viper *Cerastes cerastes* venom

**A.F. Wahby [a], El-Sayed M.E. Mahdy [b], Hatem A. EL-mezayen [b], Walaa H. Salama [a], Nihal M. Ebrahim [a], Azza M. Abdel-Aty [a,*], Afaf S. Fahmy [a]**

[a] *Department of Molecular Biology, National Research Center, Dokki-Cairo, Egypt*
[b] *Department of Chemistry, Faculty of Science, Helwan University, Egypt*

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Hemorrhage;
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**Abstract** Hyaluronidase "venom spreading factor" is a common component of snake venoms and indirectly potentiates venom toxicity. It may cause permanent local tissue destruction at the bite site/systemic collapse of the envenomated victim. The present study was performed to assess the benefits of inhibiting the hyaluronidase activity of Egyptian horned viper, *Cerastes cerastes* (Cc). The aqueous extracts of some medicinal plants were screened for their inhibitory effect on hyaluronidase activity of Cc venom. The results revealed that the *Rosmarinus officinalis* (Ro) extract is the most potent hyaluronidase inhibitor among the tested extracts. The Ro extract is more potent inhibitory effect on the hyaluronidase activity than the prepared rabbit monoclonal antiserum of previously purified hyaluronidase enzyme from Cc venom (anti-CcHaseII). In addition, the Ro extract is efficiently inhibited the activity of hemorrhagic toxin previously purified from Cc venom, and it also neutralized the edema inducing activity of the Cc venom *in vivo*. Furthermore, the Ro extract markedly increased the survival time of experimental mice injected with lethal dose of Cc venom up to 7 h in compared to mice injected with venom alone or with venom/anti-CcHaseII (15 ± 5, 75 ± 4 min), respectively. Our findings imply the significance of plant-derived hyaluronidase inhibitor in the neutralization of local effects of Cc venom and retardation of death time. Therefore, it may use as a therapeutic value in complementary snakebite therapy.



* Corresponding author.
E-mail address: azzasdm@hotmail.com (A.M. Abdel-Aty).
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## 1. Introduction

Snake's envenomation is a serious socio-medical problem, especially in the places where snakes are abundant, with an estimated 1.8–2.5 million incidences per year, a mortality level of 100,000–125,000 persons annually and more than 100,000

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individuals suffering from severe complications, which may end in amputation of the attacked limb [22,36]. In Egypt, approximately 1000–10,000 envenomings per year with about 11–100 persons are estimated to die annually [22].

The Egyptian desert horned viper (genus *Cerastes*) is the best known, most distinctive and most abundant venomous snakes of the great deserts of North Africa and the Middle East. This viper is popular among snake-keepers and thus encountering snakebite is not rare outside the area of its original distribution [11,43]. Envenomings by *Cerastes* viper inflict prominent local tissue damage, which disrupts the extracellular matrix and basement membrane surrounding blood vessels and capillaries that are essential for rapid dissemination of target-specific toxins. Therefore, locally acting factors, especially hyaluronidases, hemorrhagic metalloproteinases and myotoxins (enzymatic/non-enzymatic) have been received more attention [2,16]. These spreading factors are facilitating the diffusion of toxins into the circulation leading to systemic effects, which include spontaneous hemorrhage–cerebral hemorrhage being the most serious manifestation defibrinogenation, disseminated intravascular coagulation, and cardiovascular shock secondary to hypovolaemia, vasodilation, and direct effects on the myocardium, acute renal failure and acute respiratory distress syndrome [48]. It is likely that inhibition of these enzymes will not only minimize local tissue damage such as hemorrhage, edema, necrosis and inflammation in site of the bite, but will also retard the distribution of lethal toxins; hence their importance in the management of snakebite.

Although the available antivenom neutralizes the active venom components, stopping further damage but do not reverse the damage already done [26–28,52]. It is important to search for different venom inhibitors, either synthetic or natural, which would complement the action of antivenom, particularly in relation to the neutralization of local tissue damage. Hence, hyaluronidase is an important target for minimizing envenomation-associated morbidity, so the inhibition of hyaluronidase not only prevents local tissue destruction but also retards the diffusion of toxins into the blood, resulting in delay in time to death in severe cases of envenomation [13,14,53].

Compounds such as proteins, glycosaminoglycans, polysaccharides, flavonoids, alkaloids, antioxidants, anti-inflammatory agents, and synthetic organic have been investigated as hyaluronidase inhibitors. [12–14,23,25,29,33,35,40,44,53]. In addition, aqueous, methanolic or ethanolic extracts prepared to different parts of numerous plant species have been investigated as hyaluronidase inhibitors [9,15,18,30,38,39,47]. In previously study, novel hyaluronidase CcHaseII (33 kDa) of the most dangerous horned viper *Cerastes cerastes* was purified and characterized in a set of biochemical assays. CcHaseII enhanced one hundred percent of hemorrhagic activity of the potent purified hemorrhagic SVMP of corresponding venom and the antiserum prepared against it showed highly cross-reactivity with many of the Egyptian snake venom proteins [51]. This study aims to screen the aqueous extracts of different common medicinal herbs for their inhibitory effect on the hyaluronidase activity of Cc venom. Select the most potent inhibitor among the tested extracts as a natural inhibitor and studies its role for inhibition of hemorrhagic activity, edema inducing activity and lethality of the Cc venom.

## 2. Materials and methods

### 2.1. Collection of medicinal plants

Plants names and their families: (*Cuminum cyminum, Foeniculum vulgare, Pimpinella anisum*; Apiaceae), (*Ceratonia siliqua, Glycyrrhiza glabra, Tamarindus indica*; Fabaceae), (*Mentha peprinta, Ocimum basilicum, Origaum majorana, Rosmarinus officinalis, Thymus monglicus*; Lamiaceae), (*Hibiscus sabdariffa*; Malvaceae*; Sesamum inddicum*; Pedaliaceae), (*Hordeum vulgare*; Poaceae), (*Nigella sativa*; Ranunculaceae), and (*Curcuma longa, Zingiber officiale*; Zingiberaceae). All plants for the present study were obtained from Agricultural Research Center, Dokki, Egypt.

### 2.2. Extraction of plants

Air-dried plant material collected, washed thoroughly with water, shade-dried and grounded into coarse powder. Five grams of the powdered shade dried plant material was soaked in 50 ml distilled water (w/v) overnight at room temperature with constant stirring. The obtained extract was filtered using Whatman filter paper No. 1, the residue obtained was re-suspended in freshly distilled water (1:2 ratios). The process was repeated three times. Then, crude extracts were centrifuged at 3000 rpm for 10 min, and were stored in tightly stopper bottles at 4 °C for further study.

### 2.3. Animals

Male Swiss albino mice weighting 20 ± 1 g were used for lethality test. Albino and Swiss rabbits weighting (1, 1.5 kg) were used for hemorrhagic test and antiserum preparation, respectively. The animals were obtained from the laboratory animal (National Research Centre). They housed in stainless steel cages at room temperature (28–32 °C) under a light period of 16–18 h daily and fed on standard commercial feed. The experiments were carried out in the same lab in accordance with the institutional guidelines for animal care and use.

### 2.4. Snake venom and chemicals

Venom of *C. cerastes* (Cc) was milked from adults collected from the field. The venom was lyophilized and stored at −20 °C. Samples were thawed and centrifuged before use. CcHaseII, hyaluronidase enzyme previously purified and characterized from Cc venom [51] Rooster comb hyaluronic acid (HA) and cetyltrimethylammonium bromide (CTAB) were obtained from Sigma. All other chemicals and reagents were of analytical grade. The buffers were prepared according to Gomorie [17] and Blanchard [6] and the final pH was checked by pH meter (Hanna, pH 211 Microprocessor pH meter).

### 2.5. Immunization of rabbits and preparation of CcHaseII antiserum

According to a low dose and low volume immunization protocol assessed by Chotwiwatthanakun et al. [10] with slight modification. Two male Swiss rabbits (1.5 kg) were injected intramuscularly with dose containing 10 μg of CcHaseII dissolved in 0.5 ml isotonic saline. The primer dose was emulsified