

Selective Toxicity of Persian Gulf Stonefish (*Pseudosynanceia Melanostigma*) Venom on Human Acute Lymphocytic Leukemia B Lymphocytes

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HIGHLIGHTS

- Cytotoxicity of Persian Gulf Stonefish venom on ALL B-lymphocytes.
- ROS acceleration in ALL B-lymphocytes due to Persian Gulf Stonefish venom treatment.
- Mitochondrial membrane damage as a consequence of Persian Gulf Stonefish venom treatment.

Keywords:

Acute lymphoblastic leukemia
Stonefish (*Synanceia verrucosa*) venom
ROS
Cytotoxicity

ABSTRACT

Persian Gulf Stonefish (*Pseudosynanceia melanostigma*) is one of the poisonous fish which is naturally found in Indian Ocean and Persian Gulf. The venom, which is isolated from this species, is suspected to use as an anticancer agent. In this study, we showed the cytotoxic effect of stonefish crude venom on lymphocytes, which obtained from acute lymphoblastic leukemia (ALL) patients and normal donors. Our results demonstrated that crude venom of Persian Gulf Stonefish could affect cancerous lymphocytes by reactive oxygen species (ROS) generation and mitochondrial membrane damage without any significant effect on normal cells.

Introduction

Stonefish (*Synanceia verrucosa*) is a member of the genus *Synanceia*, which is usually observed in shallow waters of the Pacific and Indian Oceans. *Pseudosynanceia melanostigma*, the Persian Gulf stonefish, is a special species of stonefish that is naturally found in the western Indian Ocean and the Persian Gulf, where dominates in muddy bottoms of the coast (Sutherland, 1983).

The venom of these poisonous fishes is a protein accumulated in their dorsal stings, which can produce intense pain, respiratory arrest, cardiovascular damage, muscle paralysis and convulsions, and even leading to death (Diaz, 2015).

This venom is composed of various proteins,

involving the cardiotoxic cardioleputin, the proteinaceous verrucotoxin (Ziegman and Alewood, 2015) and the hemolytic stonustoxin (SNTX) (Poh et al., 1991).

Many studies revealed the medicinal properties of each subunit of stonefish's venom (Carlson et al., 1971; Church and Hodgson, 2000). In a study, researchers showed that this venom has cytolytic effects against numerous cell types (Kreger, 1991). Sri Balasubashini et al. (2006) also described the anticancer effects of venom by using in vivo cancer model. They revealed that the venom, induced apoptosis in Ehrlich's ascitis carcinoma (EAC), splenocytes and murine P388 leukemic cells (Rajeshkumar et al., 2015).

Acute lymphoblastic leukemia is an abnormal condition of immature B-cell precursors release to the bloodstream that commonly affects children under 6 years old (Schwab and Harrison, 2011). Cases with B-ALL diagnosis usually have bone marrow (BM) failure, cytopenias with or without leukocytosis (Pui et al., 2015).

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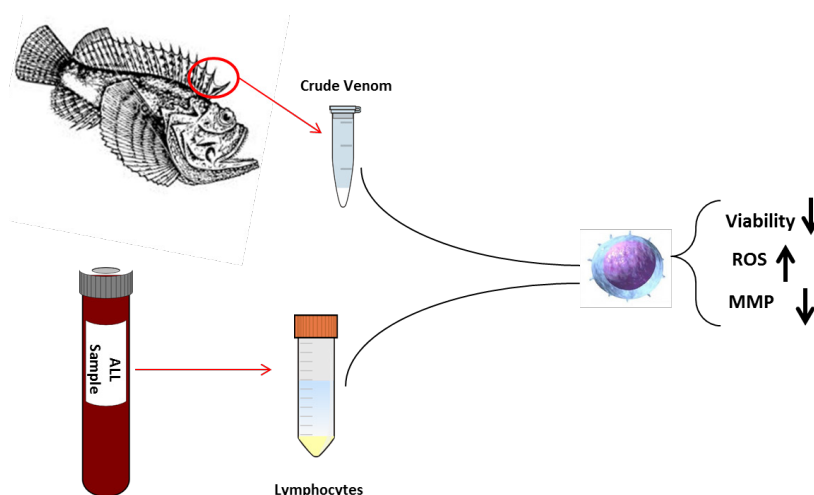


Figure 1. Schematic presentation of the effect of Stonefish crude venom on B-lymphocytes.

Pediatric acute lymphoblastic leukemia (ALL) is curable in 80% of cases, yet the remaining 20% are refractory and fatal. Furthermore, all chemotherapeutic agents have severe acute and persistent side effects (Hunger and Mullighan, 2015). As it has already shown the stonefish venom result in cell lysis in certain types of cells, thus it could be applicable to induce cell death in cancerous lymphocytes obtained from ALL patients (Fig. 1).

Materials & Methods

Chemicals

Trypan blue, 2',7'-dichlorofluorescein diacetate (DCFH-DA), Rhodamine123, bovine serum albumin (BSA), N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES), were purchased from Sigma-Aldrich Co. (Taufkirchen, Germany). RPMI1640 and FBS (Fetal Bovine serum) were purchased from Gibco, Life Technologies, Grand Island, NY. Ficoll-paque PLUS was obtained from Ge Healthcare Bio-Science Company.

B-Lymphocyte isolation

Blood samples were obtained from the ALL patient and healthy donors during a routine diagnosis. B-lymphocytes were isolated immediately using Ficoll gradient centrifugation (Lohan et al., 2014).

Cytotoxicity assay

The effect of Stonefish crude venom on lymphocytes obtained from healthy donors and patients with ALL was investigated using MTT assay (Mosmann, 1983).

The optical density was read at 580 nm wavelength in an ELISA plate reader after 4-hr incubation of the plates with MTT in an incubator (37 °C and 5% CO₂ air). All determinations were confirmed using replication from at least three identical experiments.

Determination of ROS

ALL and healthy B-lymphocytes (1×10^6 cells) were treated with various final concentrations of Stonefish crude venom for 2, 4 and 6 hours. H₂DCFDA (10 μM) was used to measure intracellular reactive oxygen species (Kalyanaraman et al., 2012). ROS concentration, was then assayed by a fluorescence spectrophotometer at EXλ = 488 nm and EMλ = 527 nm.

Determination of the collapse of mitochondrial membrane potential (MMP)

Mitochondrial redistribution of cationic fluorescent dye, rhodamine123 (Rh 123), from mitochondria into the cytosol has been used for the determination of the collapse of MMP. The cytosolic Rh 123 fluorescence intensity was determined using fluorescence spectrophotometer at the EXλ = 490 nm and EMλ = 535 nm (Andersson et al., 1987).

Results

Cell viability

As shown in Fig. 2, following 12-hr treatment, a significant decline in cell viability was observed for Stonefish crude venom in lymphocytes from ALL patients. Our results with MTT assay demonstrated that 200, 500 and 600 μg/

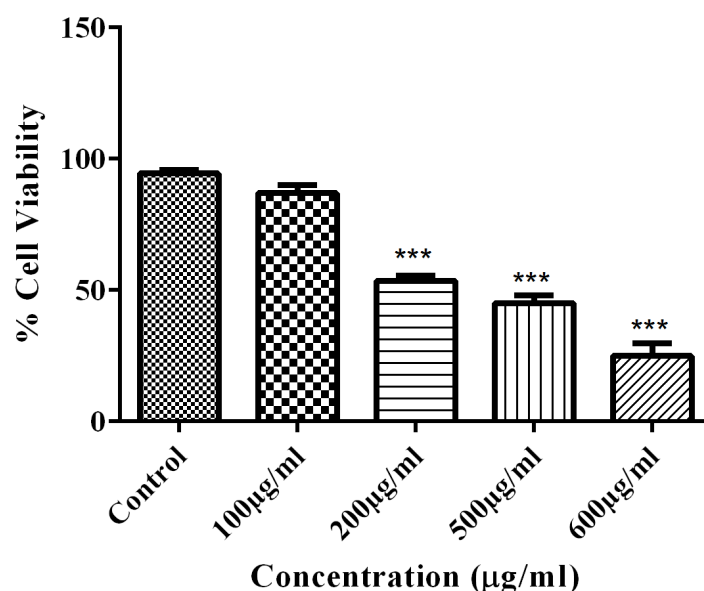


Figure 2. Effects of Stonefish crude venom on cell viability in ALL and healthy B lymphocytes. Cell viability, B-lymphocytes from ALL and healthy donors, at 1×10^6 cells/well, were seeded on 96-well plates. Stonefish crude venom at 100, 200, 500 and 600 $\mu\text{g/ml}$ concentrations were incubated for 12 hours. The absorbance representing the viability of B-lymphocytes was determined by the ELISA reader at 570 nm. Data presented as mean \pm SD. The significant level was $p < 0.05$ ($n = 3$).

ml of Stonefish crude venom can significantly ($P < 0.05$) diminish cell viability. The absorbance representing the viability of B-lymphocytes was determined by the ELISA reader at 570 nm. Data presented as mean \pm SD. The significant level was $p < 0.05$ ($n = 3$) (Fig. 2).

Determination ROS

In our study, Stonefish crude venom in three different concentration 125, 250 and 500 $\mu\text{g/ml}$ ($1/2 \text{ IC}_{50}$, IC_{50} and 2 IC_{50}) caused a significant ($p < 0.05$) increase in intracellular ROS levels in ALL B-lymphocytes but not in healthy B-lymphocytes (Fig. 3).

Determination of MMP

For further investigation of Stonefish venom toxicity mechanisms, we examined the effect of the crude venom on $\Delta\Psi\text{m}$. As shown in Fig. 4, Stonefish crude venom significantly ($p < 0.05$) decreased the MMP in a time-related manner in mitochondria obtained from cancer lymphocytes of ALL patients without any significant effect on healthy lymphocytes (Fig. 4).

Discussion

The Persian Gulf stonefish is one of the most venomous fish in the world. Its dorsal stings comprised of the

venom which is the mixture of different toxins such as cardioleptin, stonustoxin (SNTX) and verrucotoxin (Khoo, 2002).

Acute lymphocytic leukemia (ALL) is the most frequent pediatric cancer around the world. Despite numerous advanced treatment, ALL is a hardly curable disease. Besides, unwanted side effects of usual drugs to normal cells frequently make even complex problems (Hospitalfor and Children, 1982, American Cancer Society, 2015). In this study, we showed that stonefish crude venom in different concentrations could cause cytotoxicity only on cancerous lymphocytes. Our results also showed that stonefish crude venom could augment ROS level to the critical point and directly affect mitochondria only in cancerous lymphocytes.

Conclusion

These observations lead us to the conclusion that stonefish venom triggers cytotoxicity in cancerous lymphocytes via ROS generation and direct intervention of mitochondria. Although, further investigations must be considered to reveal the exact mechanisms of actions.

Competing Interests

The authors declared that there are no competing interests.

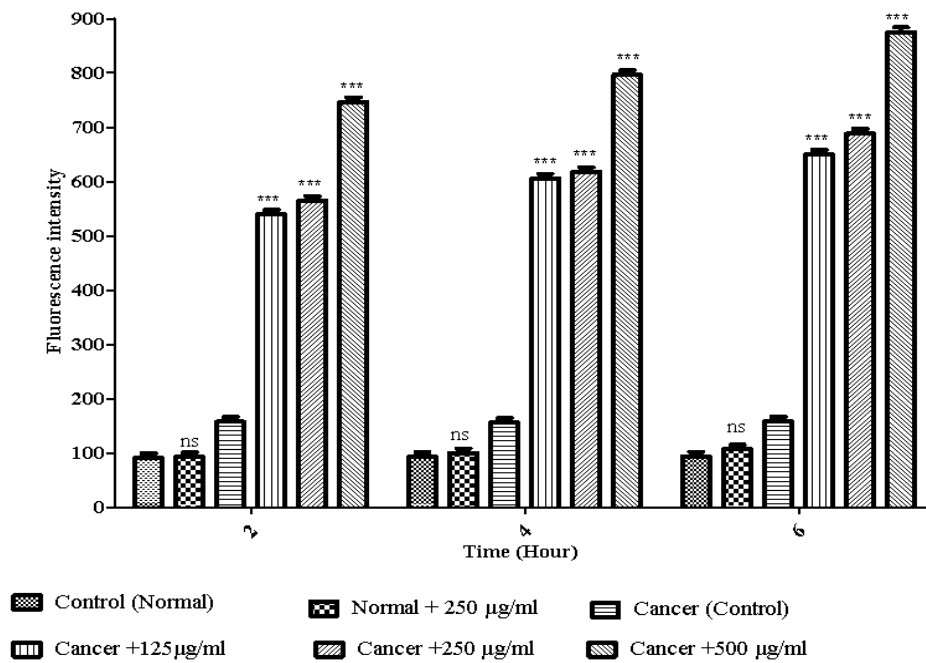


Figure 3. Effects of Stonefish crude venom on ROS formation in ALL and healthy B-lymphocytes. Stonefish venom induced ROS generation in ALL but not in healthy B-lymphocytes. Changes mean of fluorescence intensity in ROS generation in ALL and healthy B-lymphocytes treated with Stonefish venom for 2–6 h summarized in graph. Data presented as mean ± SD. The significant level was $p < 0.05$ ($n = 3$).

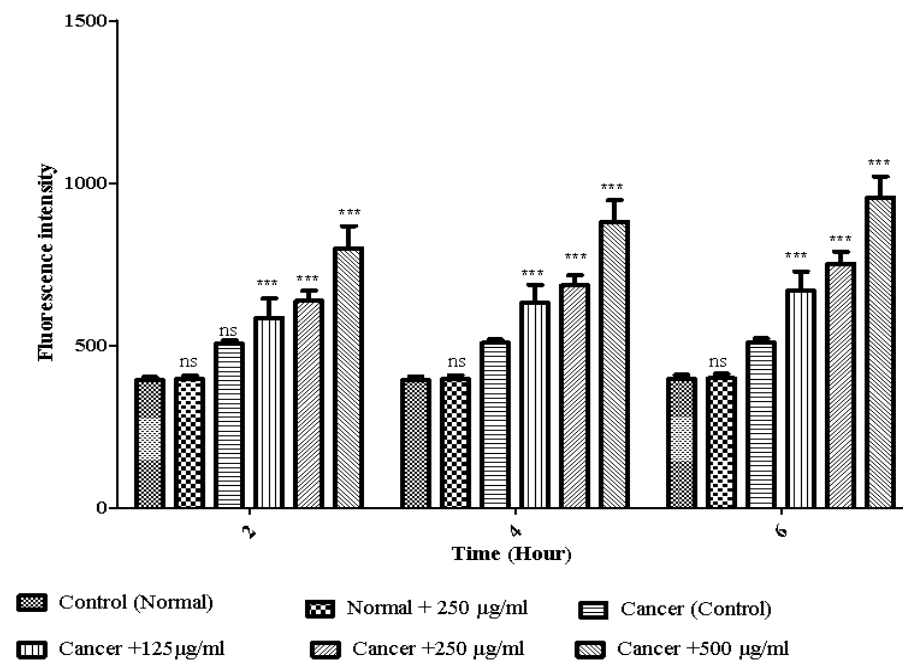


Figure 4. The effect of Stonefish crude venom on $\Delta\Psi_m$ of healthy and ALL B-lymphocytes. Freshly isolated purified B-lymphocytes were incubated with different concentrations of Stonefish crude venom for 2–6 h. $\Delta\Psi_m$ was measured by Rhodamine 123 as described above. Data presented as mean ± SD. The significant level was $p < 0.05$ ($n = 3$).

References

- American Cancer Society (2015). Leukemia: What is acute lymphocytic leukemia? available at: <http://www.cancer.org/cancer/leukemia-acute/lymphocytic-in-adults/detailed-guide/leukemia-acute-lymphocytic-what-is-all> (accessed 2016-29-11).
- Andersson, B. S., Aw, T. Y. and D. P. Jones, (1987). "Mitochondrial transmembrane potential and pH gradient during anoxia." *American Journal of Physiology-Cell Physiology*, **252**: C349-355.
- Carlson, R. W., Schaeffer, R. C., La Grange, R. G., Roberts, C. M. and F.E. Russell, (1971). "Some pharmacological properties of the venom of the scorpionfish *Scorpaena guttata*." *Toxicon*, **9**: 379-391.
- Church, J. E., and W. C. Hodgson, (2000). "Dose-dependent cardiovascular and neuromuscular effects of stonefish (*Synanceja trachynis*) venom." *Toxicon*, **38**: 391-407.
- Diaz, J. H. (2015). "Marine Scorpaenidae Envenomation in Travelers: Epidemiology, Management, and Prevention." *Journal of Travel Medicine*, **22** (4): 251-258.
- Hospitalfor, R. and S. Children, (1982). "Childhood acute lymphoblastic leukaemia : a review." *Journal of the Royal Society of Medicine*, **75**(June): 464-473.
- Hunger, S. P. and C. G. Mullighan, (2015). "Acute lymphoblastic leukemia in children." *New England Journal of Medicine*, **373**(16): 1541-1552.
- Kalyanaraman, B., Darley-Usmar, V., Davies, K. J., Dennery, P. A., Forman, H. J., Grisham, M. B., Mann, G. E., Moore, K., Roberts, L. J. and H. Ischiropoulos, (2012). "Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations." *Free Radical Biology and Medicine*, **52**(1): 1-6.
- Khoo, H. E. (2002). "Bioactive proteins from stonefish venom." *Clinical and Experimental Pharmacology and Physiology*, **29**: 802-806.
- Kreger, A. (1991). "Detection of a cytolytic toxin in the venom of the stonefish (*Synanceja trachynis*)." *Toxicon*, **29**: 733-733.
- Lohan, S., Kalanta, A., Sonkusre, P., Cameotra, S. S. and G. S. Bisht, (2014). "Development of novel membrane active lipidated peptidomimetics active against drug resistant clinical isolates." *Bioorganic and Medicinal Chemistry*, **22**(17): 4544-4552.
- Mosmann, T. (1983). "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays." *Journal of immunological methods*, **65**(1): 55-63.
- Poh, C. H., Yuen, R., Khoo, H. E., Chung, M., Gwee, M. and P. Gopalakrishnakone, (1991). "Purification and partial characterization of stonustoxin (lethal factor) from *Synanceja-horrída* venom." *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, **99**: 793-798.
- Pui, C. H., Mullighan, C. G., Evans, W. E. and M. V. Relling, (2015). "Pediatric acute lymphoblastic leukemia : where are we going and how do we get there?" *Blood*, **120**(6): 1-3.
- Rajeshkumar, R. K., Vennila, R., Karthikeyan, S., Prasad, N. R., Arumugam, M., Velpandian, T. and T. Balasubramaniam, (2015). "Antiproliferative activity of marine stingray *Dasyatis sephen* venom on human cervical carcinoma cell line." *Journal of Venomous Animals and Toxins including Tropical Diseases*, **21**(1):1.
- Schwab, C. and C. J. Harrison (2011). "Acute lymphoblastic leukaemia." *Methods in Molecular Biology*, **730**: 99-117.
- Sutherland, S. K. (1983). "Genus *Synanceja* (Linnaeus), stonefishes: *S. verrucosa* (Bloch & Schneider) & *S. trachynis* (Richardson)". In *Australian Animal Toxins, The Creatures, Their Venoms and Care of the Poisoned Patient*, Oxford University Press Melbourne, pp. 400-410.
- Ziegman, R. and P. Alewood, (2015). "Bioactive components in fish venoms." *Toxins*, **7**: 1497-1531.