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D. Isaac Dhinakaran
Centre for Marine Science and Technology,Manonmaniam Sundaranar University, Rajakkamangalam 629502, Kanyakumari District, Tamil Nadu, India.
Email: isaacdhina@yahoo.co.in
Phone No: +919442076754

QR Code for Mobile users

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Pharmacological Potentials of Sea Cucumber *Holothuria Atra* Extracts from the Indian Ocean

D. Isaac Dhinakaran[a*] and A.P. Lipton[b]

[a]Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam 629502, Kanyakumari District, Tamil Nadu, India.
[b]Marine Biotechnology Laboratory, Central Marine Fisheries Research Institute, Vizhinjam 695521, Kerala, India.

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 Holothuroidae. 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 *H.atra* 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.

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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 ja*ponicus, 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 scabraside 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* Sp*1* 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 chondroiton 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

(*Holothuria atra*):

Holothuria atra specimens with a size range of 10 to 30 cm in length and 30 to 180 g weight were collected from fishing nets operated off Kanyakumari (8° 03' and 8° 35' of the north Latitudes and 77° 15' and 77° 36' of the east longitudes) in the Indian Ocean. Immediately upon collection, *H. atra* specimens were dissected to remove the internal organs and packed using ice and kept at –80°C for extraction. The skin portion was peeled off and stored in methanol in separate containers. The body wall layer of *Holothuria atra* was used to prepare extracts. The biologically active compounds were extracted based on their polarity using methanol an organic solvent as per the method with appropriate modifications [20]. About 200g of frozen samples were homogenized with deionized water and methanol. The mixture was continuously stirred in the dark at 4°C for 24 h. Then it was centrifuged at 5000 rpm for 15 minutes. The supernatant of 250 ml was collected and filtered. The extracts thus collected were freeze-dried and kept at -80 °C, while the insoluble solid materials were re-extracted with methanol (100%). The organic extracts were combined and the solvents were removed by rotary evaporation at 40°C under low pressure to avoid degradation of compounds.

Experimental animals:

Twenty four whistar albino rats of either sex, weighing approximately 120-180g were selected. They were procured from the Department of Pharmacology, Sankaralingam Bhuvaneswari Pharmacy College Sivakasi (Tamilnadu). The experimental mice were maintained on pellet diet (Gold Mohur brand) at room temperature in separate cages. Drinking water was given by ad *libitum*. After acclimatization to the experimental conditions for a week, the rats were divided into six groups. Each group comprised of four rats of either sex. Prior to the start of the experiments, these animals were kept fasting and employed in the study. For the *in vivo* immunomodulatory study Swiss albino mice of both sexes were used. The experimental animals were procured from animal house of Sangaralinkam Bhuvaneswari College of Pharmacy (Regd.No.622/02/C/CPCSEA) used for the present study.

Anti inflammatory activity (Carrageenan induced rat paw edema):

Anti-inflammatory activity was evaluated by injecting 0.05 ml of 1%w/v carrageenan (Sigma) subcutaneously into the sub plantar region of the right hind paw of albino rats. The induced paw edema was measured [21]. Rats were divided into four groups of four individuals each. The control group was given saline (1 ml / kg) and the standard drug Diclofenac sodium (10mg / kg) administered to group II animals served as the standard reference. Group III and IV animals were treated with *Holothuria atra* extract at the dose level of 100 and 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.

Analgesic Activity (Tail Immersion Method):

The analgesic activity was assessed by measuring the sensitivity by placing the tip of the tail (last 1-2 cm) of adult albino rats gently in warm water maintained at 55 ± 2^0 C. Only active rats (tail flicking within 5 sec) were selected for this study. The selected rats were divided into four groups of four numbers/individuals each. The Group I (control group) received normal saline and the Group II (standard reference group) was treated with (10mg/kg) p.o of Pentazocine. Group III and IV individuals were treated with *H. atra* extract at the dose level of 100 and 200mg / kg (p.o) respectively. All the doses were administered orally. After drug treatment, the basal reaction time of all groups of animals was recorded at different time intervals like 0, 1, 2 3, h and the values were expressed as Mean ± SD of 4 animals in each group [23]. The data was analyzed by one way ANOVA followed by Dunnett's test.

Antipyretic activity:

Antipyretic activity was carried out by using Brewer's yeast induced hyperpyrexia method using digital Telethermometer [24]. Whistar rats of either sex weighing 120-180gm were selected. The animals were divided into four groups of four animals each. They were fasted for 24h before inducing pyrexia. Pyrexia was induced in albino rats by injecting 15% (M/V) aqueous suspension of Brewer's yeast into the nape of neck subcutaneously at 10 ml/mg of suspension to initiate pyretic action. The initial rectal temperature of each animal was recorded by digital thermometer. After 18 hours, the animals developing 0.5^0C rise in the rectal temperature were selected for further studies. The Group I (control group) received normal saline and the Group II (standard reference group) was treated with (25 mg / Kg) p.o of Paracetamol orally. Group III and IV animals were treated with *H. atra* extract at the dose level of 100 and 200mg / kg (p.o). The rectal temperature was recorded at 1, 2, 3, 4 hours after administration of the test drug/ extracts.

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 cm^3) 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 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-lV 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 Percentage radical scavenging = (Control OD - Sample OD/Control OD)* 100

Antioxidant capacity was expressed in IC_{50} 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 prostoglandin 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 chloronotus* 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 fumigata* 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].

Treatment	Group	**Dose (mg/kg)**	Increase in paw volume (ml)					
			0 h	1 h	2 h	3 h	4 h	5 h
Control	**I**	0.586±0.004	0.928±0.005	0.988±0.005	0.939±0.005	0.847±0.005	0.725±	0.586±0.005
Diclofenac sodium	**II**	**10**	0.597±0.004 ns	0.767±0.007 *	0.748±0.008*	0.648±0.009*	0.617±0.008*	0.598±0.008*
Holothuria atra	**III**	100	0.588±0.001 ns	0.756±0.007*	0.708±0.007*	0.644±0.008*	0.620±0.008*	0.570±0.008*
	IV	200	0.572±0.007 ns	0.825±0.006	0.713±0.006	0.673±0.007*	0.631±0.008	0.582±0.008*

Table 1: Antiinflammatory activity using methanolic extracts of *Holothuria atra*
P value calculated by one way ANOVA followed by Dunnett's test ns – non significant; *p<001 (significant), n=4, values are mean±SEM

Treatment	Group	Dose (mg/kg)	Reactiontime (sec) afterdrug administration			Percentage inhibition (%)		
			1 hr	2 hr	3 hr	1 hr	2 hr	3 hr
Control	I	-	1.73±0.48	2.72±0.25	2.85±0.29	0.0	0.0	0.0
Pentazocine	II	10	7.5±0.65**	9.25±0.48**	15.25±0.42**	73.07	80.20	99.59
Holothuria atra	III	100	4.5±0.43*	5.33±0.41**	6.44±0.43**	55.30	63.00	75.10
Control	IV	200	5.7±0.22**	6.15±0.71**	7.70±0.64**	61.07	70.66	84.35

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

Antipyretic activity:

The methanol extracts *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 analysed 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 gastro 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 glycosphingolipids from sea cucumbers *Stichopus chloronotus* for neuritogenic avtivity may lead to the development of therapeautic products for neurological disorders [34].

Treatment	Group	Dose (mg/kg)	Initial temp. (^{0}C)	Rectal temperature ^{0}C in hour ± SEM				
			37.53±0.09	38.15±0.16	38.16±0.09	38.18±0.09	38.20±0.09	37.23±0.1
Control	I							
Paracetamol	II	45	37.39±0.13*	38.70±0.17*	37.96±0.20**	37.77±0.27 **	37.70±0.34 *	37.50±0.31**
Holothuria atra	III	100	35.42±0.07	35.92±0.11*	35.63±0.1*	35.56±0.27**	35.47±0.25**	35.33±0.24 **
	IV	200	35.38±0.08	35.22±0.10	35.88±0.21 *	35.75±0.20**	35.54±0.25*	35.26±0.30**

Table 3: Antipyretic activity using methanolic extracts of *Holothuria atra*
P value calculated by one way ANOVA followed by Dunnett's test Mean ± SEM, (n=4), ns – non significant, *P<0.05, **P<0.01 (significant)

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].

Treatment	Group	Dose (mg/kg)	Meanlocomotoractivity scores in ten minutes		Percentage of locomotor activity	Nature of action
			Before treatment	After treatment		
Control	l	-	163.5±1.708	162.5±1.708	0.6	No actiom
Caffeine	ll	30	170.5±2.218	224±3.163	24	Stimulant
Holothuria atra	lll	100	155±1.10	173.4±1.708	0.4	Stimulant
	lV	200	165±1.290	235±13.329	20	Stimulant

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 magnificient 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].

Time minutes	in Group	control	25mg/kg *H.atra*	50mg/k *H.atra* g	100mg/kg *H.atra*
3	l	0.42 ± 0.26	0.83 ± 0.45	1.24 ± 0.24*	1.81±0.30**
6	II	0.37 ± 0.29	0.85 ± 0.36	1.05±0.20*	1.32±0.05**
9	III	0.30 ± 0.33	0.78 ± 0.42	1.15 ± 0.28*	1.58±0.53**
12	IV	0.25 ± 0.27	0.75 ± 0.34	1.20 ± 0.51*	1.65±0.35**

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

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 (IC_{50}) was evaluated. The IC_{50} 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].

Extracts	Concentration µg/ml	Inhibition(%)	IC_{50} Value
Holothuria atra	25	27.60	300
	50	36.35	
	75	64.00	
	100	84.66	
	125	95.72	

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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