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The cardiotoxicity of crude tentacle-only extract from the Persian Gulf jellyfish "*Cassiopea* sp." in isolated rat heart

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ABSTRACT

The upside-down jellyfish produces venom with some biological activities. In the present study, direct cardiotoxicity of crude tentacle-only extract from the Persian Gulf jellyfish "*Cassiopea* sp." was assessed by a Langendorff isolated perfused rat heart system. Treatments were performed with concentrations of 50, 20, 10, 5, and 2.5 μg/ml of crude tentacle-only extract (CTOE) on isolated rat hearts for 60 min. Then, the hemodynamic parameters of heart rate, left ventricular end-diastolic pressure, left ventricular systolic pressure, left ventricular developed pressure, and coronary flow were evaluated. Lactate dehydrogenase (LDH) levels as well as histopathological examinations were also investigated. Based on the ECG findings, treatments in a dose-dependent pattern changed cardiac electrical activity and decreased coronary effluent. The higher concentrations of CTOE produced severe bradycardia, atrioventricular dissociation, complete atrioventricular block, and ultimately cardiac arrest. Ventricular end-diastolic pressure was also significantly increased by high concentrations of CTOE. At high CTOE concentrations, scatter lymphocytic infiltration and wavy fibers were found in the histopathological examinations. Treatment with concentrations of 2.5–10 μg/ml caused a considerable increase in LDH levels within 30 min compared with baseline levels. In conclusion, CTOE from the Persian Gulf upside-down jellyfish had significant direct cardiotoxicity effects on isolated rat hearts.

Introduction

The jellyfish venom can produce a broad spectrum of biological activities such as neurotoxic, dermonecrotic and hemolytic effects (Tibballs, 2006; Taheri et al., 2013a). Envenomation can inflict a burning sensation, crucial pain, swelling, nausea, red streaks, abdominal pain, abundant sweating, respiratory problems, muscle cramps (Tibballs, 2006; Taheri et al., 2013b), and nerve tissue damage (Yanagihara and Shohet, 2012).

Acute heart failure is known as the most important cause of death by some jellyfish venoms (Ramasamy et al., 2005a,b; Liang et al., 2012). The cardiovascular toxicities are considered the major toxic effect of some jellyfish venoms (Winter et al., 2007; Beilei et al., 2012).

The well-known "upside-down jellyfish", *Cassiopea* sp., produces a rapid-acting venom. It is injurious to humans and has a fatal effect to the victims (Nabipour et al., 2015). The upside-down jellyfish, a member of the phylum Cnidaria, are marked by extensively branched mouth-arms, which feature numerous mouth openings. Their main characteristic is having special cells harboring nematocysts. Nematocysts have a complex mixture of venoms that are highly active and structurally diverse. The nematocysts capsules are discharged under the effect of adequate chemical and mechanical stimuli evoked by prey organisms (Kass-Simon and Scappaticci, 2002). Nematocyst venom of *Cassiopea* exhibited dermonecrotic, hemolytic, cytolytic and neurotoxic effects (Radwan and Burnett, 2001).

Recently, a numbers of the upside-down jellyfish "*Cassiopea* sp." have dramatically increased in the Persian Gulf, Bushehr, Iran (Nabipour et al., 2015). During spring season, this protected area is visited by tourists in large numbers, as well as beachgoers during

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the summer months. The abundance of the upside-down jellyfish in the Nayband Bay may be a danger for beachgoers and importantly impacts the tourism industry. The cardiovascular effects of the Persian Gulf jellyfish "*Cassiopea* sp." have not been investigated yet. The main aim of the current study was to examine the cardiotoxic effects of CTOE from the Persian Gulf jellyfish "*Cassiopea* sp." in isolated rat hearts by a Langendorff isolated perfused rat heart system. The technique of isolated heart perfusion is a sensitive and predictive model for the understanding of the fundamental physiology of the heart including its contractile function, coronary blood flow regulation, cardiac metabolism and to assess the potential for drugs to cause cardiotoxicity in humans (Bell et al., 2011; Henderson et al., 2013).

Material and methods

Ethical statement

This study was approved by the Medical Ethics Committee of Bushehr University of Medical Sciences and Health Services, Bushehr, Iran. All animal work was carried out in accordance with the National Ethical Guidelines for Animal Research in Iran (2005) under a Project License approved by the Animal Care and Use Committee of Bushehr University of Medical Sciences–Iran (Protocol #: D/P/3758). All animals were maintained in a climate-controlled environment on a 12 h light/12 h dark cycle. Food and water were provided ad libitum and all efforts were made to minimize suffering.

Preparation of CTOE from the jellyfish

All specimens were collected in 2014 from the Nayband coast, in the Persian Gulf, the southwest of Bushehr Province, Iran. Identity of the organism was verified by Professor Brenden Holland from the University of Hawaii (Nabipour et al., 2015). Nematocysts and tentacles were separated as described by Bloom et al. (1998). Briefly, the tentacles were excised manually from living specimens as soon as possible after capture and then placed into small glass containers of seawater with ice packs, and immediately transferred to the laboratory in Bushehr. Then, they were homogenized using a homogenizer (IKA, Germany). The resultant homogenate was kept at 4 °C for 2 days in order to carry out the autolysis of the tissues (Burnett et al., 1992), the tissues were then centrifuged (Eppendorf, Germany) at 12,000×*g* for 15 min at 4 °C to remove the sediments. The final supernatant was freeze-dried (Christ, UK) and stored at −80 °C until usage (Mustafa et al., 1995).

The protein concentration

The protein concentration was determined by the Bradford (1976) method, using a UV/visible spectrophotometer (Cecil, England). A standard curve was constructed using bovine serum albumin (BSA) as the standard protein.

Molecular weight determination by SDS-PAGE electrophoresis

A total of 10 µl of freeze dried venom was subjected to SDS-PAGE at 25 °C, according to the method described by Laemmli (1970). (***Resolving Gel* (pH *8*):** percentage of gel 12.5%; 3.1 ml Acrylamide: bisacrylamide (30: 0.8% w/v); 3 ml Tris-Cl pH: 8.8; 38 µl SDS 20%; 1.3 ml dH_2O; 5 µl APS 10%; 7.5 ml TEMED. ***Stacking Gel* (pH *6.8*):** percentage of stack 6%; 1 ml Acryl: bisacryl (30: 0.8% w/v); 630 µl Tris-Cl pH 8.8, 1 M; 25 µl SDS 20%; 3.6 ml dH_2O; 24 µl APS 10%; and 5 ml TEMED). Proteins were stained with Coomassie Brilliant Blue. The molecular weights were evaluated by comparison to Bio-Rad markers at the ranges of 225, 150, 102, 76.52, 38, 17, and 12 kDa (Fig. 1).

Anesthetized rat preparation

Ten female Sprague-Dawley rats (200 ± 20 g, provided by the Laboratory Animal Center of the Bushehr University of Medical Sciences, Iran) were anaesthetized with pentobarbital sodium (50 mg/kg; i.p.), which was supplemented as required. The rats were then heparinized with intravenous heparin (500 IU/kg).

Langendorff perfused heart planning

Formerly, Krebs–Henseleit solution was prepared from the following chemicals obtained from Merck (NaCl 118.5 mmol/l; $NaHCO_3$ 25.0 mmol/l; KCl 4.7 mmol/l; KH_2PO_4 1.2 mmol/l; $MgSO_4$ $7H_2O$ 1.2 mmol/l; Glucose H_2O 11.1 mmol/l; and $CaCl_2$ $2H_2O$ 1.8 mmol/l). The solution was then filtered for subsequent procedures (Bastos et al., 2006).

After a bilateral thoracotomy of each animal, a 4 ml ice-cold Krebs–Henseleit buffer was poured onto the heart; the heart was then rapidly excised, and immediately cannulated via the aorta. The heart was fixed into a Langendorff perfusion system and retrogradely perfused with gassed (95% O_2 and 5% CO_2) Krebs–Henseleit solution at 37 °C (Pourkhalili et al., 2009).

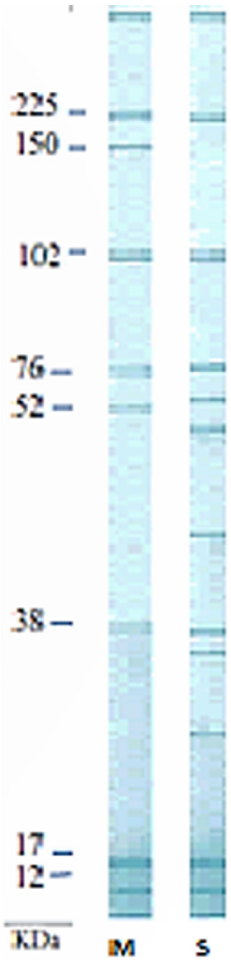

Fig. 1. SDS-PAGE (12% polyacrylamide gel stained with Coomassie brilliant blue) analysis of protein standard marker (M), the Persian Gulf jellyfish "*Cassiopea* sp." venom sample (S).