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ANALGESIC ACTIVITY OF *DICTYOPTERIS AUSTRALIS* (SONDER) ASKENASY (BROWN SEAWEED) FROM PAMBAN, RAMANATHAPURAM DISTRICT, TAMIL NADU, INDIA.

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Abstract:

The present study was aimed to determine the analgesic activity of methanolic extract of Dictyopteris australis (Sonder) Askenasy collected from Pamban, Ramanathapuram District, Tamil Nadu, India. The dried and powdered Dictyopteris australis was extracted in methanol to estimate the analgesic activity. The analgesic activity was assessed on intact rat by tail immersion method. Diclofenac Sodium in the dose of 100mg/kg was used as standard drug. Methanolic extracts of Dictyopteris australis were given in the doses of 200 and 400mg/kg. Control group received normal saline solution. All the doses were given orally. Results explained that both the doses of methanolic extracts of Dictyopteris australis had potent analgesic activity. From the observations, it was concluded that methanolic extract of Dictyopteris australis at 200mg/kg was found to have more effect as compared to 400mg/kg methanolic extract.

Key words: *Analgesic, Seaweeds, Dictyopteris, Methanolic extract, Pamban.*

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INTRODUCTION:

Marine organisms are potentially inexhaustible sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. Among the various marine organisms, seaweeds or marine macro algae were found to over 5000 species of green algae, 1500 species of brown algae and with over 4000 species of red algae. Human has used the sea for many years as a productive source for several economically useful materials, especially to supplement diet [1]. The chemistry of seaweeds has interested many researchers in order to develop new drugs. The knowledge about the chemical composition of seaweeds is an essential element for assessing chemotaxonomic, chemical ecology and natural products studies, including that directed towards evaluating the pharmacological roles. In recent years an increasing number of seaweed natural products have been reported to display various pharmacological activities [2, 3, 4]. In most cases, the evaluation of analgesic potential of methanol crude extracts from different seaweeds has been carried out by *in vivo* tests [5, 6]. Seaweeds derived compounds have played an important role in the development of several clinically useful drugs. The current study focused on the useful property of *Dictyopteris australis* such as its analgesic effects which have never been reported. Hence the present was intended to examine using a tail immersion method conducted in Wistar albino rats. The animal model offers a distinct advantage for testing new substances under controlled conditions.

MATERIALS AND METHODS:

Collection of Plant Sample

Dictyopteris australis (Sonder) Askenasy (Fig.1) is brown seaweed belonging to Phaeophyceae member focused in the present study for the analgesic potential. *Dictyopteris australis* was collected from Pamban coast, Ramanathapuram district in the south east coast of Tamil Nadu, India. The collected plant samples were rinsed with marine water to remove debris and epiphytes. The entire epiphytes were removed using soft brush. The plants were brought to the laboratory. In the laboratory, the plants were once again washed in freshwater and stored in refrigerator for further analysis [7].

Fig.1: Natural Habit of *Dictyopteris australis* (Sonder) Askenasy

Preparation of methanol extract

For the preparation of methanol extract of *Dictyopteris australis*, the collected plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered sample was packed in Soxhlet apparatus and extracted with methanol for 8h separately. The excess amount of methanol was evaporated and fine methanol crude powder was prepared and stored in the refrigerator for the analgesic activity [8].

Experimental Animals

Wistar albino rats (160-200g) of either sex were procured from Venkateswara Enterprises, Bangalore, Karnataka, India. The selected animals were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 35±1°C, relative humidity 45-55% and light/dark cycle 12/12h. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% *Arachis* oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conduct between 10.00 and 17.00h and were in accordance with the ethical guidelines of the International Association for Study of Pain [9]. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute toxicity test

Acute oral toxicity study was performed as per OECD-423 guidelines [10]. Wistar albino rats (n=6) of either sex selected through random sampling technique was used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract (50% methanolic extract) was administered orally at the dose level of 5mg/kg body weight by gastric intubation and observed for 14 days. If mortality is observed in 2 out of 3 animals, then the dose administered would be assigned as toxic dose. If mortality is observed in 1 animal, then the same dose would be repeated again to confirm the toxic dose. If mortality is not observed, the procedure would be repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. According to the results of acute toxicity test, the doses were chosen for experiments.

Table.1: Analgesic activity of methanol extracts of *Dictyopteris australis* (Sonder) Askenasy

Animal groups	Pre-analgesic (seconds)	1 hour (seconds)	2 hour (seconds)	3 hour (seconds)	4 hour (seconds)
Control	2.00±0.20	2.25±0.13	2.25±0.13	2.25±0.13	2.50±0.15
100mg/kg Diclofenac sodium	1.75±0.12	3.00±0.20	5.75±0.47	8.50±0.11	6.50±0.11
200mg/kg Methanol extract	2.20±0.20	3.75±0.32	4.75±0.22	5.75±0.47	3.75±0.47
400mg/kg Methanol extract	1.75±0.13	2.50±0.11	3.25±0.22	3.00±0.09	3.00±0.10

Analgesic activity by Tail immersion method

In the present study, analgesia was assessed according to the method of Luiz *et al.* [11]. Wistar albino rats Mice divided in the groups of six each were held in position in a suitable restrainer with the tail extending out. 2-3cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cutoff time for immersion was 180 seconds to avoid the injury of the tissues of tail. 0.2 ml of 0.9% NaCl solution was administered to control animals; plant extracts in doses of 200 and 400mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and standard drugs and then 1h, 2h, 3h and 4h after the administration. The criterion for analgesia was post drug latency which was greater than two times the predrug average latency as reported by Janssen *et al.* [12]. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

RESULTS AND DISCUSSION:

In the tail immersion test, the standard analgesic drug (100mg/kg Diclofenac sodium) as well as the test drugs of methanolic extract of *Dictyopteris australis* obtained the doses of (200 and 400mg/kg) showed a significant reductions in the number of tail flick of mice as compared to the control mice. Acute toxicity studies showed that the methanolic extracts did not cause any mortality up to 2000mg/kg and were considered as safe. The control group at pre analgesic, 1h, 2h, 3h and 4h showed hot water reaction time in sec is 2.00±0.20, 2.25±0.13, 2.25±0.13, 2.25±0.13 and 2.50±0.15 respectively. The corresponding mean volumes in Diclofenac sodium (100 mg/kg) treated group were 1.75±0.12, 3.00±0.20, 5.75±0.47, 8.50±0.11 and 6.50±0.11 respectively indicating the significant analgesic activity of Diclofenac sodium from 1h onwards when compared to control. Methanolic extract of *Dictyopteris australis* in both the doses of 200mg/kg and 400mg/kg had produced significant increase in hot water reaction time in dose depended manner from 1h to 4h. 200mg/kg methanolic extract of *Dictyopteris australis* has taken 5.75±0.47 sec in 3h whereas 400mg/kg methanolic extract showed 3.25±0.22 sec at 2h. The methanolic extract of *Dictyopteris australis* in both doses 200mg/kg and 400mg/kg had also produced significant analgesic effect with the mean hot water reaction time in dose dependent manner (Table 1 and Figure 2). Among the two different concentration of methanol extract studied, 200mg/kg concentration of methanolic extract showed the highest activity compared to 400mg/kg methanolic extract of *Dictyopteris australis*.

The present study is the first report to demonstrate the analgesic activity of methanolic extract of *Dictyopteris australis* which produces in appropriate animal models (tail immersion model). Brown algae are considered the most important source of many biologically active metabolites. Although there are relatively few studies demonstrating possible analgesic agents found in red [5] and green [6] seaweeds. Many studies have found interesting biological activities in polar fractions from seaweeds and similar results were also obtained in the present study. The data indicated that the methanolic extract of the brown seaweed *Dictyopteris australis* produced a dose dependent analgesic effect. The search for new metabolites from marine organisms has resulted in the isolation of some compounds such as terpenes, peptides and sulphated carbohydrates that exhibit analgesic effects [13, 14, 15]. The analgesic activity observed may be associated with the presence of such compounds and other secondary metabolites in the methanolic extract of *Dictyopteris australis.*

CONCLUSION:

The results of the present study recommended that the methanolic extract of *Dictyopteris australis* possesses the analgesic activity in both the doses of 200mg/kg and 400mg/kg. Among the two doses, 200mg/kg methanolic extract was found to be the best result. Further chemical analysis on the composition of methanolic extract of *Dictyopteris australis* is necessary to isolate and identify bioactive compounds that may have applications in therapeutic field of pain.

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