Pharmacological Potentials of Sea Cucumber

*Holothuria Atra* Extracts from the Indian Ocean

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**Abstract**

Drugs derived from marine organisms are currently used to cure infectious diseases. Marine invertebrates collected from the intertidal regions of the Indian coast have shown promising biological activities. Sea cucumbers are soft-bodied worm-like echinoderms which belong to the class Holothuroidea. Anti-inflammatory activity was detected using Carrageenan induced rat paw edema method. The analgesic activity was performed using tail immersion method. Antipyretic activity was analysed using Brewer's yeast induced hyperpyrexia method. CNS was determined using Locomotor activity. Immunomodulatory activity was tested using Carbon clearance test. Antioxidant activity was measured using DPPH method. Pharmacological studies showed that the anti-inflammatory bustle of methanol extracts in *Holothuria atra* at the concentration of 100 and 200 mg/kg, p.o on rats showed significant decrease in the paw thickness in a dose dependent manner when compared to that of the control, at the 5th hour of administration. Similarly the highest inhibition rate seen in *H. atra* extracts was 84% showed analgesic activity. Then antipyretic, immunomodulatory and CNS depressant activities were found to be moderate. The methanol extract of *Hatra* showed high antioxidant activity with *IC*\(_50\) value of 300. Overall results provide information that sea cucumber *H. atra* could be explored as a potential source of high-value bioactive metabolites and could be used in the pharmaceutical industry.

**Keywords:** *Holothuria atra*, Anti inflammatory, Analgesic, Antipyretic, DPPH.
INTRODUCTION

Marine invertebrates in particular are promising organisms for the synthesis of novel bioactive compounds. It is an adaptation strategy to thrive in the extreme environmental conditions of the sea and as a defense strategy to escape from predators by the marine invertebrates especially soft bodied animals like Seacucumbers [1]. The antimicrobial peptides seen in sea cucumbers like Cucumaria frondosa such as steroidal glycosides and polyhydroxylated sterols indicate remarkable activity against microbes. Therapeutic properties and medicinal benefits of sea cucumbers can be linked to the presence of a wide array of bioactives especially triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycans [2]. Secondary metabolites obtained from using polar extracts of Holothuria scabra are the sulfated triterpene glycosides, scabraside A and B and they have effective cytotoxicity against four human tumor cell lines [3]. Triterpene glycosides, scabraside A and B exhibit significant in vitro cytotoxicity against four human tumor cell lines such as the human leukemia (HL-60, MOLT-4), human lung cancer (A-549) and human hepatoma (BEL-7402) cells [4]. The polar extract of the sea cucumber Stichopus japonicus, collected from Jeju Island, Korea, has led to isolation of five new fatty acid derivatives (1, 4 - 7) along with known compounds (2 - 3, 8 - 14) lyso-PAF analogue and nucleosides [5]. Triterpene glycosides are the predominant secondary metabolites of sea cucumbers (holothurians) and are responsible for their general toxicity. These glycosides have been reported to possess a wide spectrum of biological effects, including cytotoxic, antifungal, hemolytic, and immunomodulatory activities. More than 100 of these glycosides have been described, and the majority are lanosterol type triterpenes with an 18(20) lactone and a sugar chain linked to the C-3 of the aglycone [6]. New antifungal active triterpene glycosides of sea cucumber Holothuria scabra were identified as scabrades A, echinoside A and holothurin A1 [7]. Giant red sea cucumbers, Parastichopus californicus, are commercially harvested in the U.S. Pacific Northwest. The chemical characterization of freeze-dried edible tissues from P. californicus demonstrated that these products have valuable nutritional properties. It is composed of 68% protein, 12% ash, 9% carbohydrate, and 5% lipids, while the body wall was composed of 47% protein, 26% ash, 15% carbohydrate, and 8% lipids. The body wall components are used in nutraceutical and pharmaceutical applications [8]. Sea cucumber extract of Stichopus Sp1 reduced human osteoblast cell viability in a concentration dependent manner, it potentially promotes osteoblast functional activity. It is essential and could be used in systemic modulator of human bone metabolism [9]. The antioxidant potential of Atlantic sea cucumber Cucumaria frondosa Gunnerus (Cucumaridae), a widespread species in coastal waters of the North Atlantic Ocean was found at by detecting the presence of total phenols and flavonoids using the ethyl acetate extracts [10]. Fucan sulfates were isolated from chloroform/methanol extract of the body wall of the sea cucumber Stichopus japonicas. They are the potent inhibitors of osteoclastogenesis [11]. Sulfated polysaccharide, a metabolite from the body wall of the sea cucumber Stichopus japonicus has the ability to regulate the cell proliferation rate in neurodegenerative disorders [12]. The novel glycosaminoglycan isolated from the body wall of sea cucumber has appeared as a potentially useful therapeutic component for antithrombotic applications. They have a molecular weight of around 70 kDa from the body wall of sea cucumber Thelenata ananas which consisted of GalNAc, GlcUA, fucose and ester sulfate [13]. The low molecular weight sulphated polysaccharides are noted from sea cucumbers with efficient anticoagulant activities and several pharmacological properties [14]. The chondroitin and glucosamine components of holothuria were reported to be important cartilage building blocks and other bioactivities including anti-inflammatory and anti tumor activity properties [15]. The extract LPS obtained from Stichopus japonicus induced inflammatory response via blocks the MAPK signaling pathway in murine macrophages, showed in vitro with anti-inflammatory potential [16]. Seacucumbers are characteristic with the presence of appreciable amounts of triterpene (4,4,14-trimethylsterol) oligoglycosides (saponins). In Cucumaria frondosa the presence of oligosides and Holostane glycosides showed antitumour, antifungal, and immunomodulatory properties [17]. Three species of sea cucumbers Holothuria edulis, H. scabra Jaeger, and Stichopus horrens obtained from Malaysia have high content of proteins. Significant differences in the protein binding patterns were noticed with the molecular weight range from 20 kDa - 125 kDa [18]. An aqueous fraction of the edible sea cucumber Holothuria edulis has been shown to deliver a strong cytotoxic effect against the human HL-60 leukemia cell line. The up regulation of Bax and caspase-3 protein expression was observed while the expression of Bcl-xL protein was down regulated in ESC-AQ treated HL-60 cells [19]. Holothuria atra is commonly called as the black sea cucumber or lolly fish. There are 20 species of sea cucumbers in the Indian Ocean along the south east coast of India. Therefore, the aim of this present study to investigate the Pharmacological studies of Sea cucumber Holothuria atra extracts from the south east coast of India.

MATERIALS AND METHODS

Collection and extract preparation of Sea cucumber
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d into four groups of four individuals
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ivity was assessed by measuring the
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d to detect the CNS
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36
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reference. Group III and IV animals were treated with
administered to group II animals served as the standard
drug Diclofenac sodium (10mg / kg)
each. The control group was given saline (1 ml / kg) and

Rats were divide
albino rats. The induced paw edema was measured [21].
o.05 ml of 1%w/v carrageenan (Sigma) subcutaneously
Anti

study.
(Regd.No.622/02/C/CPCSEA) used for the present
Sangaralinkam Bhuvaneswari College of Pharmacy
animals were procured from animal house of
albino mice of both sexes were used. The experimental
study. For the
these animals were kept fasting and employed in the
rats of either sex. Prior to the start of the experiments,
divided
experimental conditions for a week, the rats were
maintained on pellet diet (Gold Mohur brand) at room
Sivakasi (Tamilnadu). The experimental mice were
procured from the Departme

200mg / kg (p.o). All the doses were administered orally. After 30 minutes of drug treatment carrageen
was injected subcutaneously into the rats to induce inflammation into the sub planter region of the right
hind paw. The thickness of right paw was measured before and after carrageenan injection at time intervals
0, 1, 2, 3, 4, 5 hours [22]. The data were analyzed by one way ANOVA Dunnett’s test.

**Central Nervous System (CNS) Depressant activity:**
Effect of Locomotor activity was used to detect the CNS depressant activity. A computerized locomotion
detection system (actophotometer) equipped with photosenser was used to measure spontaneous
 locomotor activity and rearing [25]. In the experiment, each rat was individually placed in a transparent cage

(25X48 X 18 cm²) before the administration of extract and the locomotor activities were recorded for 10 minutes. The animals were divided into four groups. The Group I served as an untreated control, Group II was treated with standard Caffeine (30 mg / kg, i.p). Group III and IV animals were treated with Holothuria atra extract at the dose level of 100 and 200mg / kg (p.o). Sodium lauryl sulphate at 1.0% was used as a suspension medium. Basal reaction time was noted before and 30 minutes after the administration of treatment. The locomotor activity was observed and the percentage of changes in the activity was recorded.

**In-vivo Immunomodulation study (Carbon clearance test)**

Swiss albino mice were divided into four groups, each containing 6 animals. Group I (control) was given 1.0% sodium carboxy methyl cellulose in water (0.3ml/mouse) for 5 days. Group II-IV were given different concentrations of methanol extract of H. atra at the dose of (25, 50 and 100 mg/kg, p.o.) for 7 days. At the end of 7th day, after 48 h, mice were injected via the tail vein with carbon ink suspension (1:50 dilution of Indian ink, Camel, 10 μl/gm body wt.). Blood samples were withdrawn (in EDTA solution 5 μl) from the retro-orbital vein at 3 to 12 min. Then 25-μl sample was mixed with 0.1% sodium carbonate solution (2 ml) and its absorbance at 650 nm was determined [26]. Results were expressed as the arithmetic mean ± S.E.M. of six mice. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett’s test. The values expressed as P< 0.05, and P< 0.001 were considered significant.

**Antioxidant activity (DPPH Scavenging Assay):**

Antioxidant activity of the extracts was measured in terms of radical scavenging ability by using the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH), as described [27]. Different concentrations of extracts at 0.1ml and standard ascorbic acid were taken in different test tubes. To this 2.5 ml of 0.1mM methanolic solution of DPPH was added and shaken vigorously. The tubes were kept at room temperature for 20 minutes. The control was prepared as above using the DMSO instead of the extract. 100 μl of extracts in 2.5 ml methanol was used as blank. The changes in the absorbance of the samples were measured at 517nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula

\[
\text{Percentage radical scavenging} = (\text{Control OD} \times \text{Sample OD/Control OD}) \times 100
\]

Antioxidant capacity was expressed in IC50 values. All measurements were carried out in triplicates.

**RESULTS AND DISCUSSION**

**Anti-inflammatory activity:** The anti inflammatory effect was demonstrated by the inhibitory effect of carrageenan induced paw edema is depicted in (Table 1). The results were comparable with that of standard Diclofenac sodium (10mg).The anti inflammatory activity was determined using the standard mean and standard error values from 0 to 5 hrs followed by one way ANOVA Dunnett’s test. The H. atra extract had high activity at 100mg and at 200mg levels; though only gradual increase in value was noticed. Both the concentrations showed significant (p<001) values. These results indicated the important anti-inflammatory effect which inhibited almost 50% of the induced edema. The body wall extracts of H. atra suppressed the acute and chronic inflammation strikingly in rats. The anti inflammatory action of the Holothuria atra was maximal at 100 and 200mg/kg. The release of histamine serotonin and prostaglandin could be related to reduce the inflammation. Stichopus japonicus and Stichopus chloronotous sea cucumbers found in Korea have potent melanin biosynthesis inhibitory activity. They possessed fatty acids with effective antitumor, anticoagulant and antiinflammatory activities [28]. Sea cucumbers such as Apostichopus japonicas, Cucumaria frondosa, Stichopus chloronotous and Holothuria scabra were reported to contain high-value components and bioactives including lectins, peptides, glycoprotein, glycosphingolipids and essential fatty acids [29].

**Analgesic activity:**

The extracts of Holothuria atra exerted a dose dependent increase in latency time when compared with the control groups is shown in (Table 2). In H. atra the percentage inhibition measured for 100mg was as 55.30%, 63%, and 75.10%. At 200 mg the percentage the inhibition was 61.07%, 70.66 and 84.355% respectively. The reference standard drug pentazocine and control values were compared. The results were statistically significant (p<0.001). It was denoted that the Holothuria atra exerted analgesic activity. The occurrence of compounds in H. atra such as isoquinoline alkaloids and phenolic compounds could show analgesic activity. Holothuria tubulosa, Leptogorgia ceratophyta, Coscinasterias tenuispina and Phallusia fumigated extracts were used using dichloromethane and methanol to assess their analgesic activity Compounds such as triterpene glycosides, glycosaminoglycans and lectins seen in sea cucumbers acted as analgesics [30]. The H. atra extracts of 100mg/kg showed analgesic activity of 75%. It was compared with the standard drug pentazocine. It suggested a possibility of analgesic potency of extracts. Analgesic activity of Sea cucumber Stichopus japonicas extracts at a dosage of 300 mg/kg was studied on rats and it was compared with the drugs such as morphine, aspirin and paracetamol. They showed efficacy of 50% [31].
Antipyretic activity:
The methanol extracts of *H. atra* were given orally to group 3 and 4 at 100 and 200mg dosages respectively. The difference in temperature between 0 hour and at the end of 4 hours was compared and analyzed with that of the standard drug paracetamol. Significant value noted at *p<0.05* and at **p<0.01** thus indicated high significance. The extracts showed a constant and steady level decrease in temperature at 100 and 200mg dosages of *Holothuria* extracts as shown in (Table 3).

Sea cucumber extracts (SCE) unlike paracetamol, consistently showed marked antipyretic effects in all the animal models used such as rabbits and guinea pigs. It also exhibited anti-anaphylactic, and gastrointestinal protective effects [32]. Sea cucumber *Holothuria edulis* extracts indicated the presence of neurostimulators agents which act on the central nervous system [33]. The fatty acids seen in *Holothuria atra* could act as the substrate for the biosynthesis of eicosanoids which are known to mediate inflammation and regulate CNS. Based on this mode of action, compounds that inhibit PLA2 activity have been targeted as potential therapeutic agents in the treatment of inflammation and neurological disorders.

The *in vitro* analysis of glycosylphosphatidylinositol from sea cucumbers *Stichopus chloronotus* for neuritogenic activity may lead to the development of therapeautic products for neurological disorders [34].

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose (mg/kg)</th>
<th>Increase in paw volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>0.586±0.04</td>
<td>0.928±0.005</td>
</tr>
<tr>
<td>0 h</td>
<td>0.988±0.00</td>
<td>0.939±0.00</td>
</tr>
<tr>
<td>1 h</td>
<td>0.847±0.00</td>
<td>0.725±0.00</td>
</tr>
<tr>
<td>2 h</td>
<td>0.757±0.00</td>
<td>0.586±0.005</td>
</tr>
</tbody>
</table>

**Table 1:** Antinflammatory activity using methanolic extracts of *Holothuria atra*

P value calculated by one way ANOVA followed by Dunnett's test ns – non significant; *p<0.01 (significant), n=4, values are mean±SEM

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose (mg/kg)</th>
<th>Reactiontime (sec) afterdrug administration</th>
<th>Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>1</td>
<td>1.73±0.48</td>
<td>0.0</td>
</tr>
<tr>
<td>1 h</td>
<td>2.72±0.25</td>
<td>2.85±0.29</td>
<td>0.0</td>
</tr>
<tr>
<td>2 h</td>
<td>3.75±0.15</td>
<td>4.55±0.10</td>
<td>0.0</td>
</tr>
<tr>
<td>3 h</td>
<td>4.55±0.10</td>
<td>5.55±0.10</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Table 2:** Analgesic activity using methanolic extracts of *Holothuria atra*

P value calculated by one way ANOVA followed by Dunnett's test N=4, values are Mean ± SEM *P<0.01, **P<0.001 (significant), values are compared with control group

Central Nervous System (CNS) Depressant activity:
Locomotor activity was considered as an index of alertness and a decrease in it indicated sedative activity as shown in (Table 4). Moreover, the effect on locomotor activity was less for *H. atra* extracts. The results were compared with the control and the stimulant drug caffeine at the dose of 30mg as the standard. The CNS depressant activity of the methanol...
H. atra revealed significant depression pattern in the test for locomotor activity in rats. The reduced locomotor activity assessed by actophotometer was found to be extract-dependent. The H. atra extract showed the maximum effect of 20%. Similarly Psolusosides A and B isolated from the holothurian Psolus fabricii a triterpene glycoside acts as an inhibitor in regulation of the central nervous system on rats. It was involved in inhibition cholesterol affinity [35]. It is found that Holothuria atra was effecting CNS regulatory action. It was compared with that of caffeine. The extract was shown to contain a complex mixture of structurally different brominated pyrrole alkaloids. It could be used as a stimulant in reducing the growth of neurological disorders. The in vitro analysis of glycosphingolipids from sea cucumbers Stichopus chloronotus for neuritogenic avtivity may lead to the development of therapeautic products for neurological disorders [36].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Mean locomotor activity scores in ten minutes</th>
<th>Percentage of locomotor activity</th>
<th>Nature of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>I</td>
<td>-</td>
<td>163.5±1.708</td>
<td>0.6</td>
<td>No action</td>
</tr>
<tr>
<td>Caffeine</td>
<td>II</td>
<td>30</td>
<td>170.5±2.218</td>
<td>2.4</td>
<td>Stimulant</td>
</tr>
<tr>
<td>Holothuria atra</td>
<td>III</td>
<td>100</td>
<td>155±1.10</td>
<td>0.4</td>
<td>Stimulant</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>200</td>
<td>165±1.290</td>
<td>0.8</td>
<td>Stimulant</td>
</tr>
</tbody>
</table>

Table 4: CNS locomotor activity using methanolic extract of Holothuria atra

In-vivo Immunomodulation activity:
Sea cucumber species of H.atra have shown moderate immunomodulator activity at a concentration of 25, 50 and 100 mg/ml as compared with control in (Table 5). The in vivo immunomodulatory study with carbon clearance test on mice showed that these extracts acted as immune stimulant. Holothuria atra showed significant inhibition effect at 50 and 100mg of the extract with potent values showing P < 0.001 significant and then maximum effect at P < 0.05 seen statistically significant at the dosage of 25 and 50mg of extracts. Frondoside A from sea cucumber Cucumaria frondosa and Stichopus japonicas an immunostimulant of cell-based immunity including phagocytosis have significant effect on amplification of humoral immune activity or adjuvant properties [37].

Antioxidant activity:
The antioxidant activity of extracts was evaluated by their ability to scavenge free radicals by using DPPH assay were monitored in (Table 6). The extract concentration that caused scavenging of 50% of DPPH (IC50) was evaluated. The IC50 value of Holothuria atra was 300. In the present study methanolic extracts exhibited higher DPPH scavenging capacity. The inhibition effect increased at higher concentrations with promising antioxidant activity. In general the antioxidants play the important role of protecting the human body against damage by reactive oxygen species [38]. Furthermore previous epidemiological studies have shown that the intake of natural antioxidants has been associated with reduced risks of cancer and other diseases associated with oxidative damages [39].

<table>
<thead>
<tr>
<th>Time minutes</th>
<th>in Group</th>
<th>control 25mg/kg H.atra</th>
<th>50mg/kg H.atra</th>
<th>100mg/kg H.atra</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>I</td>
<td>0.42 ± 0.26</td>
<td>0.83 ± 0.45</td>
<td>1.24 ± 0.24*</td>
</tr>
<tr>
<td>6</td>
<td>II</td>
<td>0.37 ± 0.29</td>
<td>0.85 ± 0.36</td>
<td>1.05±0.20*</td>
</tr>
<tr>
<td>9</td>
<td>III</td>
<td>0.30 ± 0.33</td>
<td>0.78 ± 0.42</td>
<td>1.15 ± 0.28*</td>
</tr>
<tr>
<td>12</td>
<td>IV</td>
<td>0.25 ± 0.27</td>
<td>0.75 ± 0.34</td>
<td>1.20 ± 0.51*</td>
</tr>
</tbody>
</table>

Table 5: Effect of Holothuria atra extracts on carbon clearance in mice
Values are mean ± SEM (n=6) * P < 0.05, ** P < 0.001

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Concentration µg/ml</th>
<th>Inhibition(%)</th>
<th>IC50 Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holothuria atra</td>
<td>25</td>
<td>27.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>36.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>64.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>84.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>95.72</td>
<td>300</td>
</tr>
</tbody>
</table>

Table 6: DPPH radical scavenging activity of Holothuria atra
CONCLUSION
This study is a preliminary evaluation of pharmacological studies of H. atra extract. Therefore it could be concluded that sea cucumber can be explored as a potential source of high-value bioactive metabolites and could be used in the pharmaceutical industry. The results of the present study showed that it acts as an effective antioxidant with significant pharmacological properties such as anti-inflammatory, analgesic, antipyretic, and immunomodulatory activities. Further studies of isolation and structure elucidation of compounds in H. atra are currently in progress.

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REFERENCES


