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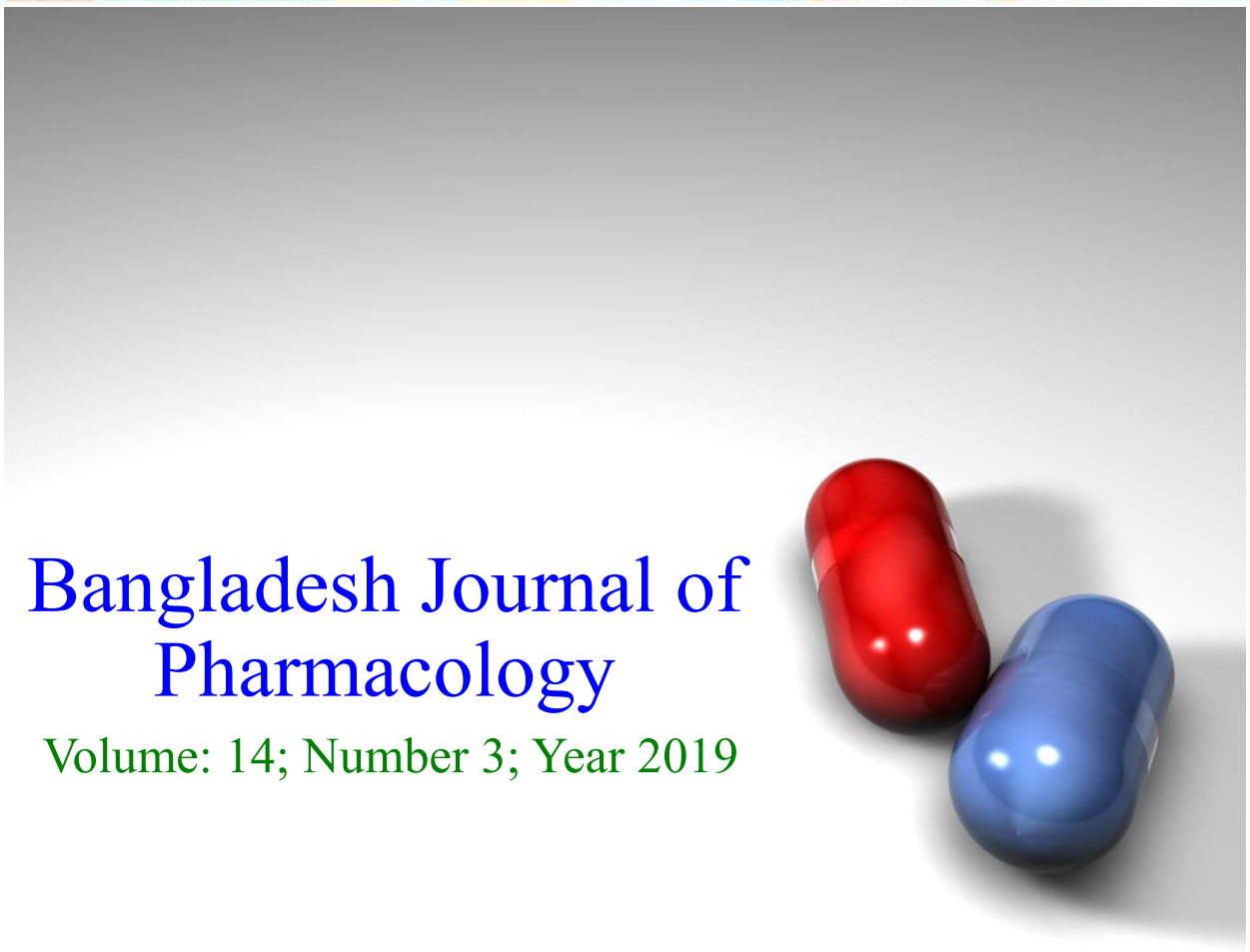

Bangladesh Journal of Pharmacology

Volume: 14; Number 3; Year 2019

Cite this article as: Kumari KA, Dagha RC, Gawali PG, Jadhav BL. Anti-inflammatory effect of *Actinia tenebrosa*. Bangladesh J Pharmacol. 2019; 14: 125-26.

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088; DOI: 10.3329/bjp.v14i3.41472
Bangladesh J Pharmacol 2019; 14: 125-126

Letter to the Editor

Anti-inflammatory effect of *Actinia tenebrosa*

Sir,

Sea anemone belonging to phylum cnidarian, possess radial symmetrical body with tentacles that surround a central mouth opening. Each nematocyst in the tentacles is heavily loaded with venom, used for defense against predators. The venom consists of numerous proteins, peptides, and chemical agents such as protease inhibitors, neurotransmitters (Chi et al., 2012), different peptides (Diochot et al., 2004; Kozlov et al., 2009; Monastyrnaya et al., 2002), actinoporins (Hu et al., 2011) and ion channel modulators (Abita et al., 1977) which seem to be potentially useful biologically active molecules. Sea anemone extracts also shows promising anti-bacterial property (Thangaraj et al., 2018).

There are few studies on *A. tenebrosa* for their cytolytic proteins (Anderluh and Maček, 2002; Maček, 1992) and haemolytic proteins (Norton et al., 1990; Simpson et al., 1990). Other than this, to the best of our knowledge, there is no report of any bioactivity from them.

Current work focuses on the *in vitro* anti-inflammatory activity of whole body methanolic extract of sea anemone *A. tenebrosa*.

A. tenebrosa were identified and collected post-monsoon in the month of October, 2017 from Wayangani beach area located at 16°55′40′′N and 73°16′56′′E Ratnagiri District, Maharashtra, India. Fresh sample was washed, dried at 40° C and pulverized. The dried sea anemone *A. tenebrosa* was homogenized to fine powder. 10g of this fine powder was immersed in 200 ml of methanol and maintained for 2 days. The solvent then was filtered through Whatman filter paper No. 1 (11 µm). It was then concentrated by using rotary flask evaporator (Buchi, Japan) to get the residues. The resulted compound was stored at 4°C for further use.

The protein denaturation inhibition bioassay was performed according to Sakat et, al. (2010) with some modifications where diclofenac sodium salt (Sigma Aldrich, USA) was used as standard. In 3 mL reaction mixture, 450 µL 1% w/v bovine serum albumin (HiMedia, India) was mixed with 50 µL of different concentrations of the methanolic extracts of the sample (assay concentrations: 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL and 600 µg/mL) and were incubated at 37°C for 20 min and then heated at 57°C for 5 min. After cooling the test tubes, 2.5 mL phosphate buffer saline (pH 6.3) was added to each tube and absorbance was read at 660 nm in UV-1800 visible spectrophotometer (Shimadzu Scientific, Japan). A control was used where no drug was added. Percentage protein denaturation inhibition at different concentra-tion of the extract and standard was calculated as per given equation. The assay was performed in triplicates.

$$\%\text{Inhibition} = [(A_c - A_{sample})/A_c] \times 100$$

Where, A_c = absorbance of the control, A_{sample} = absorbance of the extract

The data are represented as mean ± SD using One-way Anova analysis with SPSS 23.0 statistical software, significance was set at $p<0.05$. The half maximal inhibitory concentration (IC_{50}) value was calculated using Microsoft Excel 2010 package.

Table I

Anti-inflammatory activity of *A. tenebrosa*

Concentration (µg/mL)	*A. tenebrosa*		Diclofenac sodium	
	Protein denaturation inhibition (%)	IC_{50} value (µg/mL)	Protein denaturation inhibition (%)	IC_{50} value (µg/mL)
100	6.9 ± 0.3		43.2 ± 0.5	
200	10.2 ± 0.4		52.5 ± 1.1	
300	13.5 ± 0.4		61.7 ± 0.7	
400	18.1 ± 0.6	1090.3 ± 5.9	75.3 ± 0.4	174.4 ± 6.3
500	23.2 ± 0.3		82.0 ± 0.4	
600	29.5 ± 0.1		97.0 ± 0.2	

Data are mean ± SD; n=3

This study examined the *in vitro* anti-inflammatory property of *A. tenebrosa* using the whole body methanolic extract by protein denaturation inhibition bioassay (Table I). As shown, the methanolic extracts of whole body *A. tenebrosa* exhibits *in vitro* anti-inflammatory activity but with lower percentage inhibition even at high concentrations (range: 6.9 ± 0.3 - 29.5 ± 0.1% inhibition) with an IC_{50} value of 1090.3 ± 5.9 µg/mL. Conversely, diclofenac sodium salt, the standard, showed approximately 6 times more profound anti-inflammatory activity (range: 43.2 ± 0.5 - 97.0 ± 0.2% inhibition) with an IC_{50} value of 174.4 ± 6.3 µg/mL.

The peptides from nematocyst of sea anemones are reported to show anti-inflammatory activity. The IC_{50} value of *A. tenebrosa* extract when compared with IC_{50} value of standard shows that the whole body methanolic extract has less anti-inflammatory property. Different species of sea anemone are reported to exhibit antitumor activity, anti-parasitic activity, antimicrobial activity (Thangaraj et al., 2011), analgesic activity (Andreev et al., 2008), antiviral activity, anti-hypersensitivity activity (Driscoll et al., 1989), autoimmune activity (Diochot et al, 2004) and anti-inflammatory activity (Sintsova et al, 2015) from their nematocysts.

To the best of our knowledge no studies on whole body extract of *A. tenebrosa* has been reported for its bioactivity. Current study is the first ever attempt for extracting bioactive compound from whole body of *A. tenebrosa* for its anti-inflammatory property. Our results indicate that there is a decrease or synergistic effect taking place when whole body of sea anemone is considered for its bioactivities and thus we emphasize the need for isolation and purification of metabolites and polypeptides from nematocysts rather than considering whole body extracts to evaluate the true bioactive potency of *A. tenebrosa.*

The authors acknowledge Dr. Swapna Mohite and Mr. Bahar Mahakal, College of Fisheries, Shirgaon, Ratnagiri in collecting the specimen from the coast. Mr. Sandeep Kolte, Ms. Kiran Saroj and Ms. Snehal Vichare, University of Mumbai for their support in research activities.

The authors have declared that there is no conflict of interest.

Komal Arvind Kumari, Romil Champak Dagha, Poonam Gautam Gawali and Bhaskar Laxman Jadhav

Department of Life Sciences, University of Mumbai, Mumbai 400098, India.

Corresponding author: Bhaskar Laxman Jadhav
email: head@lifesciences.mu.ac.in; drbljadhav@gmail.com

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