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Central nervous system depressant properties of reef associated gastropods, *Drupa margariticola* and *Trochus tentorium* from Gulf of Mannar, Southeastern India

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ABSTRACT

The 100% acetone fraction of the gastropods, Drupa margariticola and Trochus tentorium tested for their Central Nervous System (CNS) depressant properties on swiss mice model showed promising results. The locomotor activity of the animal was reduced by the extracts of the D. margariticola and T. tentorium. But the activity was found to be dose dependent, the higher the dose the lower the activity. In control, the activity was found to be very negligible. The standard Chlorpromazine treated animal exhibited a nearly 67.86% reduction in locomotor activity. The 100 mg/kg dose of the 100% column-purified fraction of D. margariticola and T. tentorium exhibited a reduction of locomotor activity by 72.46 and 67.44 % respectively. D. margariticola (50 and 100 mg / kg) and T. tentorium (100 and 200 mg / kg) caused a significant ($p<0.001$) reduction in locomotor activity in the dose dependant manner. This fact suggests that the compound responsible for the CNS depressant action was distributed between both extracts, although the 100% acetone fraction of the D. margariticola was the strongest.

Keywords: Marine mollusks, partial purification, CNS depressant properties.

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. 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 [1]. 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 [2]. 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.

The marine environment comprises 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 [3]. The most interesting phyla with respect to pharmacologically active marine compounds include bacteria, fungi, algae, sponges, soft corals, tunicates, molluscs or bryozoans [4]. 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.

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* [4], [5]. The biomedical potential 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 term of desired and undesired consequences [6], [7]. 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 Osteo Arthritis Sciences Inc., for the treatment of rheumatoid arthritis and osteoarthritis respectively [8]. Among them manoalide, a sesterterpene isolated from the sponge *Luffariella variabilis* [9] was found to have a selective anti-inflammatory profile [10]. (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 [11].

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 Central Nervous System (CNS) depressant properties of marine molluscs is available. In the present study 100% column purified extracts of Molluscs *Drupa margariticola* and *Trochus tentorium* of Tuticorin coast, Southeastern India were evaluated for their CNS depressant activity in animal model such as adult Swiss mice.

EXPERIMENTAL SECTION

Extraction

The crude extracts of *Drupa margariticola* and *Trochus tentrorium* were separately 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 various tests.

Animal Model

Adult Swiss mice of either sex weighing between 18-25g and 150-200g respectively were used. The animals were housed under standard environmental conditions (temperature of 22 ± 1 ^{0}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 extracts were 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.

Central Nervous System (CNS) Depressant

Locomotor activity

Spontaneous locomotor activity and rearing were measured using a computerized locomotion detection system equipped with photosenserea as described previously [12]. Thirty minutes after the administration of vehicle or test compound, a mouse was individually placed in a transparent cage (25X48 X 18 cm^3) and locomotor activity and rearing were recorded for 60 minutes. To evaluate the interaction between Test compounds and chlorpromazine, animals were divided into six groups. Group I served as an untreated control, group II and III were treated with Test compounds at concentration of 50 and 100 mg / kg (*D. margariticola* extract) 100 and 200 mg / kg (*T. tentorium* extract) (Tween 80 suspended) and group IV was treated with standard of Chlorpromazine (3mg / kg, i.p). The control group was administered only 10% v/v Tween 80 suspension. The locomotor activity was observed after 30 minutes for 10 minutes and the percentage change in the activity was calculated.

Statistical Analysis

Drug effects were assessed by ANOVA followed by the Dunnet's *t*-test. The level of significance was set at $p<0.05$.

RESULTS

Acute toxicity (LD50)

The intraperitoneal LD_{50} was found to be 375 and 425 mg / kg of *Drupa margariticola* and *Trochus tentorium* extracts respectively in 48 hrs of observation. Oral administration of doses up to 0.75g / kg (*Drupa margariticola)* and 1.25g / kg (*Trochus 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.

Central Nervous System (CNS) Depressant activity of the 100% acetone fractions of the Gastropods

The result of evaluation of the locomotor activity is presented in Tables 1 and 2 and that of Chlorpromazine induced sleeping time. The locomotor activity of the animal was reduced by the extracts of the *D. margariticola* and *T. tentorium*. But the activity was found to be dose dependent, the higher the dose the lower the activity. In control, the activity was found to be very negligible. The standard Chlorpromazine treated animal exhibited a nearly 67.86% reduction in locomotor activity. The 100 mg / kg dose of the 100% column-purified fraction of *D. margariticola* and *T. tentorium* were exhibited a reduction of locomotor activity by 72.46 and 67.44% respectively. *D. margariticola* and *T. tentorium* (50, 100 and 100, 200 mg / kg respectively) caused a significant ($p<0.001$) reduction in locomotor activity in the dose dependant manner (Tables 3 and 4).

Table. 1. Evaluation of locomotor activity using actophotometer for CNS action

Marking	Control	Test t (200mg/kg p.o)	Test 1 (300mg/kg p.o)
H	275	261	374
B	300	272	308
T	268	281	287
C	224	251	310
HB	287	263	298
HT	256	269	312
Average	268.33	266.17	314.83

Table. 2. Locomotor activity in scores after drug treatment in 10mts

Marking	Control	Test t (200mg/kg p.o)	Test 1 (300mg/kg p.o)
H	268	92.0	104.0
B	306	83.0	87.0
T	260	88.0	78.0
C	221	79.0	93.0
HB	285	94.0	72.0
HT	258	97.0	87
Average	266.33	88.83	86.83
SEM		6.85	11.23

Table 3. Evaluation of locomotor activity (CNS depressant) of 100% acetone column purified fraction of *Drupa margariticola* using actophotometer

Groups	Treatment	Reduction of locomotor activity in Percentage
Group 1	Control	0
Group 2	*Drupa margariticola* 50 mg / kg p.o	66.41 ± 2.54
Group 3	*Drupa margariticola* 100 mg / kg p.o	72.45 ± 1.91
Group 4	Standard (Chloropromazine 3 mg / kg p.o)	67.86 ± 1.09

*p< 0.001 statistically significant

Table 4. Evaluation of locomotor activity (CNS depressant) of 100% acetone column purified fraction of *Trochus tentorium* using actophotometer

Groups	Treatment	Reduction of locomotor activity in Percentage
Group 1	Control	0
Group 2	*Trochus tentorium* 100 mg / kg p.o	67.44 ± 3.42
Group 3	*Trochus tentorium* 200 mg / kg p.o	75.04 ± 2.44
Group 4	Standard(Pentazocine 10 mg / kg p.o)	67.86 ± 1.09

**p< 0.001 statistically significant*

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. Marine organisms are of considerable current interest as a new and promising source of biologically active compounds. They produce a variety of metabolites, some of which can be used for drug development [13]. Current research activities, while primarily within the academic laboratories have generated convincing evidence that marine drug discovery has an exceedingly bright future [1]. The pharmaceutical industry now accepts the world's oceans as a major frontier for medical research. 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 [14].

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.

In the present study, 100% acetone column-purified extracts of *D. margariticola* and *T. tentorium* exhibited CNS depressant activity in mice tested by actophotometer and Chlorpromazine induced sleeping time. The 100% acetone extracts caused a dose dependent reduction in motor activity in mice. The possible mechanism of CNS depressant activity by the

Acetone extracts of *D. margariticola* and *T. tentorium* may be attributed to the enhancement of GABA (Gamma Amino Butyric Acid) in brain). The same type of result was reported, but this finding has promising activity [15]. The data suggest that the *D. margariticola* and *T. tentorium* have significant ($p<0.001$) CNS depressant properties in mice.

CONCLUSION

The 100% Acetone column-purified fractions of *Drupa margariticola* and *Trochus tentorium* have possible CNS depressant effect. Further studies are needed to evaluate the real usefulness of these extracts in the therapy of pain release.

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