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Original Article

Screening of Analgesic Activity of Methanol Extract of *Hypnea flagelliformis* Greville ex J.Ag.(Red seaweed) in Koothankuzhi Coast Tamilnadu India

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ABSTRACT

In the present study, an attempt was taken to determine the analgesic activity of methanolic extract of *Hypnea flagelliformis* Greville ex J.Ag. in Koothankuzhi coast, Tamil Nadu, India. **Methods:** The dried powdered *Hypnea flagelliformis* Greville ex J.Ag. was extracted in absolute methanol to estimate the analgesic activity. The analgesic activity was predicted on intact rats by tail flick latency in tail immersion method. Diclofenac Sodium in the dose of 100mg/kg was used as standard drug. Methanolic extracts of *Hypnea flagelliformis* Greville ex J.Ag. were given in the doses of 200 and 400mg/kg. Control group received normal saline solution. All the doses administered orally. **Results:** Results showed that both the doses of methanolic extracts of *Hypnea flagelliformis* Greville ex J.Ag. had analgesic activity. **Conclusion:** From the results, it was concluded that methanolic extract of *Hypnea flagelliformis* Greville ex J.Ag. at 400mg/kg was found to have more effect as compared to 200mg/kg methanolic extract.

***Key words*:** Analgesic, Seaweeds, *Hypnea*, Koothankuzhi.

1. INTRODUCTION

The marine ecosystem is the richest source of both biological and chemical diversity in the world. In recent years, a number of novel metabolites with effective pharmacological properties have been discovered from the marine organisms. Among the various marine organisms, the seaweeds popularly known as macroalgae are considered to be a rich source of bioactive compounds suitable for

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therapeutic and medical applications. Emerging trend of increasing new molecules from seaweeds promotes the marine science towards the potential research area of drug discovery. Commercially available varieties of seaweeds are the futuristically promising plants, act as one of the most important marine resources. Being a plant of unique structure and biochemical composition, seaweeds could be explored for various purposes in the form of food, medicine and cosmetics[1, 2].

Production of safe and potent analgesic, anti-inflammatory and anti-ulcer drugs from natural origin has recently been concentrated. Due to wide abundance of seaweeds could be a potential source of new therapeutic compounds[3, 4]. The red algae has been reported to contain active compounds that may help to prevent various diseases[5]. Natural compounds derived from seaweeds would be safer to be used as therapeutics as they were taken as food and used in traditional medicines since time immemorial[6]. Hence, in the present study, an effort has been taken to analyze the analgesic activity of the methanolic extract of *Hypnea flagelliformis* Greville ex J.Ag. collected from Koothankuzhi coast, Tamil Nadu, India by tail immersion test.

2. MATERIALS AND METHODS

1.1. Collection of Plant Sample

Hypnea flagelliformis Greville ex J.Ag. is red seaweed belonging to Rhodophyceae member showed much attention in the present study for analgesic activity. *Hypnea flagelliformis* Greville ex J.Ag. were collected from Koothankuzhi coast 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].

1.2. Preparation of methanol extract

For the preparation of methanol extract of *Hypnea flagelliformis* Greville ex J.Ag., 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].

1.3. 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).

1.4. Acute toxicity test

Acute oral toxicity study was performed as per OECD-423 guidelines[10]. 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 5 mg/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.

1.5. Analgesic activity by Tail immersion method

In the present study, analgesia was assessed according to the method of Luiz *et al.*[11]. Rats 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.

3. RESULTS AND DISCUSSION

In the tail immersion test, the standard analgesic drug (100mg/kg Diclofenac sodium) as well as the test drugs of methonolic extract of *Hypnea flagelliformis* Greville ex J.Ag. obtained the doses of (200 and 400mg/kg) showed a significant reductions in the number of tail flick of rats as

compared to the control rats. The control group at pre analgesic, 1h, 2h, 3h and 4h showed hot water reaction time in sec is 2.0±0.7, 2.3±0.4, 2.3±0.4, 2.3±0.4 and 2.5±0.5 respectively. The corresponding mean volumes in Diclofenac sodium (100 mg/kg) treated group were 1.8±0.8, 3.0±0.7, 5.8±1.4, 8.5±1.1 and 6.5±1.1 respectively indicating the significant analgesic activity of Diclofenac sodium from 1h onwards when compared to control. Methanolic extract of *Hypnea flagelliformis* Greville ex J.Ag. in both the doses of 200mg/kg and 400mg/kg had formed significant increase in hot water reaction time in dose depended manner from 1h to 4h. 200mg/kg methonolic extract of *Hypnea flagelliformis* Greville ex J.Ag. has taken 3.50±0.14 sec whereas 400mg/kg methanolic extract showed 4.75±0.17 sec at 4h. The methanolic extract of *Hypnea flagelliformis* Greville ex J.Ag. 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 1).

Table 1: Analgesic Effect of methanolic extract of *Hypnea flagelliformis* Greville ex J.Ag. by Tail Immersion method

Animal groups	Pre Analgesic (seconds)	1 hour (seconds)	2 hour (seconds)	3 hour (seconds)	4 hour (seconds)
Control Normal saline	2.0±0.7	2.30±0.4	2.30±0.4	2.30±0.4	2.50±0.5
100mg/kg Diclofenac sodium	1.80±0.8	3.00±0.7	5.80±1.4	8.50±1.1	6.50±1.1
200mg/kg methanol extract	2.00±0.04	2.25±0.03	3.00±0.02	3.25±0.12	3.50±0.14
400mg/kg methanol extract	2.10±0.06	3.00±0.03	3.75±0.01	4.50±0.11	4.75±0.17

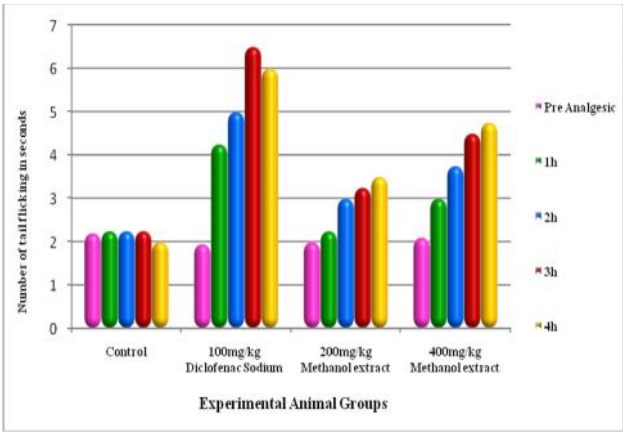

Fig 1: Analgesic activity of methanol extract of *Hypnea flagelliformis* Greville ex J.Ag.

4. CONCLUSION

From the present study, it can be concluded that the methanolic extract of *Hypnea flagelliformis* Greville ex J.Ag. was confirmed with both central and peripheral analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

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