



## EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF THE PLANT *MARSILEA QUADRIFOLIA* ON ALBINO RATS USING CARRAGEENAN AND HISTAMINE INDUCED PAW EDEMA

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### ABSTRACT

The present study investigates the anti-inflammatory activity in Methanolic extract of *Marsilea quadrifolia* using Carrageenan and histamine induced paw edema in albino rats. Traditional healers used the plant for the treatment of various diseases and there have been literature supporting that it contains anti-inflammatory property. Dried leaves of *M. quadrifolia* was powdered and extracted with the solvent methanol using soxhlet apparatus, the concentrations of 200 and 400mg/kg were used for this inflammatory activity. The paw edema was induced by subplantar injection of 0.1ml of 1% w/v Carrageenan and 0.1ml of 1% w/v histamine and edema volume was recorded using a Plethysmometer. For this activity Diclofenac sodium (25mg/kg) was used as a standard drug, methanol extract of the plant *M. quadrifolia* 200 and 400mg/kg was set as test groups. The anti-inflammatory activity is more effective in 400mg/kg of the methanolic extract of *M. quadrifolia* both in Carrageenan and histamine induced group than that of 200mg/kg.

**Keywords:** *Marsilea quadrifolia*, Antiinflammatory, Carrageenan, Histamine.

### INTRODUCTION

*Marsilea quadrifolia* is a pteridophyte belonging to Family Marsileaceae commonly known as European water clover. In eastern parts of India it is known as Sushni. The plant is widely distributed throughout India. Juice made from the leaves is diuretic and febrifuge and also used to treat snake bite and applied to abscesses etc (Duke J.A and Ayensu. E.S1985). The plant is anti-inflammatory, diuretic, depurative, febrifuge and refrigerant (Schofield. J.J 1989). Plant spacifics vitiated pitta, cough, bronchitis, diabetes, psychiatric diseases, eye diseases, diarrhea and skin disease.

Inflammation is normal and necessary protective response to harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents and ischemia (Goldyne *et al.*, 1984) it is caused by variety of stimuli including physical damage, UV radiation, microbial attack, and immune reactions. The classical key features of inflammation are Redness, Warmth swelling and Pain.

Inflammation cascades can lead to the development of diseases such as chronic asthma, arthritis, multiple sclerosis, inflammatory bowel disease and psoriasis many of these disease are debilitating and are becoming increasingly common in our ageing society rheumatoid arthritis are the major inflammatory diseases affecting people worldwide (woolf and Pflieger 2003).

The carrageenan induced paw edema is believed to be a biphasic response. The first phase is attributed to the release of histamine, serotonin and kinin and the second phase is related to the release of prostaglandin and bradykinins (Vane, 1987). All these mediators produce inflammation when they are injected subcutaneously in the rat paw (Vogel *et al.*, 1997). Histamine is an important inflammatory mediator, potent vasodilator substance and also increases the vascular permeability (Cuman *et al.*, 2001). So, it can be used as an inflammatory agent.

After the careful survey of literature regarding the pharmacological activities of *Marsilea quadrifolia* the anti-inflammatory activity has not reported till date. So the present study aimed to evaluate the anti-inflammatory potency of 200mg/kg and 400mg/kg methanolic extracts of *M. quadrifolia* were studied against carrageenan and histamine induced inflammation.

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## MATERIALS AND METHOD

### Collection and Preparation of Plant Extract:

The plant materials were collected from Kanyakumari Dist. The plant *M. quadrifolia* was washed thoroughly, it was shade dried and then coarsely powdered. The powder was passed through sieve no.40 and stored in an air tight container for further use. The powder was then extracted with methanol using Soxhlet apparatus for 72 hrs. The extract was dried and stored in dessicator, the extract obtained 200 and 400 mg/kg was suspended in 1% (w/v) of aqueous carboxymethyl cellulose for administration to animals.

### Experimental Animals:

Albino winstar rats (160-200g) of either sex were used for experimental study. The animals were housed in cages and are provide with light. All the animals were acclimatized to laboratory environment for 1 week to 10 days before the experiment. Animals were fed with hygienic feed, pure water ad libitum and kept under temperature  $22 \pm 2^\circ\text{C}$ .

### Carrageenan Induced rat paw Edema:

The effect of oral administration of 200 and 400mg/kg of the methanolic extract of the plant *M. quadrifolia*, 25mg/kg Diclofenac sodium on the hind paw edema induced by subplantar injection of 0.1ml carrageenan (1% w/v) was evaluated according to the method described by Winter *et al.*, (1962). In short, 0.1ml of 1%w/v carrageenan was injected into the subplantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured after 1hr, 2hr and 3hrs using plethysmometer. Animals were treated with test extract 1hr before the carrageenan injection.

The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibited effect of the drugs under investigations was calculated based upon the percentage inhibition of inflammation.

### Histamine- induced paw edema in mice:

The activity of 200 and 400mg/kg of methanolic extracts of the plant *M. quadrifolia* and 25mg/kg of the standard drug diclofenac sodium were evaluated by using histamine-induced paw edema model. The rats were divided into four groups comprising of six animals in each group (n=6). The animals were starved overnight and deprived of water only during the experiment. After 1hr of drug administration, histamine 0.1% was injected subplantar into the right hind paw of each mice to induce edema. The paw volume was measured at 1hr, 2hr and 3hrs respectively. The anti-inflammatory effect was expressed as percent inhibition of edema.

The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibited effect of the drugs under investigations was

calculated based upon the percentage inhibition of inflammation. Percentage inhibition was calculated using the formula

$$\text{percentage inhibition} = Vc - \frac{Vt}{Vc} \times 100$$

Where,  $Vc$  = paw volume in control group.

$Vt$  = paw volume in test group.

## RESULT AND DISCUSSION

### Inhibition of Carrageenan induced paw edema in rats:

The anti-inflammatory activity of the methanolic extract of the plant *Marsilea quadrifolia* was calculated by increasing the paw edema in rats by carrageenan and histamine inducing method. In carrageenan inducing method the inhibition increased progressively with time in the control group. *M. quadrifolia* extract at the dose of 200 and 400mg/kg caused significant inhibition in paw edema by 20.45% and 42.61% in first hour, 31.81% and 50.55% in second hour and 37.52% and 55.58% in third hour after carrageenan injection. Oral administration of Diclofenac sodium(25mg/kg) also significantly reduced the edema by 47.16%, 51.65% and 57.88% in first, second and third hour respectively (Table 1 and Fig 1.).

### Inhibition of histamine induced paw edema in rats

A significant reduction in the edema of rat treated with the extract was observed by the histamine induced paw edema method. The test extract at the dose 200mg/kg show gradual increase in the inhibition by 13.91%, 19.39% and 28.23% at first, second and third hour. The extract of 400mg/kg shows 29.47%, 35.46% and 44.9% by first, second and third hour respectively. The control and the standard group also shows increase in inhibition by the time interval of 1hr, 2hr and 3hr (Tab 2 & Fig 1).

In comparing the carrageenan and histamine inducing paw edema method, the standard drug Diclofenac sodium(25mg/kg) and the methanolic extract of the plant *M. quadrifolia* at a dose of 200mg/kg and 400mg/kg shows high rate of inhibition of edema caused by carrageenan than the edema caused by histamine(fig 1.) The potent antiinflammatory activity of methanolic extract of *M. quadrifolia* due to the presence of bioactive compounds like flavonoids(chlorogenic acid), phytosterols( $\beta$ -sitosterol) and flavonol (Quercetin) (Loizou *et al.*, 2010, Parul and Deepak, 2007). It is reported that carrageenan induce the inflammation by escalating PE2 release and leukocytes migration. It is moreover increase the expression of COX-2 in skeletal muscle, epidermis and inflammatory cells in air-pouch models, suggestive of that production of prostaglandin E2 is connected through the expression of cyclooxygenase-2 (Sedgwick and Lees 1986) (Nantel *et al.*, 1999).

**Table 1:** Effect of methanolic extract of *Marsilea quadrifolia* on carrageenan induced rat paw edema

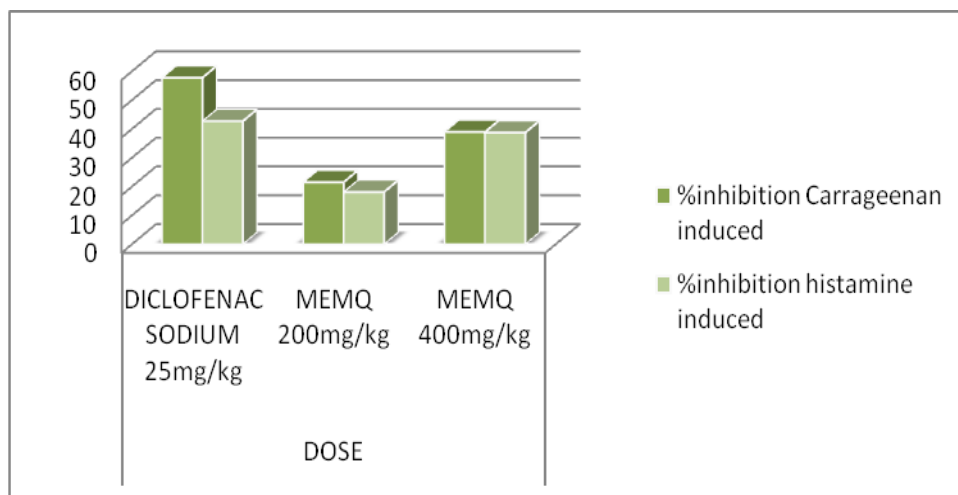
| Treatment                                   | Dose (mg/kg) | Increase in paw volume(ml) |                    |                    | % inhibition       |                    |                    |
|---|--------------|----------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|   |              | 1 <sup>st</sup> hr         | 2 <sup>nd</sup> hr | 3 <sup>rd</sup> hr | 1 <sup>st</sup> hr | 2 <sup>nd</sup> hr | 3 <sup>rd</sup> hr |
| Control                                     | -            | 0.352±0.032                | 0.455±0.056        | 0.565±0.069        | -                  | -                  | -                  |
| Diclofenac sodium                           | 25           | 0.186±0.041                | 0.220±0.062        | 0.238±0.030        | 47.16              | 51.65              | 57.88              |
| Methanolic extract of <i>M. quadrifolia</i> | 200          | 0.280±0.052                | 0.310±0.060        | 0.353±0.061        | 20.45              | 31.87              | 37.52              |
|   | 400          | 0.202±0.072                | 0.225±0.055        | 0.251±0.072        | 42.61              | 50.55              | 55.58              |

Each value represent the mean ± SEM n = 6

**Table 2:** Effect of methanolic extract of *Marsilea quadrifolia* on histamine induced rat paw edema

| Treatment                                   | Dose (mg/kg) | Increase in paw volume(ml) |                    |                    | % inhibition       |                    |                    |
|---|--------------|----------------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|   |              | 1 <sup>st</sup> hr         | 2 <sup>nd</sup> hr | 3 <sup>rd</sup> hr | 1 <sup>st</sup> hr | 2 <sup>nd</sup> hr | 3 <sup>rd</sup> hr |
| Control                                     | -            | 0.302±0.052                | 0.423±0.032        | 0.588±0.061        | -                  | -                  | -                  |
| Diclofenac sodium                           | 25           | 0.173±0.021                | 0.233±0.071        | 0.302±0.032        | 42.72              | 44.92              | 46.83              |
| Methanolic extract of <i>M. quadrifolia</i> | 200          | 0.260±0.032                | 0.341±0.012        | 0.422±0.073        | 13.91              | 19.39              | 28.23              |
|   | 400          | 0.213±0.033                | 0.273±0.054        | 0.324±0.043        | 29.47              | 35.46              | 44.9               |

Each value represent the mean ± SEM n = 6



**Fig 1:** Effect of methanolic extracts of *M. quadrifolia* on carrageenan and histamine induced rat paw edema dose Vs % inhibition

## CONCLUSION

Inflammation induced via carrageenan involves three distinct phases of the discharge of the mediator; as well as serotonin & histamine in the primary phase (0- 2 h); kinins released in the second phase (3 h) and PG in the 3rd phase (>4 h) (Singh *et al.*, 1996). The methanolic extract of *M.quadrifolia* was effective in this phases at the dose of 200 and 400mg/kg.

The methanolic extract of *M. quadrifolia* shows the presence of Linolenic acid, a - 3 (18:3, n-3) fatty acid (allcis-9, 12, 15 octadecatrienoic acid), is progressively metabolized in the body to 6, 9, 12, 15octadecatetraenoic acid (18:4, n-3), stearadonic acid (20:4, n-3) and eicosapentaenoic acid (20:5, n-3). The end product, eicosapentaenoic acid, has the capacity to competitively inhibit the formation of prostaglandins and leukotrienes derived from arachidonate while serving as a substrate for synthesis of prostaglandins with three double bonds and leukotrienes with five double bonds, which are anti-inflammatory. This could be possible mechanism for the antiinflammatory activity of linolenic acid (Lee *et al.*, 1984).

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