

In vivo Anti-inflammatory bustle of reef associated mollusc, *Trochus tentorium*

C.Chellaram and J. K. P. Edward

Abstract

The 100% acetone fraction of the gastropod, *Trochus tentorium* tested for its anti-inflammatory assets on isolated albino rats shown promising results. The acute toxicity was noted, the LD50 was found to be 425mg/kg in 48 hrs of observation. Oral administration of doses up to 1.25g/kg did not show any toxic symptom in mice. The extract of *T. tentorium* at the concentration of 100 and 200mg/kg, p.o shown significant ($p < 0.01$) decrease in the paw thickness, 41.15 and 73.6% respectively at the 5th hour of the experiment. This fact suggests that the compound responsible for the anti-inflammatory action of the *T.tentorium* was the strongest with safety than standard drug, Diclofenac sodium.

Key words: Anti-inflammatory drug, marine gastropod, reef,

Introduction

Marine organisms comprises approximately a half of the total biodiversity, thus offering a vast source to discover useful therapeutics. In the recent years, a significant number of novel metabolites with potent pharmacological properties have been discovered from marine organisms. So far few marine derived products are currently in the market and several marine natural products are now in clinical trials (Fenical, 1997). This environment encompasses of complex ecosystems and many of its inhabitants especially sessile or low moving benthic animals are known to possess bioactive compounds as a common means of defense which are frequently difficult to synthesize. These compounds are usually a part of highly toxic defense mechanisms which is a reflection of the highly competitive and solute environment in which the organism resides (Grabley and Thierjcke, 1999).

The most interesting phyla with respect to pharmacologically active marine compounds include bacteria, fungi, algae, sponges, soft corals, tunicates, molluscs or bryozoans (Faulkner, 2000). Among the marine invertebrates, the molluscs are a potential source of bioactive substances. The bioactive compounds isolated from the gastropods are considered to have a role in the chemical defense of the animals against their predators. Many promising lead compounds have been reported from marine sources having anti-inflammatory activity. Compounds isolated from marine organisms such as manoalide, pseudopterosins, topsentins and scytonemin have all been studied extensively, while debromohymenialdisine was investigated by both Smith Kline Beecham and OsteoArthritis Sciences Inc. (Mayer and Lehmann, 2001) for the treatment of rheumatoid arthritis and osteoarthritis respectively. Among them manoalide, a sesterterpene isolated from the sponge *Luffariella variabilis* was found to have a selective anti-inflammatory profile (Potts and Faulkner, 1992). Since Non-steroidal compounds and sphingosine derivatives were reported to have significant anti-

inflammatory activity and some of them have even entered into the clinical trial, the new sphingosine derivative and the cembrenoid diterpene obtained from soft corals of *Sinularia crassaa* and *Lobophytum* species respectively were evaluated for their anti-inflammatory activity (Loukaci, *et al.*, 2000). Molluscs in the oceans are a common sight and are virtually untapped resource for the discovery of novel compounds. Many studies on bioactive compounds from molluscs exhibiting antitumour, antileukaemic, antibacterial and antiviral activities have been reported worldwide, like *Phyllidae* sp.(Ilangedone *et al.*, 1999), bivalves (Chellaram *et al.*, 2004) and gastropods (Kagoo *et al.*, 1992). Bioactive metabolites from molluscs such as sea hare (Schmitz *et al.*, 1993), *Chromodoris* sp. (Morris *et al.*, 1990), *Onhidella* sp. (Ireland *et al.*, 1993) were isolated and structurally elucidated.

The severe side effects of steroidal and non-steroidal anti-inflammatory drugs have lead to the search of new anti-inflammatory agents. Scanty literatures concerning the anti-inflammatory properties of marine molluscs are available. In the present study crude and column purified extracts of Mollusc, *Trochus tentorium* of Tuticorin coast, Southeastern India was evaluated for its Anti-inflammatory activity in various animal models such as adult Swiss mice and albino rats.

Materials and Methods

Study Area

The Molluscan samples were collected by hand picking using SCUBA diving from the intertidal area at a depth of 5- 7 m in Tuticorin coastal waters (Lat 8°45 and Long 78°13'E) of southeast coast of India.

Extraction

The crude extract of *Trochus tentorium* was subjected to column chromatography (silica gel) using eluants of increasing solvent polarities of hexane, hexane-acetone (0-100%) and acetone-Methanol (0-100%) to get several fractions. Of these, active fraction of the 100% acetone was used for the test.

Test animals

Adult Swiss mice and albino rats of either sex weighing between 20-25g and 150-175g respectively were used. The animals were housed under standard environmental conditions (temperature of 22 ± 1 °C with an alternating 12 hrs light-dark cycle and relative humidity of 60 ± 5 %) in the Department of Pharmacology, SRM college of Pharmacy, Chennai, fed with standard diet and water *ad libitum*. Prior approval of Institutional Animal Ethics Committee (IAEC) was obtained.

Acute toxicity studies

For toxicity studies, the partial purified extract of *T. tentorium* was suspended in saline containing 1% propylenglycol and administered intraperitoneally to six groups of ten mice and orally to another five groups of ten mice. The mice were kept under observation for 48 hrs. The test compounds in the range of 50 to 1000 mg / kg were administered and the mortality rates were observed after 48 hrs.

Anti-inflammatory activity

Carrageenan-Induced Rat Paw Edema

Rats were divided in to 5 groups of 6 animals each. The control group was injected with saline (1 ml/kg) into the sub-plantar region of the right hind paw. Anti-inflammatory activity was evaluated by injecting carrageenan (Sigma, 0.05 ml of 1% w/v) subcutaneously into the sub-plantar region of the right hind paw. The induced paw edema was measured. One hour prior to carrageenan injection, group II and III were treated with test compound at the dose level of 100 and 200mg / kg p.o.. Saline (1 ml/kg) given to group I was used as carrageenan treated control and the standard drug Diclofenac sodium (10mg / kg) was administered to group IV rats. All the doses were administered orally. The thickness of right paw was measured before and after carrageenan injection at time intervals 0, 1, 2, 3, 4 and 5 hours respectively. Percentage increase of paw edema thickness was calculated using the method of Duwiejua *et al.* (1994)

Statistical Analysis

The results are expressed as mean \pm S.E.M. Dunnet's t-test was used to verify the statistical significance at $p < 0.05$ between the treated and control groups.

Results

Acute toxicity (LD₅₀)

The intraperitoneal LD₅₀ was found to be 425 mg/kg of *Trochus tentorium* extracts in 48 hrs of observation. Oral administration of doses up to 1.25g/kg did not show any toxic symptom in mice. Administration of 1, 10 and 100 mg / kg, p.o. of the extract and doses of 1 and 10 mg/kg, i.p. did not provoke any significant change in their general behavior.

Anti-inflammatory potential of the 100% acetone fractions of the Gastropod

The anti-inflammatory bustle of the 100% acetone column purified extracts of *T. tentorium* was experimented on albino rats. *T. tentorium* at the concentration of 100 and 200 mg/kg, p.o showed significant decrease in the paw thickness in a dose dependent manner when

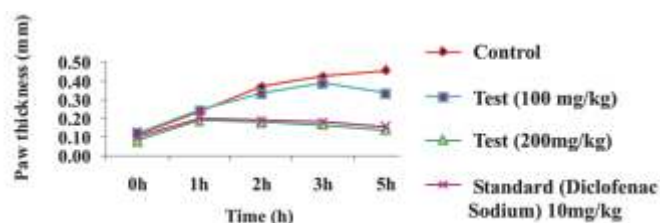


Fig. 1. Anti-inflammatory effect of 100% acetone column purified extracts of *Trochus tentorium* against carrageenin induced paw edema in albino rats

compared to that of control, at the 5th hour of experiment as indicated in Fig.1. The results were comparable with that of standard Diclofenac sodium (69.05%). The percentage inhibition of paw thickness was found to be 41.5 and 73.6 at concentration of 100 and 200 mg / kg p.o. of *T. tentorium* respectively (Fig. 2)

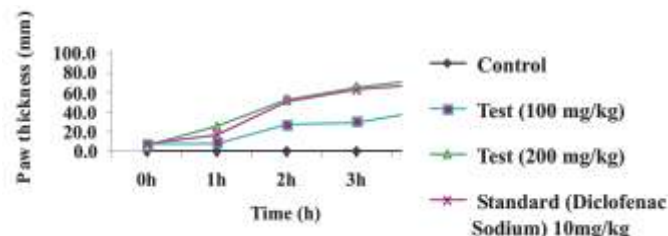


Fig. 2. Inhibition of paw volume of the albino rats treated with 100% acetone column purified extracts of *Trochus tentorium*

Extract *Trochus tentorium* exhibited a significant ($p < 0.001$) reduction of paw thickness at 5th hour in carrageenan induced paw edema when compared to that of control and standard drug.

Discussion

The emergence of this new field, sometimes called as marine pharmacology has been of enormous interest in the popular press. It is quite clear that marine compounds have the potential to treat a wide array of diseases in addition to cancer. In recent years, significant numbers of novel metabolites with potent pharmacological properties have been discovered from the marine organisms. Although there are only a few marine-derived products currently on the market, several robust new compounds derived from marine natural products are now in the clinical pipeline with more clinical development. While the marine world offers an extremely rich resource for novel compound it also represents a great challenge that requires inputs from various scientific areas to bring the marine chemical diversity up to its therapeutic potential. The pseudopterins, a series of diterpenoid glycosides isolated from the Caribbean Sea whip *Pseudopterogorgia elisabethae*, show impressive anti-inflammatory properties on the skin (Look *et al.*, 1986).

The anti-inflammatory effect is demonstrated by its inhibitory effect of Carrageenan induced paw edema. Subcutaneous injection of carrageenan into the rat paw produces plasma extravasation and the inflammation is characterized by increased tissue water and plasma metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathway (Gamache, *et al.*, 1986). There are biphasic effects in carrageenan induced edema. The first phase begins immediately after injection and diminishes in 1 hour and the second phase begins after 1 hour. It is suggested that the early hyperemia of carrageenan induced edema results from the release of histamine and serotonin (Kulkarni, *et al.*, 1986). On the other hand, the delayed phase of carrageenan induced edema results mainly from the potentiating effect of prostoglandins on mediator release, especially of bradykinin.

The Carrageenan induced paw edema method is generally used to evaluate the effect of Non-steroidal Anti-inflammatory Drugs (NSAIDs). Chellaram and Edward (2009) had reported that the acetone extract of *Drupa margariticola* exerted potential anti-inflammatory effect against Carrageenan-induced inflammation. A study on the anti-inflammatory activity of the gorgonian (sea whip) *Pseudopterogorgia*

elisabethae has resulted in the identification of Pseudopterin, which possessed potent anti-inflammatory activities and it was reported that it was non-toxic at 300 mg/kg (Look *et al.*, 1986). Novel anti-inflammatory drugs were isolated and characterized from corals (Shin and Fenical, 1991) and sponge (Pastor *et al.*, 1999). The active components were identified as sesterterpenes which caused *in vivo* rat paw edema inhibition. It was observed that the increase in the paw thickness was inhibited to about 73.60% by the 100% acetone column-purified fractions of *T. tentorium* at a concentration of 200 mg/kg, whereas standard anti-inflammatory drug, Diclofenac sodium (10 mg/kg) inhibited the paw thickness to 64.96%.

The present study revealed that the gastropod's compounds are effective against carrageenan induced edema. This revealed that the column fractions (*T. tentorium*) are potent inhibitors of exudative and proliferate phase of inflammation. However the ulcerogenic activity of these compounds was not studied, which otherwise might have provided valuable information with respect to the efficacy and safety of these compounds, when compared to Diclofenac sodium. So it can be inferred that, upon further purification, these gastropods extracts may be more potent than the standard drug. In conclusion, the 100% Acetone column-purified fractions of *Trochus tentorium* has possible anti-inflammatory effect. Further studies are needed to evaluate the real usefulness of these extracts in the therapy of pain release.

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