Original Article

# Analgesic, Anti-inflammatory and Antipyretic Activity of the Methanol Extracts of Brown Alga *Lobophora variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveir

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## ABSTRACT

**Objective:** To determine the analgesic, anti-inflammatory and antipyretic activity of the methanol extracts of marine brown alga *Lobophora variegata* through *in vivo (*animal) models.
**Methods:** The analgesic activity of methanol extracts of *L. variegata* has been evaluated by the acetic acid induced writhing reflux in mice. The anti-inflammatory activity was observed in Wister albino rats, by i.p route, one hour prior to carrageenin injection. 0.1mL, 1% carrageenin was injected. Swelling of carrageenan induced foot was calculated at 0, 1, 2, 3, 4 and upto 24 hrs by using a digital plethysmometer. Lessening in left paw volume is recorded for 24 hrs and it was evaluated with Indomethacin (10 mg/kg p.o.) and control using the formula. The antipyretic activity was carried out by the suspension of Brewer's yeast (15%) in saline (0.9%). The rats were divided into 4 groups where either sex was taken for consideration. The thermocouple was introduced into the rectum by 2cm deep and the corresponding temperatures were measured. A subcutaneous injection of 20% w/v of brewer's yeast (10 mL/kg) in distilled water was used to induce Pyrexia. The basal rectal temperature was measured. At 1, 2 and 4 hours the rectal temperature was measured and compared using Paracetamol (150 mg/kg) as a standard drug.
**Results:** In the present study, it was found that the analgesic activity of *L. variegata,* at a dose of 10 mg/kg body weight on acetic acid induced writhing reflex could be clearly observed and nearly, 67.10% of the animals were protected. In addition, the study also demonstrated that the extract of *L. variegata* inhibited PMA-induced inflammation. The antipyretic activity of *L. variegata*, was studied in Brewer's yeast induced pyrexia on Wistar rats where Paracetamol (150 mg/kg) was taken as a standard drug. The methanol extract of *L. variegata* on 10 mg/kg body weight has shown significantly

($p<0.01$) decrease in an elevated body temperature as compared with the standard drug.
**Conclusion:** From the present study it has been concluded that the methanol extract of the brown alga *L. variegata* has potent anti-inflammatory activity, significant antipyretic effect and substantial analgesic activity with no serious toxic effect at moderate doses. From this study, it has been found that the extract of *L. variegata* can be used for the discovery of life saving drugs.

**Keywords**: Analgesic; Anti-inflammatory; Antipyretic; *Lobophora variegata*; Wistar albino rats; Paracetamol; Brewer's yeast and Pyrexia.

## INTRODUCTION

Brown algae are one of the most interesting phyla pertaining to pharmacologically active compounds and was investigated widely in the last decade[1]. Inflammation involves with various diseases like rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gout, migraine. Nonsteroidal anti-inflammatory drugs (NSAID) and steroidal anti-inflammatory drugs (SAID) are extensively used for the treatment of these ailments. It is well known that these kinds of drugs have some adverse side effects.

However, there are many natural products which exhibit anti-inflammatory and analgesic properties and possess relatively low incidences of side effects[2]. An increasing number of studies on marine flora and fauna are demonstrating that many compounds produced by marine life have useful pharmacologoical activities. Among these organisms, the macroalgae are well suited to be a rich source of bioactive substances suitable for therapeutic applications[3-12]. Several studies have reported the antioxidant properties of extracts[13] or chemical components[14-16]. *Ecklonia cava* polysaccharide exihibited anti-inflammatory activity facilitated by the inhibition of NO and prostaglandin E2 production[17,18].The mechanism may involved the suppression of inflammation by antioxidant constituents. Based on these ethanobotonical evidences, the present study attempts to evaluate the biological screening of methanol extract of marine brown alga *L. variegata* for its analgesic, anti-inflammatory and antipyretic activity using laboratory animal model.

Pyrexia is triggered as a secondary impact by infection, malignancy or other diseased states; wherein, there is an unexpected increase in the core temperature above the normal level[19]. Consequently, it is the body's natural resistance to create an atmosphere where an infectious agent or a damaged tissue cannot survive[20,21] The regulation of body temperature requires a subtle equilibrium between the production and loss of heat. As the temperature regulating structure is administrated by a nervous feedback mechanism, whenever the body temperature becomes very high, it dilates the blood vessels and increase sweating to reduce the temperature; but when the body temperature becomes very low, hypothalamus protect the internal temperature by 'vasoconstriction'. Under the influence of fever, this set point is elevated and a drug like paracetamol does not

influence body temperature when it is elevated by factors such as exercise or an increase in ambient temperature[22].

Several plants and their products are claimed and proved to possess antipyretic properties[23]. 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 the core temperature closely) is about 0.6ºC higher than the oral temperature and is about 1.4ºC higher than the axillary temperature. The generally accepted normal limits of rectal temperature in adults are 36.1ºC and 37.8ºC; the body temperature is higher in infants. If the core temperature rises by more than a few degrees in man, mental changes occur. The working of many tissue enzymes is also adversely affected and hyperpyrexia may result in death. However, core temperature below 40.5ºC is generally borned by many individuals[24].

Based on Ayurvedic studies, the pyrexia originates from a combination of indigestion, seasonal variations and significant alterations in daily routine[25]. These fever is often accompanied by aches and pains which all leads to morbidity and mortality[26]. However, antipyretic medication can be very effective at reducing the body temperature, which may include the affected person's comfort[27]. At high temperature casued by high fever frequently increases the disease progression by increasing tissue catabolism, dehydration and existing complaints as found in HIV[28,29].

A natural antipyretic agent with reduced or non-toxicity is essential. Further, as health care costs continue to escalate, the attraction for low cost remedies has stimulated consumers to re-evaluate the potential of alternatives[30-32].Therefore, the present investigation was focused to study the analgesic, anti-inflammatory and antipyretic activities of the methanol extract of *Lobophora variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveiraobtained from the Mandapam Village on the South East Coast of Tamil Nadu, India. Recently, we studied the antioxidant activity of *L. variegata* against various free-radicals. We have elucidated the chemical structure of active component present in the methanol extract using $^{1}H$, $^{13}C$ NMR and LC-MS[33]. In addition, we also prepared the silver nano particles using the methanol extract of *L.variegata* as a template and evaluated the anti-microbial properties of biosynthesized silver nano particles[34].

## MATERIALS AND METHODS

### Collection of Sample

The experimental brown alga *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C.Oliveira was obtained from Mandapam, in the South East coast of Tamil Nadu, India. The collected algae samples rinsed with marine water to remove debris and epiphytes. The entire epiphytes were removed by soft brush. In the laboratory, the alga was once again washed in seawater and was stored in a refrigerator for further analysis[35].

### Preparation of methanol extract

For the preparation of extract, the collected alga was washed in sea water thoroughly and placed on blotting paper and the samples are shade dried at room temperature. The dried samples were powdered then stored in a refrigerator for further use. Powdered samples (about 3g) of weight were packed in a soxhlet apparatus and were extracted with methanol for 8 hrs separately. The solvent methanol was evaporated and the crude powder was prepared and stored in the refrigerator for the antipyretic activity[36].

### Animals

Wister albino rats (180-220 g) were selected for writhing analgesic method and Wistar albino rats (180-220 g) of either sex were used for examining analgesic activity using Eddy's hot plate method and carrageenan induced rat paw edema anti-inflammatory effects. They were housed in wll protected polypropylene cages and maintained in standard laboratory conditions. Animal experiments were performed in accordance with the CPCSEA norms and are approved by the Institute of Animal Ethical Committee (IAEC) No: IAEC/KMCP/152/FT/4379/2013-2014.

### Acetic acid writhing test

The methods of Koster *et al.*, Williamson *et al*., (1996) and Garcva *et al.,* (2004)[37-39] were used. Six mice were used in a groupper dose of methanol extract or drug. The animals were kept individually in transparent perspex cages (dimension: 25 × 15 × 15cm) for about 30 minutes to acclimatize to their new environment before the commencement of the experiment. Control mice were pre-treated with normal saline in a volume of 10mL/kg of body weight and after 15 minutes, each mouse was injected with 0.2 mL of 3% acetic acid. 5 minutes after the administration of acetic acid, the writhes were counted for 20 minutes. The animals in the other groups were pre-treated with algae extract and after 15 minutes injected them intraperitoneally with 0.2mL of 3% acetic acid. All experiments were performed between 08:00 and 16:00 hrs atambient temperature of 22±1°C. The ability of the algal extract was significantly reduced and the number of acetic acid-induced writhes was taken as an analgesic activity.

### Assessment of anti-inflammatory activity

The anti-inflammatory activity was examined in rats, according to the method of Winter *et al*, (1962)[40].Threee rats per group were formed and were treated with vehicle, the extract of *L. variegata* (10 mg/kg) through i.p route, one hour prior to carrageenin injection.0.1 mL of 1% carrageenin was injected into the subplantar region of the left hind paw of each rat animal. Swelling of carrageenan induced foot was measured at one hour inverval upto 24 hrs by using digital plethysmometer. The right paw was injected with 0.1mL of the vehicle. Reduction in left paw volume is measured for 24 hrs and the result was compared with Indomethacin (10 mg/kg p.o.) and control. Inflammation or paw volume in rats was calculated by taking the difference (Vt-Vo) between final (Vt) i.e. after carrageenan and initial volume (Vo) i.e. before carreageenan administration, for each group at different time intervals. The anti-inflammatory effect was calculated as percentage inhibition in edema, obtained at each time interval for all groups, using the following formula and reported.

% inhibition of edema = [{(Vt-Vo) control-(Vt-Vo) treated}/(Vt-Vo) control] × 100.

### Treatment protocol for Analgesic studies

Group-I: Treated as normal control received 10 mL/kg/body weight of normal saline through orally.

Group-II: Treated as a standard control received 10mg/kg/body weight of diclofenac sodium through Intraperitonealy.

Group-III: Treated as treatment control received 10mg/kg/body weight on *L. variegata* with 2 mL of sterile water administered through orally.

The methanol extract of brown alga *L. variegata* was administered one hour prior to the acetic acid administration. The on set on writhing was noted. The number of abdominal contractions, trunk twist and extension of hind limbs as well as the number of animals showing such response

during a period of 10 minutes were recorded.

### Experimental animals and requirements for antipyretic studies

Wistar albino rats in 180-200g weight range were purchased from Venkateswara Enterprises, Bangalore, Karnataka, India. The animals were housed in a departmental animal house under standard conditions (26 ± 2°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 excess of water. The composition of diet was 10% protein, 4% Arachisoil, 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 the experimentation. All the experiments were conducted between 08.00 and 16.00 hrs and were in accordance with the ethical guidelines of the International Association for Study of Pain[41]. All experiments were conceded out according to the guidelines for care and use of experimental animals and are approved by the Institutional animal ethical committee (IAEC) No: IAEC/KMCP/152/FT/4379/2013-2014 framed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Treatment protocol for the Anti-pyretic studies

The body weights of the animals were measured and were randomly divided into 4 groups of six animals each as follows:

Group I: Animals served as control normal saline (10 mL/kg)

Group II: Animals were treated with yeast via subcutaneous injection (10 mL/kg).

Group III: Animals were administered with yeast (10 mL/kg) and the standard drug paracetamol 150 mg/kg b.w.), orally.

Group IV: Animals were administered with yeast (10 mL/kg,) and 10 mg/kg of methanol extract of *Lobophora variegata* with 2 mL of sterile water administered through orally.

### Antipyretic activity using the Yeast induced pyrexia method

Brewer's yeast (15%) in saline (0.9%) was prepared as suspension. Six rats of either sex were taken in a group and four groups were formed. The thermocouple was inserted for 2 cm into the rectum and the temperature was measured. A subcutaneous injection of 20% w/v of brewer's yeast (10 mL/kg) in distilled water was injected to induce Pyrexia. Also, the basal rectal temperature was measured before the injection of yeast. The sight of injection was massaged in order to spread the suspension beneath the skin. The experimental room temperature was set at 22-24°C, immediately after yeast administration, food was withdrawn and the increase 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 (150 mg/kg) was chosen as a reference drug. The 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[42-43].

### Statistical analysis

Data present in the investigation are expressed as mean ± SEM; the same were analyzed using the one way ANOVA followed by Newman keul's multiple range tests to determine the significance of the

difference between the control group and rats treated with the extracts by using SPSS 17.0.

## RESULTS

### Analgesic studies

The present study included an evaluation of the analgesic property of the experimental alga *vide* Materials and Methods. The methanolic extract of *L.variegata* (10 mg/kg body weight) showed significant analgesic activity by reducing the number of acetic acid-induced writhes (Table.1).

The analgesic activity of *L.variegata* at a dose of 10 mg/kg body weight on acetic acid induced writhing reflex could be clearly observed and nearly 67.10% of the animals were protected as against *L.variegata* whereas 84.22% by Diclofenac (10 mg/kg body weight), a standard peripherally acting analgesic drug. The observed results are statistically significant at $p<0.001$.

### Antipyretic Studies

The antipyretic potential of methanol extract of *L. variegata* was evaluated by determining its effect on yeast-induced pyrexia in albino rats.Table.2 shows that animals treated with methanol extract of *L. variegata* possess significant antipyretic property. The result showed that the *L. variegata* drug at a dose of 10 mg/kg body weight was able to reduce yeast induced body temperature from 41.61°C to 39.83°C at 1 hr in the animals and maintained the tendency up to 3 hours (38.67°C) (Fig.1). After 3 hrs, the treated algal drug exhibited a body temperature was 38.67°C while that of the normal control animals was 37.61°C. The antipyretic activity of the algal control was equal to that observed for the paracetamol (drug) control.

## DISCUSSION

The studies demonstrated that the extracts, as well as structurally diverse compounds obtained from marine brown and green seaweeds have been shown to inhibit inflammation[44-45]. The active components in the seaweed extracts may act as a competitive inhibitors of cyclo-oxygenase and/or lipoxygenase in an inflammation reaction, resulting in decreased production of prostaglandins and leukotrienes[46].

In the present study, we demonstrated that the methanol extract of *L. variegata* inhibited PMA-induced infla-mmation. Topical application of PMA induces a long-lasting inflammatory response, resulting from protein kinase-C (PKC) activation associated with a transient increase in prostanoid production and marked cellular influx[47]. This high prostaglandin level is likely due to cyclooxygenase (COX) induction[48]. The mouse skin was treated PKC activator, such as PMA, toinduce the formation of free radicals *in vivo*[49]. Therefore, the potential inhibition of reactive oxygen species generation by *Ecklonia cava* is consistent with the inhibition of NF-jB-dependent cytokines and inducible nitric oxide synthase and COX-2 expression, and thus reduced inflammation. The tail-flick response is believed to be a spinally mediated reflex[50].

The analgesic test used in the present study was chosen in order to test the different nociceptive stimuli, namely cutaneous thermic and chemical visceral stimuli[51]. In acetic acid induced abdominal writhing cause's analgesia by liberating endogenous substances. Based on the percentage inhibition, the number of writhes obtained with different doses of *L. variegata* methanolic extract, it was found that the intensity of the analgesic effect was similar to that of the diclofenac. Diclofenac and

related drugs can inhibit cyclooxygenase in peripheral tissues, thus interfering with mechanical transduction in primary afferent nociceptors[52]. The ostagladina amplify the pain mechanism and enhance vascular permeability while the leukotriens contract smooth muscle of blood vessels. Prostaglandins also enhance the vascular permeability and mediate pro-inflammatory and allergic responses[53-54]. The results of the present investigation revealed that all the doses of the methanol extract of *L. variegata* produce a significant antinociceptive effect which may be due to blockade or the release of endogenous substances that stimulate pain in nerve endings similar to paracetamol and other NSAIDs.

The present study establishes the anti-inflammatory activity of methanol extract of *L.variegata*. It is evident that phytochemicals obtained from *L. variegata* are commonly used to induce acute inflammation and are believed to be bi-phasic. The first phase is due to the release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome. Based on this, it would be argued that the suppression of its phase may be due to inhibition of release of early mediators, such a histamine, serotonin and action in second phase may be explained by an inhibition of cyclo-oxygenase. These mediators take part in inflammatory response and are able to stimulate nociceptive and thus reduce pain. It has been reported that a second phase of edema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the antiedematous effect of natural products[55]. Based on these reports, it can be inferred that the inhibition effect of the methanol extract of *L. variegata* on phytochemicals induced inflammation in rats may be due to inhibition of the mediators responsible for inflammation.

Several inflammatory mediators produce algesthesia by peripheral and spinal sensory fiber sensitization through protein kinase activation, including PKC[56]. Algesthesia is associated with the formation of edema and erythema by PMA treatment[57], and thus the main active constituents in the methanol of *L. variegata* may inhibit the pathway that mediates the pain, edema, and erythema associated with inflammation. An herbal medicine is considered toxic if the $LD_{50}$ is lowerthan 5g/kg body weight[58]. Thus, *L. variegata* extracts are not toxic and can be safely used by humans at moderate doses, since no mortality at 5 g/kg body weight was recorded. The methanol extract of *L. variegata* antagonized the pain produced by acetic acid analgesic test method; it is possible that the seaweed produces its analgesic activity both peripherally and centrally.

Many studies have found interesting biological activities in polar fractions of marine algae[59-61]and similar results were also obtained in our study. The *L. variegata* extract contains phenols, tannins, reduced carbohydrates and other sugars. Also, the results showed a weak presence of triterpenes and steroids. Some of these compounds such as phenols, terpenes and steroids have been reported to possess anti-edimatous effects[62-67]. In agreement with these reports, it is possible that such compounds are present in the methanolic extract of *L. variegata* and are able to inhibit the synthesis, release or action of inflammatory mediators involved in inflammation.

The non-specific test, acetic acid induced writhing in rat, represents a model of peripheral nociception and is widely used for analgesic screening. The local irritation provoked by chemical substances in the intraperitoneal cavity induces the release of endogenous mediators such as bradykinin, P, PGI2, IL-Ib, TNF-α and IL-8[68-70]. These

mediators stimulate the nociceptive neurons that are sensitive to nonsteroidal anti-inflammatory drugs and opioids[71, 72].

The treatment of animals with the methanol extract given up and PO, have shown significant inhibition of the writhing induced by 0.8% acetic acid solution. The maximum reduction, by po, in the number of contortions was 64.55%, although the dose required being higher than the acetic acetylsalicyclic acid (ASA) dose required for a similar effect. Nevertheless, all doses given ip were found to be more potent. These doses showed percentage reductions in the number of writhing of over 65%.

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[73-75]. The analgesic activity observed may be associated with the presence of such compounds and other secondary metabolites in the methanol extract of *L. variegata*, which are able to inhibit the release of endogenous mediators in response to acetic acid.

Plants are the major source of drugs in Indian medicine, Ayurveda, Siddha, Unani and Homeopathy [76], and other ancient systems in the world. The earliest description of the curative properties of medicinal plants can be found in Rig-Veda, CharakaSamhita and SushrutaSamhita give an extensive description on various medicinal herbs[77]. With the emerging worldwide interest in adopting and studying traditional systems and exploiting the potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential. In this regard, an attempt was made in the present work to study detailed pharmacological action, particularly the antipyretic activity of marine brown alga *L. variegata*.

The elevation of body temperature during such condition 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, an injection into experimental animals, induce the production of pro-inflammatory cytokines (e.g., IL-1β, IL-6, IFN-α and TNF) which stimulate the release of local PG (produced by the activity of COX) that leads to the elevation of body temperature[78]. 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-1β, while lowering the thermoregulatory set-point by blocking COX production of PGE2[79]. The extract administration resulted in the lowering of temperature, but the decrease of temperature was not comparable with the standard antipyretic which showed significant antipyretic effect. Similar results were also reported by Gautham and Onkarappa (2013); Prashith Kekuda *et al*., (2013)[80, 81].

Antipyretic are the agents which reduce the elevated body temperature. The yeastinduced fever is called pathogenic fever. Its etiology includes production of prostaglandins which set the thermoregulatory center lower temperature[82]. Antipyretic activities of various marine algae were previously tested in *Ulva rigida*[83,84]. Since the antipyretic activity is commonly mentioned as a characteristic of drug or compound, which has an inhibitory activity on prostaglandins biosynthesis, the yeast induced hyperpyrexia

in the rat model was employed to investigate the antipyretic activity of the extract[85]. Yeast induced pyrexia is called pathogenic fever, which is due to the production of prostaglandins (PGE2) which set the thermoregulator center at a higher temperature[86].

The methanol extract of *L. variegata* showed significant antipyretic activity. The animals were also favoured by injection of Brewer's yeast suspension (10 mL/kg body weight) subcutaneously in back below the nape of the neck for the antipyretic activity. The methanol extract of *L.variegata* showed significant decrease in elevated body temperature as compared to standard drug paracetamol. The possible mechanism of antipyretic action may be due to the inhibition of prostaglandin as that of paracetamol by blocking the cyclo-oxygenase enzyme activity[87]. There are several mediators for pyrexia and the inhibition of any one of these can be responsible for the antipyretic effect[88]. Inhibition of any of these mediators may bring about antipyresis.

Antipyretics have been shown to suppress fever by inhibiting prostaglandin synthetase, resulting in the blockade of the synthesis of prostaglandin in the brain or suppressing the rise of interleukin-1α production subsequent to interferon production. The intraperitoneal administration of the methanol extract of *L. variegata* has significantly attenuated rectal temperature of yeast induced pyrexia in rats and comparable to that of the standard drug paracetamol. So, inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as in the case of paracetamol. Also, there are several mediators or multiprocesses underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about the antipyresis[89]. Thus, it can be postulated that the methanol extract of *L. variegata* contains pharmacologically active principles that interfere with the release of prostaglandins. This may be attributed to the presence of the various bioactive compounds present in the methanol extract of *L. variegata* which are involved in the 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. Flavonoids like baicalin have been shown to exert antipyretic effect by suppressing TNF-α[90] and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in the reduction of prostaglandin levels thus reducing the fever and pains[91]. The present study also correlates the study of Zakaria *et al.,* (2007)[92]in the sense that the compounds like flavonoids and saponins are suggested to act synergistically to exert the observed pharmacological activity. Flavonoids are known to target prostaglandins which are responsible for pyrexia[93]. The presence of flavonoids in the methanol extract of *L.variegata* may be contributory to its antipyretic activity. This potentiality supports the earlier traditional claims as a pediatric antipyretic remedy.

## CONCLUSION

In the present investigation, it has been revelaed that the methanol extract of the brown alga *L. variegata* is may bepotential anti-inflammatory source at moderate doses. In addition, the result suggests that the active constituents in this extract could inhibit chemical mediators responsible for inflammation and that the inhibitory role in the migration of leucocytes to the site of inflammation is a strong sign in this study. This could therefore support the anti-inflammatory property of the *L. variegata*. Therefore, the results possess significant analgesic activity as compared to

the control group in the pain model *in vivo*, with no serious toxic effect at moderate doses. From the result we obatined, we here suggest that the methanol extract of *L. variegata* inhibits the inflammatory mediators like bradykinine and substance which are involved in stimulation of pain, sensory neurons and inflammations, and the current results strengthen the claims of the health care industry and indigenous medicine that *L. variegata* can be used as a remedy for inflammation-related symptoms. Further identification of bioactive compounds is very impotant that may have applications in therapeutic fields of inflammation and pain release. Also, the present study delivers evidences for the methanolic extract of *L. variegata* showing significant antipyretic activity which could partly contribute to its ethnomedical use. However, further investigation is required for isolation and its mode of action is suggested for the development of a new drug in the treatment of pyrexia and analgesia. It is well understood that, the rich diversity of marine biota with its unique physiological adaptations to the harsh marine environment provides a fruitful source for the discovery of life saving drugs.

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## Conflict of interest statement

We declare that we have no conflict of interest.

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**Table 1.** Analgesic activities of methanol extract of *L. variegata* on acetic acid induced writhing reflux in mice

| Treatment | Dose (mg/kg) | No. of writhing | % reduction in reaction time |
|---|---|---|---|
| Group I Normal saline | Inject 1% v/v acetic acid 1 ml/100 g of body weight | 38.0 ± 3.5 | - |
| Group II Standard | 10 mg/kg I.P.Diclofenac sodium | 6.0 ± 0.8 | 84.21%** |
| Group III Methanol extract of *L. variegata* | 10 mg/kg Administered through orally. | 12.5 ± 3.0 | 67.10%** |

Values are expressed as Mean±SEM

**Values were considered significant at p<0.001.

**Table 2:** The effect of methanol extract of *Lobophora variegata* on the body Temperature on yeast induced pyrexia

| Group | Rectal Temperature | | | |
|---|---|---|---|---|
| | 0hr | 1hr | 2hr | 3hr |
| Group I (Control) | 38.30 ± 0.8 | 37.50 ± 0.80 | 37.70 ± 0.80 | 37.61 ± 0.50 |
| Group II Negative control (10 ml/kg) | 41.54 ± 0.23 | 42.18 ± 0.18 | 39.30 ± 0.15 | 39.19 ± 0.26 |
| Group III Positive control(150 mg/kg) | 41.43 ± 0.19 | 39.70 ± 0.18 | 38.50 ± 0.23* | 37.65 ± 0.38 * |
| Group IV Treatment control (10 m/kg) | 41.61 ± 0.02 | 39.83 ±0.20 | 39.42 ± 0.16* | 38.67 ± 0.42 * |

Values are expressed as Mean ±SEM. n = 6 in each group,

*values are significant (p< 0.01) different from pyrexia control (G2)

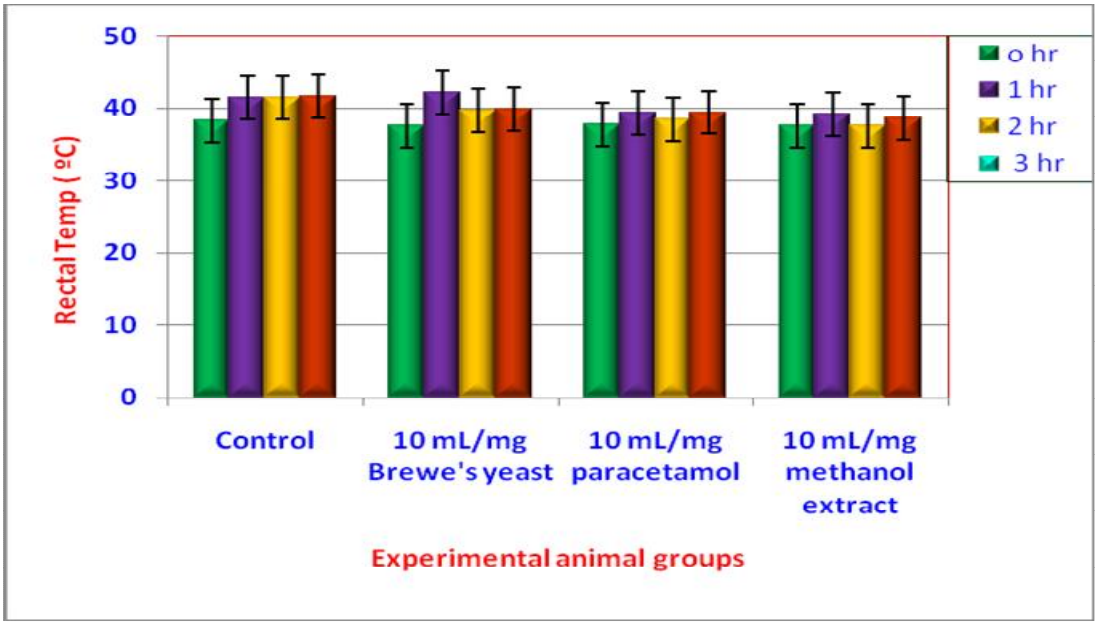


**Figure 1:** Antipyretic effect of methanol extract of *L. variegata*.