RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2013.15.21

Anti-inflammatory, Anti-ulcer, Antipyretic, Analgesic and Cns Stimulant Activities of Marine Bryozoan *Zoobotryon verticillatum*

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ABSTRACT

Objective: To evaluate the anti-inflammatory, antiulcer, antipyretic, analgesic and CNS stimulant activities of crude extract of marine bryozoan *Zoobotryon verticillatum*. **Methods:** The bryozoan *Z. verticillatum*, collected by snorkeling from Tuticorin coastal waters, was washed thoroughly with sterile seawater, rinsed with sterile distilled water, air-dried for 24 h at room temperature, macerated in a warring blender and extracted repeatedly with diethyl ether. The pooled extracts was cold steeped overnight at -18°C, filtered with Whatman No.1 filter paper and evaporated to dryness in a rotary evaporator. The extract was investigated for pharmacological properties by following standard methods using adult albino rats. **Results:** In the anti-inflammatory assay, extract at 100 mg kg^{-1} showed significant activity. The analgesic activity of the extract at 200 mg kg^{-1} was comparable to that of positive control (Pentazocine) and was highly significant ($p<0.001$) when compared to negative control. The extracts showed dose dependent and significant ($p<0.01$) antipyretic activity. The bryozoan extract, at 200 mg kg^{-1}, showed Central Nervous System (CNS) stimulant activity (120.8%), comparable to that of positive control (Caffeine). The bryozoan extracts exhibited dose dependent anti-ulcer activity. Extracts showed significant ($p<0.001$) reduction in ulcer index, enhanced pH and serum calcium level, decreased volume of gastric juice, total acidity, free acidity and alkaline phosphatase, indicating the anti-ulcer property. **Conclusion:** The study indicated that the bryozoan *Z. verticillatum* extract possess pharmacological properties.

Key words: *Zoobotryon verticillatum,* bryozoan, anti-inflammatory, antiulcer, antipyretic, analgesic, CNS Stimulant

Pharmacologia 4 (1): 15-21, 2013

INTRODUCTION

Nature offers wide scope as plants and microbes have been the source of medications from ancient days. In this context, the rich diversity of marine organisms, due to their unique physiological adaptations to the harsh marine environment, produce natural products which offer a good source of pharmacologically active agents with the potential to produce valuable therapeutic entities (Thakur *et al*., 2005; Glaser and Mayer, 2009).

The number of natural products isolated from marine organisms exceeded 18,000 in 2007 (MarinLit, 2007). Though a wide range of useful drugs including antibiotics, analgesic, anti-inflammatory, anticoagulants, CNS depressants, antipyretic agents etc., have been isolated from marine organisms, only a few marine derived products are in the market and several of them are in clinical trials (Thakur *et al*., 2005).

Inflammation is a tissue directed response to noxious and injurious external and internal stimuli which is mediated by arachidonic metabolites (Taranalli *et al*., 2009). Most of the conventional anti-inflammatory drugs, particularly steroids and cyclooxygenase inhibitors, are often associated with adverse side effects including gastro-intestinal irritation, ulcers, hypertension and cardiac abnormalities. So, there is a continuous search for the alternative anti-inflammatory agents from natural sources in order to avoid inherent problems associated with currently used anti-inflammatory drugs (Patil *et al*., 2010). Many anti-inflammatory compounds have been reported from marine environment such us africanene from the soft coral *Sinularia erecta* (Reddy *et al*., 1999), Cacospongiolide B from sponge (Pastor *et al*., 1999) palinurin from the Mediterranean sponge *Ircinia variabilis*, palinurines A, B from the fungus *Cunninghamella* sp. (El-Sayed *et al*., 1999) and Pseudopterin from gorgonian (sea whip) *Pseudopterogorgia elisabethae* (Look *et al*., 1986).

Gastric ulcer is a one of the most widespread diseases in the world (Alkofahi and Atta, 1999) and peptic ulcer is a serious gastrointestinal disorder and it is common in India (Patil *et al*., 2010). Anti-ulcer drugs have some side effects and hence, a constant search is on to find a new effective anti-ulcer compound. Also, the

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current analgesic agents have adverse side effects like gastric lesions caused by NSAIDs and tolerance and dependence induced by opiates Patil *et al.* (2010).

The most interesting organisms of pharmacological significance inhabiting the complex ecosystems of the marine environment are bacteria, fungi, algae, sponges, soft corals, tunicates, molluscs and bryozoans (Faulkner, 2000). Among them, the bryozoans also have proved to be an excellent source of novel and biologically active compounds (Prinsep *et al.*, 2004). So, the present study was aimed at to study the pharmacological potential especially the CNS stimulant, anti-inflammatory, analgesic, antipyretic and antiulcer activities of the crude diethyl ether extract of the bryozoan *Zoobotryon verticillatum.*

MATERIALS AND METHODS

Extraction: The bryozoan *Zoobotryon verticillatum*, collected by snorkeling along Tuticorin coastal waters (Lat 8°45 and Long 78°13'E) of southeast coast of India, were immediately brought to the laboratory, washed thoroughly with sterile seawater, rinsed with sterile distilled water, air-dried for 24 h at room temperature, macerated in a warring blender and extracted repeatedly with diethyl ether. The extracts were then pooled, cold steeped overnight at -18°C, filtered with Whatman No.1 filter paper and evaporated to dryness in a rotary evaporator (Becerro *et al.*, 1994; Riguera, 1997; Wright, 1998). The concentrated extract was used for assessment of pharmacological studies. One percent sodium lauryl sulphate was used as a vehicle for bryozoan diethyl ether extract.

Experimental animals: Albino rats of either sex weighing between 120-180 g were used for the experiments after prior approval of Institutional Animal Ethics Committee (IAEC) of Department of Pharmacology, SB college of Pharmacy, Sivakasi. The animals were maintained under standard environmental conditions (temperature of 22 ± 1^0C with an alternating 12 h light-dark cycle and relative humidity of $60 \pm 5\%$) and were fed with standard diet and water *ad libitum.*

Anti-inflammatory activity: The carrageenan (Sigma, 0.05 mL of 1% w/v), injected subcutaneously into the sub-plantar region of the right hind paw of albino rats, induced paw edema was measured according to the method of Winter *et al.* (1962). Four groups of rats with four individuals each were used. The Group I which served as control was given saline (1 mL kg^{-1}) and the Group II served as standard and received standard drug Diclofenac sodium (10 mg kg^{-1}). The Group III and IV received bryozoan test extracts at 100 and 200 mg kg^{-1} p.o. one hour before Carrageenan injection. Then after one hour, carrageen was injected subcutaneously into the sub planter region of the right hind paw and the thickness of right paw was measured before and after carrageenan injection at time intervals 0, 1, 2, 3, 4, 5 h. The percentage increase in paw edema thickness was calculated (Duwiejua *et al.*, 1994).

Analgesic activity: The assessment of analgesic activity was carried out by measuring the sensitivity of the tip of the tail (last 1-2 cm) of adult albino rats placed gently in warm water maintained at 55 ± 2°C and the active rats flicking the tail within 5 seconds were selected for the study. The active rats were divided into four groups of four animals each. The Group I was the control and received normal saline. The Group II was the standard reference group and received Pentazocine (4 mg kg^{-1}). The Group III and Group IV animals received bryozoan extracts at 100 mg and 200 mg kg^{-1}, respectively. The basal reaction time of all groups of animals after treatment was recorded at different time intervals of 0, 1, 2 and 3 h (Turner, 1965; Kulkarni, 1999).

Antipyretic activity: Antipyretic activity was carried out by using Brewer's yeast induced hyperpyrexia using digital Telethermometer (TNCO) (Asha and Pushpangadan 1999; Rawat and Malviya 2010). The Group I was the control and received normal saline. The Group II was standard reference group and treated with Paracetamol (45 mg kg^{-1}). The Group III and Group IV were given bryozoan extracts at 100 and 200 mg kg^{-1}, respectively. The rectal temperature was recorded at 1, 2, 3, 4 h after administration of the test drug/extracts.

Central nervous system (CNS) stimulant activity: The spontaneous locomotor activity and rearing were measured in a computerized locomotion detection system (actophotometer) equipped with photosenser (Asakura *et al.*, 1993). The rats were individually placed in a transparent cage ($25 \times 48 \times 18$ cm^3) and the locomotor activity and rearing were recorded for 10 min. The animals were divided into four groups with Group I serving as an untreated control. The Group II was treated with standard Caffeine (4 mg kg^{-1}, i.p.,) and group III and IV were treated with bryozoan extracts at a dosage level of 100 and 200 mg kg^{-1}. The locomotor activity was again observed after 30 min of drug administration for 10 min and the percentage of changes in the activity was recorded.

Anti-ulcer activity: Albino rats (120-180 g) were starved for 48 h with access only to water. During this time, they were housed singly in cages with raised bottoms of wide wire mesh in order to avoid cannibalism and coprophagy. Animals were divided into four groups of four animals each. The Group I (control group) received normal

saline and the Group II (standard reference group) was treated with Ranitidine (13.5 mg kg^{-1}). Group III and Group IV were treated with bryozoan extracts at 100 and 200 mg kg^{-1}, respectively (Suzuki *et al.*, 1976). All the animals received the test extracts for three days and on the fourth day, under ether anesthesia, a midline abdominal incision was made. The pylorus was ligated (Shay *et al.*, 1945) and care was taken that neither damage to the blood supply nor traction on the pylorus occurred. The abdominal wall was closed by suturing.

The animals were deprived of food and water post operatively and the animals were sacrificed after 19 h of Pyloric ligation. Blood samples were withdrawn from the marginal tail vein and subjected to estimation of serum alkaline phosphatase and serum calcium. Alkaline Phosphatase was estimated by Kind and King's method (Kind and King, 1954). Serum calcium was estimated by Ortho Cresolphthalein Complexone (OCPC) method (Schwarzenbach, 1955, Biggs and Moorehead, 1974). The stomach was dissected out along the greater curvature and examined for lesions. The mucosa was then washed and extent of ulceration was scored (Kunchandy *et al.*, 1985). The gastric juice was collected from the stomach, centrifuged at 3000 rpm for 30 min and pH, free acidity, total acidity were estimated. The results were compared with control.

Statistical analysis: The values were expressed as Mean±SD of 4 animals in each group. The data were then analyzed by one way ANOVA followed by Dunnett's test.

RESULTS

Anti-inflammatory activity: In anti-inflammatory assay, increase in paw edema in control was observed till the 2nd hour after Carrageenan injection (Table 1). The increase in paw volume was 0.398. After 5 h, the paw volume got decreased in all experiments including that of negative control. But, the decrease was significant in positive control and that of 100 mg kg^{-1} bryozoan extract group. The increase in paw volume was higher in 200 mg kg^{-1} extract (0.243 mm) than that of 100 mg kg^{-1} group (0.153 mm) at the end of 1st hour. At the end of 5th h, the 100 mg kg^{-1} extract was significantly low (0.001 mm) than the initial level. When compared to the negative control, the positive control and that of extract groups showed significant reduction in paw volume at the end of 5th h. Both positive control and that of extracts were significant ($p<001$) when compared to the negative control.

Analgesic activity: The reaction time increased over the time in positive control (Pentazocine) until the observation at the end of 3 h (Table 2). In the extracts also, the reaction time increased over the time, though lower than the positive control. The extract at 200 mg kg^{-1} was comparable to that of positive control. When compared to the negative control, the reaction times in positive control and that of extracts were highly significant ($p<0.001$) except that of 100 mg kg^{-1} dose at the end of 1st h wherein it was significant ($p<0.01$).

Antipyretic activity: The positive control and that of bryozoan extracts showed dose and time dependent activity (Table 3). Gradual decrease in temperature was noticed over the time. The reduction in temperature with extract at 200 mg kg^{-1} was comparable to that of positive control. The activity of test extracts at 100 and 200 mg kg^{-1} at 4 h was significant ($p<0.01$) when compared to the control.

Central nervous system (CNS) stimulant activity: The positive control, caffeine showed 131.4% stimulation of locomotor activity (Table 4). The bryozoan extract, at

Table 1: Anti-inflammatory activity of diethyl ether extract of bryozoan *Zoobotryon verticillatum*

		Increase in paw Paw edema (mm)					
Treatment	Dose (mg kg^{-1})	0 h	1 h	2 h	3 h	4 h	5 h
Control	-	0.584±0.008	0.927±0.009	0.982±0.007	0.937±0.005	0.849±0.007	0.712±0.007
Diclofenoc sodium	10	0.599±0.007^{ns}	0.765±0.004*	0.742±0.007*	0.643±0.008*	0.615±0.008*	0.595±0.008*
Bryozoan	100	0.599±0.006^{ns}	0.752±0.006*	0.736±0.007*	0.653±0.01*	0.613±0.008*	0.598±0.008*
	200	0.578±0.007^{ns}	0.821±0.006*	0.715±0.006*	0.636±0.007*	0.604±0.007*	0.588±0.007*

n=4; values are Mean±SEM; ns-Non-significant; *p<001 compared to control

Table 2: Analgesic activity of diethyl ether extract of bryozoan *Zoobotryon verticillatum*

		Reaction time (seconds) after drug administration		
Treatment	Dose (mg kg^{-1})	1 h	2 h	3 h
Control	-	2.65±0.48	2.75±0.25	2.5±0.29
Pentazocine	4	6.5±0.65**	9.25±0.48**	12.25±0.48**
Bryozoan	100	5.5±0.65*	6.75±0.48**	7.75±0.48**
	200	6.5±0.65**	8.25±0.75**	9.75±0.86**

n=4; values are Mean±SEM; *p<0.01, **p<0.001 compared with control

Table 3: Antipyretic activity of diethyl ether extract of bryozoan *Zoobotryon verticillatum*

Treatment	Dose (mg kg^{-1})	Initial rectal temp. (°C)	Initial pyrexia (°C)	Rectal temperature °C (Mean±SEM) 1 h	2 h	3 h	4 h	Reduction of Temp. (°C)
Control	-	37.53±0.09	38.15±0.16	38.16±0.09	38.16±0.09	38.15±0.09	38.17±0.1	--
Paracetamol	45	37.59±0.23	38.20±0.17	37.96±0.14^{ns}	37.78±0.14^{ns}	37.72±0.14^{ns}	37.59±0.11^{ns}	0.61
Bryozoan	100	37.29±0.08	37.93±0.11	37.69±0.1*	37.53±0.11**	37.46±0.20*	37.34±0.17**	0.59
	200	37.42±0.08	38.08±0.13	37.83±0.11^{ns}	37.65±0.11*	37.57±0.1^{ns}	37.42±0.07**	0.66

n=4; values are Mean±SEM; ns-non significant; *p<0.05, **p<0.01 when compared to control

Table 4: CNS stimulant activity of diethyl ether extract of bryozoan *Zoobotryon verticillatum*

Treatment	Dose (mg kg^{-1})	Mean locomotor activity scores in ten minutes: Before treatment	After treatment	(%) increase in locomotor activity
Control	-	163.5±1.708	163.5±1.708	0
Caffeine	4	170.5±2.218	224±3.163	131.4
Bryozoan	100	201±1.291	200.5±1.708	-0.3
	200	173±1.291	209±13.329	120.8

n=4; values are mean±SEM

Table 5: Anti-ulcer activity of diethyl ether extract of bryozoan *Zoobotryon verticillatum*

Treatment	Dose (mg kg^{-1})	Volume of gastric juice	pH	Free acidity (mEq L^{-1})	Total acidity (mEq L^{-1})	Ulcer scores (UI L^{-1})	Serum alkaline phosphate (mg dL^{-1})	Serum calcium (mg dL^{-1})	(%) of ulcer inhibition
Control	-	9.50±0.129	2.40±0.06	96.65±0.221	119±0.183	2.50±0.287	48.81±0.08	9.74±0.041	--
Ranitidine	13.5	2.75±0.171*	4.89±0.013*	33±0.183*	58.50±0.129*	0.25±0.143*	12.78±0.081*	11.57±0.53*	90
Bryozoan	100	5.50±0.129*	3.74±0.089*	45.00±0.183*	66.70±0.388*	0.75±0.143*	23.53±0.068*	10.75±0.047*	70
	200	3.60±0.183*	4.57±0.017*	34.35±0.222*	63.20±0.183*	0.50±0*	14.49±0.072*	11.72±0.039*	80

n=4; values are Mean±SEM; *p<0.001 compared with control group

200 mg kg^{-1}, showed 120.8% stimulant activity, comparable to that of positive control. But, the extract at 100 mg kg^{-1} dosage showed no stimulant activity and the activity was almost flat down by 0.3%. The results indicated the stimulant activity of the bryozoan extract at higher concentrations.

Anti-ulcer activity: The bryozoan extracts showed dose dependent anti-ulcer activity (Table 5). The ulcer score decreased significantly ($p<0.001$) in both positive control and bryozoan extracts. Ranitidine showed 90% inhibition closely followed by 200 mg kg^{-1} of extract with 80%.

The volume of gastric juice was comparatively low in both positive control and test extracts. The 200 mg kg^{-1} dosage was comparable to that of positive control. The free and total acidity got reduced significantly when compared to negative control and the 200 mg kg^{-1} was comparable to that of positive control. The pH got increased in positive control and test extracts indicating the anti-ulcer activity against gastric pyloric ulcers. The increase in pH in 200 mg kg^{-1} dosage was significant and comparable to that of positive control. Also, the serum alkaline phosphatase level decreased in positive control and test extract treated animals and the serum calcium level increased. In both these parameters, the 200 mg kg^{-1} extract was comparable to that of positive control.

DISCUSSION

In the present study, the diethyl ether crude extract of the marine bryozoan *Zoobotryon verticillatum* showed good anti-inflammatory, anti-ulcer, antipyretic, analgesic and CNS stimulant activities. Marine compounds like astaxanthin, bolinaquinone, cacospongionolide B, clathriol B, conicamin, cycloamphilectene 2, elisabethadione, plakohypaphorine, pourewic acid A, methylpourewate B, cadlinolide C, petrocortyne A, petrosaspongiolides M-R, pseudopterosin N, pseudopterosin R and seco-pseudopterosin E were earlier reported as anti-inflammatory compounds in pharmacological studies (Mayer and Hamann, 2002, 2004, 2005; Mayer and Lehmann, 2000). The sponges *Haliclona*, *Petrosia* and *Discodemia* produced powerful anti-cancer and anti-inflammatory agents (Blunt *et al.*, 2004). The marine compound Manoalide isolated from the sponge *Luffariella variabilis,* inhibited inflammation (Glaser and Jacobs, 1986, 1987) and novel sesterterpenes type anti-inflammatory drugs were isolated and characterized from corals (Shin *et al.*, 1991) and sponge (Pastor *et al.*, 1999).

The dosage level of 100 and 200 mg kg^{-1} in which significant anti-inflammatory activity observed was comparatively lower than that of potent anti-inflammatory activity at 300 mg kg^{-1} concentration of Pseudopterin compound isolated from gorgonian

(sea whip) *Pseudopterogorgia elisabethae* (Look *et al*., 1986). But, the dosage in the present study was higher when compared to anti-inflammatory activity of marine soft corals *Sinularia crassa* and *Lobophytum* sp. derived sphingosine derivative and cembrenoid diterpene at 5 mg and 10 mg kg^{-1} dosage level (Radhika *et al*., 2005).

The bryozoan extract in the present study showed increasing analgesic activity with increasing extract concentration and the 200 mg kg^{-1} dosage was comparable to the standard drug. This coincided with the similar observation made with the ascidian *Eudistoma viride* extracts (Rajesh RP. Bioactive natural products from marine ascidians. Ph.D. Thesis, Manonmanium Sundaranar University, Tirunelveli. India; 2008). It was substantiated by the observation of analgesic activity in the acetone and methanol extracts of scallop *Minnivola pyxidata* (Jayaseeli AA. A study on some aspects of biology of scallops in Gulf of Manner, Southeast coast of India. Ph.D. Thesis. Manonmanium Sundaranar University; 2004). Similar analgesic activities were reported from pseudopterosins isolated from the gorgonian *Pseudopterogorgia elisabethae* (Look *et al*., 1986; Roussis *et al*., 1990) and Egyptian Red Sea sponge extracts (Fakhr *et al*., 2006).

The bryozoan *Zoobotryon verticillatum* indicated the presence of CNS stimulant potential. Similar stimulant activities were reported in the marine invertebrates *Melibe rangi, Ircina ramose, Leptodius arassimanus* and *Acanthaster planci* (Bhakuni and Rawat, 2005). The present result was further substantiated by the good CNS stimulant activity of the seaweed extracts of *Sargassum tenerrimum* and *Caulerpa sertularioides* and the ethanolic extracts of *Pocockiella variegata, Sargassum cinereum* from India (Kamat *et al*., 1991, 1994) and the excellent CNS stimulant activity of about 19.5 and 203.5% in *Laurencia papillosa* extract at the concentrations of 100 and 200 mg kg^{-1} (Arul Senthil K. Bioactive natural products from seaweeds of southeast coast of Tamil Nadu. Ph.D. Thesis, Manonmaniam Sundaranar University, Tamil Nadu, India; 2008).

Many marine organisms like *Cypraea errones* and *Cypraea arabica* (Kumar SS. Studies on the Cowries (Mollusca: Gastropoda: Cypraeidea) of Gulf of Mannar, Southeast coast of India. Ph. D Thesis, Manonmaniam Sundaranar University, Tirunelveli; 2003) were shown to possess antipyretic activity. The concentration in the present study was much higher than that of 10 mg kg^{-1} reported for *Cypraea errones* and *Cypraea arabica* (Kumar SS. Studies on the Cowries (Mollusca: Gastropoda: Cypraeidea) of Gulf of Mannar, Southeast coast of India. Ph. D Thesis, Manonmaniam Sundaranar University, Tirunelveli; 2003). The reduction in temperature was prominent in the 1st hour and stabilized after that. This may be due to the fact that the test extracts and standard drug may be metabolized within one hour (Lane *et al*., 2002).

Ranitidine and bryozoan extract (100 and 200 mg kg^{-1}) showed reduced ulcer index, enhanced pH, decreased volume of gastric juice, total acidity and free acidity. The increase in serum calcium level observed in the present study was important as deficiency of calcium may be a new wire-puller of aphthous ulcer (Lingyong *et al*., 2009). Alkaline phosphatase lead to bone diseases, liver diseases and gastrointestinal lumen and it plays a role in tissue necrosis, associated with gastrointestinal ulceration (Obi *et al*., 2000). So, the decrease in alkaline phosphatase level in the current study indicated that the bryozoan extracts possess anti-ulcer characteristics.

The antiulcer and anti-inflammatory activities observed in bryozoan extract substantiated the report that some compounds possess both antiulcer and anti-inflammatory activities and this additional effect may help in complementing the natural anti-inflammatory activity (Patil *et al*., 2010). The study indicated the presence of anti-inflammatory, analgesic, antipyretic, anti-ulcer and CNS stimulant activities. Among these, the extract showed prominence in anti-ulcer and CNS stimulant activities.

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