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# EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF MURRAYA KOENIGII LEAVES IN EXPERIMENTAL ANIMALS

**ARUNKUMAR J**
Department of Pharmacology, MADURAI MEDICAL COLLEGE AND HOSPITAL

**Abstract :** Objectives- To evaluate the Anti-inflammatory effect of Methanolic extract of Murraya koenigii leaves on carrageenan induced paw edema in albino rats.Materials and methods-Thirty adult male albino rats weighing 175-200 grams were selected and allocated in to five groups of six animals each. The control group received vehicle 2 gum acasia (10mlkg),Standard group received aspirin (200mgkg) and test groups received Methanolic extract of Murraya Koenigii leaves (100mgkg, 200mgkg, 400mgkg per oral respectively) 60 mts before giving subplantar injection of 0.1ml of 1 carrageenan into left hind paw of the rats. The anti-inflammatory effect is estimated by measuring paw volume using plethysmograph. The results were tabulated and analysed with suitable statistical method. Results- Murraya Koenigii leaves showed statistically significant reduction of rat paw edema in a dose dependant manner. Maximaum inhibition occurred at the dose of 400mg kg-1(50.81) after 4th hour of carrageenan injection.(p0.001). The results were comparable to that produced by standard drug aspirin.Conclusion- Murraya Koenigii leaves has anti-inflammatory activity which is comparable to aspirin. Further studies are essential to prove the anti-inflammatory activity of Murraya Koenigii in human.

**Keyword :**Anti-inflammatory, Carrageenan , Murraya koenigii , Paw edema

## INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues. Cardinal signs of inflammation are rubor (redness), tumor (swelling), calor (heat), dolor (pain) and funtio laesa (loss of function). Inflammation may be acute or chronic depending on the nature of the stimulus or the damaged tissues. Although it is a defense mechanism that helps body to protect itself against infection, burns, toxic chemicals, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. Drugs that are currently used for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. These drugs carry potential toxic effects like peptic ulcer, renal failure etc. Many medicines of plant origin had been used without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs**(1)**.

**Figure – 1 Murraya Koenigii**

Murraya koenigii L. (curry tree),belonging to family Rutaceae, is a tropical to sub-tropical tree native to India**(2) (Figure-1)**. **Indian names:** karivempu, karuveppilei (Tamilnadu). karepaku (Andhra Pradesh); karibeva (Karnataka); kariveppilei (Kerala); The leaves are used as a spice in different curries and impart a very good flavour to the preparations. Traditionally, the plant is used as tonic, stomachic, and carminative. Fresh juice of the root is taken to relieve pain associated with kidney. Previous Phytochemical investigations on this plant revealed the presence of carbazole alkaloids **(3 - 6)**. Antioxidant, anti-tumour, antimicrobial, anti-inflammatory, anti-trypanocidal and mosquitocidal, antioxidant, anti-tumor activities have been indicated for some of these alkaloids.

**Aims and objectives :-**

The present study is aimed to evaluate the anti-inflammatory activity of Methonalic Extract of Murraya Koenigii leaves (MEMK). The anti-inflammatory activity was evaluated by subplantar injection of 0.1ml of 1% carrageenan into the left hind paw of the rats, in order to confirm the medicinal properties of the plant. The study was undertaken after obtaining approval of Institutional Animal Ethics Committee.

**Ref.No :- Roc No 12677/ E1 / 5 / 2012**

## MATERIALS AND METHODS

**Plant material**

The leaves of Murraya koenigii were collected from the local gardens of Madurai, Tamilnadu. The leaves were identified and authenticated by Professor of Botany, American College, Madurai.

**Preparation of leaves extract**

Extract was prepared in order to study their anti-inflammatory activity. The leaves were dried under shade and were ground to form the smooth powder. The dried powder (total 100g) was loaded into Soxhlet extractor with glass thimble in 2 batches of 50 g each and was subjected to extraction for about 48 hrs with methanol. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to give a semisolid syrupy consistency residue of 16.8g. The methanol extract was stored in a closed bottle and kept in a refrigerator at temperature below 4°C until tested.

**Selection of animals, caring and handling :-**

A total of 30 healthy albino male Wistar rats (175–200 g), bred locally in the central animal house of Madurai Medical College, Madurai were selected for the study. They were housed under controlled conditions of temperature of 22±2C, relative humidity of 30–70% and 12 h light–12 h dark cycle. Animals were fed with sterile commercial pelleted rat chow supplied by Hindustan Lever Ltd. (Mumbai, India) and had free access to water ad libitum. Animals were kept under fasting for overnight and weighed before the experiment. The animals were acclimatized to laboratory conditions for 7 days before commencement of experiment.

**Study design :**

The rats were randomly allocated into five groups of six each for testing anti-inflammatory activity. Group I (control) received of 2% gum acacia. Group II received Aspirin 200mg/ kg, Group III, IV and V received Methanol extract of Murraya koenigii 100 mg/ kg, 200 mg/kg, 400 mg/kg po respectively.

**Determination of the drug dosage and dosing schedule (7 - 8)**:-

Doses were selected and determined according to the previous acute toxicity studies of methanolic extract of Murraya koenigii. Three different doses were selected 100 mg/kg, 200 mg/kg and 400 mg/ kg for anti-inflammatory activitiy.

**METHODOLOGY :-**

**CARRAGEENAN-INDUCED PAW EDEMA( 9-12 ) :-**

Anti inflammatory activity was assessed by carrageenan-induced hind paw edema method in rat. 30 Albino male wistar rats were weighed and divided into 5 groups containing 6 animals each. Among these groups one was kept as control, one as standard and rests as test groups. A mark was made on left hind paw just beyond knee-joint of each animal of all groups, so that every time the paw was dipped in the plethysmograph up to the fixed mark to ensure constant paw volume. The control group received 2% gum acacia, the standard group received aspirin (200 mg/kg) & the test groups received Methanol extract of Murraya koenigii 100 mg/kg, 200 mg/kg, 400 mg/kg po suspended in 2% gum acacia and administered orally. After 60 mts carrageenan solution (1%w/v) was injected by sub planter region in all the groups. After the administration of carrageenan solution the paw volume was measured at 0, 1 ,2 ,3, 4 hrs respectively by using the plethysmograph. The percentage of edema inhibition was calculated by using the formula,

Percentage of edema inhibition = Vc- Vt / Vc× 100 Where,

Vc= Mean paw volume in control group

Vt = Mean paw volume in drug treated groups.

**STATISTICAL ANALYSIS**

The results were tabulated and expressed as Mean ± SEM, analysed for statistical significance using one way ANOVA, followed by post hoc Dunnet's test for multiple comparison. P < 0.05 was considered statistically significant.

**RESULTS**

The anti-inflammatory effects of the Methanolic Extract of Murraya Koenigii Leaves in Carrageenan induced hind paw edema of rats are shown in **Table 1 & Figure 2.**

**Table-1;Effect of Murraya koenigii on carrageenan induced hind paw edema in rats**

| Groups | Increase in paw volume (Mean±SEM) (ml) | | | | |
|---|---|---|---|---|---|
| | 0 hr | 1 hr | 2 hrs | 3 hrs | 4 hrs |
| Control (Gum acacia) | 0.38 ± 0.00 | 0.48 ± 0.01 | 0.57 ± 0.01 | 0.62 ± 0.02 | 0.61 ± 0.02 |
| Aspirin 200mg/kg | 0.35 ± 0.01 | 0.35 ±0.01** | 0.31 ±0.01** | 0.25 ±0.02** | 0.23 ±0.01** |
| MEMK 100 mg/kg | 0.38 ± 0.00 | 0.46 ± 0.01 | 0.53 ± 0.01 | 0.56 ± 0.02 | 0.53 ± 0.02 |
| MEMK 200mg/kg | 0.37 ± 0.00 | 0.45 ± 0.01 | 0.48 ± 0.01* | 0.38 ±0.00** | 0.34 ±0.01** |
| MEMK 400mg/kg | 0.36 ± 0.00 | 0.43 ± 0.01 | 0.44 ± 0.01** | 0.35 ±0.01** | 0.30 ±0.03** |

Each value is the Mean ± S.E.M. for 6 rats, * indicates P < 0.01; ** indicates P < 0.001; compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

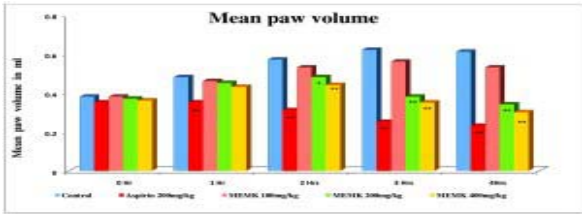
Standard drug: Aspirin -200mg/kg b.w.

MEMK 100: Methanolic extract of Murraya koenigii at dose 100mg/kg b.w.

MEMK 200: Methanolic extract of Murraya koenigii at dose 200mg/kg b.w.

MEMK 400: Methanolic extract of Murraya koenigii at dose 400mg/kg b.w.

**Figure-2;Effect of Murraya koenigii on carrageenan induced hind paw edema in rats**




Each value is the Mean ± S.E.M. for 6 rats, ***** indicates P < 0.01; ****** indicates P < 0.001; compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test. Standard drug: Aspirin 200mg/kg b.w.

MEMK 100: Methanolic extract of Murraya koenigii at dose 100mg/kg b.w.

MEMK 200: Methanolic extract of Murraya koenigii at dose 200mg/kg b.w.

MEMK 400: Methanolic extract of Murraya koenigii at dose 400mg/kg b.w.

**Percentage of inhibition in hind paw edema of rats are shown in Table -2 and Figure - 3.**

**Table – 2; Percentage of Inhibition of rat paw edema by MEMK**

| Groups | Percentage of Inhibition of paw edema | | | |
|---|---|---|---|---|
| | 1 hr | 2 hrs | 3 hrs | 4 hrs |
| Control (Gum acacia) | 0% | 0% | 0% | 0% |
| Aspirin 200mg/kg | 27.08% | 45.61% | 59.67% | 62.3% |
| MEMK 100 mg/kg | 4.1% | 7.01% | 9.83% | 13.11% |
| MEMK 200mg/kg | 6.2% | 15.78% | 38.7% | 44.26% |
| MEMK 400mg/kg | 10.4% | 22.8% | 43.54% | 50.81% |

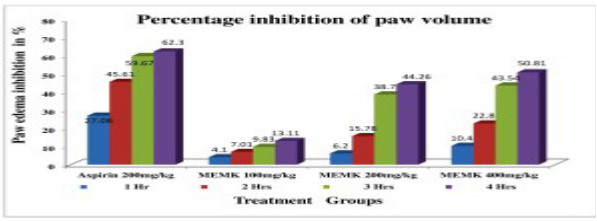
Standard drug: Aspirin-200mg/kg b.w.

MEMK 100: Methanolic extract of Murraya koenigii at dose 100mg/kg b.w.

MEMK 200: Methanolic extract of Murraya koenigii at dose 200mg/kg b.w.

MEMK 400: Methanolic extract of Murraya koenigii at dose 400mg/kg b.w.

**Figure - 3; Percentage of Inhibition of rat paw edema by MEMK**




Standard drug: Aspirin-200mg/kg b.w.
MEMK 100: Methanolic extract of Murraya koenigii at dose 100mg/ kg b.w.
MEMK 200: Methanolic extract of Murraya koenigii at dose 200mg/ kg b.w.
MEMK 400: Methanolic extract of Murraya koenigii at dose 400mg/ kg b.w.

Basal mean paw volume was comparable in all the groups. There was a gradual increase in the paw volume noted in the control group after sub-plantar injection of Carrageenan. Among the test groups, animals those received lower dose of extract 100 mg/kg b.w also showed gradual increase in the mean paw volume upto 4hrs after carrageenan injection. So at the dose of 100mg/kg bw Methanolic Extract of Murraya Koeinigii Leaves did not show significant antiinflammatory activity when compared to control group. At the dose of 200mg/kg b.w , at 2nd hour after carrageenan administration ,animals showed significant reduction in mean paw volume when compared to control group.( p <0.01). At 3rd & 4th hrs after carrageenan administration these group of animals showed further reduction in mean paw volume when compared to control group of animals.(p<0.001). At the dose of 400mg/kg b.w , at 2nd ,3rd & 4th hour after carrageenan administration these group of animals showed statistically significant reduction in mean paw volume when compared to control group of animals.(p<0.001). However, Standard drug Aspirin (200mg/kg bw) treated animals were showed maximum reduction of mean paw volume at all the time duration when compared to the control group.(p< 0.001).

The standard drug Aspirin produced maximum inhibition of paw edema (62.3%) at 4th hour after carrageenan administration while Methanolic Extract of Murraya Koenigii at the doses of 400 & 200mg/kg b.w produced significant inhibition of paw edema 50.81% & 44.26% respectively at 4th hour after carrageenan administration among the test groups.**(Table -2 & Figure -3)** Hence, the standard and test compounds produced significant percentage of decrease in paw edema volume as compared to the control. Here, Compound Murraya koenigii has shown statistically significant (p<0.001) inhibitory effect in paw edema at the dose of 200 & 400 mg kg-1 at 3rd & 4th hr after carrageenan injection as compared to the control. So synthesized compounds produced significant inhibition of Carrageenan induced paw edema at the doses of 200mg/kg b.w & 400mg/kg b.w.

**DISCUSSION:-**

Inflammation can be classified as either acute or chronic inflammation. Acute inflammation is the initial response of the body to injurious stimuli and is achieved by increased movement of plasma and leukocytes from the blood into the injured tissues. The process of acute inflammation is initiated by cells already present in the tissues. This is characterized by marked vascular changes, including vasodilatation and increased capillary permeability which are induced by the actions of the various inflammatory mediators. Chronic inflammation is a prolonged inflammatory response that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissues from the inflammatory process . Carrageenan induced paw edema is a biphasic response **(13-15)**.

The first phase was mediated through the release of Histamine, serotonin & Kinin, where as the second phase is related to the release of prostaglandin and slow reacting substances which peak at 4 hour. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal antiinflammatory agents. Generally NSAIDs strongly inhibit the second phase of carrageenan-induced edema while some others inhibit both phases. Aspirin seems to block both phases. The methanol extract of *M. keinegii* at 400 mg kg-1 showed 50.81% reduction in edema which was comparable to that of 200 mg kg-1 of the standard drug Aspirin. The most widely used primary test to screen new anti-inflammatory agent's measures the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. The significant anti-inflammatory effect shown by the methanol extract of *Murraya koenigii* leaves may be due to inhibition of prostaglandinlike substances. The anti-inflammatory activity of methanol extract of *M.koenigii* may be due to a combination of different biologically constituents rather than any single compound, the most interesting being the alkaloids, the steroids, the flavonoids and the triterpenoids.

The presence of steroids, triterpenoids, alkaloids, and flavanoids was confirmed by the preliminary pytochemical analysis. Recent reports have also indicated that many flavonoids possess anti-inflammatory activity **(6)**.

**CONCLUSION**

The present study demonstrates that the extract of *Murraya koenigii* leaves can produce significant anti-inflammatory activity. However, to know the exact mechanism by which *Murraya koenigii* leaf extract produce this effect is a subject of further studies on isolation and fractionation of the active components.

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