

*Full Length Research Paper*

# Anti-inflammatory and analgesic effects of coral reef associated gastropod, *Trochus tentorium* from Tuticorin coastal waters, Southeastern India

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The aim of this work was to investigate extensively, the biomedical potential of the mollusc *Trochus tentorium* which are abundantly associated with coral reef of the Tuticorin coastal water. The 100% acetone fraction of the gastropod tested for its analgesic effect on Swiss mice model and anti-inflammatory activity on albino rat showed promising results. *T. tentorium* at the concentration of 25 and 50 mg/kg (p.o) showed significant decrease in the paw thickness (41.15 and 73.6%, respectively) at the 5th hour of the experiment. The 100% column-purified fraction of the *T. tentorium* (200 mg/kg p.o) exhibited significant ( $p < 0.001$ ) inhibition of 79.22% against acetic acid induced abdominal constrictions. The dose of 25 mg/kg showed the inhibitions in the writhings of 67.86% ( $p < 0.001$ ) of animals when compared to the standard (diclofenac sodium), and 56.83% (50 mg/kg) inhibition was observed. These facts suggest that *T. tentorium* is a potential source for anti-inflammatory and analgesic compounds.

**Key words:** Analgesic activity, anti-inflammatory activity, mollusc, southeastern India.

## INTRODUCTION

Marine environment continuously provides broad and structurally diverse array of pharmacologically active compounds to mankind. These compounds are indispensable for the cure of deadly diseases. Marine organisms comprise approximately a half of the total biodiversity, thus offering a vast source to discover useful therapeutics. In 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 (Chellaram et al., 2004). Since 1970, significant

advances have been made in marine drug discovery. Academic researchers began to collaborate with pharmacologists in 1989 and the potential of the oceans became clear with many unique bioactive substances being extracted from marine plants and invertebrates (Fenical, 1997). Most of the compounds initially discovered were not effective in treating diseases but some were found to possess important biochemical properties that helped in the understanding of human diseases. These compounds referred to as pharmacological probe, have the potential to revolutionize our understanding of the underlying biochemistry of disease (Monks et al., 2002).

The most interesting phyla with respect to pharmacologically active marine compounds include bacteria, fungi, algae, sponges, soft corals, tunicates, mollusks and bryozoans. Among the marine invertebrates, the

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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 (Faulkner, 2000)

The only compound that shows significant therapeutic antiviral activity is Ara-A, a semi synthetic drug based on the arabinosyl nucleosides isolated from the sponge *Tethya crypta* (Bergmann et al., 1985). The anti-inflammatory and analgesic actions of aspartame may involve similar mechanism of actions as that of aspirin possibly through the interference of prostaglandin (PG) synthesis. The co-administration of aspartame with opiates or non-steroidal anti-inflammatory drugs (NSAIDs) may have clinical significance both in terms of desired and undesired consequences (Kanarek et al., 1991). 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 debromohyemialdisine 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). Scanty literature concerning the anti-inflammatory and analgesic properties of marine molluscs is available. In this study, 100% partial purified fraction of gastropod, *Trochus tentorium* of Tuticorin coast, Southeastern India were evaluated for their analgesic and anti-inflammatory activity in various animal models such as adult Swiss mice and albino rats.

## MATERIALS AND METHODS

### Extraction

The crude extract of *T. tentorium* was separately subjected to column chromatography (silica gel) using eluants of increasing solvent polarities of hexane, hexane-acetone (0-100%) and acetone-methanol (0 to 100%) to get several fractions. Of these, the active fraction of the 100% acetone was used for these two tests. Adult Swiss mice and albino rats of either sex weighing between 18 and 25 g and 150 to 200 g, respectively were used. The animals were housed under standard environmental conditions (temperature of  $22 \pm 10^\circ\text{C}$  with an alternating 12 h light-dark cycle and relative humidity of  $60 \pm 5\%$ ) in the Department of Pharmacology, SRM college of Pharmacy, Chennai, and 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 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 h. The tested compounds in the range of 25 to 500 mg/kg were administered and the mortality rates were observed after 48 h (Kulkarni et al., 1986).

### Anti-inflammatory activity

#### Carrageenan-induced rat paw edema

Rats were divided into 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 III and IV were treated with the test compound (*T. tentorium* extract) at the dose level of 25 and 500 mg/kg (p.o). Saline (1 ml/kg) given to group II was used as carrageenan treated control and the standard drug, diclofenac sodium (50 mg/kg) was administered to group V rats. All the doses were administered orally. The thickness of right paw was measured before and after carrageenan injection at time intervals of 0, 1, 2, 3, 4 and 5 h, respectively. Percentage increase of paw edema thickness was calculated (Kulkarni et al., 1986).

### Analgesic activity

#### Chemical induced (acetic acid) writhing method

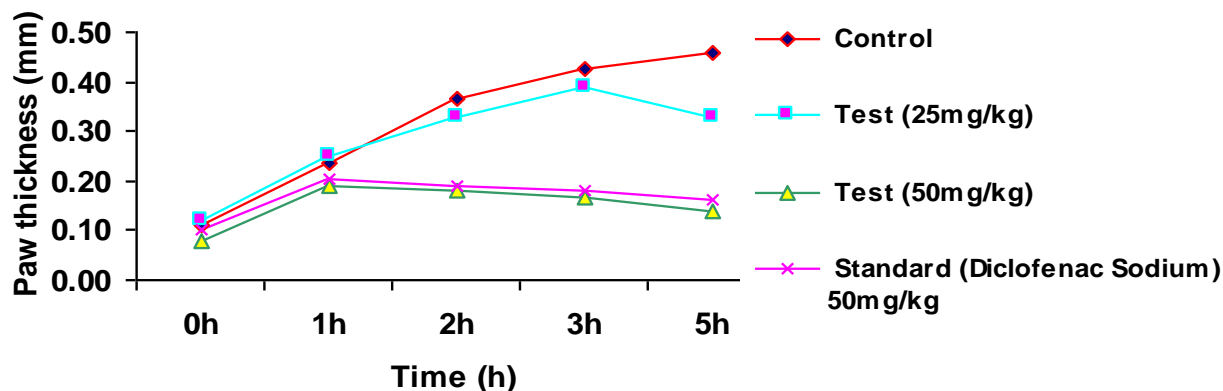
The test was carried out using the method of Koster et al. (1959). Different concentrations of the column-purified extract of *T. tentorium* (25 and 50 mg/kg) were given intraperitoneally. Thirty minutes after treatment, the mice were injected intraperitoneally with 0.2 ml of 0.6% acetic acid solution to induce characteristic writhing. The number of writhings occurring between 5 and 15 min after injection was recorded. Diclofenac sodium (50 mg/kg) was used as a reference drug while animals in the control group received normal saline. Anti-nociceptive response was assessed by counting the number of writhes (constriction of abdomen, turning of trunk (twist) and extension of hind limbs) of the mice subjected to column-purified extracts.

#### Analgesic activity by hot plate method

The hot plate method described by Turner (1965) was used to evaluate the analgesic activity. The animals were dropped gently on a plate maintained at  $53 \pm 0.5^\circ\text{C}$ . Reaction time was taken as the interval between the distant the animal reaches the hot plate till the moment the animal licks its forepaws or jumps out. Measurements were carried out 15 min before and 30 min after oral administration of the test compounds (100% acetone column-purified fraction of *T. tentorium* at the concentration of 25 and 50 mg/kg body weight p.o). The control group was administered with normal saline, while the standard reference group was treated with 50 mg/kg (p.o) of pentazocine. Values are expressed as mean  $\pm$  S.E.M of 6 animals in each group.

### Statistical analysis

The results are expressed as mean  $\pm$  S.E.M. Dunnet's t-



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

test was used to verify the statistical significance at  $p < 0.05$  between the treated and control groups.

## RESULTS

### Acute toxicity ( $LD_{50}$ )

The intraperitoneal  $LD_{50}$  was found to be 425 mg/kg of *T. tentorium* extract in 48 h of observation. Oral administration of doses up to 500 mg/kg (*T. tentorium*) did not show any toxic symptom in mice. Administration of 1, 10 and 100 mg/kg (p.o.) of the extracts 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 fraction of the gastropod

The anti-inflammatory effect of the 100% acetone column purified extracts of *T. tentorium* was experimented on albino rats. The extract at 25 and 50 mg/kg (p.o) was able to inhibit paw thickness to 41.5 and 73.6%, respectively as compared with that of the standard reference drug, diclofenac sodium (69.05%) at the 5th hour of the experiment (Figure 1). Extract of *T. tentorium* exhibited a significant ( $p < 0.001$ ) reduction of paw thickness at the 5th hour in carrageenan induced paw edema when compared to that of control and standard drug (Figure 2).

### Analgesic properties of the 100% acetone fraction of the gastropod

Results obtained in the analgesic activity using acetic acid induced method are shown in Tables 1 and 2. The 100% acetone column purified extracts of the *T. tentorium* showed significant ( $p < 0.001$ ) percentage of (79.22) inhibition, against acetic acid induced abdominal

constrictions at the dose of 25 mg/kg (p.o.). The dose of 25 and 50 mg/kg of *Trochus* extract showed the inhibitions in writhings of  $67.86 \pm 2.69$  and  $79.22 \pm 3.18\%$ , respectively ( $p < 0.001$ ). This observation suggests that the 100% acetone fraction of *Trochus* compounds is responsible for the strong analgesic action. Results of analgesic study of hot plate method showed that test compounds increases animal reaction time to hot plate (Tables 3). At 30 min, the mean reaction time for extract (25 mg/kg) group was  $4.5 \pm 0.55$  s, when compared to the control group ( $2.67 \pm 0.52$  s) and pentzaoicene treated groups ( $11.5 \pm 1.22$  s), respectively. The increase of percentage in reaction time after 30 min of drug administration (jump response) was shown (Table 4). The difference between the mean reaction time and increased percentage of jump response of test animals in the treated groups, control and standard groups were statistically significant ( $p < 0.001$ ).

## DISCUSSION

Although, initiated in the late 1970s, natural drug discovery from the world's oceans has been accelerated by the chemical uniqueness of marine organisms and by the need to develop drugs for contemporary, difficult to cure diseases. The pharmaceutical industry now accepts the world's oceans as a major frontier for medical research (Fenical, 1997). The emergence of this new field, sometimes called 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 in the market, several robust new compounds derived from marine natural products are now in the clinical pipeline with more clinical development (Chellaram et al., 2009).

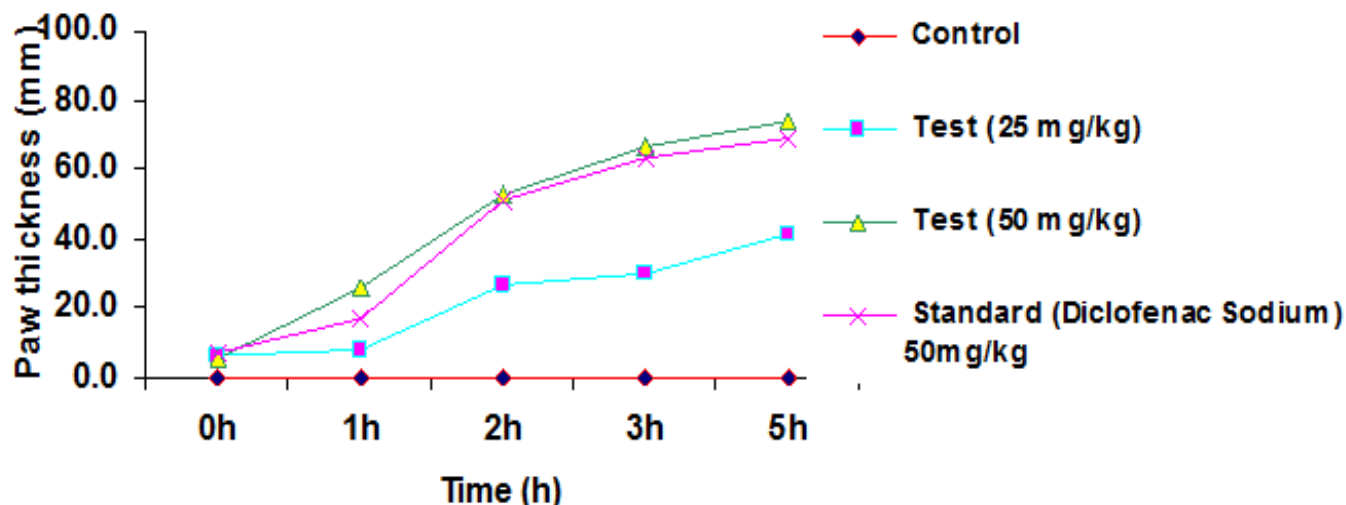


Figure 2. Inhibition of paw volume of the albino rat treated with 100% acetone column purified extracts of *T. Tentorium*.

Table 1. Evaluation of analgesic activity of 100% acetone column purified fraction of *T. tentorium* using chemical induced (acetic acid) method.

Group	Treatment	Number of writhing in 10 min (Average)
1	Control	51.33 ± 3.3
2	<i>T. tentorium</i> 25 mg / kg p.o	15.67 ± 1.63 <sup>a*</sup>
3	<i>T. tentorium</i> 50 mg / kg p.o	10.67 ± 1.38 <sup>b*</sup>
4	Standard (diclofenac sodium 50 mg/kg)	22.17 ± 1.34 <sup>c*</sup>

Values are expressed as Mean ± S.E.M of 6 animals in each group (n = 6).

<sup>a</sup> Comparison of Group II vs. Group I; <sup>b</sup>, comparison of Group III Vs Group I; <sup>c</sup>, comparison of Group IV Vs Group I; \*P < 0.001 is statistically significant.

Table 2. Analgesic activity of 100% acetone column purified fraction of the *T. tentorium* on chemical induced (acetic acid) method.

Group	Treatment	Percentage inhibition of writhing (%)
1	Control	-
2	<i>T. tentorium</i> 25 mg/kg p.o	67.86±2.69 <sup>a*</sup>
3	<i>T. tentorium</i> 50 mg/kg p.o	79.22±3.18 <sup>b*</sup>
4	Standard (diclofenac sodium 50 mg/kg p.o))	56.83±3.78 <sup>c*</sup>

Values are mean ± SD of each test (n = 6); \* p > 0.001 statistically significant.

Table 3. Analgesic activity of the 100% acetone column purified fraction of *T. tentorium* using hot plate method.

Group	Treatment	Reaction time after 30 min of drug administration (in sec)
1	Control	2.67 ± 0.52
2	<i>T. tentorium</i> 25 mg/kg p.o	4.5 ± 0.55 <sup>a*</sup>
3	<i>T. tentorium</i> 50 mg/kg p.o	7 ± 0 <sup>b*</sup>
4	Standard (pentazocine 50 mg/kg p.o))	11.5 ± 1.22 <sup>c*</sup>

Values are Mean ± S.E.M of 6 animals in each group (n = 6).

<sup>a</sup>, comparison of Group II Vs Group I; <sup>b</sup>, comparison of Group III vs. Group I; <sup>c</sup>, comparison of Group IV vs. Group I; \*p < 0.001 statistical significant.

**Table 4.** Analgesic activity of the 100% acetone column purified fraction of *T. tentorium* using hot plate method (Increase in reaction time).

Group	Treatment	Percentage increase in reaction time after 30 min of drug administration (%)
1	Control	0
2	<i>T. tentorium</i> 25 mg/kg p.o	58.07 ± 13.52 <sup>a*</sup>
3	<i>T. tentorium</i> 50 mg/kg p.o	137.6 ± 20.02 <sup>b*</sup>
4	Standard (pentazocine 50 mg/kg p.o)	283.33 ± 40.82 <sup>c*</sup>

Values are Mean ± SD of each test (n = 6); \*p < 0.001 statistically significant.

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).

The carrageenan induced paw edema method is generally used to evaluate the effect of NSAIDs (Phadke and Anderson, 1988). Kumar (2003) had reported that the methanolic extracts of *Cypraea erronea* and *Cypraea Arabica* exerted moderate anti-inflammatory effect against carrageenan-induced inflammation at a dose of 100 mg/kg. 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 from corals (Shin and Fenical, 1991) and sponges (Pastor et al., 1999). The active components were identified as ses-terterpenes 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 fraction of *T. tentorium* at a concentration of 50 mg/kg, whereas standard anti-inflammatory drug, diclofenac sodium (50 mg/kg) inhibited the paw thickness to 64.96%.

This study revealed that, the gastropod's compounds are effective against carrageenan induced edema. This revealed that the column fraction of *T. tentorium* was 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 gastropod extract may be more potent than the standard drug. The analgesic effect of 100% acetone column-purified fraction of *T. tentorium* was investigated. The important results obtained in this study were central and peripheral analgesic activities demonstrated by the inhibitory action on the acetic acid induced writhings and hot plate models. The hot plate

method was found to be suitable in the evaluation of centrally acting analgesic action but not for peripherally acting analgesic action (Mandal et al., 2005). In order to distinguish between the central and peripheral analgesic action of 100% acetone fraction of *T. tentorium*, acetic acid induced writhing repose in mice was used to examine the effect. The abdominal constriction response induced by acetic acid is a very sensitive procedure that enables the detection of antinociceptive activity of compounds in laboratory animals (Collier et al., 1968). Since the inhibition percentage of acetic acid induced writhings was superior to that shown by the reference drugs in this study, this indicates a promising analgesic activity. In conclusion, the 100% acetone column-purified fraction of *T. tentorium* have potential analgesic and 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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