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Research Article

An *in vivo* study for the effect of *Citrus reticulata* (*Rutaceae*) fruit peels extracts on the onset of toxicity of *Cerastes cerastes* venom in Albino mice

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Abstract

Venom of *Cerastes cerastes* has been extracted and its toxicity was investigated in the presence of aqueous and methanolic extracts of *Citrus reticulata (Rutaceae)* fruit peels. The decline in the mean survival time of the male albino swiss mice were used to deduce the venom property in the presence and absence of aqueous and methanolic extracts of *Citrus reticulata (Rutaceae)* fruit peels. The aqueous and methanolic extracts of *Citrus reticulata (Rutaceae)* fruit Peels significantly decrease the mean survival time compared to the venom alone. From these results it was evident that the toxicity of *Cerastes cerastes* venomis increased significantly in the presence of *Citrus reticulata* in a dose dependent manner

Keywords: *Citrus reticulata; Cerastes cerastes*; Venom; Toxicity

Introduction

Snakebites are severe socio-medical difficulty that lead to morbid and fatal affect on victims in Libya and other North African countries [1,2]. Immediate antivenom treatment is crucial and *vital* to avoid morbidity and mortality [3]. The oxidative trauma condition, which result from snake bite envenomation is another measurement of kidney destruction and severe renal failure [4], connected with the antioxidant defense system, that might be subject for treatment by antioxidant therapy [5]. ROS (Reactive oxygen species) are engaged in many inflammatory reactions, thus influencing the physiology of the cells and participate a significant function in the pathological conditions [6]. As have been free radical, ROS are involved in harming cellular components, and they play an important function in venom induced toxicity, as reported among envenomed mice [7]. Ascorbic acid is an antioxidant that has been reported to have useful effects on a number of cancer types [8,9] and could be concerned in alleviation of Reactive oxygen species cellular damage, produced during exposure to toxins, metabolism and carcinogens [10]. In addition to augmentation of protease inhibitor effects concerned in preventing organ efficient injure [11,12]. *Citrus reticulata* (Rutaceae) is commonly known as narangi or santra (orange). It is a small spiny tree with thick top of slim branches, extensively grown in Egypt, Tunisia and Libya [13]. Mandarin is a collection name for this class of orange with thin, loose peel. The name 'tangerine might be applied as an interchange name to the entire group, but in trade, it is usually limited to the types with red-orange skin. The fruit has aphrodisiac, laxative, tonic and astringent properties [14,15]. It is also used to alleviate vomiting [16,17]. The fruit peel controls the skin moisture, rough and softens hard skin and possess a cleaning effect on oily skin [18]. Chemical composition of the volatile oil of the fruit peels of this species has been reported [19-23]. The effects of the volatile oil of *C. reticulata* has been studied against *Saccharomyces cerevisiae* [24], pathogenic fungi, *Paenibacillus larvae*, *Schistosoma mansoni*, *Aspergillus flavus*, and other microorganisms [25-30]. Very recently, the volatile oil of *C. reticulata* also demonstrates anticancer activity [31-33]. The main aim of the current study is to investigate the effects of *Citrus*

reticulata (Rutaceae) fruit peels extracts on the toxicity of *Cerastes cerastes* venom in albino mice.

Materials and Methods

Collection of plant material and preparation of aqueous extract

The oranges were bought from a shop in Tripoli (February 2019), and the *Citrus reticulata* was identified and authenticated by a botanist. Orange rinds were peeled off carefully with the help of a sharp razor blade, and each rind sample was cut into smaller pieces and 30g mass of the sample was taken. The sample was initially rinsed with distilled water, and the fresh peels (30g) were added to 30ml hot distilled water. In addition, another 30g of the fresh peels were macerated in cold 99% methanol for three hours at room temperature (28-30 °C), the mixture was then filtered under vacuum and the filtrate was stored at 4 °C and used when appropriate [34].

Experimental models

Albino mice (Swiss type) of either sex weighing approximately 18–28g (2 to 2.6 month old) were utilized for investigational purpose. They were kept in cages made from polypropylene in air-conditioned room with the temperature retained at 25±2 °C, and twelve hours sporadicing dark and light cycles. The mice were supplied with drinking water ad libitum and an adequate diet during the study. The authorization for the experimental procedures was obtained from the Animal Ethics Committee.

Venoms

Cerastes cerastes venom was extracted by means of physical stimulation and was gained in liquid forms, from the Faculty of Science, Zoology Department, University of Tripoli (Libya) and kept at –20 °C until utilize. A 7.5µl aliquot from the venoms was added to eight hundreds microliter of normal saline. A dosage of hundred microliter (100 nanogram) was administered to the male Swiss Albino mice.

Acute toxicity study

Acute toxicity was commonly performed to determine the LD_{50} value in experimental animals. The intend of doing acute toxicity study is to establish the therapeutic index of a methanolic and aqueous extracts of *Citrus reticulate* and to guarantee the *in-vivo* safety. The acute toxicity experiment was done in mice, in which all animals were overnight fasted prior to treatment and given food one hour after aqueous and methanolic extracts administration, with the period observation of common behavior at 0.5, 1, 8, 12 and 24 hours. The number of animals that died after taken the extracts was monitored daily for 7 days [35,36].

Intoxication of venom by *Citrus reticulata* extracts

The animals (albino mice) used in this study were divided to ten groups, each of them is of six mice (male or female). Five groups were used to investigate the aqueous extracts, while the other were used for methanolic extract. The first group received only hundred microliter (hundred microgram of total protein) of the *Cerastes cerastes* venom (LD99 5µg/kg). Groups 2 to 4 were used as treatment groups and given an equivalent amount of the *Cerastes cerastes* venom with 50µl, 100µl and 200µl of aqueous *Citrus reticulate* extracts intraperitoneally (30g/30ml), respectively. Group 5 was given 100µl of the *Cerastes cerastes* venom and polyvalent anti-snake venom (ASV) was bought from India from Haffkine Bio-Pharmaceuticals Company. The number of death was recorded within twenty-four hours. Similar experiments were repeated in the same manner with the methanolic extract using groups 6 to 10.

Statistical analysis

The difference among various control group and treated groups were analyzed using ANOVA method of one-way. The obtained results were dealt with using unpaired Student's test. All results were articulates as the mean±SEM of the number of experiments performed, with P value less than 0.05 showing significant difference among groups.

Results and discussion

Acute toxicity study

With the growing amount of research about naringin as a component of the orange and its potential utilize within the pharmacological and food industries, illuminating its toxicological outline becomes increasingly significant. In the present study, the *Citrus reticulata* extracts were found to be safe up to 200mg/kg orally. This present study is compared with other previous studies in which an oral single dose of 16g/kg of naringin did not produce acute oral toxicity in rats [37].

Acute toxicity of *Cerastes cerastes* venom and its reaction with aqueous and methanolic Citrus reticulata extracts and antivenom

The *Cerastes cerastes* venom at the dose five micrograms per kilogram (LD_{99}) produces 100% mortality in mice. The aqueous and methanolic *Citrus reticulata* extracts significantly decrease the mean survival times by 3, 5 and 6 times for 50, 100 and 200µl (30g /30mL), respectively when compared with the venom alone which was 3.1±0.3 hours. ASV was established to be efficient and showing mean survival of 2-days for 5-mice and absolute survival of one mouse. The *Cerastes cerastes* toxins contain of cardiotoxin, neurotoxin, proteins and enzymes. The victim may die from respiratory troubles which is the main cause of death. Assisted ventilation and ASV can save life in a lot of cases [38-40].

It has been reported that the citrus species contain glycosides and flavonones in huge amounts, and they play a main function in treating a range of pathological conditions. Hesperidin and naringein, are the major components of the citrus fruits. Intestinal microorganism are able to convert naringin into naringenin (an aglycone part). They established to have metal chelating effect, antioxidant, antidiabetic, antiviral, antiallergic, antiestrogenic, antimicrobial, ischemic heart disease adipolytic activity, anti-inflammatory, antiobesity, hypoxia, anti-cancer and hepatoprotective activity. Because of all these pharmacological action, both naringenin and naringin are assumed to be useful as a food supplement [41-47]. The accelerated death could be related to the interactions of *Citrus reticulata* components

(which were mainly polyphenolic components) with snake venom which is not consistent with the previous studies reporting that secondary metabolites polyphenol are competent to inhibit PLA_2 [48]. In the literature, it has been reported that naringin which is a flavonoid that is contained in grapefruit and recognized for its various biochemical activities and pharmacological effects on a secretory phospholipase A ($sPLA_2$) of *Crotalus durissus cascavella*, is concerned in the releasing of arachidonic acid in phospholipid membranes [48]. $sPLA_2$ was incubated with naringin in a ratio of 1:1 mole at 37 °C and a distinct decrease in the ultraviolet absorption signal and a changes of the circular dichroism spectra suggesting a significant effect of PLA_2 structure and function [48]. The obtained results are for the whole extract of *Citrus reticulate* and not for naringin or naringenin and this could be explained for the lack of association between pharmacological and enzymatic activities in which the chemical modification of some amino acids induced by naringin, in particular aromatic amino acids and histidines, affected the toxin's ability to interact with the pharmacological receptor, but did not lead to eliminate of this function. Our results and those described by Cardoso *et al.* expressed that enzymatic activity of sPLA2 is not crucial for pharmacological activities of this sPLA2 which was isolated from *C. d. cascavella* venom [49].

Conclusion

The present study confirmed that the aqueous extract of peeled *Citrus reticulate* accelerate the onset of toxicity of *Cerastes cerastes* venomis in a dose-dependent effect.

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