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Study of synergistic anti-inflammatory activity of *Murraya koenigii* and *Aegle marmelos*

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ABSTRACT

The curry leaf tree is native to India, Sri Lanka, Bangladesh and the Andaman Islands. The leaves are particularly associated with South Indian famous cuisines. The study investigate the anti-inflammatory activities of the leaves of Murraya. Aegle marmelos has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India Bangladesh and Sri Lanka. Methanolic extracts were prepared separately from Murraya koenigii & Aegle marmelos. Individual & synergistic anti-inflammatory activity of both the plants were studied by carrageenan induced inflammation & paw edema on albino wistar rats. Individual doses of above two extracts produced anti-inflammatory property. But combined doses of above two extracts produced pronounced synergistic anti-inflammatory activity. When combined dose is increased the degree of inhibition is much better & comparable to reference drug. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibition suggesting that the MEM_K and MEA_B may possess arachidonate COX inhibitory property. These extracts can be used to isolate pure compound that can be utilized for rational designing of anti-inflammatory drugs with significant synergism.

Key words: Carragenan, Paw Edema, Cyclooxygenase, Synergism, Comparable Inhibition

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INTRODUCTION

Murraya koenigii is known as '*curry patta*' in hindi and widely used as spice and condiment in India and other tropical countries. It belongs to the family Rutaceae [1]. Traditionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhoea, dysentery; insect bites and also used to allay heat of body [2]. Previous phytochemical investigations on this plant revealed the occurrence of carbazole alkaloids [3, 4, 5, 6]. Anti-oxidant, anti-tumour, anti-microbial, anti-inflammatory, anti-trypanocidal and mosquitocidal activities have been indicated for some of these alkaloids [7, 8, 9, 10, 11]. The study, therefore, intends to investigate the anti-inflammatory activities of the leaves of *Murraya koenigii* by studying the effects of methanol extracts of the plant on carrageenan induced inflammation in experimental animal models, in order to validate the folk medicinal use of this plant. The curry leaf tree is native to India, Sri Lanka, Bangladesh and the Andaman Islands. Later spread by Indian migrants, they now grow in other areas of the world where Indian immigrants settled. Widely cultivated, the leaves are particularly associated with South Indian cuisines. The curry leaf tree is native to India, Sri Lanka, Bangladesh and the Andaman Islands. Later spread by Indian migrants, they now grow in other areas of the world where Indian immigrants settled. Widely cultivated,

the leaves are particularly associated with South Indian cuisines. The major constituents responsible for the aroma and flavor has been reported as pinene, sabinene, caryophyllene, cadinol and cadinene. The bark and the roots are used as a stimulant by the physicians. They are also used externally to cure eruptions and the bites of poisonous animals. The green leaves are stated to be eaten raw for curing dysentery, and the infusion of the washed leaves stops vomiting. Curry leaves are also used in calcium deficiency. It has Vitamin A, Vitamin B, Vitamin C, Vitamin B2, Calcium and iron in plenty. Its nutritional value benefits both the young and the old alike. Women who suffer from calcium deficiency, osteoporosis etc can find an ideal natural calcium supplement in curry leaves. Fresh juice of curry leaves, with lime juice and sugar is an effective medicine in the treatment of morning sickness, nausea and vomiting due to indigestion and excessive use of fats [12]. One or two teaspoons of juice of these leaves mixed with a teaspoon of lime juice may be taken in these conditions. The curry leaves, ground to a fine paste and mixed with buttermilk, can also be taken on an empty stomach with beneficial results in case of stomach upsets. Also used as laxative. Boils and similar eruptions appear on skin during summer. Most of the boils remain painful. Curry leaves come handy in treating such conditions. A paste made of curry leaves is applied on these persistent boils for quick relief [13]. Along with mint leaves and coriander leaves, curry leaves can be used in treating excessive pitta conditions. Curry leaves can be used with effective result to treat burn, bruises and skin erruption. Cataract development can be prevented by using fresh juice of curry leaves. Kidney pain can be cured by using juice of root of *Murraya koenigii*. It can be used in preventing premature greying of hair. Indian Medicinal plants are considered as a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailmets [14, 15]. *Aegle marmelos* is another indian medicinal plant; which has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant [16, 17]. Various phytoconstituents have been isolated from the various parts of *Aegle marmelos* [18]. Phytoconstituents isolated from various parts of *Aegle marmelos*: Leaf- Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol, Marmesinine. Bark -Skimmianine, Fagarine, Marmin Fruit- Marmelosin, Luvangetin, Aurapten, Psoralen, Marmelide, Tannin. The different parts of Bael are used for various therapeutic purposes, such as for treatment of Asthma, Anaemia, Fractures, Healing of Wounds, Swollen Joints, High Blood Pressure, Jaundice, Diarrhoea, Typhoid & troubles during Pregnancy [19, 4]. *Aegle marmelos* has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India Bangladesh [human tumor] and SriLanka [20, 21]. The main usage of the parts of this tree is for medicinal purposes. The unripe dried fruit is astringent, digestive, stomachic and used to cure diarrhoea and dysentery [22]. Sweet drink prepared from the pulp of fruits produce a soothing effect on the patients who have just recovered from bacillary dysentery [12]. Ripe fruit is a good and simple cure for dyspepsia. The pulp of unripe fruit is soaked in gingelly oil for a week and this oil is smeared over the body before bathing. This oil is said to be useful in removing the peculiar burning sensation in the soles. The roots and the bark of the tree are used in the treatment of fever by making a decoction of them. The leaf part of the plants have been claimed to be used for the treatment of inflammation, asthma, hypoglycemia, febrifuge, hepatitis and analgesic. The mucilage of the seed is a cementing material. The wood takes a fine polish and is used in building houses, constructing carts, agricultural implements. A yellow dye is obtained from the rind of the unripe fruits. The dried fruits, after their pulp separated from the rind are used as pill boxes for keeping valuable medicines, sacred ashes and tobacco. In Homeopathic treatments it is largely used for conjunctivitis, styes, rhinitis, coccygodynia, nocturnal seminal emission with amorous dreams, chronic dysentery. Ayurveda prescribes the fruit of the herb for heart, stomach, intestinal tonic, chronic constipation and dysentery; some forms of indigestion, typhoid, debility, cholera, hemorrhoids, intermittent fever, hypocondria, melancholia and for heart palpitation. The unripe fruit is medicinally better than the ripe fruit. Leaf poultice is applied to inflammation; with black pepper for edema, constipation and jaundice. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammatory diseases are very common throughout the world. Rhematoid arthritis is one of the oldest known diseases of mankind affecting the majority of population no substantial progress has been made in achieving a permanent cure and different types of Rheumatic diseases are a major cause of morbidity of the working force [23]. Inflammation results in the liberation of endogenous mediators like serotonin, histamine, bradykinin, prostaglandins etc. These mediators even in small quantities can elicit pain response. Anti inflammatory drugs make up about half of analgesics, relieving pain by reducing inflammation as opposed to opiods which affect the central nervous system. The greatest disadvantage in presently available potent synthetic drugs for the treatment of inflammation lies in their toxicity and reappearance of symptoms after discontinuation. Therefore the screening and development of drugs for their anti inflammatory activity is still in progress and there is much hope for finding anti inflammatory drugs from medicinal plants. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine.

MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal (s) under the prescribed guidelines and recommendations. It includes in it all the steps from field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization protocol , usage of instruments, preparation of reagents, selection of specific solvents for extraction , formation of protocols and final execution of standardized protocol. All these requires good build of mind and soft technical and to handle the materials and procedures in a true scientific manner. This Study was approved by Institutional Ethical Committee of Geethanjali College of Pharmacy, Cheeryal. Healthy adult albino wistar rats weighing 200-250 grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory animal diet) and water ad libitum. Animals were housed within the departmental animal house and the room temperature was maintained at 27^{o} C. The leaves of *Murraya koenigii* and *Aegle marmelos* were collected from the local area of Keesara, Rangareddy District, Hyderabad, India in the month of January. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored and taken up for extraction process. The plants material was dried under shade and powdered mechanically. The 50 gm of powder sample was defatted with petroleum ether (60-80°c), and then extracted with methanol by using soxhlet apparatus. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The solvent was removed by a vacuum rotary evaporator and dried under reduced pressure. The yield of the methanol extract was 9.4%. The dried extract was stored in refrigerator until further studies [24]. Male Albino wistar rats were divided into four groups containing of six animals of each group-1 received extracts of 200 mg/kg body weight (100mg each extract), group-2 received extracts of 100 mg/kg body weight, (50mg each extract) group-3 received standard drug (diclofenac) and group- 4 received control drug (Carragenan). Carragenen induced paw edema is a suitable experimental animal model for evaluating an anti edematous effect. Edema developed following injection of Carragenan serves as an index of acute inflammatory change, was and can be determined from differences in the paw volume measured immediately after Carragenan injection and then every hour for 6 hours. Edema induce by caragenan is believed to be a bi-phasic: the first phase (1h) involves the release of serotonin and histamine and the second phace (over 1h) is mediated by prostaglandins, cyclooxygenase products. Continuity between the two phases is provided by kinins. The anti-inflammatory activity was determined using a carragenan induced paw edema model. Six albino wistar (200-250 g) either sex, were randomly divided into 4 groups [12].

RESULTS AND DISCUSSION

In carragenan induced paw edema activity, the paw volumes and percentage of inhibition of the control, standard and test compounds are shown in table. The tests compounds are diclofenac at a dose of 5mg/kg body weight for anti-inflammatory activity. Presently diclofenac showed 80% inhibition of inflammation at 2 hours when compared to control. Methanolic extracts of *Murraya koeniigi* leaves (200mg/kg) shown significant inhibition of inflammation with 30% and 10% respectively at 2 hours when compared with control. Combined doses of above two extracts produced synergistic anti-inflammatory property. When combined dose is increased, the degree of inhibition is much better & comparable to reference drug.

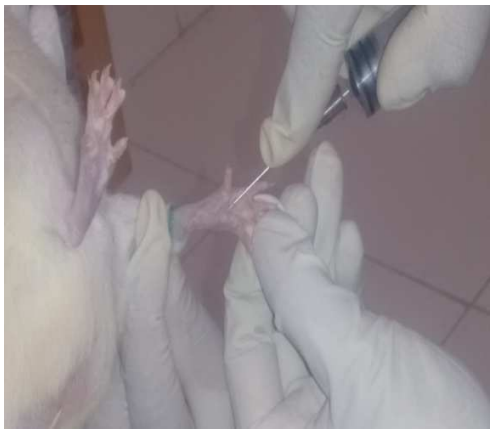

Fig No 4.2:-Giving Injection to the paw

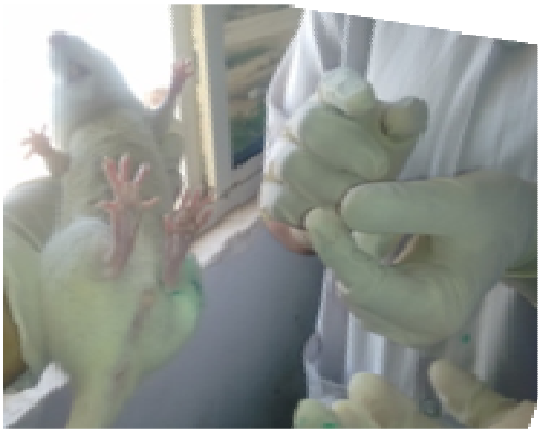

Fig No 4.3:- Comparison of two paws

Fig No 4.4:-Control Paw

Fig No 4.5:- Test Paw

Table: Effect of extracts of *Murraya koeniigi* and *Aegle marmleos* on paw edema volumes

GROUPS	DOSE (MG/KG)	CHANGE IN PAW VOLUME (ML) MEAN± SEM & PERCENTAGE INHIBITION					
		0 MIN		1 HOUR		2 HOUR	
		R	L	R	L	R	L
CONTROL	--	0.2± 0.03	0.1± 0.02	0.2± 0.02	0.7± 0.03	0.2± 0.02	0.8± 0.02
STANDARD (DICLOFENAC SODIUM)	10	0.2± 0.03	0.3± 0.04	0.2± 0.02	0.4± 0.04	0.2± 0.03	0.4± 0.04
MEM_K	200	0.2± 0.02	0.2±0.03	0.2± 0.03	0.4±0.02	0.2± 0.02	0.6±0.03
MEA_M	200	0.2± 0.03	0.3±0.02	0.2± 0.02	0.5±0.03	0.2± 0.03	0.7±0.02
MEM_K+MEA_M	200 (100+100)	0.2± 0.02	0.2± 0.02	0.2± 0.03	0.3± 0.02	0.2± 0.02	0.4± 0.02
MEM_K+MEA_M	400 (200+200)	0.2± 0.03	0.1± 0.02	0.2± 0.03	0.2± 0.02	0.2± 0.03	0.3± 0.02

The results are expressed as mean ± S.E.M differences in mean value between groups were analyzed by a one-way ANOVA. Statistical significance was assessed as $p < 0.001$. Here MEM_K: Methanolic extract of *Murraya koenigii.* MEA_M: Methanolic extract of *Aegle marmelos*. Current anti-inflammatory drugs such as opioids and non-steroidal

anti-inflammatory drugs are not useful in all cases because of their side effects and low potency. As a result, search for other alternatives became necessary and imperative. Therefore, the present study is aimed at evaluating the scientific basis for the traditional use of *Murraya koenigii* and *Aegle marmelos* leaves using carragenan induced rat paw edema for anti-inflammatory activity. Carrageenan has been widely used as a harmful agent able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity. Carrageenan induced rat paw edema is a suitable model to predict the value of anti-inflammatory agents, which act by inhibiting the mediators of acute inflammation. Carrageenan induced hind paw edema in rat is a biphasic event. The early phase (90-180min) of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase (270-360min) is associated with activation of kinnin like substances; i.e., prostaglandins, proteases and lysosome. Methanolic extract of *Murraya koenigii* and *Agles marmelos* leaves inhibited carrageenan induced rat paw edema formation at both early and later phase. These results tends to suggest that the inhibitory of the extract on edema formation is probably due to the inhibition of the synthesis and/or release of the inflammatory mediators, especially the cyclooxygenase (COX) products [Singh S et al. 1996]. The carrageenan induced paw edema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibition due to its COX-dependent mechanism, thus, it is suggested that the MEM_K and MEA_B may possess arachidonate COX inhibitory property [24, 25].

CONCLUSION

Through such findings we can conclude that both MEM_K and MEA_M have anti-inflammatory activity in carragenan-induced paw edema in rats. This extracts has showed that decrease in paw edema volume when compared to control and standard drugs. Therefore, the anti-inflammatory effects observed in this study may be due to activity of one or a combination of some of the identified constituents. It may suggest that the inhibition of the systhesis and/or release of the inflammatory mediators, especially the cyclooxygenase products. The carragenen induced paw edema test is effectively controlled with the arachidonate COX inhibitors due to its COX-dependent mechanism. The extracts can be used to isolate pure compound that can be utilized for rational designing of anti-inflammatory drugs with significant synergism.

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