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Research Article

# EVALUATION OF ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *MYXOPYRUM SMILACIFOLIUM* (WALL.) BLUME

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**ABSTRACT**

**Objective:** The aim of this study was to evaluate the anti-inflammatory and antipyretic activity of ethanolic extract of *Myxopyrum smilacifolium* (EEMS) leaves in experimental animals.

**Methods:** Plant material was collected from Meenadom, Kottayam district, Kerala, India, in the month of March 2014. The leaves were washed and dried under shade at room temperature. After 1-month, the leaves were powdered. The powder was weighed (50 g) and was extracted by successive solvent extraction process using ethanol. The total yield of the ethanolic extract was 4.6%. Phytochemical screening was carried out for the detection of the phytoconstituents by simple qualitative methods. The acute toxicity studies carried out as per OECD guidelines 423. The dosing was designed as per the acute toxicity study. The anti-inflammatory activity was performed by carrageenan and formalin induced paw edema model at two different doses, 200 mg/kg and 400 mg/kg. The antipyretic activity was performed by Brewer's yeast induced hyperpyrexia model at two different doses. Wistar rats weighing (150-200 g) of either sex were used for these studies.

**Results:** The results of anti-inflammatory study revealed that the ethanolic leaves extract of *M. smilacifolium* inhibited the inflammation in carrageenan and formaline induced paw edema method. The ethanolic leaves extract of *M. smilacifolium* possesses a significant antipyretic effect in yeast induced elevation of body temperature in experimental rats.

**Conclusion:** EEMS leaves shows a dose dependent increase in the anti-inflammatory, antipyretic and immunomodulatory activities.

**Keywords:** Ethanolic extract of *Myxopyrum smilacifolium*, Carrageenan, Formalin, Brewe's yeast.

## INTRODUCTION

Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent [1]. Inflammation has two main components - cellular and exudative. The cellular component involves the movement of white blood cells from blood vessels into the inflamed tissue. The white blood cells, or leukocytes, take on an important role in inflammation [2]. Non-steroidal anti-inflammatory drugs, usually abbreviated to NSAIDs, are drugs with analgesic, antipyretic and anti-inflammatory effects - They reduce pain, fever and inflammation. The most common side-effect of NSAID therapy is gastric or duodenal ulceration that may cause anemia due to the blood loss [3].

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive [4].

*Myxopyrum smilacifolium* is a large scandent shrub. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, notalgia, consumption, fever, otopathy, neuropathy and cuts and wounds [5].

## METHODS

### Collection of plant material

The fresh leaves of *M. smilacifolium* were collected from Meenadom, Kottayam district, Kerala, India, in the month of March 2014. The plant material was identified and authenticated by Mr. Rogimon. P Thomas, Assistant Professor, Department of Botany, CMS College, Kottayam and a herbarium specimen was deposited in our college (DPS/MGU/RIMSR/2014/HERB6).

### Preparation of extract

The freshly collected leaves of *M. smilacifolium* were washed thoroughly with distilled water and dried in open air at room temperature. The dried samples were finely powdered. 50 g of dry plant powder were packed well in soxhlet apparatus and extracted with 500 ml of ethanol for 72 hrs. The ethanolic extract was concentrated and dried using rotary evaporator. It was kept in desiccators until used.

### Phytochemical screening

Phytochemical screening was carried out for the detection of tannins, alkaloids, flavonoids, glycosides, sterols, and saponins by simple qualitative methods [6].

### Experimental animals

Healthy Wistar albino rats weighing about (130-150 g) of either sex were procured from Trivandrum. The animals were maintained under standard conditions of relative humidity and temperature. The animals were acclimatized for 10 days under laboratory conditions before carrying out the experiments. The animals were housed in the animal house approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA)-Registration number-1702/po/c/14/CPCSEA.

### Evaluation of anti-inflammatory activity

The anti-inflammatory activity was determined by two experimental models namely carrageenan-induced paw edema and formalin induced paw edema. The dosage of the test drug was designed based on the oral acute toxicity studies carried out on the plant. The experimental animals were divided into four groups in both the models with six animals in each group.

**Carrageenan-induced paw edema**

The first group treated orally with carboxy methyl cellulose. The second group was treated with diclofenac sodium (10 mg/kg) p.o. as a standard drug. Group 3 and Group 4 was treated orally with ethanolic extracts of leaves of *M. smilacifolium* 200 mg/kg and 400 mg/kg respectively. After 30 minutes of treatment, the rats were challenged with intra plantar injection of 0.1 ml of 1% w/v solution of carrageenan into the sub plantar region of left paw. The paw was marked with ink at the level of lateral malleolus. The paw volume was measured at 0, 1, 3, 5 and 24 hrs after carrageenan injection using a plethysmograph. The difference between initial and subsequent reading gave the actual edema volume. The anti-inflammatory activity in animals that receive ethanolic extract of *M. smilacifolium* (EEMS) and diclofenac (10 mg/kg) was compared with that of vehicle control groups. The percentage inhibition of edema was calculated as follows:

Percentage inhibition of edema = 1–Vt/Vc×100

Where, Vt is the inflammatory increase in paw volume in drug - treated rats, and Vc is the inflammatory increase in paw volume in control group of rats. Percentage inhibition of edema is proportional to anti-inflammatory activity [7,8].

**Formalin induced paw edema**

The animals were divided into four groups. The first group was treated orally with Carboxy methyl cellulose. The second group was served as standard and treated with diclofenac sodium (10 mg/kg) p.o. Group 3 and Group 4 was treated orally with ethanolic extract of leaves of Myxopyrum smilacifolium 200 mg/kg and 400 mg/kg respectively. After 30 minutes, the inflammation was induced by intra plantar injection of 0.1 ml of 1% w/v solution of formalin into the sub plantar region of left paw. The paw was marked with ink at the level of lateral malleolus. The paw volume was measured at 0, 1, 3, 5 and 24 hr. after formalin injection by using a Plethysmograph. The difference between initial and subsequent reading gave the actual edema volume. The anti-inflammatory activity in animals that receive ethanolic extract of Myxopyrum smilacifolium and diclofenac (10 mg/kg) was compared with that of vehicle control groups. The percentage inhibition of edema was calculated as follows:

Percentage inhibition of oedema = 1 – Vt/Vc × 100

Where Vt is the inflammatory increase in paw volume in drug-treated rats, and Vc is the inflammatory increase in paw volume in control group of rats. Percentage inhibition of edema is proportional to anti-inflammatory activity [9,10].

**Antipyretic activity**

*Brewer's yeast induced hyperpyrexia method*

Animals of either sex were divided in to four groups containing six in each group for this experiment. Before yeast injection the basal rectal temperature of rats was recorded and after recording animals were given subcutaneous injection of 10 ml/kg of 15% w/v yeast suspended in 0.5% w/v methyl cellulose solution for elevation of body temperature of rats. Rats were then returned to their housing cages. At the 18 hrs after yeast injection, the vehicle, standard drug and test drugs were administered in to different groups. Propylene glycol at dose of 5 ml/kg was administered orally to the control groups of animals and paracetamol at dose of 150 mg/kg was administered orally to standard group of animals. The EEMS was administered orally to test groups of animals respectively. Rectal temperature was recorded by clinical thermometer at 0, 1, 2, 3 hrs after drug administration and tabulated [11,12].

**Statistical analysis**

Results were expressed as mean ± standard error of mean, (n=6). Statistical analysis were performed with one-way analysis of variance followed by Dennett's test. $p<0.05$ was considered to be statistically significant. *$p<0.05$, **$p<0.01$ when compared with control group.

## RESULTS

**Percentage yield**

The percentage yield of powdered leaves of *M. smilacifolium* extract was obtained by soxhlet extraction process with solvent ethanol. The yield was 4.8% w/w.

**Preliminary phytochemical screening**

The qualitative chemical investigation of ethanolic extracts of leaves carried out to check the presence of various phytoconstituents in extract. It is observed from the phytochemical study that carbohydrates, steroids, terpenoids, flavonoid, tannins and polyphenols were present in the extracts.

**Carrageenan induced rat paw edema volume and percentage inhibition**

The injection of carrageenan, the phlogistic agent, caused localized edema starting at 1-hr after injection. The swelling increased progressively to a maximum volume of 2.22±0.01 ml at 5$^{th}$ hr after the carrageenan injection. In pre-treated with diclofenac (10 mg/kg, p.o.) had a significant reduction of rat paw volume at 1$^{st}$ hr after the diclofenac administration and continued up to 5$^{th}$ hr with the percentage of inhibition of 15.12%, 40.69%, 94%, at 1, 3 and 5$^{th}$ hr respectively compared to the control. The EEMS extract at dose of 200 and 400 mg/kg, p.o. exhibited the anti-inflammatory effect at 1$^{st}$ hr after administration and continued up to 5$^{th}$ hr. The inhibition of rat paw edema of the EEMS at the doses of 200 and 400 mg/kg, p.o. at 1, 3 and 5$^{th}$ hr were 6.21%, 19.87%, 38.76%, 10.8%, 32.79% and 52.70% respectively when compared with the control. Hence, EEMS 400 mg/kg had maximum percentage inhibition (Tables 1 and 2).

**Formalin induced rat paw edema volume and percentage inhibition**

The injection of formalin, the phlogistic agent, caused localized edema starting at 1$^{st}$ hr after injection. The swelling increased progressively to a maximum paw volume of 2.24±0.03 ml at 5th hr after the formalin injection. In pre-treated with diclofenac (10 mg/kg, p.o.) had a significant reduction of rat paw volume at 1$^{st}$ hr after the diclofenac administration and continued up to 5$^{th}$ hr with the percentage of inhibition of 15.12%, 41.53%, 70.08%, at 1, 3 and 5$^{th}$ hr respectively compared with the control. The EEMS extracts at doses of 200 and 400 mg/kg, p.o. exhibited anti-inflammatory effect at 1$^{st}$ hr after administration and continued up to 5$^{th}$ hr. The inhibition of rat paw edema of the EEMS at the doses of 200 and 400 mg/kg, p.o. at 1, 3 and 5$^{th}$ hr was 8.32%, 27.86%, 42.56%, 10.83%, 33.33% and 54.46% respectively when compared with the control. Hence, EEMS 400 mg/kg had maximum percentage inhibition (Tables 3 and 4).

**Antipyretic activity using Brewer's yeast induced hyperpyrexia method**

The effect of methanolic extract of *M. smilacifolium* plant on yeast induced hyperpyrexia has been shown in Table 5. Treatment with extracts at dose of 200 mg/kg and 400 mg/kg body weight (BW) and paracetamol at dose of 150 mg/kg decreased body temperature of yeast induced rats. The results obtained from both standards and extracts treated groups were compared with the control group. A significant reduction in the yeast elevated rectal temperature was observed in the EEMS at dose of 400 mg/kg.

## DISCUSSIONS

Presently anti-inflammatory effect of *M. smilacifolium* was studied using carrageenan induced paw edema models in experimental animals. The carrageenan induced paw edema is frequently used as an experimental model for acute inflammation. The carrageenan induced paw edema shown biphasic response; first phase is mediated by release of histamine and serotonin while the second or delayed phase is related to neutrophil infiltration and release of other neutrophil derived mediators, eicosanoid release and production of free radicals [13]. The results of acute inflammatory study revealed that the ethanolic leaf extract of *M. smilacifolium* 400 mg/kg inhibited the inflammation.

**Table 1: Carrageenan induced rat paw edema volume**

| Treatment | Before treatment | After treatment | | | | |
|---|---|---|---|---|---|---|
| | | 0-hr | 1-hr | 3 hrs | 5 hrs | 24 hrs |
| Carrageenan | 0.35±0.01 | 0.41±0.01 | 1.19±0.09** | 1.86±0.4** | 2.22±0.01** | 0.41±0.01** |
| Carrageenan+diclofenac | 0.32±0.01 | 0.37±0.01 | 1.01±0.01** | 1.1±0.01** | 0.67±0.01** | 0.35±0.01** |
| Caraageenan+EEMS 200 mg/kg | 0.34±0.023 | 0.41±0.008 | 0.91±0.008* | 1.3±0.063** | 0.92±0.01* | 0.43±0.014 |
| Caraageenan+EEMS 400 mg/kg | 0.34±0.01 | 0.39±0.01 | 1.071±0.03** | 1.25±0.01** | 1.05±0.02** | 0.38±0.01** |

Values are presented as mean±SEM for six animals, by one-way analysis of variance followed by Dunnett's test (n=6). *p<0.05, **p⍰0.01 significantly different compared with control. SEM: Standard error of mean, EEMS: Ethanolic extract of *Myxopyrum smilacifolium*

**Table 2: Percentage inhibition of paw volume using carrageenan induced paw edema**

| S. No. | Groups | Percentage inhibition of oedema at different time intervals (%) | | |
|---|---|---|---|---|
| | | 1-hr | 3 hrs | 5 hrs |
| 1 | Standard (diclofenac sodium 10 mg/kg) | 15.12 | 40.86 | 69.94 |
| 2 | Test (EEMS 200 mg/kg) | 6.21 | 19.87 | 38.76 |
| 3 | Test (EEMS 400 mg/kg) | 10.8 | 32.79 | 52.70 |

EEMS: Ethanolic extract of *Myxopyrum smilacifolium*

**Table 3: Formalin induced rat paw edema volume**

| Groups | Before treatment | After treatment | | | | |
|---|---|---|---|---|---|---|
| | | 0-hr | 1-hr | 3 hrs | 5 hrs | 24 hrs |
| Group 1 (control) | 0.39±0.04 | 0.61±0.07 | 1.28±0.06** | 1.83±0.03** | 2.24±0.03** | 0.69±0.02** |
| Group 2 (standard) | 0.42±0.047 | 0.62±0.02 | 1.02±0.02** | 1.07±0.03** | 0.67±0.01** | 0.51±0.04** |
| Group 3 (EEMS - 200 mg/kg) | 0.45±0.034 | 0.78±0.021 | 0.99±0.03** | 1.23±0.068* | 0.98±0.023** | 0.76±0.05* |
| Group 4 (EEMS - 400 mg/kg) | 0.45±0.01 | 0.64±0.01 | 1.07±0.03** | 1.22±0.02** | 1.02±0.01** | 0.57±0.01** |

Values are presented as mean±SEM for six animals, by one-way analysis of variance (ANOVA) followed by Dunnett's test (n=6). *p<0.05, **p⍰0.01 significantly different compared with control. SEM: Standard error of mean, EEMS: Ethanolic extract of *Myxopyrum smilacifolium*

**Table 4: Percentage inhibition of paw volume**

| S. No | Groups | Percentage inhibition of oedema at different time intervals (%) | | |
|---|---|---|---|---|
| | | 1-hr | 3 hrs | 5 hrs |
| 1 | Standard (diclofenac sodium 10 mg/kg) | 15 | 41.53 | 70.08 |
| 2 | Test (EEMS 200 mg/kg) | 8.32 | 27.86 | 42.56 |
| 3 | Test (EEMS 400 mg/kg) | 10.83 | 33.33 | 54.46 |

EEMS: Ethanolic extract of *Myxopyrum smilacifolium*

**Table 5: Antipyretic activity**

| S. No | Treatment | Dose | Rectal temperature in °C before yeast injection | Rectal temperature after 18 hrs of yeast injection | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0-hr | 1-hr | 2 hrs | 3 hrs |
| 1 | Control propylene glycol | 5 ml/kg | 37.16±0.75 | 40.66±0.51 | 40.33±0.81* | 39.83±0.40** | 39±0.89* |
| 2 | Standard paracetamol | 150 mg/kg | 37.33±0.51 | 40.75±0.51 | 38.66±1.03* | 37.83±0.40** | 37.33±0.51** |
| 3 | EEMS | 200 mg/kg | 37.55±0.43 | 40.42±0.56 | 39.98±0.53* | 38.86±0.43** | 38.23±0.6* |
| 4 | EEMS | 400 mg/kg | 37±0.632 | 40.53±0.63 | 39.1±0.54** | 38.33±0.51** | 37.55±0.54** |

Values are presented as mean±SEM for six animals, by one-way analysis of variance followed by Dunnett's test (n=6). *p<0.05, **p⍰0.01 significantly different compared with control. SEM: Standard error of mean, EEMS: Ethanolic extract of *Myxopyrum smilacifolium*

The EEMS 400 mg/kg showed more inhibition of inflammation than 200 mg/kg ethanolic extract, suggesting the inhibition of inflammatory mediators. The preliminary phytochemical analysis of the EEMS leaves showed the presence of flavonoids, phenolic compounds, steroidal glycosides and triterpenoids. Flavonoids and triterpenoids possess anti-inflammatory activity and the constituent is present in *M. smilacifolium* [14,15]. These phytochemical constituents may be responsible for the anti-inflammatory activity.

Formalin induced paw edema is mediated by an early release of substance bradykinin (neurogenic phase) followed by tissue mediated response involving release of histamine, serotonin, prostaglandin and bradykinin 34. The ethanolic leaf extract of *M. smilacifolium* 400 mg/kg BW showed more inhibition of inflammation than 200 mg/kg ethanolic extract, suggesting that reduction of paw volume may be due to the inhibition of these substances.

The present results showed that the ethanolic leaf extract of *M. smilacifolium* 400 mg/kg BW possesses a significant antipyretic effect in yeast induced elevation of body temperature in experimental rats. It was revealed that the extract showed dose dependent antipyretic activity. Flavonoids are known to target prostaglandins which are involved in the pyrexia [16]. Hence, the presence of flavonoids in the ethanolic leaf extract of *M. smilacifolium* plant may be contributory to its antipyretic activity.

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