



Anti-inflammatory potential of coral reef associated gastropod, *Drupa margaritcola*

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Abstract: The anti-inflammatory effect of the 100% acetone column purified extracts of gastropod, *Drupa margaritcola* was experimented on albino rats shown promising result. The acute toxicity was noted, the LD₅₀ was found to be 375 mg/kg in 48 hrs of observation. Oral administration of doses up to 0.75g/kg did not show any toxic symptoms and did not provoke any significant change in their general behavior. The extract of *D. margaritcola* at the concentration of 50 and 100mg/kg, p.o showed significant decrease in the paw thickness, 36.5 and 72.9 % respectively at the 5th hour of the experiment. The 100% column fraction of *D. margaritcola* is potent inhibitors of exudative and proliferate phase of inflammation and might have provided valuable information with respect to the efficacy and safety of these compounds, when compared to standard drug, Diclofenac sodium. So it can be inferred that, upon further purification, these gastropod extract may be more potent than the standard drug. The 100% acetone column-purified fraction of *Drupa margaritcola* has possible anti-inflammatory effect.

Keywords: Anti-inflammatory drug, Mollusc, reef.

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. 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 have our understanding of human diseases. These compounds referred to as pharmacological probe, have the potential to revolutionize our underlying bio chemistry of disease (Monks *et al.*, 2002). There is always been a pressing need for the development of new pharmaceuticals. Even today, our inability to cure cancer, AIDS, Alzheimer's disease and arthritis demonstrated the continuing importance of new drug discovery. The growing incidence of drug-resistant infectious disease alone suggests that a major investment is needed to combat this problem. Regarding the natural sources

for drugs, the marine environment has great frontier. Marine ecosystems are recognized recently as potentially contain novel new drugs (Fenical, 1997).

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 & Feeney, 1951; Bergmann & Burke, 1955). The anti-inflammatory action 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 term of desired and undesired consequences (Bergmann *et al.*, 1985; 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 debromohymenialdisine was investigated by both Smith Kline Beecham and OsteoArthritis Sciences Inc. (Mayer & Lehmann, 2001) for the treatment of rheumatoid arthritis and osteoarthritis respectively. Among them manoalide, a sesterterpene isolated from the sponge *Luffariella variabilis* (De Silva, 1980) was found to have a selective anti-inflammatory profile (Potts & 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).

Many studies on bioactive compounds from molluscs exhibiting antitumour, antileukaemic, antibacterial and antiviral activities have been reported worldwide. The severe side effects of steroidal and non-steroidal anti-inflammatory drugs have lead to the search of new anti-inflammatory agents. Scanty literature concerning the anti-inflammatory potential of marine molluscs is available. In the present study 100% column purified extract of Molluscs, *Drupa margaritcola* of Tuticorin coast, Southeastern India was evaluated

Fig 1. Anti-inflammatory effect of 100% acetone column purified extracts of *D. margariticola* and Diclofenac sodium against carrageenin induced paw edema in albino

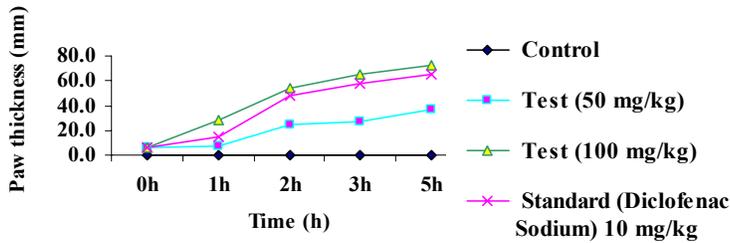
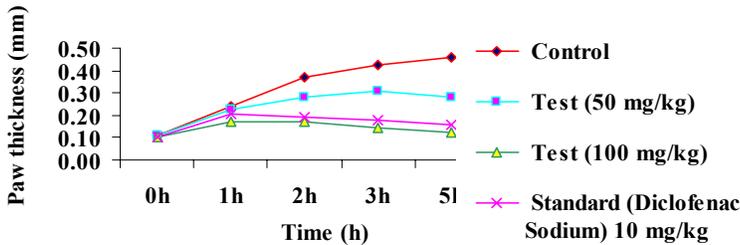


Fig 2. Inhibition of paw volume of the albino rats treated with 100% acetone column purified of *D. margariticola*



for its anti-inflammatory activity in various animal models such as adult Swiss mice and albino rats.

Materials and Methods

Extraction

The crude extract of *Drupa margariticola* was isolated and subjected to column silica gel column chromatography using eluants of step gradient method like, 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 100-200g 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 $60 \pm 5\%$) 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 extracts was suspended in saline containing 1% propylenglycol and administered intraperitoneally to six groups of ten mice and orally to another five groups of ten albino rats. The rats 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 according to the method of Kulkarni *et al* (1986). One hour prior to carrageenan injection, group II and III were treated with test compound at the dose level of 50 and 100mg / 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.

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 and discussion

Acute toxicity (LD_{50})

The intraperitoneal LD_{50} was found to be 375 mg / kg in 48 hrs of observation. Oral administration of doses up to 0.75g / kg did not show any toxic symptom in the animals. 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 fraction of the *Gastropods*:

The anti-inflammatory effect of the 100% acetone column purified extracts of *D. margariticola* was experimented on albino rats. The extract at the concentration of 50 and 100 mg / kg, p.o showed significant decrease in the paw thickness in a dose dependent manner when 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. The percentage inhibition of paw thickness was found to be 36.5 and 72.99 at concentration of 50 and 100 mg / kg p.o. respectively (Fig.2). Extracts of *D. margariticola* 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. 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. Current research activities, while primarily within the academic laboratories have generated convincing evidence that marine drug discovery has an exceedingly bright future (Fenical, 1997). In recent years, significant numbers of novel metabolites with potent pharmacological properties have been discovered from the marine organisms. The pseudopterosins, 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 Carrageenan induced paw edema method is generally used to evaluate the effect of Non-steroidal Anti-inflammatory Drugs (NSAIDs) (Phadke & Anderson, 1988). Kumar (2003) had reported that the methanolic extracts of *Cypraea erronea* and *C. arabica* exerted moderate anti-inflammatory effect against Carrageenan-induced inflammation at a dose of 10 mg/kg. 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 72.99% by the 100% acetone column-purified fractions of *D. margarticola* at a concentration of 100mg/kg, whereas standard anti-inflammatory drug, Diclofenac sodium (10mg /kg) inhibited the paw thickness to 64.96%.

Conclusion

The present study shows that the gastropod's compounds are effective against carrageenan induced edema. This revealed that the 100% column fraction of *D. margarticola* is 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 100% acetone column-purified fraction of *Drupa margarticola* 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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