A new method for venom extraction from venomous fish, Green Scat

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Abstract

Scatophagus argus argus (Green Scat) is a pretty aquarium fish. Its hard spines are venomous and can cause painful injury. In this study 60 specimens of Green Scat were collected periodically from coastal waters of Boushehr (south of Iran) from May 2011 to April 2012. Anatomical features of venomous spines were investigated. Scat venom was extracted from the spines in a new manner for keeping the specimens alive. The nature of venom was tested by SDS-PAGE. Ethical issues and animal welfare principles such as rapid and instantaneous anesthetizing, post operation disinfection and fast recovery of the specimens was practiced in order to minimize the complications. This method enhanced the purity and quantity of venom as demonstrated by 12 separated proteins in electrophoresis. New ethical issues were developed to surviving the specimens and prolong viability as well.

Keywords: *Scatophagus argus argus*, Venomous spine, Venom extraction, Viability, Ethical issues, Boushehr

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Introduction

S. argus argus is a pretty venomous fish belonging to the family Scatophagidae. Some of its synonyms are *Cacodoxus argus, Chaetodon argus, C.atromaculatus, Ephippus argus, S. argus atromaculatus.* It can inflict painful wounds with partial paralysis during handling (Herre, 1935; Cameron and Endean, 1977). So many people such as fishermen and those who keep this fish in aquarium are at risk. Treatment of the wound is often done by soaking the injured area in hot water. Dorsal fin and some of the ventral fin spines contain cells that produce venom (Endean, 1961) with hemolytic and some other toxic properties (Sivan *et al*., 2007, 2010). In fact *S. argus* as a venomous fish had been less considered in contrast to other species (Ghafari, 2013).

Many venum extraction methods have been developed based on the anatomy of spines and consistency of venom content in different fishes (Endean, 1961; Saunders and Tokes, 1961; Carlisle, 1962). In this investigation we excogitated a novel extraction method in order to enhance the quantity and quality of the venom as well as ethical issues.

Material and methods

Sampling

Sixty specimens of *S. argus argus* (Fig. 1) were collected periodically (both sexes) by trap from the Boushehr coastal waters of Persian Gulf (Fig. 2) from May 2011 to April 2012. Total lengths of the specimens were measured and recored. The live specimens were packed in plasitic bags containing seawater and pressured oxygen, and transferred to the Pasteur institute of Iran (Tehran) by plane. At the venom and toxin laboratory of Pasteur Institute, the fishes were acclimated and kept in aquariums with 25°C seawater for further studies.

Venom preparation (new method)

The specimens were anesthetized in a sterile solution of 0.125 g/L of clove (*Caryophillus aromaticus*) powder for 3-4 minutes. After confirmation of general anesthesia, the dorsal fin spines (11 spines) and anal fin spines (3 spines) were cut just at the base.

The specimens recovered completely in 10 minutes. The surface layer and derma of spines were trimmed and cleaned completely. Then the spines were cut into small pieces using a hand–held mortar, made of cast iron with liquid nitrogen. The fragments were powdered in porcelain with liquid nitrogen. Then the pieces were homogenized at 5000 rpm in 0.9% NaCl for 5-10 minutes on ice and then centrifuged at 4000 rpm for 15 minutes at 4°C. The pellet was discarded and the supernatant liquid was filtered against 5KDa filter (Millipore) at 4°C and then lyophilized with a freeze dryer (Christ, Alpha 1-2 LD Plus – Germany).

Vertical section of spine

A small fish was selected to study the vertical section of the spines.

A dorsal spine (Fig. 4) was cut vertically and the tissue details was observed in an optical Loop (Wild Heerbrugg-Switzerland).

SDS-PAGE

Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to standard method (Laemmli, 1970). The venom samples were loaded on to a 12% polyacrylamide gel.

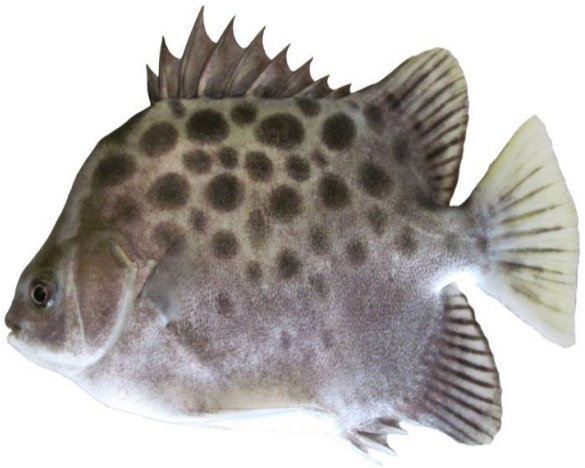

Figure 1*: Scatophagus argus argus* isolated from coastal waters of Boushehr before cutting spines.

Figure 2: Study areas in Boushehr city, Boushehr province, south of Iran (29° N,51° E).

Results

Follow up the viability and compatibility

The average temperature and pH of the fish holding pool during one month of the survey were maintained at 31°C and 7.45, respectively. Microscopic analysis revealed phytoplanktons (green algae from Desmids and chlamydomonas) (Fig.3).

Figure 3: Some phytoplanktons in the pool water.

Figure 4: Cleaned spines, before cutting to small pieces.

Vertical section of spine

Total length of the examined fish was 5cm. The length and diameter of spine were 11mm and 4mm, respectively. Two collecting ducts were observed alongside the spine. Blood vessels within bony central axis were observed too (Fig. 5). A schematic presentation of the vertical section of spine is demonstrated in Fig. 6.

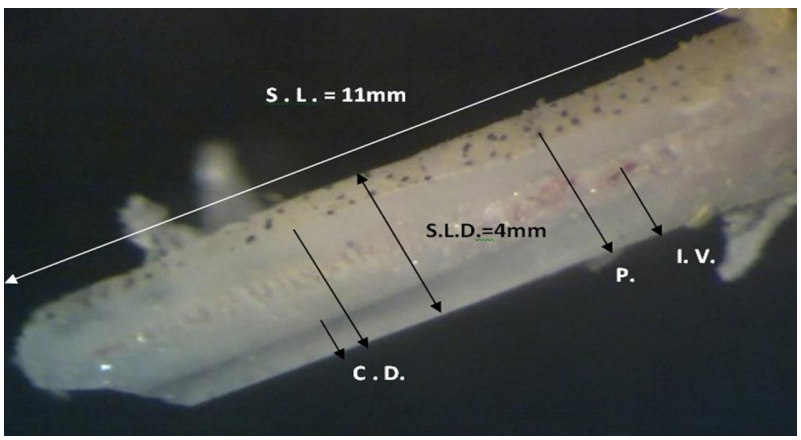

Figure 5: Vertical section of green scat dorsal spine: Total length of fish was 5cm, S.L. (spine length=11mm), L.S.D. (spine large diameter= 4mm), C.D. (two collecting ducts that collect the venom produced by venomous cells, I.V. (Internal bony blood vessel),p. (pigments), (zoom lens=50).

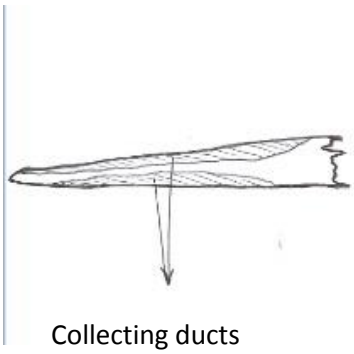

Figure 6: A schematic figure of the spine´s vertical section.

SDS-PAGE

In this new method the concentration was 10 µg/µl (Bradford, 1976) and SDS-PAGE revealed 12 separated proteins (Fig.7).

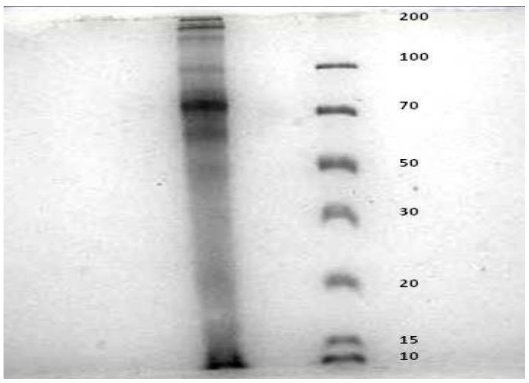

Figure 7: Electrophoretic profile of *Scatophagus argus argus* venom. The venom was analyzed by SDS PAGE using 12% polyacrylamide gel and stained with Comassie brilliant blue. Numbers at right corresponds to molecular markers.

Discussion

Discoveries of toxins and venoms, especially from marine resources, are racing ahead because of their extremely complex and unique action on various mammalian physiological systems. Venom secretion is part of an organism's defense and predatory mechanisms, whose specificity have been extended over a million years of evolution. Scat as a venomous fish which had been less considered so far.

Despite a contemplation regarding to simple venom extraction from the spines of a venomous fish, scat is an exception and hard challenge. Basically, there are three different methods for extracting venom from venomous fish.

In this study because of anatomical feature of the spines, we could not exploit current extraction methods. We could not use the rubber sheet method (Endean, 1961), and needle method (Saunders and Tokes, 1961) because the spines were very hard, inflexible and had an impenetrable structure. As there was no liquid venom in the spine, sponge method (Carlisle, 1962) was not used as well. A homemade device used for collection of a little amount of liquid was not applicable in this case, due to the lack of liquid venom in the internal lateral ducts.

In earlier years, Sivan *et al*. (2010) conducted some studies on scat with a different method of venum extraction. They cut the spines with derma and basal tissues 3-5 mm from their base. According to low quality SDS-PAGE results documented in Sivan *et al*'s study (2010), it is supposed that this method of

extraction led to excess protein fragments, lack of high molecular weight proteins, as well as dark background of unresolved polypeptides.

There are some chemical materials used for fish anesthetizing such as MS 222 (Donald and Andrew, 2009), Benzocaine, 2-phenoxyethanol and tricaine methanesulfonate (Donald and Andrew, 2009; Ghanawi *et al*., 2011). In earlier studies, clove oil was used in fish anesthetizing (Soto and Burhanuddin, 1995; Anderson *et al*., 1997; Munday and Wilson, 1997; Keene *et al*., 1998; Peake, 1998).

The natural material that we used in this study (clove powder) was appropriate because of its biosafety for these animals and lack of possible disadvantages. Clove powder as a natural source has several benefits including rapid desensitizing and disinfection of injured tissue with minimum complications as well as fast recovery of specimens.

Our fish specimens were anesthetized in about 3-4 minutes and recovered in about 10 minutes without any side effects. Cutting the spines just below the base avoid major lesion, and lead to fast regeneration of dermal tissue.

In spite of Sivan *et al*. (2010) method (decapitation before cutting the spines), the specimens remained alive in our study. Viability of specimens after removing the spines depend on many factors including the conditions of transportation, the cutting method, natural source of analgesic material, recovery conditions, and maintaining optimum environmental conditions in the holding pool.

In this study a novel method developed to improve extraction efficiency regarding quantity and quality of proteins.

The viability of specimens and quality of extraction are strongly corresponded together. The extraction of venom from the spines while the specimens are alive affects the venom quality. This issue is very obvious when the quality of SDS-PAGE result of this study is compared with Sivan *et al*. (2010) results.

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