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Anti-Nociceptive Assets of Coral Associated Gastropod, *Drupa margariticola*

[1]C. Chellaram and [2]J.K. Patterson Edward
[1]Department of Biomedical Engineering, Vel Tech Multi Tech Dr. RR. Dr. SR. Engineering College, Chennai-600 062, Tamil Nadu, India
[2]Suganthi Devadason Marine Research Institute, Tuticorin-628 001, Tamil Nadu, India

Abstract: The extract of gastropod, *Drupa margariticola* tested for their analgesic assets using chemical (acetic acid) induced and hot plate method on Swiss mice model showed promising results. One hundred percent column purified extracts of the *D. margariticola* (25 and 100 mg kg^{-1} p.o.) exhibited significant ($p<0.001$) writhing inhibition of 65 and 78.57%, respectively against acetic acid induced abdominal constrictions. The result of hot plate study, in 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 ($p<0.001$). At 30 min, the mean reaction time for extracts (50 mg kg^{-1} p.o.) group was 6.67±1 sec, when compared to control group (2.67±0.52 sec) and pentazocine treated groups (11.5±1.22 sec) for 100 mg kg^{-1} p.o. These facts suggest that the 100% acetone fraction of the *D. margariticola* was shown the strongest analgesic action. The important results obtained in present 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.

Key words: Toxicity, analgesic, marine mollusk, crude, purified extracts, 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 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. Since 1970 significant advances have been made in marine drug discovery (Sung *et al*., 2004; Karuso and Scheuer, 2002). 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; Faulkner, 2000). 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 (Chellaram *et al*., 2004). These compounds referred to as pharmacological probe, which has the potential to revolutionize the underlying bio chemistry of disease (Monks *et al*., 2002; Mandal *et al*., 2005). 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 (Gavagnin *et al*., 2003).

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 (Avila *et al*., 2000). Only few promising compounds were reported from marine sources having analgesic activity. Compounds isolated from marine organisms such as manoalide and pseudopterosins, have all been studied extensively, while debromohymenialdisine was investigated by both Smith

Corresponding Author: Dr. C. Chellaram, Department of Biomedical Engineering, Vel Tech Multi Tech Dr. RR. Dr. SR. Engineering College, Chennai 600062, Tamil Nadu, India

Kline Beecham and Osteoarthritis Sciences Inc. Mayer and Lehmann (2001) and Kokke *et al.* (1984) for the treatment of rheumatoid arthritis and osteoarthritis, respectively. 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 drugs have lead to the search of new analgesic response agents (Tsukamoto *et al.*, 1998). Scanty literature concerning the analgesic properties of marine molluscs is available. In the present study, 100% column purified extract of Mollusc, *Drupa margariticola* of Tuticorin coast, Southeastern India was evaluated for their Analgesic assets in animal model adult Swiss mice.

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 extracts of *Drupa margariticola* 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, the 100% acetone was used for various tests.

Animals: Adult Swiss mice either sex weighing between 18-25 g was used. The animals were housed under standard environmental conditions (temperature of 22±1°C with an alternating 12 h light-dark cycle and relative humidity of 60±5%) in the Department of Pharmacology, SRM College of Pharmacy, Chennai (during 3rd to 8th (9 am to 5 pm) October, 2008) 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 extracts were suspended in saline containing 1% propylenglycol and administered intraperitoneally to 6 groups of 10 mice and orally to another 5 groups of 10 mice (Ray and Yaksh, 2008). The mice were kept under observation for 48 h. The test compounds in the range of 50 to 1000 mg kg^{-1} were administered and the mortality rates were observed after 48 h.

Anti-nociceptive activity

Chemical induced (acetic acid) writhings method: The test was carried out using the method of Koster *et al.* (1959). Different concentrations of the column purified extracts of *Drupa margariticola* (25 and 50 mg kg^{-1} p.o.) was 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 (20 mg kg^{-1} p.o.) 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 and extension of hind limbs) of the mice subjected to column purified extracts.

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±0.5°C. Reaction time was taken as the interval between the instant, 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 test compounds (100% acetone column purified fraction of *Drupa margariticola* at the concentration of 25 and 50 mg kg^{-1} p.o.). The control group was administered with normal saline while the standard reference group was treated with (100 mg kg^{-1} p.o.) of pentazocine. Values are expressed as Mean±SEM of 6 animals in each group.

Statistical analysis: Values are expressed as Mean±SEM statistical significance was determined using the ANOVA followed by Dunnet's t-test. Values with $p<0.05$ were considered significant.

RESULTS

Acute toxicity (LD_{50}): The intraperitoneal LD_{50} was found to be 375 mg kg^{-1} of extracts in 48 h of observation. Oral administration of doses up to 0.75 g kg^{-1} did not show any toxic symptom in mice. Administration of 1, 10 and 100 mg kg^{-1} p.o. of the extracts and doses of 1 and 10 mg kg^{-1}, i.p. did not provoke any significant change in their general behavior.

Analgesic properties of the 100% acetone fractions of the gastropods: The doses of 25 and 50 mg of the extract shown the number of writhings are 16.50±1.38 and 10.67±1.63, respectively, when control and standard drug shown 51.33±3.3 and 22.17±1.34, respectively (Table 1). One hundred percent acetone column purified extracts of the *Drupa margariticola* shown significant ($p<0.001$) percentage of 78.57 inhibition, against acetic acid induced abdominal constrictions at the dose of 50 mg kg^{-1} p.o. (Table 2). The dose of 25 mg kg^{-1} of the extract shown the

Table 1: Evaluation of analgesic activity of 100% acetone column purified fraction of *Drupa margariticola* using chemical induced (acetic acid) method

Groups	Treatments	No. of writhings in 10 min (Avg.)
1	Control	51.33±3.30
2	*Drupa margariticola* 25 mg kg^{-1} p.o.	16.50±1.38a*
3	*Drupa margariticola* 50 mg kg^{-1} p.o.	10.67±1.63b*
4	Standard (Diclofenac sodium 50 mg kg^{-1} p.o.)	22.17±1.34c*

Values are expressed as Mean±SEM of 6 animals in each group (n = 6). a: Comparison of group II vs. group I, b: Comparison of group III vs. group I, c: 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 *Drupa margariticola* on chemical induced (acetic acid) method

Groups	Treatments	Inhibition of writhings (%)
1	Control	-
2	*Drupa margariticola* 25 mg kg^{-1} p.o.	65.39±3.19a*
3	*Drupa margariticola* mg kg^{-1} p.o.	78.57±3.18b*
4	Standard (Diclofenac sodium mg kg^{-1} p.o.)	56.82±3.78c*

Values are Mean±SD of each test (n = 6). a: Comparison of group II vs. group I, b: Comparison of group III vs. group I, c: Comparison of group IV vs. group I. *p>0.001 statistically significant

Table 3: Evaluation of analgesic activity of the 100% acetone column purified fraction of *Drupa margariticola* using hot plate method

Groups	Treatments	Reaction time after 30 min of drug administration (sec)
1	Control	2.67±0.52
2	*Drupa margariticola* 25 mg kg^{-1} p.o.	4.50±0.84a*
3	*Drupa margariticola* 50 mg kg^{-1} p.o.	6.67±1.03b*
4	Standard (Pentazocine 100 mg kg^{-1} p.o.)	11.50±1.22c*

Values are Mean±SEM of 6 animals in each group (n = 6). a: Comparison of group II vs. group I, b: Comparison of group III vs. group I, c: Comparison of group IV vs. group I. *p<0.001 statistical significant

Table 4: Analgesic activity of the 100% acetone column fraction of the *Drupa margariticola* using hot plate method

Groups	Treatments	Percentage increase in reaction-time after 30 min of dmg administration (sec)
1	Control	0
2	*Drupa margariticola* 25 mg kg^{-1} p.o.	58.5±13.94a*
3	*Drupa margariticola* 50 mg kg^{-1} p.o.	136.0±22.17b*
4	Standard (Pentazocine 100 mg kg^{-1} p.o.)	203.0±40.82c*

Values are Mean±SD of each test (n = 6). a: Comparison of group II vs. group I, b: Comparison of group III vs. group I, c: Comparison of group IV vs. group I. *p<0.001 statistically significant

inhibition of the writhing is 65% (p<0.001). This observation suggests that the compounds responsible for the analgesic action, while the 100% acetone fraction has strong action.

A result was analgesic study for the hot plate method shown that the test compounds increases the animal reaction time for the hot plate (Table 3). At 30 min, the mean reaction time for extracts (50 mg kg^{-1}) group was 6.67±1 sec, when compared to control group (2.67±0.52 sec) and pentazocine treated groups (11.5±1.22 sec) for 100 mg kg^{-1} p.o. 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. Current research activities, while primarily within the academic laboratories have generated convincing evidence that marine drug discovery has an exceedingly bright future (Fenical, 1997). The pharmaceutical industry now accepts the world's oceans as a major frontier for medical research.

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 pseudopterosins, a series of diterpenoid glycosides isolated from the Caribbean Sea whip *Pseudopterogorgia elisabethae* show impressive analgesic properties on the skin (Look *et al*., 1986).

In the present study, the analgesic effect of 100% acetone column purified fractions of *D. margariticola* was investigated. The important results obtained in present 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. In order to distinguish between the central and peripheral analgesic action of 100% acetone fraction of *D. margaritcola*, acetic acid induced writhing repose in mice was used to examine the effect. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. The number of writhing movements during a 30 min observations are 16.50±1.38

and 10.67±1.63, respectively. 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. The reason for this activity is that the mollusk *Drupa margariticola* is a corallivorous animals and it has strong defense substances for their surviving among the competitive marine environment, so naturally this animals having some potential substances.

In conclusion, the 100% acetone column-purified fractions of *Drupa margariticola* have possible analgesic effect. Further studies are needed to evaluate the real usefulness of these extracts in the therapy of pain release.

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