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Sub-acute toxicity of the alien *Cassiopea andromeda* (forsskal, 1775) jellyfish venom, in rats

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ABSTRACT

The sub-acute toxicity of the crude tentacle-only extract from the alien Cassiopea andromeda (forsskal, 1775) jellyfish was studied in rat within 21 days after exposure. For sub-acute toxicity, changes in weights and pathological parameters were studied. The intraperitoneal LD_{50} value of venom was estimated 104.0 µg/kg BW in male mice. For sub-acute toxicity, 3 doses of crude venom (0.0125, 0.025 and 0.05 µg/ml/day) were administered intraperitoneally to female rats, once daily for 21 days. Increase in size and weights were observed in the spleens in all groups. The Scab-like spots were seen on skin of all the rats. Histopathological examinations of the rat kidney, heart, liver and spleen tissues indicated that there were different demonstrable abnormalities and alterations in the microscopic examinations in comparison to their control groups, especially at a higher dose of 0.05 µg/ml/day.

Key words: Sub-acute toxicity, *Cassiopea andromeda* jellyfish, Histopathological examinations

INTRODUCTION

Cnidarians are the primary phylum of typically toxic animals. They are characterized by the presence of nematocysts (Cnidocysts), the secretions of specialized cells that exist mainly in their tentacles [1]. Cnidocysts, contain a complex mixture of extremely active and structurally diverse toxins. The cnidocyst capsules are discharged in response to adequate chemical and mechanical stimuli elicited by prey organisms [2].

Previous investigations have shown that cnidarian venoms have vasoactive compounds such as 5-HT, catecholamines, histamine, and histamine liberators; neuroactive mixtures such as quaternary ammonium compounds, certain amino acids and small peptides; and proteins including enzymes, such as proteases, phospholipases, and cholinesterases [3-5]. These enzymes play an important role in numerous pathophysiological effects, such as hemolytic, mytotoxic and neurotoxic injuries in their envenomed victims [6].

Jellyfish belong to phylum Cnidaria. In recent years, it has been observed a significant increase in Jellyfish blooms in marine ecosystems throughout the worldwide [7]. Correspondingly, a significant increase of the jellyfish

envenomation to both swimmers and fishermen and the resulting public health hazards is occurring in the affected areas. Envenoming by Jellyfish can produce an immediate burning feeling, severe pain, swelling, red streak, nausea, abdominal pains [8-10]. In severe envenomations, it may perhaps the fever, respiratory distress, nausea, emesis, abdominal colic, diarrhea [11], immediate cardiac and respiratory arrests, delayed renal failure [12], and nerve tissue disorders [13], in their victims. Their venoms have a wide spectrum of biological activities [1, 14].

The so called 'upside-down jellyfish', *Cassiopea andromeda*, appears to be venomous [15, 16]. Limited research has been reported on *Cassiopea* venoms [17]. The components of jellyfish venoms and their mode of actions are still far from our understanding.

Previous studies revealed that the *Cassiopea* venom has hemolytic and proteolytic activities [18].

Recently, a population of *C. andromeda* has increased intensely in Nayband Bay (Bushehr/ Iran). This protected biodiversity area is visited by a lot of tourists and bathers, and consequently, increase in number of envenomations [16].

The purpose of current study is evaluation of Sub-acute toxicity of the *C. andromeda* crude venom on rat kidney, heart, spleen and liver tissues.

Statistical analysis

Data were expressed as mean ± SD. Statistical analysis was carried out using t-test and f-test methods. Level of statistical significance was considered at values of ($p < 0.05$).

MATERIALS AND METHODS

Chemicals

All chemicals and solvents used for extraction and analysis of samples purchased from Sigma (MO, USA) and Merck (Germany) Chemical Companies, USA.

Sample collection

All specimens of *C. andromeda* were collected from the Nayband bay, in the North (27º 30´ S, 52º 35´ E) of bushehr-Iran (Fig.1).

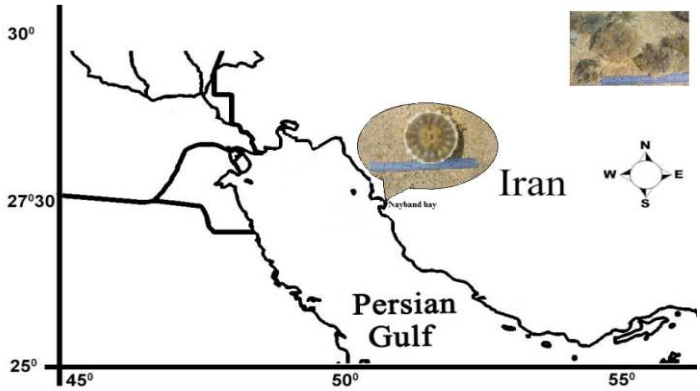

Fig. 1. Map of the study area (A population of the upside-down jellyfish *Cassiopea andromeda* in Nayband Bay, Bushehr-Iran)

Specimens were guessed right by marine biologists M. Moradi and Professor I. Nabipour from our institute. Then, the identities of the species were verified by Professor B. Holland from the University of Hawaii [16].

Extraction of the crude venom

Separation of tentacles was performed according to *Bloom* et al., (1998) method [19]. Briefly, the tentacles were excised manually from specimens, immediately after capture by trawl, and directly placed into small glass containers filled with third part of seawater and then, transported in the ice bags to the Persian Gulf Marine Biotechnology Research Center laboratory from the Persian Gulf Biomedical Research Center, Bushehr University

of Medical Sciences, Iran. subsequently, after homogenization (IKA homogenizer, Germany), kept in 4°C for 2 days for the autolysis of the tissues and release of toxins [20], and then centrifuged (Eppendorf, Germany) at 12,000×g for 15 min in 4°C to remove the sediments. The resultant supernatant was freeze-dried (Christ, UK) and kept at −80°C until analysis [21].

The median lethal dose (LD_{50})

The LD_{50} of the crude venom was measured by injecting appropriate dilutions of the sample intravenously into the caudal vein of 18.5- 22 grams male albino mice (n: 4-fold serial dilutions with sterile saline and 4 mice per dilution). The mortality rate was measured within 24 hours, according to Wiltshire et al. (2000) method [22], and the result was expressed as µg/kg of animal body weight.

Sub-acute toxicity

Twenty four female Wistar rats (weighing approximately 160–190 g), from the animal house of Bushehr University of Medical Sciences, bushehr-Iran, were randomly divided into four groups (n= 6). The LD_{50} (IV mouse) was estimated at 104.0 µg/kg BW in a 24 hours observation period. According to LD_{50} value, three groups of animals were respectively received 0.5 ml venom at the doses of (0.05, 0.025 or 0.0125 µg/ml/day, ip), for 21 days. Also, one group of animals was given saline as a control group. Also, the body weights were measured weekly. Animals were observed for general behavioral and signs of abnormalities during the study. The study was permitted by the Medical Ethics Committee of Bushehr University of Medical Sciences and Health Services, Bushehr-Iran; and written informed consent was obtained from all subjects of study. All animal work was carried out in accordance with the National Ethical Guidelines for Animal Research in Iran (2005) under a Project License which was approved by the Animal Care and Use Committee of Bushehr University of Medical Sciences- Iran, according to Protocol: D/P/3758. All animals were kept in a climate-controlled environment at 25°C on a 12h light/ 12h dark cycle. Adequate food and water were obtainable during all experimental process and all efforts were made to minimize suffering.

Biopsy and histological study

Samples were taken at end of treatment, after anesthetizing the rats by Ketamine (50 mg/Kg I.M). It was performed by sterilized surgical set from declared area of tissues (Kidney, Heart, Liver and spleen). The specimens were fixed with 10% formalin and referred to the histopathology lab. For the light microscopic study, samples were intake and molded by alcohol (ethanol) and Paraffin, respectively. There were sectioned three microns thickness by a rotary microtome and stained normally (H & E). The microscopic slide photos were also taken by microscope equipped with a Moticam camera model A352 (Netherland) in a high resolution (resolution*100). Furthermore, the measurable parameters such as the number of fibroblastic cells, blood vessels, wound zones; necrotic tissues and diameter of epidermis were evaluated using photomicrographs with software image tool (version 8). The obtained results were involved the observed changes on histopathological changes such as congestion and tissue damages in different groups.

RESULTS AND DISCUSSION

Morphological studies

Figure (2), shows the light micrographs sections of classical liver lobules in control and test groups. As seen, in the high dose of test groups, hepatocytes were lost their regular arrays. There were seen many abnormal spaces with congestion between liver cells more than other groups. Meanwhile, liver cells highly lost their cellular arrays. Cellular nucleus was extremely heterochromatic and pyknotic; and the total tissue arrangement were lost in the high dose of test groups (Fig. 2).

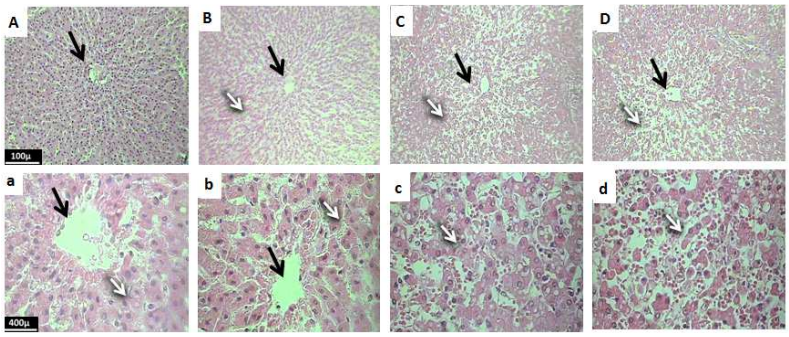

Fig. 2. Photomicrograph of hepatic tissues cross section in different groups treated with *Cassiopea andromeda* (forsskal, 1775) jellyfish venom. (figures.1 (A and a): No treated as control group); (figures. 1(B and b): 0.0125 µg/ml/day, ip); (figures. 1(C and c): 0.025 µg/ml/day, ip); (figures. 1(D and d): 0.05 µg/ml/day, ip). Central vein (white arrow), Hepatocyte plates (black arrow). (Top micrographs with low magnificatin ×100 and down micrographs with high magnification×400. Normal staining (H & E))

Also, there were seen a lot of pathological changes such as congestion, irregularity, nuclear density, dispersion tissue in lineal (Fig. 3) and renal tissues (Fig. 4) in high dose of crude venom.

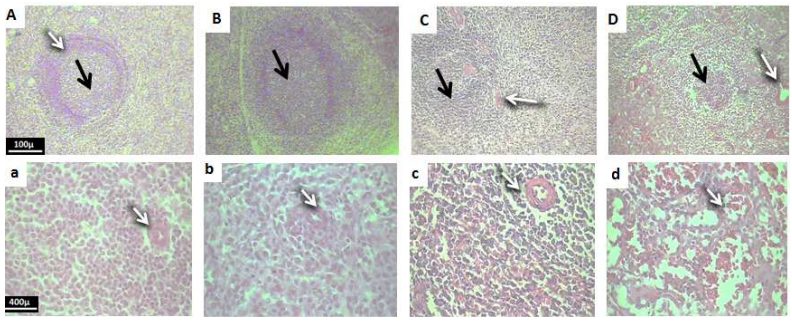

Fig. 3: Photomicrograph of Lineal tissues cross section in different groups. Treated with with *Cassiopea andromeda* (forsskal, 1775) jellyfish venom. (figures.1 (A and a): No treated as control group); (figures. 1(B and b): 0.0125 µg/ml/day, ip); (figures. 1(C and c): 0.025 µg/ml/day, ip); (figures. 1(D and d): 0.05 µg/ml/day, ip). Central arteriol (white arrow), white pulp (black arrow), (Top micrographs with low magnificatin ×100 and down micrographs with high magnification×400. Normal staining (H & E))

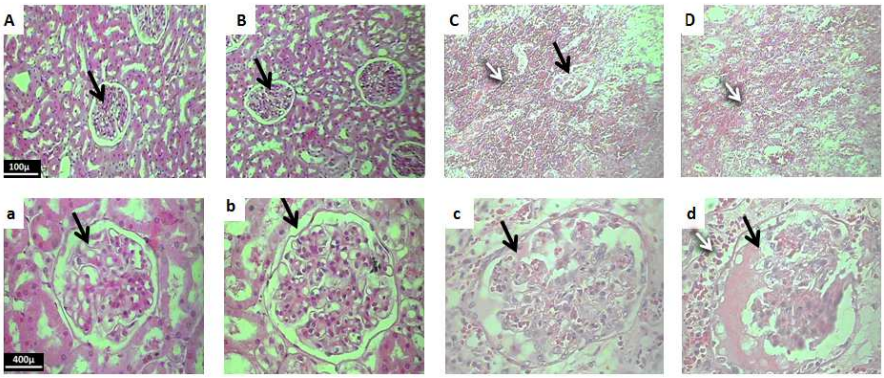

Fig. 4 Photomicrograph of renal tissues cross section in different groups treated with *Cassiopea andromeda* (forsskal, 1775) jellyfish venom. (figures.1 (A and a): No treated as control group); (figures. 1(B and b): 0.0125 µg/ml/day, ip); (figures. 1(C and c): 0.025 µg/ml/day, ip); (figures. 1(D and d): 0.05 µg/ml/day, ip). Renal corpuscle (black arrow), Congestion (white arrow). (Top micrographs with low magnificatin ×100 and down micrographs with high magnification×400. Normal staining (H & E))

As seen in figure (5), no significant changes in cardiac tissues exposed to different doses were observed, except a congestion manner in high dose of crude venom (Fig. 5).

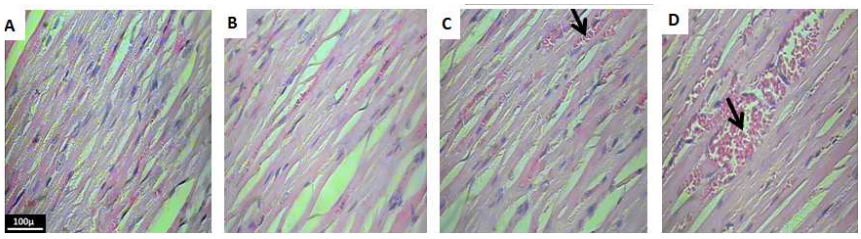

Fig. 5: Photomicrograph of cardiac tissues cross section in different groups. treated with *Cassiopea andromeda* (forsskal, 1775) jellyfish venom. (figures.1 (A and a): No treated as control group); (figures. 1(B and b): 0.0125 µg/ml/day, ip); (figures. 1(C and c): 0.025 µg/ml/day, ip); (figures. 1(D and d): 0.05 µg/ml/day, ip). Congestion (black arrow. Magnification ×100 and staining (H & E)

Morphometical study

For the assessment of sub-acute toxicity of the venom on renal tubule, the morphometric of light micrographs such as means of small and large diameters of renal corpuscles, distal and proximal convoluted tubules, collecting duct, thick and thin segments of henle loop, and changes in the thickness of urinary filtration barrier were analyzed using Image Tool (version 3) software. Also, the diameter of kidney tubules were measured (Table 1).

Table1. Morphometric effect of *cassiopea andromeda* crude venom on renal tubule and corpuscle in rat

Renal tubules and Corpuscle	Diameter (µm)			
	Dose (µg/ml/day)			
	Control group (slain normal)	D_1(0.0125)	D_2 (0. 025)	D_3 (0.05)
Renal corpuscle	158.21 ± 13.34	161.24 ± 10.34	163. 22 ± 6.18	198.21 ± 18.34*
Proximal convoluted tubule	70.21 ± 8.35	72.45 ± 9.35	69.73 ± 3.12	99.71 ± 12.35*
Distal convoluted tubule	71.56 ± 0.78	74.51 ± 11.78	82.45 ± 13.17	98.12 ± 9.12*
Collecting duct	135.02 ± 14.28	141.02 ± 7.52	146.02 ± 1.50	165.02 ± 0.53*
Loop of Henle, thick segment	65.02 ± 15.30	66.14 ± 0.29	67.27 ± 1.23	70.18 ± 8.11
Loop of Henle, thin segment	25.16 ± 1.28	27.13 ± 5.16	28.25 ± 1.85	30.16 ± 11.09

Data were analyzed with t-test and f-test methods and were expressed as mean ±SD.
**Significant difference with the control group; $p < 0.05$; n = 10.*

As shown in table (1), the mean diameter of renal corpuscles in the test groups (D_1, D_2, and D_3) were dose-dependently, increased in all groups, but it was significant only, in the D_3 group compared to the control group ($p < 0.05$).

Although, the differences in mean diameter of proximal convoluted tubules between all groups (D_1, D_2, D_3), only D_3 group (higher dose), had a significant increase compared to the control group ($p < 0.05$).

About the mean diameter of distal convoluted tubules in the test groups (D_1, D_2, and D_3), it can be said that, although dose-dependent increase in the test groups, but it had considerably increased only in D_3, compared to the control group ($p < 0.05$).

The results of study have also shown dose-dependent increase in mean diameters of collecting duct, henle loops and thick and thin segments in their control and test groups (D_1, D_2, and D_3). Correspondingly, they had considerably increased only in D_3 groups, compared to their control groups ($p < 0.05$).

In a comparable study, Crude *C. andromeda* and *C. xamachana* venoms activated liver cells exceeding doses of 50 and 10 µg protein/ml, respectively [23]. Also, crude *Chironex fleckeri* and *Chrysaora quinquecirrha* venoms had been active at 3±5 fold lower protein concentrations in similar assays [24].

A morphological study by Liang et al., (2012) [25], on isolated rat hearts after 30 minutes of perfusion with 180 mg venom from the jellyfish *Cyanea capillata* was demonstrated that venom induced the wavy fibers, irregular myocyte diameters, and interstitial edema on rat heart tissues.

Changes in organ weights

According to our observations, the size and mean of spleen masses in samples were higher than their control groups. The mean± SD of spleen weights in test groups (D_1, D_2, and D_3) were 0.63±0.1, 0.8±0.28, and 1.12±0.38 grams,

compared to the control groups (0.57±0.21 gr), respectively. There were no significant differences in other organ weights compared to their control groups.

Some general behavioral and aspects of venom

Feel severe pain after injection, and subsequently, restlessness and confusion were seen in almost all animals. Minutes after the injection, an aggressive feel, or a sense of libido with violence against its related animal, and with the passing of time, apathy and lethargy were also perceived. Dermonecrosis was noticeable in site of injection. Outwardly, on the skin of all the rats, scab-like spots were seen all over their bodies, especially on their backs (Fig. 6).

Fig 6. Scab-like spots on the skin of rats after 21 days exposure by *Cassiopea andromeda* jellyfish venom

Pain production, bluing, eschar and dermonecrosis in site of injection by venom, is similar to results of Radwan et al., (2001) [23].

CONCLUSION

Histopathological examinations of the rat kidney, heart, liver and spleen tissues indicated that there were different demonstrable abnormalities and alterations in the microscopic examinations in comparison to their control groups, especially at a higher dose of 0.05 µg/ml/day. Increase in size and weight were observed in the spleens in all groups. The Scab-like spots were seen on skin of all the rats.

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Conflict of interest disclosure

The contents of the manuscript have not been published previously, or submitted elsewhere for consideration, nor are they in press. All of the authors have seen and approved the manuscript. There is no conflict of interest.

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