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Extracts of *Citrus Reticulata (Rutaceae)* Fruit Peels Accelerate the onset of Toxicity of *Cerastes Cerastes* venom in Albino Mice

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Abstract

To investigate the effect of aqueous and methanolic extracts of *Citrus reticulata (Rutaceae)* Fruit Peels on *Cerastes cerastes* venom. 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 mortal impact on victims, and in Libya and other North African countries [1,2]. Immediate antivenom treatment is crucial and vital to avoid morbidity and mortality [3]. The oxidative stress status, which result from snake bite envenomation is another measurement of kidney impairment and acute renal failure, connected with the antioxidant defense system that might be subject for treatment by antioxidant therapy [4,5]. Reactive oxygen species (ROS) are involved in the inflammatory reactions, thus affecting the cellular physiology and play an important role in the pathological conditions [6]. These 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 types of cancer and could be concerned in alleviation of ROS cellular damage, produced during metabolism and exposure to toxins and carcinogens, in addition to augmentation of protease inhibitor effects concerned in preventing organ functional damage [8-12]. *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 properties and astringent [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*, pathogenic fungi, *Paenibacillus larvae*, *Schistosoma mansoni*, *Aspergillus flavus*, and other microorganisms [24-30]. The volatile oil of *C. reticulata* also demonstrates the anticancer activity [31-33]. In this present study, our aim was 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). The *Citrus reticulata* was identified and authenticated by a botanist. Orange rinds were peeled off carefully with the help of a sharp razor blade. Each rind sample was cut into smaller pieces and 30g mass of the sample was taken. The sample was initially rinsed with distilled water. The fresh peels (30 g) were added to 30 ml hot distilled water. In addition, another 30 g of the fresh peels were macerated in cold 99% methanol for three hours. After 3 hours of maceration at room temperature (28°C), the mixture was then filtered under vacuum and the filtrate was stored at 4°C and used to treat animals as needed [34].

Experimental Models

Swiss albino mice of either sex weighing about 18–28 g (2–2.6-month-old) used for experimental purpose. They were housed in polypropylene cages in the air-conditioned room with the temperature maintained at 25 ± 2°C, and 12 h alternating light and dark cycles. The mice were provided with a nutritionally adequate diet and drinking water *ad libitum* throughout the study. Approval by the Animal Ethics Committee for the experimental procedures obtained.

Venoms

Snake (*Cerastes cerastes*) venom was extracted by manual stimulation and was 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 800µl of normal saline. A dose of 100µl (100ng) was given to the male Swiss Albino mice.

Acute Toxicity Study

Acute toxicity was generally carried out for the determination of LD_{50} value in experimental animals. The aim of performing acute toxicity study is for establishing therapeutic index of a methanolic and aqueous extracts of *Citrus reticu late* and to ensure safety *in-vivo*. Acute toxicity test was performed in mice. All animals were fasted overnight before treatment and were given food one hour after aqueous and methanolic extracts. General behavior was also observed at 0.5, 1, 8, 12 and 24 h after administration. The number of animals that died after administration was traced daily for 7 days [35,36].

Intoxication of Venom by *Citrus Reticulata* Extracts

Five groups of mice were used in this study. The first group of six mice received only 100 µl (100 ng of total protein) of the *Cerastes cerastes* venom (LD99 5µg/kg). Groups 2-4 of six mice each (serving as treatment groups) were given an equivalent amount of the *Cerastes cerastes* venom with 50 µl, 100 µl and 200 µl of aqueous *Citrus reticulate* extracts intraperitoneally (30 g/30 ml), respectively. Group 5 of six mice received 100 µl of the *Cerastes cerastes* venom and ASV. The number of mortality was recorded within 24h. Similar experiments were repeated in the same manner with the methanolic extract.

Statistical Analysis

The difference among various treated groups and control group were analyzed using one-way-ANOVA followed using unpaired Student's 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

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 200 mg/kg orally. This present study is compared with other previous studies in which an oral single dose of 16 g/kg of naringin did not produce acute oral toxicity in rats [37].

Acute Toxicity of *Cerastes Cerastes* Venom and its Reaction with Aqueous (Methanolic) *Citrus Reticulata* Extracts and Antivenom

The *Cerastes cerastes* venom at the dose 5 µg/kg (LD_{99}) produces 100% mortility in mice. The aqueous (methanolic) *Citrus reticulata* extracts significantly decrease the mean survival times by 3, 5 and 6 times for 50, 100 and 200 µl (30g /30 mL), respectively when compared with the venom alone which was 3.1 ±0.3hours. ASV [polyvalent anti-snake venom by Haffkine Bio-Pharmaceuticals Company (India)] was found to be effective and showing mean survival of two days for five mice and complete survival of one mouse. The toxins of *Cerastes cerastes* venom are composed ofneurotoxin, cardiotoxin, enzymes and proteins. The victim might diefrom respiratory paralysis which is the major cause of death. ASVand assisted ventilation can save life in many cases [38-40].

It has been reported that the Citrus species contain flavonones and glycosides in large amounts, and they play a major role in maintaining a range of pathological conditions. Hesperidin and naringein, are the major constituents of the citrus fruits. Naringin gets converted into naringenin which is an aglycone part by the intestinal microorganism. 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 activity hepatoprotective activity. Because of all these pharmacological action, both naringin and naringenin are assumed to be importance 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 polyphenolic secondary metabolites are able to inhibit PLA_2 [48]. In the literature, it has been reported that naringin which is a flavonoid that is found in grapefruit and known for its various pharmacological effects and biochemical activities of a secretory phospholipase A ($sPLA_2$) from *Crotalus durissus cascavella*, an imperative protein involved 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 UV absorption signal and a changes of the circular dichroism spectra suggesting a significant effect of PLA_2structure 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. [49] expressed that enzymatic activity of $sPLA_2$ is not crucial for pharmacological activities of this $sPLA_2$ which was isolated from *C. d. cascavella* venom [49].

Conclusion

The present study demonstrated that the aqueous extract of peeled *Citrus reticulate* possess dose-dependent toxic activity. Further, there is need to isolate, characterize, and screen the active principles that are responsible for its toxic activity. Furthermore, there is need to find out the exact mechanism by which the *Citrus reticulata* extract exerts above effects. Further studies are needed to separate and confirm the active components and its effect as a toxic agent with the venom.

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