

SCREENING OF ANTIPYRETIC ACTIVITY OF METHANOL EXTRACT OF *GRACILARIA CORTICATA* J.AG. (RED SEAWEED) IN HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

JOHN PETER PAUL J.

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

Email: johnarock2008@yahoo.com

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ABSTRACT

Objective: To investigate the antipyretic activity of methanol extract of *Gracilaria corticata* J.Ag. collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India.

Methods: Antipyretic activity was determined by brewer's yeast induced pyrexia method. Dried powder plant materials were subjected to methanol extract at the dose of 200mg/kg and 400mg/kg body weight.

Results: Methanol extract showed potential significant antipyretic activity in both doses ($p < 0.001$ and $p < 0.01$) from 1 hour to 4 hour as compared to the standard drug paracetamol.

Conclusion: The present study has been provided the evidence for the antipyretic activity of *Gracilaria corticata* J.Ag. which could partly contribute to its ethno medical use.

Keywords: Seaweed, Antipyretic effect, *Gracilaria corticata*, Paracetamol.

INTRODUCTION

The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs have been used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments. Pain, inflammation and fever are very common complications in human beings. Several plants and their products are claimed and proved to possess antipyretic property [1]. Although the body surface temperature is ordinarily measured in clinical practice, it is the body core temperature which is physiologically important. The rectal temperature (which reflects core temperature closely) is about 0.6°C higher than oral temperature and about 1.4°C higher than axillary temperature. The generally accepted normal limits of rectal temperature in adults are 36.1°C and 37.8°C; the body temperature is higher in infants. If the core temperature rises by more than a few degrees in man, mental changes occur. It is well known that an individual with high fever is often confused and delirious. The working of many tissue enzymes is also adversely affected and hyperpyrexia may result in death. However, core temperature below 40.5°C is generally tolerated by most individuals [2].

From the time immemorial the macroscopic marine algae or seaweeds have been closely associated with human life and are being exhaustively used in numerous ways as a source of food, feed, fertilizer and medicines. Seaweeds contain more than 60 trace elements in a concentration much higher than in terrestrial plants. Seaweeds also contain protein, iodine, bromine, vitamins and substances of stimulatory and antibiotic nature [3]. The phytochemicals from seaweeds are extensively used in various industries such as food, confectionary, textile, pharmaceutical, dairy and paper mostly as gelling, stabilizing and thickening agents. In addition to vitamins and minerals, seaweeds are also potentially good sources of polysaccharides and fibres [4]. Seaweeds are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic [5], antiviral [6], antihelminthic [7], antifungal and antibacterial activities [8] have been detected in green, brown and red algae. A detailed literature reviews indicated that the antipyretic activity of *Gracilaria corticata* J.Ag. has not been

clinically evaluated so far. In the present study, the antipyretic activity of methanolic extract of *Gracilaria corticata* J.Ag. collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India on mice was reported.

MATERIALS AND METHODS

Collection of Sample

Gracilaria corticata J.Ag. (Figure 1) is red seaweed belonging to Rhodophyceae member shows much attention in the present study for antipyretic activity. *Gracilaria corticata* J.Ag. were collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu, India during the month of January 2014. 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 [9].



Fig.1: Natural Habit of *Gracilaria corticata* J.Ag.

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 antipyretic activity [10].

Experimental animals

Swiss albino rats were weighing (150-240 gm) and male albino rats (15-18 gm) were procured from Venkateswara Enterprises, Bangalore, Karnataka, India. The animals were housed in the departmental animal house under standard conditions ($26\pm 2^\circ\text{C}$ and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free access 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 conducted between 10.00 and 17.00h and were in accordance with the ethical guidelines of the International Association for Study of Pain [11]. 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).

EXPERIMENTAL PROTOCOLS

The experimental treatment was carried out as;

Group I: Control group animals Normal saline 5ml/kg

Group II: Animals were treated with Paracetamol (10mg/kg) p.o.

Group III: Animals were administered with 200mg/kg methanol extract p.o.

Group IV: Animals were administered with 400mg/kg methanol extract p.o.

ANTIPYRETIC ACTIVITY

Yeast induced pyrexia method

A suspension of Brewer's yeast (15%) in saline (0.9%) was prepared. Four groups each containing 6 rats of either sex were taken. The thermocouple was inserted 2cm deep into the rectum and the rectal temperatures were recorded. The animals were febrile by injection of brewer's yeast suspension (10mg/kg) subcutaneously in the back below the nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin. The room temperature was kept at $22-24^\circ\text{C}$. Immediately after yeast administration, food was withdrawn and the rise in rectal temperature was recorded. The measurement was repeated after 30 minutes. The dose of the test compound and standard drug was given orally. The rectal temperature was recorded again after 1, 2 and 4 hours. Paracetamol (10mg/kg) was selected as a standard drug. The various methanol extracts were dissolved in saline with the help of 2% w/v Gum acacia. The data were analyzed for significance using the unpaired two-tailed student's t-test [12-13].

RESULTS

Screening of antipyretic activity of methanol crude extract of *Gracilaria corticata* J.Ag. was studied by determining the effect on yeast-induced pyrexia in albino rats. The methanol extract of *Gracilaria corticata* J.Ag. showed the highest noticeable antipyretic activities which was also dose dependent on albino rats. The result expressed that methanol extract of different doses caused lowering of the body temperature up to 4h following its administration. The effect of methanol extract on yeast-induced pyrexia showed that the rectal temperature was markedly elevated to 41.7°C , after 18h the subcutaneous injection of yeast suspension decreased to 40.7°C within 1h of 200mg/kg methanol extract of *Gracilaria corticata* J.Ag. treatment followed by 39.6°C at 2h and further reduced to 38.2°C at 4h showing a considerable decrease in compared to paracetamol.

In contrast, 400mg/kg methanol extract also showed the decreased in temperature from 41.4°C to 39.6°C after 1h of treating with the administration of the methanol crude extract of *Gracilaria corticata* J.Ag. When the time was increased up to 4h, the results were

observed significant reduced temperature to 37.2°C . Both 200 and 400mg/kg marked antipyretic activity detected were significantly different than the controls ($p < 0.05$). Generally, for all concentration of methanol crude extract of *Gracilaria corticata* J.Ag. showed marked antipyretic activities, hence, 400mg/kg methanol extract was highly effective than 200mg/kg. This result revealed that the methanol extract of *Gracilaria corticata* J.Ag. have detectable antipyretic activity as compared with standard paracetamol.

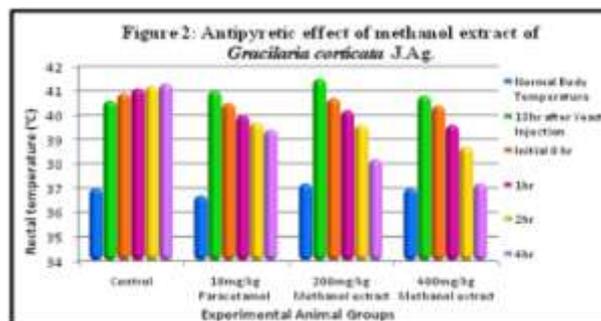


Fig.2: Antipyretic effect of methanol extract of *Gracilaria corticata* J.Ag.

DISCUSSION

Fever is a pathophysiological condition characterized by an elevation of core body temperature above normal (37°C). It results from the interaction of central nervous and immune systems and is a result of injury, infection, tumor and inflammation. The elevation of body temperature during such conditions results from the pyrogen induced upward resetting of thermoregulatory set point. Many of the exogenous substances are known to evoke fever in animal models. These pyrogens, on injection into experimental animals, induce the production of pro-inflammatory cytokines (e.g., IL-1 β , IL-6, IFN- α and TNF) which stimulate release of local PG (produced by the activity of COX) that leads to elevation of body temperature [14]. Yeast induced pyrexia in rats is a suitable and sensitive model for evaluating antipyretic effects of compounds. Yeast induces both TNF- α and prostaglandin synthesis. Antipyretics such as acetyl salicylic acid (ASA) and other NSAID reduce fever by suppressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These drugs suppress production of pyrogenic cytokines such as TNF- α and IL-1 β , while lowering the thermoregulatory set-point by blocking COX production of PGE $_2$ [15]. The extract administration resulted in lowering of temperature but the decrease of temperature was not comparable with standard antipyretic which showed significant antipyretic effect. Earlier study was also showed similar result by Gautham and Onkarappa [16, 17].

This study also correlated with the study of Zakaria *et al* [18] that the compounds present in *Gracilaria corticata* J.Ag. were suggested to act synergistically to exert the observed pharmacological activity. The results of present study indicate that the methanol crude extract of *Gracilaria corticata* J.Ag. possesses significant antipyretic effect compared to the effect of standard drug paracetamol on yeast induced pyrexia in rats. This may be attributed to the presence of various secondary metabolites present in the methanol extract which may be involved in inhibition of prostaglandin synthesis. Also, there are several mediators or multiprocessors underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis.

CONCLUSION

The present study showed dose dependent antipyretic activity of crude methanol extract of *Gracilaria corticata* J.Ag. collected from Hare island, Thoothukudi in the south east coast of Tamil Nadu. Though, the antipyretic activity exhibited by the extract was closely related to reference drugs, it was clear that the methanol crude extract possess components having pharmacological significance. Further studies are under progress to isolate pharmacologically active compounds from the methanol crude extract of *Gracilaria corticata* J.Ag. to determine other bioactivities.

Table 1: Antipyretic effect of methanol extract of *Gracilaria corticata* J.Ag.

Groups	Rectal temperature (°C)		Time after administration			
	Normal Body Temperature	18hr after Yeast injection	Initial 0hr	1hr	2hr	4hr
Control	37.0±0.02	40.6±0.17	40.9±0.31	41.1±0.13	41.2±0.18	41.3±0.14
Paracetamol	36.7±0.06	41.0±0.21	41.3±0.15	40.5±0.24	39.7±0.09	39.4±0.11
200mg/kg Methanol extract	37.2±0.03	41.5±0.11	40.7±0.11	40.2±0.18	39.6±0.08	38.2±0.11
400mg/kg Methanol extract	37.0±0.04	40.8±0.01	40.4±0.07	39.6±0.24	38.7±0.16	37.2±0.13

Significantly different from the control at P<0.05, Standard drug – Paracetamol

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