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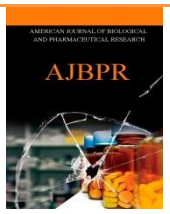

ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF GRACILARIA CORTICATA J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

Iniya Udhaya C and John Peter Paul J*

Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

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ABSTRACT

The present study was carried out to determine the analgesic activity of methanolic extract of *Gracilaria corticata* J.Ag. in Hare island, Thoothukudi, Tamil Nadu, India. The dried powdered *Gracilaria corticata* J.Ag. was extracted in absolute methanol to estimate the analgesic activity. The analgesic activity was assessed on intact mice by tail flick latency in tail immersion method. Diclofenac Sodium in the dose of 100mg/kg was used as standard drug. Methanolic extracts of *Gracilaria corticata* J.Ag. were given in the doses of 200 and 400mg/kg. Control group received normal saline solution. All the doses administered orally. Results showed that both the doses of methanolic extracts of *Gracilaria corticata* J.Ag. had potent analgesic activity. From the observations, it was concluded that methanolic extract of *Gracilaria corticata* J.Ag. at 200mg/kg was found to have more effect compared to 400mg/kg methanolic extract.

INTRODUCTION

During the past decade, the traditional system has gained importance in the field of medicine. In most of the developing countries, who are dependent on medicinal plants to meet their primary health care needs? Since the usage of these herbal medicines has increased due to their quality, safety, and efficacy in industrialized and developing countries. Increasing interest has forced researcher to screen scientifically various traditional claims. The naked nerve endings are the sensing zones for pain found in almost every tissue of the body. The pain impulses are transmitted to the central nervous system by two fibre systems. The nociceptor system is made up of small myelinated fibres of 2-5µ in diameter which conduct at 12-30m/s.

Corresponding Author

John Peter Paul, J
Email:- johnarock2008@yahoo.com

The unmyelinated fibres of 0.4-1.2 µ in diameter conduct at slower rate of 0.5-2m/s and also called dorsal root fibers. There is evidence that the synaptic transmitter secreted by primary afferents, sub serving mild pain is glutamate and transmitter sub serving severe pain is another substance. The primary mechanism involved may be synaptic inhibition at the endings of the primary conductors that transmit the pain impulses [1].

The adequate stimulus for pain receptors is not specific. Pain receptors respond to a variety of strong stimuli factors or effects originating from thermal, electrical, mechanical and chemical changes. It is suggested that the pain is chemically modulated and that stimuli in common the ability to liberate a chemical agent that stimulates nerve endings which may be due to ATP. It opens ligand gated channels on sensory neurons via P2x receptors. Capsaicin is a component responsible for burning pain. A capsaicin receptor that is non selective ion channel permits flow of Na^{+} and Ca^{2+} into nociceptive neurons

when activated producing depolarization. This channel is activated by warmth. Pain is as perception alone which does not require cortex. Nociception is the mechanism by which nervous peripheral stimuli are transmitted to the central nervous system. Pain is subjective not always associated with nociception. Polymodal nociceptors (PMN) are the main types of peripheral sensory neurons that respond to various stimuli. Chemicals stimulate by acting on PMN to cause pain include bradykinin, 5-HT and capsaicin. PMN are sensitized by prostaglandins which explains analgesic effect of aspirin like drugs particularly in inflammation [2].

Post injury pain persists after injury. The stimuli in that injured region produce exaggerated response (hyperalgesia) and stimuli such as touch cause pain (allodynia). If the nerves are damaged the pain persist after injury heals (neuropathic pain). In post injury and neuropathic pain, there is increased sensitivity of peripheral pain receptors due to local release of sensitizing substances. There is also increased transmission at the synaptic junction between the first and the second order neuron in dorsal horn. Many methods are available for evaluation of analgesic effect. In all the methods, one or other type of stimulus is applied to produce pain reaction. Of the methods available, the tail immersion test is used for the evaluation of the analgesic activity of peripherally acting drugs [3]. However, in the present study, an attempt has been taken to analyze the analgesic activity of the methanolic extract of *Gracilaria corticata* J.Ag. collected from Hare island, Thoothukudi, Tamil Nadu, India by tail immersion test.

MATERIALS AND METHODS

Collection of Plant Sample

Gracilaria corticata J.Ag. (Figure 1) is red seaweed belonging to Rhodophyceae member showed much attention in the present study for analgesic activity. *Gracilaria corticata* J.Ag. were collected from Hare island, Thoothukudi 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 [4].

Preparation of methanol extract

For the preparation of methanol extract of *Gracilaria corticata* 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. 3g 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 [5].

Experimental Animals

Wistar albino rats (160-200g) and Swiss albino mice 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 [6]. 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 [7]. Albino mice (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.

Analgesic activity by Tail immersion method

In the present study, analgesia was assessed according to the method of Luiz *et al.* [8]. 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 240 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 incubation. 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.* [9]. 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

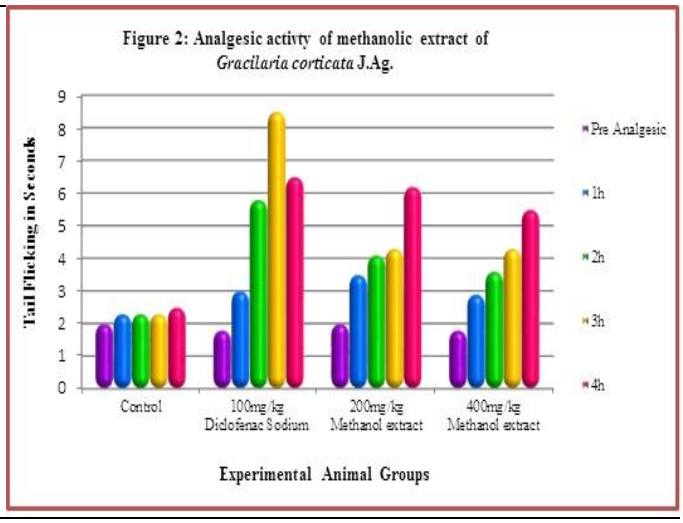
In the tail immersion test, the standard analgesic drug (100mg/kg Diclofenac sodium) as well as the test drugs of methonolic extract of *Gracilaria corticata* J.Ag. obtained the doses of (200 and 400mg/kg) showed a significant reductions in the number of tail flick of mice as compared to the control mice. 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 *Gracilaria corticata* J.Ag. 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 methonolic extract of *Gracilaria corticata* J.Ag. has taken 6.2±0.7 sec whereas 400mg/kg methanolic extract showed 5.5±1.0 sec at 4h. The methanolic extract of *Gracilaria corticata* 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 2).

The present study is the first report demonstrating that the methanolic extract of *Gracilaria corticata* J.Ag. showed analgesic effect in appropriate models (Tail immersion test). There are around 8000 species of red algae, most of which are from marine sources. Red algae are considered the most important source of many biologically active metabolites in comparison to other algal classes [10]. Although there are relatively few studies demonstrating possible analgesic agents found in red algae, the species *Vidalia obtusaloba* and *Ceratodictyon spongiosum* alone yielded two bromophenolic metabolites and one peptide [11]. Many studies have found interesting biological activities in polar fractions from seaweeds [12] and similar results were also obtained in the present study. The data indicated that the methanolic extract of the red seaweed *Gracilaria corticata* J.Ag. produced a dose dependent analgesic. The search for new metabolites from seaweeds has resulted in the isolation of some compounds such as terpenes, peptides and sulphated carbohydrates that exhibit analgesic effects [13-14]. The analgesic activity observed may be associated with the presence of such compounds and other secondary metabolites in the *Gracilaria corticata* J.Ag. methanolic extract.

Table 1. Analgesic Effect of methanolic extract of *Gracilaria corticata* 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	2.3±0.4	2.3±0.4	2.3±0.4	2.5±0.5
100mg/kg Diclofenac s**odium**	1.8±0.8	3.0±0.7	5.8±1.4	8.5±1.1	6.5±1.1
200mg/kg m**ethanol extract**	2.0±0.7	3.5±0.07	4.1±0.7	4.3±1.3	6.2±0.7
400mg/kg m**ethanol extract**	1.8±0.4	2.9±0.8	3.6±0.8	4.3±1.0	5.5±1.0

Figure 1. Natural Habit of *Gracilaria corticata* J.Ag.



CONCLUSION

In conclusion, it was confirmed that the methanolic extract of *Gracilaria corticata* J.Ag. was endowed 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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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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