ISSN: 2287-6898
International
Journal of Bio-Pharma Research
Volume 8, Issue 6 (2019) pp. 2607-2616
Research Article
Open Access

Inhibition of colon carcinoma cell lines by treatment with crude spine venom from marine catfish *Tachysurus dussumieri*

Kavitha Raja, Sharon Selvaraj and Arulvasu Chinnasamy*
Department of Zoology, University of Madras, Guindy Campus, Chennai - 600 025, India.

Abstract: Catfishes, come under the order Siluriformes, they represent as venomous fishes having venom glands in that dorsal and pectoral spines that are speared into place when threatened. The catfish, *Tachysurus dussumieri* spine venom is unsafe and having neuromuscular blocking activity, envenoms causes instant, local and intense pain, and a variable extent of bleeding. The present study was carried out in the histological section of spine and effect of spine venom on human colon cancer cell lines (HT 29 and COLO 320). Histology study of spine shows the presence of venom secretory cells. Venom was extracted from spines of catfish and the biochemical composition such as protein (1.020 mg/ml), lipid (0.955 mg/ml) and carbohydrate (0.313 mg/ml) was estimated. Protein profiles of Native and SDS page were indicating both high and low molecular weight of proteins. Toxicity on brine shrimp nauplii revealed the LC_{50} observed at 400 mg/ml. Result of spine venom on Vero cell line showed maximum cell viability up to 800 mg/ml. The cell viability on HT 29 and COLO 320 cell lines showed IC_{50} at a concentration of 300 mg/ml and 400 mg/ml, at the time of 24 h incubation. Significantly Propidium iodide staining method was observed to view damaged nucleus of cells. DNA fragmentation assay has confirmed DNA fragments due to the induction of apoptosis.

Key words: *Tachysurus dussumieri,* Histology, Spine venom, Cytotoxicity, DNA fragmentation.

Introduction

Cancer is a group of diverse disorders involving unusual cell growth with the feasiblility to attack or spread to other parts of the body. It is the foremost cause of mortality worldwide. According to WHO estimate, in 2017 cancer exemplified for 8.2 million deaths (Lopes and Beatriz, 2017). The DNA on mutating in the genome causes the cells to undergo changes which results in the cells to keep dividing to form cancers. The burden of cancer continues to largely increase with the development of the world population along with an increasing alteration to cancer causing lifestyle. According to GLOBOCAN estimates 12.7 million cancer cases and 7.6 million cancer death are esteemed to have occurred in 2008, but of this 56 % of new cases are detected and 64% of the deaths happened in economically developing world (Jemal *et al.,* 2011). More than 30 % Indians of the age group of 45 to 64 years dying due to colon cancer, hence it's has become important to go through it and find sufficient preventive measures (Alice and Elegbede, 2016). Colon cancer ranks 8th among men and 9th among women (Nandakumar, 1990). Epidemiologically, our food habits are accompanying with increased risk of colon cancer. Possibly that toxic products from colonic protein metabolism contribute to increase genotoxic stress (Hughes *et al.,* 2000).

A nature is an instrumental source for therapeutics. Contempt, the fact earth as an Oceanic planet, a numeral of technical factors has factually hindered the evolution of a marine based medication. During the last 25 years natural products were derived from marine organisms that have been the focus of much research. The compounds isolated from the natural source were highly used since the primeval times, for the treatment of various sicknesses and for the development of life (Titilade and Olalekan 2015). Fishes are major resource for bioaegis therapeutic compounds. Among the demersal resources, catfishes are an extremely large and recognized group of diverse fishes that contributes significantly to the fishery along the Indian Coast, (Marasas *et al.,* 1984), Cat fish *Tachysurus dussumieri* belong to the family Tachysuridae, order Silluriformes commonly found in deep seas.

Corresponding Author:
Mr. Arulvasu Chinnasamy,
Dept. of Zoology, University of Madras,
Guindy Campus, Chennai - 600 025, India.
E-mail: carulvasu@gmail.com

Other common characteristics of catfishes are the retractable thorny spines that are found at the front edge of the dorsal and pectoral fins. These spines are enlarged, stiffened rays, otherwise similar in form to the remaining rays that support the fins with a formidable weapon against predators. Erected spines can be locked into place, thereby enlarging the fins spine size of fish and making it hard for the predator to swallow. Spines can be sharply pointed at the tip, further protecting the fish from attack (ICAR-CIBA, 1998). Fish venoms, have limited data about their bioactive potential. Over 1600 species of catfishes may be venomous, number is far greater assessment of venomous catfish diversity is reported previously (Wright, 2009).

The spine venom from *Plotosus lineatus* due to its biochemical activities additional to their potential cytotoxicity activity together with HEp2 cells (Pachaiyappan *et al.,* 2015). The sting venom of *Potamotrygon* is toxic to mice and has nociceptive, endematogenic and proteolysis activities and hence showed bioactive properties and pharmacological (Monteiro-dos-Santos *et al.,* 2011) *Scatophagus arius* are venomous fishes with a venom apparatus that is constituted by 11 dorsal spines, a pair of ventral spine with elongated venom glands and an integumentary sheath, enveloping all these proteins it has pharmaceutical properties (Cameron and Endean, 1973). Hence, this study has been attempted for screening the anticancer properties of crude spine venom of marine catfish *Tachysurus dussumieri* on human colon cancer cell lines.

Materials and Methods

Collection of spine from *Tachysurus dussumieri:*

The marine cat fish *Tachysurus dussumieri* was collected from South East coast of Tamilnadu Kovalam, Chennai, India. The fishes were acclimatized to the laboratory condition and were maintained in large cement tanks with filled sea water and aerated by electric aerator. The cat fish was identified and authenticated based on examination of the morphological characteristics by an expert from Zoological Survey of India, (ZSI) Chennai. The spine was collected from cat fish by cutting it approximately 3-5 mm from base of dorsal and pectoral fins using cutting plier and stored at -20° C.

Histological observation of catfish spine:

The collected spine was fixed in 4 % formalin and decalcified by 4 % hydrochloric acid/formic acid completely within 24 h or it may take time depending on the size of the specimen. Once the decalcification is complete, washed in running tap water thoroughly up to 24 h. Then it is proceeded with routine paraffin embedding and stained with Hematoxylin and Eosin Followed by Dehghani *et al.,* (2010)

Extraction and Biochemical analysis of crude spine venom:

The catfish spine venom was extracted using a standard technique followed by Abirami *et al.,* (2014). The collected sample extract were homogenized with PBS buffer (0.005 M, pH 7.4). These samples were centrifuged at 4^{o}C for 15 min at 8000 rpm. Then the supernatant was collected lyophilized and stored at -20^{o}C.

Protein content of the crude spine venom was estimated by Lowry *et al.,* (1951). The lipid and carbohydrate was estimated by the standard method of Roe (1955), Barnes and Blackstock (1973) respectively. The protein profile of crude spine venom was analyzed by Native and SDS-PAGE according to the method of Maurer, (1971) and Laemmli, (1970) respectively.

Toxicity assessment of crude spine venom

Brine shrimp lethality assay:

The Brine shrimp lethality assay was performed by following method of Meyer *et al.,* (1982**)** Twenty organisms were used for each concentration of crude catfish *T. dussumeiri* spine venom. The chosen concentrations for the lethality testing were 200, 400, 600, 800 and 1000 µg/ml. The experimentation was done in 24 multi-well plates and each plate contained 20 nauplii and spine venom with 1ml of seawater in each well was subjected 24 hours exposure. Triplicates of each concentration stood to calculate the LC_{50}. The outcomes were plotted as percentage mortality versus concentration of crude spine venom.

Cytotoxcity of vero cell line:

Cytotoxic effects on normal cells of spine venom tested upon vero cell line (Green African monkey kidney cells) by MTT assay. Different concentrations 200 to 1000 µg/ml of crude spine venom were applied on vero cell line and incubated for 24 h.

Cancer cell lines and chemicals:
Human Colon adenocarcinoma cell lines (HT 29 and COLO 320) were procured from National Centre for Cell Science, Pune, India. DMEM, Trypsin-EDTA, Fetal Bovine Serum, 3-(4,5-dimethylthiazol-2yl)-2,5 diphenyl tetrazolium bromide, sodium bicarbonate, Dimethyl sulphoxide and antibiotic solution were purchased from Hi Media Laboratories. 96 well plates, 6 well plates, Tissue culture flasks (25 and 75 mm^2), Centrifuge tubes (15 and 50 ml) were purchased from Tarsons Products Pvt, Kolkata, India.

Cell viability assay:
The cell viability was assessed by MTT method as elucidated by Mosmann (1983). Human colon cancer cells, (HT 29 and COLO 320) (5 x 10^6 cells/ml) were plated in 96 well plates with medium containing DMEM, 10% FBS. The cells were incubated for 24 hours under different concentration of crude spine venom 100-500 µg/ml. After 24 hours 20 µl of MTT solution was added to each well and the cultures were further incubated for 3 hours then the formed crystals were dissolved gently by 100 µl DMSO. A micro plate reader was used to measure absorbance at 570 nm.

Cytomorphological observation:
The cytotoxicity assay was followed by the method of prabhu *et al.,* (2013). The cells were seeded into a six well plate to get attach to the substratum incubated for 24h. After, the medium was substituted with incomplete medium DMEM containing different concentration of 100 to 500 µg/ml spine venom incubated for 24 h. The cytomorphology was examined under inverted microscope

Propidium iodide staining for nuclear damage:
Cancer cells (HT 29 and COLO 320) were seeded in 6 well plate with DMEM medium containing 10 % FBS. Cells were incubated for 24 hours under 5% CO_2, 95% O_2 at 37°C. Then the control well received fresh medium and the treatment plates received different concentrations (100-500 µg/ml) of crude spine venom. Then the culture plates were incubated for treatment of spine venom. Cells were washed with PBS and fixed in methanol: acetic acid (3:1 v/v) for 10 minutes and stained with 50 µg/ml of propidium iodide stain for 20 minutes. After staining the cells were visualized immediately under Inverted Fluorescence microscope at 20X magnification (LSM 710, Carl Zeiss) followed by Keum *et al.,* (2002).

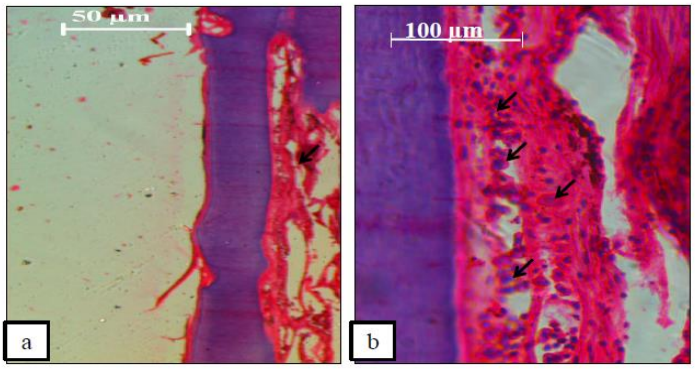
DNA-fragmentation by agarose gel electrophoresis:
Agarose gel electrophoresis was done by the method of Bortner (1995). Cancer cells (HT 29 and COLO 320) were seeded in 6 well plates with DMEM medium containing 10% FBS. The cells were incubated for 24 hours to get attached. Then, medium was removed and the control wells received again medium and treatment plates received (100-500 µg/ml) concentrations of crude spine venom containing medium. After completion of incubation time, the DNA was extracted from the HT 29 and COLO 320 as follows. DNA is isolated from the cells, further stored in the TE buffer for short time usage, 10 µg of DNA was electrophoresed in a 0.7 % agarose gel running under 90 V. in a mini gel tank containing TAE buffer. Then the gel was examined under UV transilluminator (Biorad) and photographed.

Results

Histological observation of spine:
Spines were collected from cat fish 4 - 5 cm length. Histological sections were examined by using a Light Microscopic observation. It was observed that there is presence of venom secretory cells at the serrated edges of the catfish spine in spherical shape with nucleus at the center (Fig.1).

Biochemical analysis of crude venom:
The crude spine venom *T. dussumieri* was collected and estimated the biochemical parameters. The results obtained for various biochemical constituents such as protein, lipid and carbohydrate, the quantity of proteins, lipids and carbohydrates were estimated to be 1.020, 0.955 and 0.313 mg/ml respectively.

Figure 1: Photomicrograph represents the transverse section of spine stained by Hematoxylin and Eosin. Arrow indicates the venom secretory cells



a) 10 x magnification b) 40 x magnification in Light microscope

Protein profiles of crude spine venom

The protein profile of the crude spine venom sample of *T. dussumieri* was analyzed by Native and SDS-PAGE. The non-denature proteins of spine venom have four major bands, appeared very clearly in CBB staining. The crude protein had both high molecular weight and fast moving lower molecular weight proteins. The protein profiles of the crude spine venom showed proteins band ranging from 200 to 30 kDa. The subunits of native proteins were well defined and prominent in silver staining. Thus the crude spine venom showed both low and high molecular weight protein bands (Fig. 2).

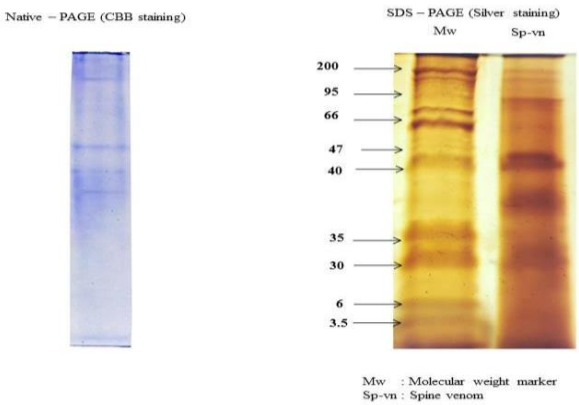


Figure 2: Protein profile of crude spine venom of cat fish

Toxicity studies:

Lethality assay: The crude spine venom was screened for apparent toxicity using brine shrimp nauplii lethality bioassay. The venom showed moderate toxicity against brine shrimp nauplii and the lethal concentration (LC_{50}) was calculated. The concentrations were fixed from 200 to 1000 µg/ml to express the lethality. Crude spine venom of *T. dussumieri* shows LC_{50} in 400µg/ml (Fig: 3) Thus this proves the toxicity increases in dose and time dependent manner, and the concentration of crude spine venom for the *in vitro* assays were fixed accordingly.

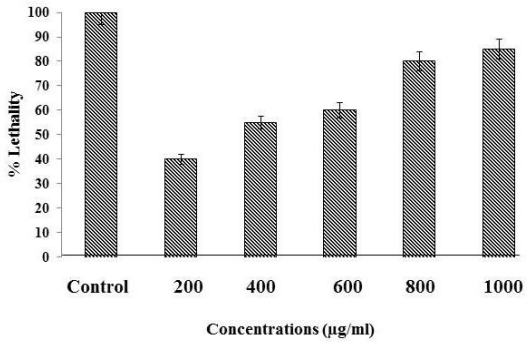


Figure 3: Toxcity assessment of crude spine venom on Brine shrimp *Artemia nauplii*

Cytotoxcity Assay: The cytotoxic assay of crude spine venom of *T. dusumieri* was tested by MTT method on African green monkey kidney cell line (Vero). The different concentrations of crude spine venom (200 to 1000 µg/ml) was tested against the vero cell line for 24 h. Though, 80 % of cells were viable up to 800 µg/ml.

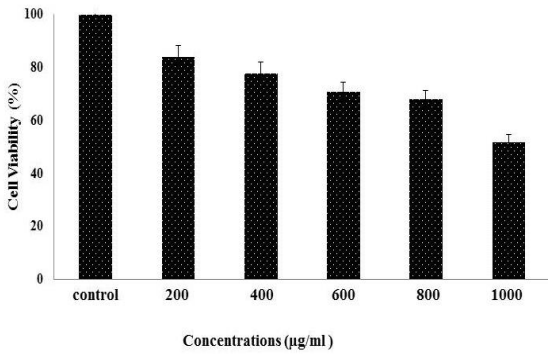
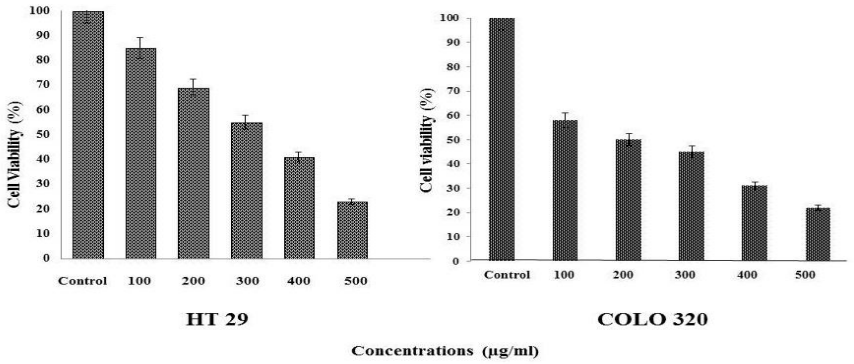


Figure 4: Cytotoxic effect on Vero cells treated with crude spine venom of catfish *T. dussumieri*

Cell viability assay:

The cell viability of crude spine venom of *T. dusumieri* was tested by MTT method using human colon cancer cell lines. The venom upon treatment hinders the growth of cancer cell lines at different concentrations in time dependent manner (Fig.5) indicated that there is decrease in the cell viability of HT 29 and COLO 320 at concentrations ranging from 100-500 µg/ml. A (IC_{50}) 50 % decrease in the viability of HT 29 and COLO 320 was obtained at the concentration of 300 µg/ml and 400 µg/ml at 24 hours incubation respectively.

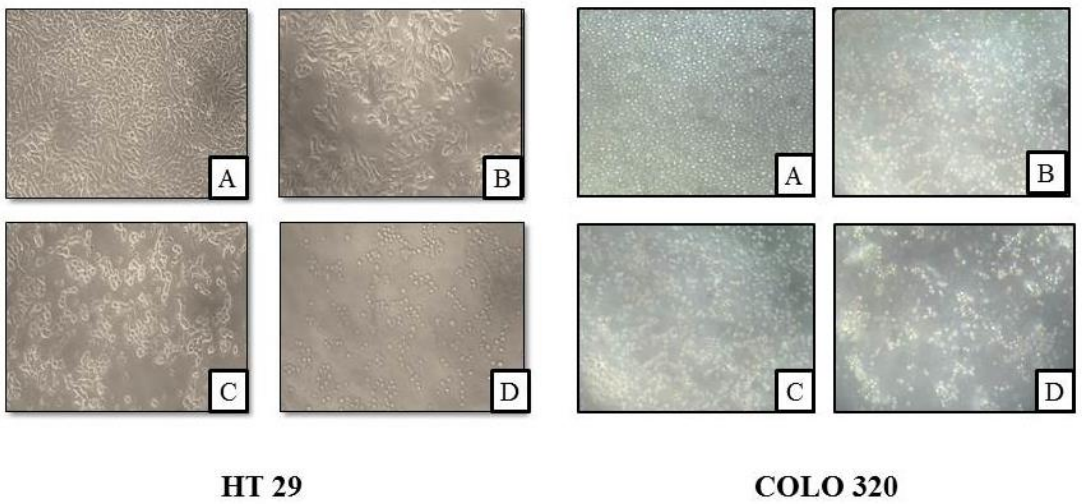
Figure 5: Effect of crude spine venom of catfish *T. dussumieri* on the cell viablity of HT 29 and COLO 320 cells



Cytomorphological observation:

Morphological variations were observed in the control and treated HT 29 and COLO 320 cells were photographed. The control cells show irregular confluent, with spherical cell morphology with even monolayer. The treated cells at 24 h showed shrink and alteration in shape (Fig.6) There was a disruption in the monolayer and cells were aggregated. This shrinkage is due to the growth inhibitory effect of *T. dussumieri* crude spine venom

Figure 6: Effect of crude spine venom of *T. dussumieri* on morphology of HT 29 and COLO 320 at 24 hr



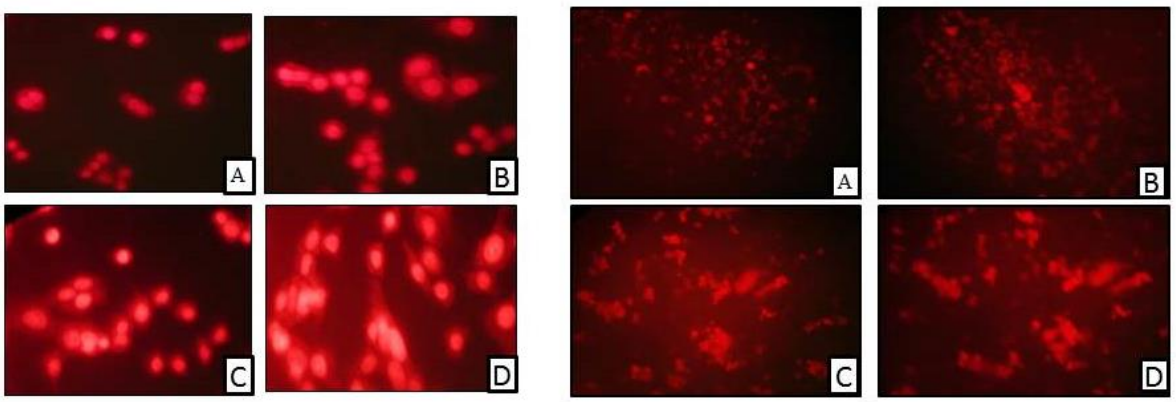
A - Control cells
B - Cells treated with minimum concentration of HT 29 and COLO 320 (100µg/ml)
C - Cells treated with IC_{50} concentration of HT 29 and COLO 320 (300 and 400 µg/ml)
D - Cells treated maximum concentration of HT 29 and COLO 320 (500 µg/ml)

Nuclear morphological observation:

The damage of nucleus was detected by the propidium iodide staining method. The propidium iodide positive cells are seen in control. The concentration of 300 µg/ml and 400 µg/ml of crude spine venom treated with cells (HT 29 and

COLO320) with exposure times 24 h. While observing nuclear damage of cells, progressive increase in the number of propidium iodide positive cells was detected in maximum concentration (Fig.7)

Figure 7: Nuclear morphological observation of HT 29 and COLO 320 cells treated with crude spine venom of *T. dussumieri* by Propidium iodide staining



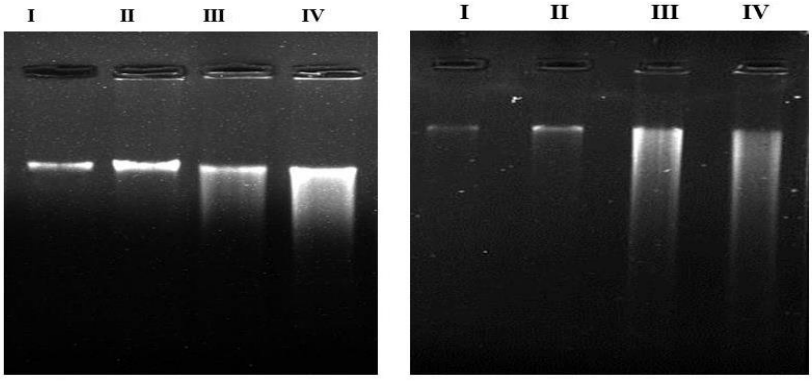
HT 29 **COLO 320**

A- Control cells
B- Cells treated with minimum concentration of HT 29 and COLO 320 (100 µg/ml)
C- Cells treated with IC 50 concentration of HT 29 and COLO 320 (300 and 400 µg/ml)
D- Cells treated with maximum concentration of HT 29 and COLO 320 (500 µg/ml)

Analysis of DNA fragmentation:

DNA-fragmentation is a distinctive assay to confirm the drug induced apoptotic cell death due cleavage in the DNA strand. HT-29 and COLO 320 cells at 24 hours of treatment with spine venom were harvested for DNA. The agarose electrograph of control cells shows a single band of intact DNA just below the well. Agarose electrograph of HT 29 and COLO 320 cells treated with crude spine venom (300 µg/ml) and (400 µg/ml) induced DNA fragmentation pattern in the form of streaks. The streaking of fragmentation in the lanes of agarose gel shows induction DNA damage causing (Fig. 8). This clearly demonstrates that the DNA damage is caused by the crude spine venom when compared to the control cells**.**

Figure 8: DNA fragmentation of HT 29 and COLO 320 cell lines treated with crude spine venom of *T. dussumieri* at 24 hr



HT 29 **COLO 320**

I - Control cells
II - Cells treated with minimum concentration of HT 29 and COLO 320 (100 µg/ml)
III - Cells treated with IC50 concentration of HT 29 and COLO 320 (300 and 400 µg/ml)
IV - Cells treated maximum concentration of HT 29 and COLO 320 (500 µg/ml)

Discussion

Many studies have been conceded out on the pharmacological activities of molecules in various species of Pisces. Venom of such animals is the strategy to survive in a specific environment, so that it exhibits very potent biological effects. Among the various classes of Pisces the venom was isolated and characterized, where most of them are proteinaceous in nature (Abirami *et al.,* 2014). Catfish venom varies from one species to another, thus its biochemical composition and activity differs from one species to another. Biologically active compounds pay much attention to marine organisms recently (Cragg *et al*., 1997).

To investigate the present study of histological observation and effect of spine venom. In previously available siluriform phylogenies and toxicological analyzes, suggest that about 1250-1625 species of cat fishes from at least 20 family of species are venomous this character optimization shows histological sections of pectoral-fins spine of catfish (Wright, 2009). The histological section of spine from *T. dussumieri* shows venom secretory cells which are similar to that of its related species of catfish.

In the present study *T. dussumieri* has good content of protein, lipid, and carbohydrate. An earlier report on catfish *Arius maculatus* was observed to have 748 µg/ml of protein (Abirami *et al*., 2014). Protein content in the *Plotosus canius* crude extracts of aqueous spine venom was found to be 0.95 mg/ml (Usman *et al.,* 2013). Our study is in line with the previous reports in crude spine venom quantification for total protein content was done and the amount of protein was observed as 1020 mg/ml. This is essentially a greater amount when compared to other catfishes. The total carbohydrate extract was observed as 313 mg/ml. Similarly, the amount of total sugars present in crude catfish sting venom was estimated as 0.025 µg/ml in *Plotosus canius* and 0.066 mg/ml in catfish *Clarias batrachus* (Benerjee and Mittal, 1976). Catfish *Arius maculatus*, *Plotosus lineatus* in the coast of Parangipettai show carbohydrate level varying from 2.15 gm to 1.98 gm. the carbohydrate content is quantitatively little higher when compare to other species (Venkaiah and Lakshmipathi, 2000). The lipids are a well-organized source of energy, which contain more than twice the energy of carbohydrate and proteins. Lipid content 9.521 mg/ml is noticed in *Clarias batrachus* (Benerjee and Mittal, 1976). A highest level of lipid was documented in *Heteropneustes fossilis* which was about 0.194 mg/ml (Mittal and whitear, 1979). The lipid content in *T. dusumieri* is estimated to be 955 mg/ml. The quantity of lipid is higher, when compared to other species such as *Clarias batrachus* and *Heteropneustes fossilis*. Accornding to (Usman *et al.,* 2013) the crude sting venom of Arius *maculatus*, revealed toxicity against brine shrimp *Artemia* nauplii. The Lethal Concentration LC_{50} value of catfish venom was found to be 2.244 mg/ml. In *plotosus lineatus*, lethal concentration of spine venom was 4µg/ml (Abirami *et al.,* 2014). Where as in our study, spine venom showed LC_{50} at 400 µg/ml.

The hemolytic toxin of 44 kDa from the venom of *Chiropsalmus quadrigatus* (Nagai *et al*., 2002) and *Chironex fleckeri* nematocysts venom contained a 20 kDa protein (Olson *et al*., 1984). The crude venom protein molecular weight was determined by SDS PAGE on 10 % polyacrylamide gel using standard protein marker. Similarly, crude spine venom of *T. dussumieri* consists of several protein bands ranging from high molecular weight of 200 kDa to 66 kDa and Low molecular weight of 35 and 30 kDa.

The catfish spine venom cytotoxin is active against colon cancer cells which are shown by MTT assay, it indicated that cytotoxin strongly inhibits cells proliferation of HT 29 and COLO 320 with an IC_{50} value. Previous literatures including the IC_{50} values of Catfish venom from *Scatophagus argus* on HeLa (Human cervical cancer cell line) IC_{50} < 10µg/ml (Sivan *et al.,* 2007), from *chiropsalamus quadrigatus* on ECV304 (transformed vascular endothelial cell) and U251 (Human maliganant glioma cells) for 24 h with IC_{50} at 49.8µg/ml and 80.3µg/ml respectively (Sun *et al*., 2002). The analysis of the data shows that *T. dussumieri* spine venom has a good cytotoxicity in comparison with other species. Our results show that spine venom contains more toxic components that have a strong cytotoxic activity against Colon adenocarcinoma cell lines HT 29 and COLO 320. The crude venom showed a dose and time dependent manner activity. Where increasing concentration of sample decrease in viability of colon cancer cells. The 50 % inhibition of cell viability was seen at 300 mg/ml and 400 mg/ml respectively, upon 24 h treatment.

The Cytomorphological observation of HT 29 and COLO 320 cell line. In both case, control cells were spherical in shape with even monolayer, whereas treated cells were shrinked and monolayer was disturbed, aggregation was seen. Accornding to (Arulvasu *et al.,* 2010) antiproliferative effect of sardine oil emulsion on A549 and HCT 15 cell lines clearly shows the morphological variations control cells are healthy apparently polygonal in nature were treated shrinkage is seen. The cytomorphology of the HT 29 and Hep2 cells treated with partially purified protein from *tachysurus dussumieri* by (Varier *et al*., 2018) after incubation of purified protein leads to destruction of monolayer with cell shrinkage forming spherical bodies which were observed in both HT 29 and Hep2 cells compared to control.

The Propidium Iodide staining of treated and untreated cells reveal the nuclear morphology which makes it permeable in dead cells and exhibit the dead cell count of both HT 29 and COLO 320. The morphology of apoptotic cells showed fragmented nuclei and highly condensed chromatin was viewed by fluorescence. There was an optimum dose and time for induction of apoptosis (Traganos *et al.,* 1975). In our study bright red emission of fluorescence showed the binding of stain to nuclei acid of cells HT 29 and COLO 320.

A significant mode of cell death after cytotoxic drug leads to apoptosis (Zhang and Wu 2008). When cells undergo apoptosis, several events like membrane blebbing, cytoplasm condensation and the activation of end nuclease/specific proteases. Later the genome will be ruptured, and smaller apoptotic bodies will be formed (Bedner *et al*., 1999). Through the process of apoptosis, unwanted or deteriorated cells can be eliminated (Mercer 1992). The nuclear fragmentation clearly demonstrates the role of apoptosis in spine venom treated cells. In our result, it was clearly observed that DNA fragmentation showed apoptosis. Hence, the cells treated with crude spine venom shows that fragment of DNA in the gel, which clearly explains the cell death of HT 29 and COLO 320 through induction of apoptosis.

Conclusion

The present study reveals that catfish *Tachysurus dussumieri* crude spine venom has a good content of protein, lipid and carbohydrate. Histology studies prove that venom secretory cells seen in the edges in the spine of catfish. Qualitatively, Native and SDS PAGE determine the high and low molecular weight protein bands from spine venom. *Tachysurus dussumieri* spine venom possesses pharmacologically active components that show toxicity on time and dose dependent manner in Brine shrimp nauplii. The involvement of cytotoxin proteins shows effective against HT 29 and COLO 320 cell lines. To confirm apoptotic nuclear damage in the cells seen through PI staining and visualizing the damage of DNA by Agarose gel electrophoresis. Concluding that *Tachysurus dussumieri* spine venom may pave a way for the isolation of novel compounds and development of new therapeutic strategies. Further research made on purification and characterization of the venom into several active components thus detailed studies could lead to the finding of new potent drug in future.

Acknowlegement

The authors are thankful to UGC-URF fund, for financial assistance for providing to carry out this work successfully.

References

1. Lopes M, Beatriz S, The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. *Acta Neuropathologica* 134.4 (2017): 521-535.
2. Jemal A, Bray F, Center, M.M., Ferlay, J., Ward, E. and Forman, D. Global cancer statistics. *CA: a cancer journal for clinicians*, *61*(2), (2011): 69-90.
3. Alice, O. D., and I. O. Elegbede. "Impact and Challenges of Marine Medicine to Man and its Environment." *Poult Fish Wildl Sci* 4.160 (2016): 2.
4. Nandakumar A, National cancer registry programme. *Indian Council of Medical Research, Consolidated report of the population-based cancer registries, New Delhi, India* 96 (1990).
5. Hughes T.R, Marton M.J, Jones A.R, Roberts C.J., Stoughton R, Armour C.D, Bennett H.A, Coffey E, Dai H., He Y.D. and Kidd M.J., Functional discovery via a compendium of expression profiles. *Cell*, *102*(1) (2000):109-126.
6. Titilade P.R. and Olalekan E.I. The importance of marine genomics to life. *Journal of Ocean Research*, *3*(1) (2015):1-13.

7. Marasas W.F.O, Nelson P.E. and Toussoun, T.A., *Toxigenic Fusarium species. Identity and mycotoxicology*. Pennsylvania State University (1984).

8. Director I. C. ICAR-CIBA Annual Report (1998):1997-1998.

9. Wright Jeremy J. Diversity, phylogenetic distribution, and origins of venomous catfishes. *BMC Evolutionary Biology* 9.1 (2009): 282.

10. Pachaiyappan A., Sadhasivam G., Arumugam S and Muthuvel A., Bio Prospecting the Enzymatic and Anticancer Potential of Spine Secretions of Marine Catfish (*Plotosus lineatus*). *Journal of Biologically Active Products from Nature*, *5*(6), (2015):406-418.

11. Monteiro-dos-Santos J., Conceição K., Seibert C.S., Marques E.E., Silva Jr, P.I., Soares A.B., Lima C and Lopes-Ferreira M. Studies on pharmacological properties of mucus and sting venom of Potamotrygon cf. henlei. *International immunopharmacology*, 11 (9), (2011): 1368-1377.

12. Cameron A. M., and Endean R. Epidermal secretions and the evolution of venom glands in fishes. *Toxicon*, 11(5), (1973): 401-410.

13. Dehghani H., Sajjadi M. M., Parto P., Rajaian H., and Mokhlesi A. Histological characterization of the special venom secretory cells in the stinger of rays in the northern waters of Persian Gulf and Oman Sea. *Toxicon*, 55(6), (2010): 1188-1194.

14. Abirami P., Arumugam M., Giji S., and Nagarajan S. Bio-prospecting of catfish sting venom Arius maculatus available along South East coast of India. *Int J Pharm Pharm Sci*, 6(1), (2014): 110-5.

15. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent *J Biol Chem* 193 (1951): 265 - 75.

16. Roe JH, The determination of sugar in blood and spinal fluid with anthrone reagent *J Biol Che*. 212 (1955): 335 – 343.

17. Barnes H, and Blackstock J, Estimation of lipids in marine animals and tissues, detailed investigation of the sulphophosphovanillin method for total lipids. *J Exp Mar Bio.* Ecol 12 (1973): 103-118

18. Maurer H R. DISC Electophoresis and related technique of ploy acrylamide Gel Electrophoresis Walter de Gruyter, *Berlin, New York* (1971): 222.

19. Laemmli UK, Clevage of structural protein during the assembly of the head of bacteriophage T4 *J Nature* 277 (1970): 680 - 85.

20. Meyer B, Ferrigni N.R, Putnam J.E, Jacobson L.B, Nichols D.E, Mclaughlin J.L. Brine shrimp a convenient general bioassay for active plants constituents. Planta Medica. 45 (5) (1982): 31-34.

21. Mossman T, Rapid colorimetric assay for cellular growth and survival. Application to proliferation and cytotoxicity assays *J Immunol Meth* 16: 65 (1-2) (1983): 55 – 63.

22. Prabhu D, Arulvasu C, Babu G, Manikandan R, and Srinivasan P. Biologically synthesized green silver nanoparticles from leaf extract of Vitex negundo L. induce growth-inhibitory effect on human colon cancer cell line HCT15. *Process Biochemistry*, 48(2) (2013): 317-324.

23. Keum YS, Kim J, Lee KH, Park KK, Surh YJ, Lee JM, Lee SS, Yoon JH, Joo SY, Cha IH, Yook JI. Induction of apoptosis and caspse -3 activation by chemopreventive (6)-paradol and structurally related compounds in KB cells. *Cancer Lett* 177 (2002): 41 - 47.

24. Bortner CD, Oldnburg NB and Cidlowski JA. The role of DNA fragmentation in apoptosis. *Trends Cell Biol* 5 (1995): 21-26

25. Cragg G. M, Newman D. J, & Snader K. M. Natural products in drug discovery and development. *Journal of natural products,* 60(1), (1997): 52-60.

26. Wright J. J. Diversity, phylogenetic distribution, and origins of venomous catfishes. BMC *Evolutionary Biology*, 9(1) (2009): 282.

27. Usman B. I, Amin S. M. N, Arshad, A., and Rahman, M. A. Review of some biological aspaects and fisheries of Grey-Eel Catfish Plotosus canius (Hamilton, 1822). *Asian Journal of Animal and Veterinary Advances*, 2(8) (2013): 154-167.

28. Banerjee T. K. and Mittal A. K. Histochemistry and the functional organization of the skin of a"

live-fish" Clarias batrachus (Linn.) (Clariidae, Pisces). *Mikroskopie,* 31(11-12) (1976): 333-349.

29. Venkaiah Y and Lakshmipathi V. Biochemical composition of epidermal secretions and poisonous spine of two freshwater catfishes. *Asian Fisheries Science,* 13(2), (2000): 183-189.

30. Mittal A. K. and Whitear M Keratinization of fish skin with special reference to the catfish Bagarius bagarius. *Cell and tissue research*, 202(2) (1979): 213-230.

31. Nagai H, Takuwa-Kuroda K., Nakao M., Oshiro N, Iwanaga S, and Nakajima T. A novel protein toxin from the deadly box jellyfish (sea wasp, Habu-kurage) Chiropsalmus quadrigatus. *Bioscience, biotechnology, and biochemistry,* 66(1), (2002): 97-102.

32. Olson C. E, Pockl E. E, Calton G. J, and Burnett J. W. Immunochromatographic purification of a nematocyst toxin from the cnidarian Chironex fleckeri (sea wasp). *Toxicon,* 22(5), (1984): 733-742.

33. Sivan G, Venketesvaran K., and Radhakrishnan C. K. Biological and biochemical properties of Scatophagus Argus venom. *Toxicon*, 50(4), (2007): 563-571.

34. Sun L. K., Yoshii Y, Hyodo A., Tsurushima H, Saito A, Harakuni T and Morine N. Apoptosis induced by box jellyfish (Chiropsalmus quadrigatus) toxin in glioma and vascular endothelial cell lines. *Toxicon*, 40(4), (2002): 441-446.

35. Arulvasu C, Dinesh D, Manikandan R, Pandi M, Srinivasan P, Radakrishnan N. and Babu G. Evaluation of antiproliferative effect of sardine oil emulsion on A549 and HCT 15 cancer cell lines. *International Journal of PharmTech Research* 2(2), (2010): 1171-1177.

36. Varier K. M, Chinnasamy A, Gajendran B, and Nagarathnam R. Isolation and characterization of a novel anticancer muscle protein from edible marine catfish tachysurus dussumeiri. *international journal of pharmaceutical sciences and research,* 9(7), (2018): 2720-2730.

37. Traganos F, Z. Darzyndiewicz T. Sharpless and M. R. Melamed. Denaturation of deoxyribonucleic acid in situ effect of formaldehyde. *Journal of Histochemistry & Cytochemistry* 23, 6 (1975): 431-438.

38. Zhang L, and Wu W. T. Isolation and characterization of ACTX-6: a cytotoxic L-amino acid oxidase from Agkistrodon acutus snake venom. *Natural product research*, 22(6), (2008): 554-563.

39. Bedner E, Li X, Gorczyca W, Melamed M. R, and Darzynkiewicz Z. Analysis of apoptosis by laser scanning cytometry. Cytometry *The Journal of the International Society for Analytical Cytology*, 35(3), (1999): 181-195.

40. Mercer W. E. Cell cycle regulation and the p53 tumor suppressor protein. Critical reviews in eukaryotic gene expression, 2(3), (1992): 251-263.

Cite this article as:

Kavitha Raja, Sharon Selvaraj and Arulvasu Chinnasamy. Inhibition of colon carcinoma cell lines by treatment with crude spine venom from marine catfish *Tachysurus dussumieri. International Journal of Bio-Pharma Research*, Volume 8, Issue 6 (2019) pp. 2607-2616.

http://dx.doi.org/10.21746/ijbpr.2019.8.6.2

Source of support: UGC, New Delhi, India.;
Conflict of interest: Nil.