WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 4, 764-776. Research Article ISSN 2277–7105

ANTI-INFLAMMATORY MECHANISMS OF CRUDE EXTRACT OF BOX JELLYFISH-CHIROPSOIDES BUITENDIJKI: IN VITRO EVALUATION OF ALBUMIN DENATURATION, ANTI-PROTEINASE, AND MEMBRANE STABILIZATION ACTIVITIES

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Article Received on
24 December 2024,

Revised on 14 Jan. 2025,
Accepted on 04 Feb. 2025

DOI: 10.20959/wjpr20254-35536

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ABSTRACT

The box jellyfish-*Chiropsoides buitendijki* were collected from Edwan village, of West Coast of Mumbai. The identification of the species was done at the CMFRI, ICAR laboratory Andheri, Mumbai. The ethical permission was sought from the MSBB for collection of samples and IAEC for using the animal for the experimentation. The samples were brought to the laboratory at Patkar-Varde College. The Methanol 80% and Acetic acid 1% crude extract of box jellyfish was prepared. The crude extract was further process to check the in-vitro anti-inflammatoryactivity by inhibition of albumin denaturation, anti-proteinase inhibitory activity, membrane stabilization by heat induced hemolysis. From the above experiment it was found the crude extract of box jellyfish showed good anti-inflammatory as well as membrane stability activity, it was also found to be effective to positive protein denaturation. So, it is concluded that the crude extract of box jellyfish-*Chiropsoides buitendijki* has effective and strong biomedical properties.

KEYWORDS: Anti-inflammatory, Membrane stability, Box jellyfish.

INTRODUCTION

Marine organisms, bioactive compounds have been extensively studied for their potential applications with medicinal properties such as, antiviral, anti-bacterial, anti-oxidant, anti-

tumour, anti-cancer, anti-inflammatory and cardio protective activities (Thitikan *et al*., 2023). Many studies investigated various marine sponges like *Aplysinacaissara*, *Haliclona* species are known for anti-inflammatory properties (Randazzo *et al*., 2001; Luciana *et al*., 2008).

Marine compounds found in cosmetic products include Estee Lauder's Resilience, which incorporates anti-inflammatory pseudopterosins derived from the soft coral *Pseudopterogorgia elisabethae* (Mayer *et al*., 1988). Research has examined the impact of harvesting soft corals and has illustrated a successful model for the sustainable use of marine species. Notably, clipped soft corals demonstrated faster branching and growth compared to the control group (Castanaro and Lasker, 2003). Furthermore, Pseudopterosin A has shown promise as a pharmaceutical candidate, having progressed to Phase II trials as a topical anti-inflammatory treatment. There is a growing demand for marine anti-inflammatory compounds in cosmetics, particularly as there is a significant need for new, sustainable, and UV-absorbing agents to replace existing sunscreen constituents, which may cause allergic reactions, irritation, or bioaccumulation. Fish oils, omega-3 fatty acids, and marine lipids are among the most recognized dietary supplements derived from the sea. Notably, the omega-3 fish oil product Lovaza has received approval from the FDA as a pharmaceutical. Out of approximately 40,000 species of algae, only a select few, such as *Chlorella vulgaris* and *Spirulina pacifica*, are utilized by the food industry (Moore, 1982).

Cnidarians have special cells called cnidocytes which gives the name to the phylum Cnidarians. Box jellyfish, belonging to the class Cubozoa, are invertebrates characterized by their distinct box-like, or cube-shaped, bodies. Certain species within this group possess highly toxic venom that is delivered through contact with their tentacles. Stings from specific species, such as *Chironexfleckeri*, *Carukiabarnesi*, and *Malo kingi*, among others, can be exceedingly painful and potentially lethal to humans. The venom of box jellyfish consists of a blend of bioactive proteins that can lead to strong hemolytic activity, cytotoxic effects, the formation of membrane pores, inflammation, in vivo cardiovascular failure, and fatal consequences in experimental animals (Badre, 2014 and Brinkman; Burnell, 2009).

There are several species of edible jellyfish, including *Lobonema smithii*, *Rhopilemae sculentum*, *Nemopile manomurai* and *Lobonemoides gracilis*, which is mostly based in Southeast Asia, produces an annual catch of more than 750,000 tones, with rising demand reaching outside of Asian markets (Nishimoto *et al*., 2008). Jellyfish species offer affordable raw materials for pharmaceuticals, nutraceuticals, and cosmetics due to high collagen and

protein content, antioxidant properties, and novel foods recognition in Europe (Li, *et al*., 2017; Omori and Nakano, 2001). Many studies are found on cnidocytes for several purposes, but very scanty study is available on their toxic potential (Moran *et al*., 2012). However, neurotoxin is found in the ectodermal gland cells. Therefore, extensive study is required not only on the nematocyst but also the whole body of jelly fish (Frazao *et al*., 2012). Earlier research has demonstrated that polysaccharides derived from *N. nomurai* and *R. esculentum* possess anti-inflammatory, antioxidant, and immunomodulatory effects. Additionally, proteins and other compounds extracted from jellyfish show notable antioxidant and various other biological activities. While there are numerous studies focused on cnidocytes for various applications, literature regarding their toxic potential remains limited (Assaw *et al*., 2010).

MATERIALS AND METHODS

a) Collection of samples

The box jellyfish-*Chiropsoides buitendijki* (Horst, R 1907) were collected during low tides from Edwan village, of West Coast of Mumbai. Animals were taken alive to the laboratory in sea water and then washed the animal two times under sea water and then rinse in distilled water. The collected samples were stored in ice cubes until they were transferred to the deep freezer at - 8^0 C at the Department of Zoology, S.S. & L.S. Patkar College of Arts & Science, and V. P. Varde College of Commerce & Economics, Goregaon west, Mumbai.

Photograph: Google Map showing the Collection site-Edwan village, of West Coast of Mumbai, Maharashtra.

b) Identification of box jellyfish

Preliminary identification was done by studying the shape and no. of tentacles and by referring the relevant literature and final confirmation of identification was done by Dr. Ramkumar, scientist, at the Central Marine Fisheries Research Institute (CMFRI), Mumbai.

c) Preparation crude extract of box jellyfish

Crude extract of *Chiropsoides buitendijki* (Horst, R 1907) was obtained following the 80 % methanol and 1% acetic acid by applying method of Braekman *et al.,* 1992 with some modifications. 10 grams of box jellyfish samples was grinded with blender and then 10 ml mixture of equal volume of 80 % methanol and 1% acetic acid, was added and kept standing for 24 hrs in a water bath at 45º C. The aliquot mixture obtained was filtered through Whatman filter paper No.1. The homogenate centrifuged at 5000 rpm for 15 minutes in cold centrifuge at -8º C (Remi centrifuge serial No. VCDX- 5983). The supernatant was collected in a conical flask and the aliquot was concentrated in at low pressure using rotary vacuum evaporator at 45^0 C. The resultant compound was subjected to Millipore filter system and finally dried in vacuum desiccators and stored at -20^0 C in a refrigerator till further use.

d) Ethical Approval

Ethical approval is sought from Maharashtra State Biodiversity Board, Nagpur, Maharashtra for collection of box jellyfish samples for research purpose (No.: MSBB/Desk-5/ /Research/ 841/2022-23) and (No.: MSBB/Desk-5/ /Research/ 397/2023-24). The voucher specimen of ***Chiropsoides buitendijki*** was submitted to the repository at the Zoological Survey of India, Western Regional Office, Pune (ZSI-WRC Misc/19), India.

In-vitro anti-inflammatory activity

1. Inhibition of albumindenaturation

The in vitro anti-inflammatory activity of unknown crude extracts of box jellyfish *Chiropsoides buitendijki* against egg albumin denaturationactivity was tested by the method proposed by (Dhara *et al,* 2016). The percentage of inhibition of protein denaturation activity was calculated using the following formula,

Percentage inhibition = (Abs control- Abs sample) X100 /Abs control

2. Anti-proteinase inhibitory activity

The in vitro anti-inflammatory activity of unknown crude extracts of box jellyfish *Chiropsoides buitendijki* against anti-proteinase inhibitory activity was tested by the

method proposed by (Dhara *et al*, 2016). The percentage of anti-proteinase activity was calculated using the following formula,

Percentage inhibition = (Abs control- Abs sample) X100 /Abs control

Membrane stabilization

Membrane stabilization test, the crude extracts of box jellyfish *Chiropsoides buitendijki* against preparation of red blood cell: (RBCs) suspension and heat-induced hemolysis was performed by the method proposed by (Mizushima *et al.*, 1968; Dhara Patel *et al*., 2016). The percentage inhibition of heat-induced hemolysis was calculated using the following formula,

Percentage Inhibition = (Abs control - Abs sample) × 100 / Abs control.

RESULTS AND DISCUSSION

Table No. 1.1: Showing the effect of crude extract of box jellyfish-*Chiropsoides buitendijki* and Standard drug diclofenac sodium on inhibition of albumin denaturation.

Sr. No.	Conc.of standard drug and crude extract	Absorbance at 660 nm standard drug	% inhibition of albumin denaturation of standard drug diclofenac sodium	Absorbance at 660 nm crude extract	% inhibition of albumin denaturation of crude extract of *Chiropsoides buitendijki*
1	100 µg/mL	0.18	51.35	0.3	18.91
2	200 µg/mL	0.15	59.45	0.2	45.94
3	300 µg/mL	0.13	64.86	0.2	45.94
4	400 µg/mL	0.10	72.97	0.1	72.97
5	500 µg/mL	0.07	81.08	0.09	75.67

(Each analysis was achieved by five replicates)

Negative control- 0.37nm

Table No. 1.2: Showing the effect of crude extract of box jellyfish-*Chiropsoides buitendijki* and standard drug diclofenac sodium on anti-proteinase activity.

Sr. No.	Conc.of standard drug and crude extract	Absorbance at 660 nm Std.	% inhibition of enzyme proteinase in anti-proteinase activity of standard drug	Absorbance at 660 nm extract	% inhibition of enzyme proteinase in anti-proteinase activity of crude extract of *Chiropsoides buitendijki*
1	100 µg/mL	0.110	13.38	0.079	37.79
2	200 µg/mL	0.044	65.35	0.056	55.90
3	300 µg/mL	0.041	67.71	0.048	62.20
4	400 µg/mL	0.034	73.22	0.044	65.35
5	500 µg/mL	0.030	76.37	0.042	66.92

Negative control- 0.127 nm

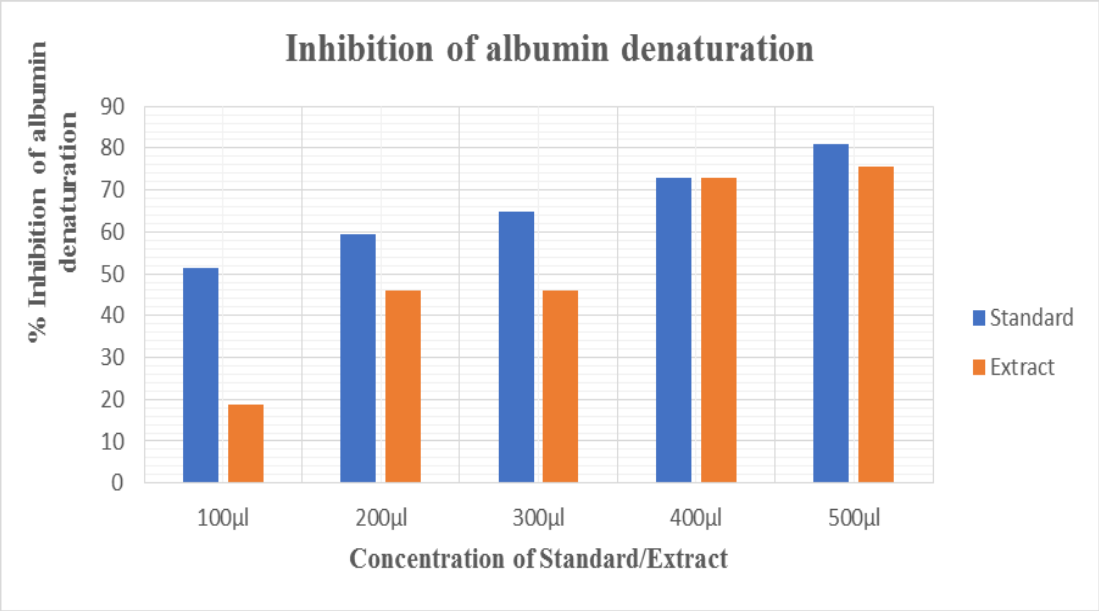
(Each analysis was achieved by five replicates)

Table No. 1.3: Showing the effect of crude extract of box jellyfish-*Chiropsoides buitendijki* Membrane stability by Heat induced hemolysis.

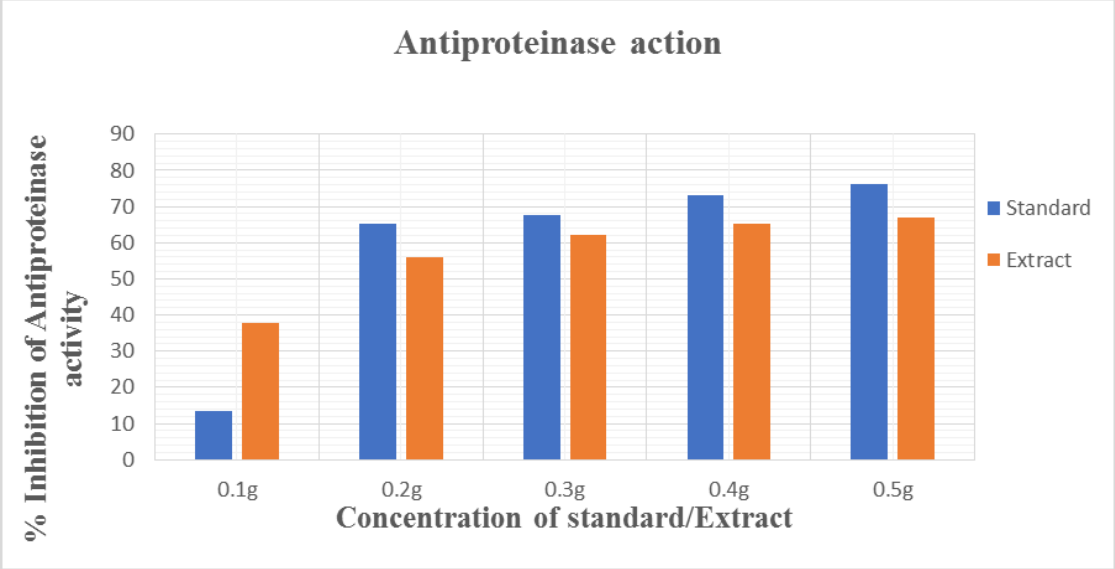
Sr. No.	Conc.of standard drug and crude extract	Absorbance at 560 nm Std.	% inhibition of RBC hemolysis of standard drug diclofenac sodium	Absorbance at 560 nm extract	% inhibition of RBCs hemolysis of crude extract of *Chiropsoides buitendijki*
1	100 µg/mL	0.22	64.51	0.43	30.64
2	200 µg/mL	0.11	82.25	0.40	33.87
3	300 µg/mL	0.09	85.48	0.35	48.38
4	400 µg/mL	0.06	90.32	0.21	66.21
5	500 µg/mL	0.05	91.93	0.09	87.09

Negative control- 0.62 nm

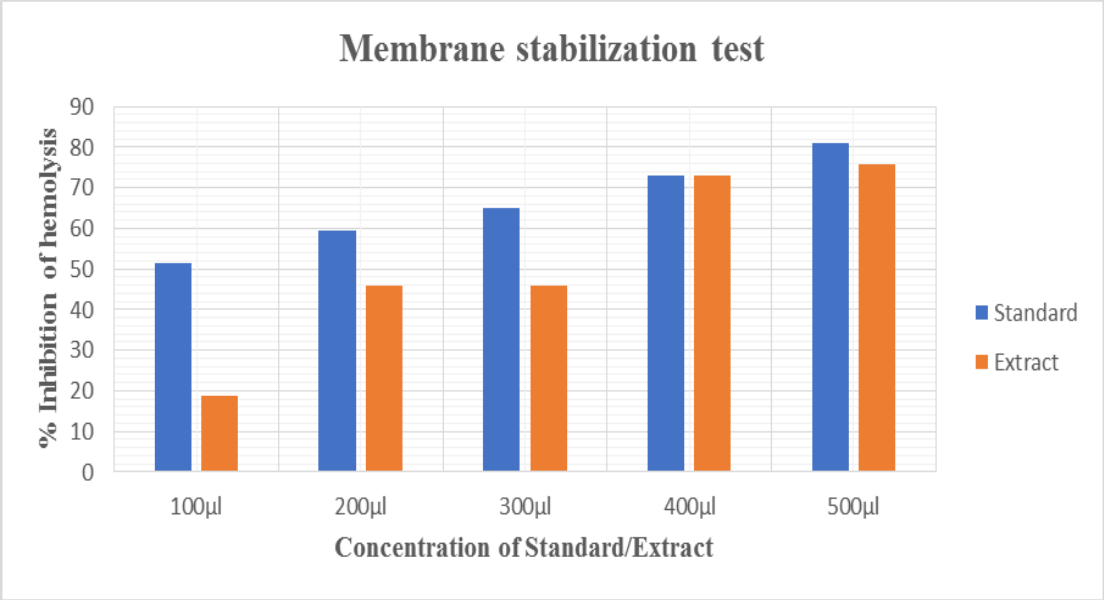
(Each analysis was achieved by five replicates**)**



Graph Showing the effect of crude extract of box jellyfish-*Chiropsoidesbuitendijki* and standard drug diclofenac sodium on inhibition of albumin denaturation.



Graph Showing the effect of crude extract of box jellyfish-*Chiropsoides buitendijki* and standard drug diclofenac sodium on anti-proteinase activity.



Graph showing the effect of crude extract of box jellyfish-*Chiropsoides buitendijki* and standard drug Diclofenac sodium on Heat-Induced Hemolysis (Membrane stabilization test).

DISCUSSION

Angela *et al.*, (2009) studied the protective effect of melatonin against the inflammatory response caused by the crude venom of *Pelagia noctiluca*. They injected rats with crude venom and observed significant inflammation, with increased MPO activity in paw tissues 5 hours post-injection. These results align with findings by Mantovani *et al*, (2006). Parkar *et al.*, (2007) found a similar inhibitory effect of morphine and diclofenac on the formalin tested mice. They have tested and confirmed the anti-inflammatory effects exhibited by the drugs morphine and diclofenac. Thitikan *et al*., (2023) studied in vitro anti-inflammatory activity against the extract of *Lobonema smithii* jellyfish. They confirmed that, the polysaccharides extracted from *Lobonema smithii* jellyfish exhibit good anti-inflammatory activity. They suggested that the jellyfish could be used as an alternative therapeutic agent against inflammation. The brown algal polysaccharides extracted from *S. cristaefolium* with an Mw of 386.1 kDa and a sulphate concentration of 9.42% reduced the production of LPS-stimulated RAW 264.7 cells. Thus, it confirmed that brown algal polysaccharide shows anti-inflammatory properties. The similar study has been carried out by Shi *et al*., (2016), Xiong *et al*., (2017), Wang *et al*., (2015), and Wang *et al*., (2019), they found the glycosidic linkage could affect anti-inflammatory activities; α-D (1 → 3)-linked glucose shows anti-inflammatory activities and anti-CAG activities (chronic atrophic gastritis). Similar results were reported by Castro *et al*., (2014) and Lee *et al*., (2013), according to their study, the

existence of monosaccharides could be a factor that affects the anti-inflammatory activities of polysaccharides, glucose, and fructose and has been found to have a good effect on inflammatory activities. Protein denaturation has been correlated with the formation of inflammatory disorders like *Rheumatoid arthritis*, diabetes and cancer. Therefore, ability of substance to prevent the protein denaturation may also help to prevent the inflammatory disorders (Maionea*et al.*, 2015). According to Das and Chatterjee, (1995), a significant level of protection was provided by proteinase inhibitors. The presence of bioactive compounds may contribute to their anti-inflammatory activity which exhibited significant antiproteinase activity in dose-dependent manner.

Leucocytes played a vital role in cellular inflammation, which is an important aspect of an inflammatory response. Stabilization of the RBCs membrane provides evidence for the mechanism of anti-inflammatory effect (Okoli *et al*., 2008). The heat induced hemolysis was effective inhibiting activity in which these cell membranes may retard or inhibit the lysis and subsequent release of the cytoplasmic contents which, in turn, minimize the tissue damage and, hence it is called, inflammatory response (Mizushima & Kobayashi, 1968). Therefore, substances that contribute significant protection of cell membrane against injurious substances are important in the event of inhibiting the progression of inflammation.

The present study was undertaken to investigate in vitro anti-inflammatory activity of the crude extract of box jellyfish *Chiropsoides buitendijki* prepared in organic solvent, 80 % methanol and 1%acetic acid to find anti-inflammatory activity. From the above experiments it was observed that Diclofenac sodium, a strong anti-inflammatory drug which induce average albumin denaturation at 65.94% inhibition, while, the crude extract of *Chiropsoides buitendijki* exhibited 68.89% inhibition. The activity of the crude extract and standard drugagainst proteinase inhibition the diclofenac sodium showed average proteinase inhibition at 85.06 %. Whereas, the crude extract of box jellyfish *Chiropsoides buitendijki*s howed at 82.02 % inhibition. The membrane stability test was performed by using heat induced hemolysis of diclofenac sodium (standard), the average heat induced hemolysis was evaluated as 82.89% inhibition. Whereas, the average heat induced hemolysis was observed in crude extract of box jellyfish at 53.23% inhibition concentration. Graphs indicated that the crude extract of box jellyfish *Chiropsoides buitendijki* showed the property of anti-inflammatory, anti-proteinase, membrane stability, and heat induced hemolysis. These properties were confirmed by evaluating the active constituents present in the crude extract of

box jellyfish *Chiropsoides buitendijki*. We have separated the crude extract by using TLC and it was confirmed that the isolated compounds contain alkaloids, steroids, glycosides, and terpenoids which have anti-inflammatory property.

From the above study, the crude extract of *Chiropsoides buitendijki* (box jellyfish) demonstrated strong albumin denaturation properties, confirming its in vitro anti-inflammatory activity. Also evaluated its anti-proteinase activity, which showed significant inhibition of proteinase enzymes, suggesting an additional anti-inflammatory mechanism. In the membrane stabilization test, the extract exhibited strong protection against heat-induced hemolysis, likely due to the release of lysosomal enzymes from neutrophils, which could help stabilize membranes at sites of inflammation.

CONCLUSION

Many marine toxins have risen in the field of novel drug discovery and proven its wide spectrum of pharmaceutical and biomedical potential. Therefore, the study suggests that, further screening of box jellyfish-*Chiropsoides buitendijki*, is required for molecular level to understand the physiology and mode of action of these compounds. The clinical study is also required which may be useful for pharmaceutical industry to manufacture the new drugs for safe performance and safety indexes to be studied to eradicate the diseases from mankind in future.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Ramkumar, scientist, at the Central Marine Fisheries Research Institute (CMFRI), Mumbai for final identification and confirmation of species. Authors are also thankful to the Director, Maharashtra State Biodiversity Board, Nagpur, Maharashtra for giving permission for collection of species.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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