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Research Article

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Olea Europaea Leaves Delay the Onset of Toxicity of *Cerastes Cerastes* Venom in Albino Mice

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Abstract

To investigate the anti-Cerastes cerastes venom effect of aqueous olive leaf extract. The mitigation in the mean survival time of the male Albino Swiss mice were used to deduce the antivenom property of the aqueous olive leaf extract after challenging with LD99 of snake venom. The aqueous extract of olive leaf significantly increases mean survival time and the protection fold, but could not protect mice from death when used alone. The higher dose, i.e., 33 g/kg was better than that of the lower dose of 15 g/kg. The anti-snake venom was found to be more effective than the aqueous olive leaf extract.

Keywords: *Cerastes cerastes*, Anti-snake venom, Aqueous olive leaf extract, Mouse

Introduction

Snakebite is a major public health problem in many African countries including Libya, Tunisia, Algeria and Egypt [1, 2]. It is a particular challenge, although, in some parts of Africa, which is home to more than 400 snake species, of which about 30 venomous species, related to four families including: viperidae, colubridae, atractaspididae, and elapidae and they are recognized to cause human deaths, as reported by the World Health Organization [1]. *Cerastes cerastes* is one of the snakes frequently related with human mortality in Libya. The *Cerastes cerastes* venom contains several enzymes showing proteolytic activity and causes multiple kinds of intoxications [3]. The toxicities cause substantial physiopathological changes in liver, skin and heart. Phospholipases A2 (PLA2) from *Cerastes cerastes* for example, has been associated with some toxicities including neurotoxicity, lung toxicity, nephrotoxicity, hepatotoxicity and cardio toxicity [4-6]. The lethal cause of snake venom mainly results from its active ingredients such as PLA2. Phospholipid hydrolysis by PLA2 releases arachidonic acid whose metabolism results in the formation of potentially toxic reactive oxygen species (ROS) and lipid peroxides [6, 7]. The increase in the activity of liver enzymes indicating injures of the heart, liver and other organs could be accredited to the synergistic action of the venom components [3, 6].

Anti-snake venom (ASV) is a specific antidote to snake venom actions and the basis of treatment. Monovalent ASV is favoured to the polyvalent kind since it is less perilous to the patient and probably to be more effective in the treatment of the specific bite; though, a species diagnosis ought to be made before the right treatment can be selected. Polyvalent ASV is usually used against snakebite, but it is pricey and contained antibodies from immunized animals; therefore, there are probabilities of adverse reactions due to activation of immune system in many patients [8, 9].

On the contrary to the impenetrability of accessibility of modern treatment in several countries of developing world where venomous snakes present, many plant species are used as the folk medicine to treat snake bites in Libya mainly as in Tarhouna. Many Libyan plants are suggested for the treatment of snake bite activity. *Olea europaea* leaves, is one of the plants that has been suggested to be used in conventional herbal medicine against snakebite. Thus, the aim of this study is to screen the anti-snake venom potential of *Olea europaea* leaves and compared with polyvalent ASV.

Materials and methods

Preparation of aqueous *Olea europaea* leave extracts

Leaves of olive trees (*Olea europaea*) were collected from the Novellien zone, Tripoli Centre, Tripoli, Libya during July 2018. The leaves (5g) were cleaned and washed with distilled water and dried at a room temperature of 25°C for 20 minutes. Dried leaves were grinded in a homogenizer (HO4A Edmund Buhler GmbH, UK) along with 15 ml of distilled water. The resulting aqueous solution was filtered using a Millipore filter (0.45 µm, GHD Acrodisc GF, UK).

Venoms

Snake (*Cerastes cerastes*,) venom were extracted by manual stimulation and were obtained in liquid forms, from the Department of Zoology, Faculty of Science, University of Tripoli (Libya) and stored at – 20 °C until use. An aliquot of 7.5µl from the venoms was added to 800 µl of normal saline. A dose of 100µl (100 ng) was given to the male Swiss Albino mice.

Experimental animals

Swiss Albino male mice (18 ± 2 g) were used for the experiments. In order to reduce the contact caused by environmental alterations and handling during behavioral studies, mice were acclimatized to the Laboratory Animal Holding Center and laboratory surroundings for three days and at least one hour before to experiments, respectively. Mice were kept under standard conditions with food (low protein diet) and water available ad libitum. The animals were housed six per cage in a light-controlled room (12 h light/dark cycle, light on 07:00 h) at 27 °C and 65% relative humidity. All experiments were performed between 11:30 and 14:00 h. Each test group consisted of at least six mice, and each mouse was used only once. All animal experiments were conducted according to guidelines set by Institutional Animal Ethics Committee of University of Tripoli.

Calculation of LD99 of *Cerastes cerastes* venom

The median lethal dose (LD99) of *Cerastes cerastes* venom was determined according to the previously developed method [10]. A range of doses of venom in 800µl of physiological saline was injected intraperitoneally using groups of six mice for each venom dose. The LD99 was calculated with the confidence limit at 99% probability by the analysis of mortality occurring within 24 hour of venom injection. The anti-lethal potentials of aqueous *Olea europaea* leave extracts were determined against LD99 of *Cerastes cerastes* venom.

Detoxification of venom by extracts

Five groups of mice were used in this study. The first group of six mice received only 100µl (100ng of total protein) of the *Cerastes cerastes* venom (LD_{99} 5µg/kg). Groups 2-4 of six mice each (serving as treatment groups) were given an equivalent amount of the *Cerastes cerastes* venom with 1 ml, 1.5 ml and 2 ml of aqueous *Olea europaea* leave extracts orally (5g/15 ml), respectively. Group 5 of six mice received 100 µl of the *Cerastes cerastes* venom and ASV. The number of mortality was recorded within 24 h.

Statistical analysis

The difference among various treated groups and control group were analysed using one-way-ANOVA followed using unpaired Student's t test. The results were expressed as the mean ± SEM of the number of experiments done, with $P < 0.05$ indicating significant difference between groups.

Results and discussion

Calculation LD99 of *Cerastes cerastes* venom

Lethality data of *Cerastes cerastes* venom was calculated. The LD_{99} of *Cerastes cerastes* venom from this study was 5µg/kg.

Acute toxicity of *Cerastes cerastes* venom and its neutralization by aqueous *Olea europaea* leave extracts and antivenom

The *Cerastes cerastes* venom at the dose 5µg/kg (LD_{99}) produces 100% mortility in mice. The aqueous *Olea europaea* leave extracts significantly increases mean survival time up to 4 ± 0.6 hours and the protection fold could not protect animals from death when *Cerastes cerastes* venom used alone. The aqueous *Olea europaea* leave extracts when used at the dose of 2 ml (5 g/15 ml) was found to be more effective against *Cerastes cerastes* venom (4 hours) when compared with 3.4 hours shown by aqueous *Olea europaea* leave extract at the dose of 1 ml (15 gm/15 ml). ASV [polyvalent anti-snake venom by Haffkine Bio-Pharmaceuticals Company (India)] was found to be more effective as compared with the aqueous *Olea europaea* leave extract showing mean survival of two days for five mice and complete survival of one mouse.

The toxins of *Cerastes cerastes* venom are composed of neurotoxin, cardiotoxin, enzymes and proteins. The victim might die from respiratory paralysis which is the major cause of death. ASV and assisted ventilation can save life in many cases [11, 12]. Nevertheless, polyvalent antivenom carries a risk of severe adverse reactions and other problems such as difficulty to manage and usage, variety of dosage and high cost. Furthermore, antivenom occasionally does not offer sufficient protection against snake envenomation, particularly local poisoning [13]. The use of plants against the effect of snakebite has long been documented, even in modern times.

In Libya, several plants are recognized against snake envenomation particularly in the North South region of Libya (mainly Tarhouna). It was observed that aqueous *Olea europaea* leave extract when given to the mice after they received snake venom of *Cerastes cerastes* venom significantly increased mean survival time and the results were found better when it was used at higher dose (2ml of 5 g/15 ml). This could be possible due to inactivation or precipitation of active venom components by the aqueous *Olea europaea* leave extract which is consistent with the result obtained with similar studies [14, 15].

The delayed survive also could be related to the interactions of AOLE components (which were mainly polyphenolic components) with snake venom which is in consistency with previous studies reporting that polyphenolic secondary metabolites are able to inhibit PLA2 [16]. For example, quercetin, kaempferol, galangin, naringenin, artemetin and other flavonoids can inhibit toxins from snake venom. They found that flavonoids usually exert their inhibitory effect through hydrophobic interactions with A and B rings and aromatic or hydrophobic amino acid residues in the protein [17-19]. In addition, another research group found that Ar-turmerone which is a phenolic compound isolated from the Curcuma longa (Zingiberaceae) plant has a strong inhibitory effect against hemorrhage and lethality caused by B. jararaca and C. d. terrificus snake venom. The effect was attributed to the interaction with PLA2 [20]. It has also been reported that 4-nerolidylcatechol (a hydroxylated phenolic compound) - an extract from Piper umbellatum and P. peltatum (Piperaceae) - is able to inhibit the myotoxic activities of PLA2s and is able to interact and inhibit the function of PLA2s. Araya and Lomonte found that caffeic acid which is one of the components of AOLE, can interact with proteins via hydrogen bonds, inhibiting enzyme function and acting as antidote [21, 22]. They found that strong interactions may cause conformational changes in the protein structure [22, 23]. The interaction of caffeic acid with snake venoms was confirmed by Shimabuku and collaborators who crystallized PrTX-I (basic Lys49-PLA2 from B. pirajai snake venom) in the presence of the inhibitor caffeic acid [24]. The electron-density map clearly signifies the presence of three caffeic acid molecules interacting with the C-terminus of the protein. It is also reported that patients with snake bite envenomation had increased oxidants (myeloperoxidase and Linoleic acid hydroperoxides) and decreased antioxidants (Human

serum paraoxonase, ARLY, and -SH) and these results demonstrate that snake bites are associated with a shift to oxidative status but therapy with antioxidants can lead to an increase in the antioxidant defense system and thus improvements in clinical symptoms [25].

Conclusion

Antivenom is the ultimate treatment for venomous snakebites and must be administered as soon as possible after a bite. First aid measures should be directed at reducing systemic toxicity by limiting lymphatic flow. Rest, splints, and evasion of movement should decrease movement of the involved extremity. Positioning of the extremity below or at the level of the heart should be individualized for snakebites with severe and potentially fatal systemic toxicity, systemic toxicity may be hindrance by positioning the extremity below the heart, while for snakebites with severe local tissue damage and less systemic toxicity, positioning the extremity below the heart could increase local toxicity. Thus, it can be concluded from the study that aqueous *Olea europaea* leave extract might has antivenom activity against *Cerastes cerastes* venom. Results are comparable with the antivenom. Further elaborative work is necessary for the better understanding of the mechanism of venom inhibition. Detailed clinical studies in this direction are necessary to confirm this claim in human beings.

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