

Evaluation of Anti Inflammatory Activity of Hydroalcoholic Leaves Extracts of Polyherbal Combination of *Vitex Negundo* and *Murraya Koenigii* against Carrageenan Induced Paw Edema in Rats

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Abstract

Vitex Negundo and *Murraya Koenigii* are popular Indian herbs which are used for various clinical disorders. The basic literature survey exposed that *Vitex Negundo* has antioxidant, antiulcer and anti-nociceptive activity and *Murraya Koenigii* possessing anthelmintic, analgesic, cooling activity, but not many scientific studies have been carried out to reveal and collaborate the anti-inflammatory activity of *Vitex Negundo* and *Murraya Koenigii* leaves. All animals were divided into five groups, six rats in each group. Group I was treated as toxicant control to observe swelling, group II was treated with Aspirin (10 mg/kg, p.o), group III and IV were treated with hydroalcoholic extract of leaves of *Vitex Negundo* and *Murraya Koenigii* at two doses (100 mg/kg, p.o.) respectively. The anti-inflammatory activity was studied by using carrageenan induced paw edema in rats. Present study showed that hydroalcoholic leaves extract of *Vitex Negundo* and *Murraya Koenigii* significantly reduce paw edema when compared with group I (Toxicant control) rats and the result were dose dependent. The toxicity study also revealed its safeness, thus the plant can be hypothesized it is nontoxic. All data were expressed as mean+SEM and were statistically analysed one way ANOVA. It is concluded that hydroalcoholic leaves extract of *Vitex Negundo* and *Murraya Koenigii* can offer protection against carrageenan induced paw edema in rats.

Keywords: *Murraya Koenigii*, *Vitex Negundo*, Anti-inflammatory, Hydroalcoholic extract

Introduction

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani. This is because of the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all plant parts to be potential sources of medicinal substances' [1].

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 [2]. 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 [3]. Previous Phytochemical investigations on this plant revealed Analgesic, Wound Healing, Antidiarrhoeal, Anthelmintic, Antibacterial, Antifungal, Antiulcer, Antiobesity, and Hypoglycaemic activities.

Vitex Negundo Linn. commonly known as Negundo, has been used by several traditional systems of medicine including Ayurveda, Unani and Siddha for different ailments. It belongs to the family Verbanaceae [4]. *Vitex Negundo* is commonly known as five leaved chaste tree (English), Nirgudi (Marathi), Nirgundi (Hindi), Indrani (Sanskrit) [5]. This plant has reported the Antipyretic, Antidiabetic, Laxative, Anticonvulsant, Antitumor, Wound Healing and Anthelmintic activities. It is also useful in leucoderma and blood disorders. An infusion of the toasted leaves is used to stop vomiting [6]. Crushed leaves are applied externally cures skin eruption and to relieve burn. The pastes of leaves are applied externally to treat the bites of poisonous animals [7]. Steam distillate of the leaves can be used as stomachic, purgative, febrifuge and anti-emetic [8]. Leaves are applied externally to bruises and eruption [9].

The present study therefore, intends to investigate the anti-inflammatory activity of hydroalcoholic leaves extracts of polyherbal

formulation of *Murraya Koenigii* and *Vitex Negundo* on carrageenan induced inflammation in experimental animal models, in order to confirm the medicinal properties of the plant.

Materials and Methods

Plant material

The leaves of *Murraya Koenigii* and *Vitex Negundo* were collected from local area of Kota, Rajasthan. The leaves of both the plants were authenticated by Department of Botany, Government College, Kota, Rajasthan.

Preparation of extract

Extracts were prepared in order to study their anti-inflammatory activity. The leaves were dried under shade and were ground to form the smooth powder. The powdered leaves of *Murraya Koenigii* and *Vitex Negundo* (80 gm each) were macerated with ethanol (50% v/v) as solvent for 7 days and after every 24 hours; the mixtures were stirred with a sterile glass rod. The extract was filtered by whatmann filter paper no.1 to obtain the filtrate. The filtrates were kept on water bath to obtain the crude extract [10].

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Group No.	Drug Treatment	1 hr	2 hr	3 hr	Average Reading	% Inhibition
		Mean ± S.D	Mean±S.D	Mean±S.D		
I	Toxicant control (Carrageenan 0.1ml, 1% sol)	2.60 ± 0.11	3.40 ± 0.09	3.35 ± 0.13	3.12
II	Carrageenan + Aspirin (10 mg/kg, p.o.)	2.49 ± 0.13	2.31 ± 0.09	2.22 ± 0.16	2.34	25
III	Carrageenan + <i>Vitex Negundo</i> (100 mg/kg, p.o.)	2.51 ± 0.11	2.43 ± 0.11	2.34 ± 0.12	2.43	22
IV	Carrageenan + <i>Murraya Koenigii</i> (100 mg/kg, p.o.)	2.56 ± 0.09	2.70 ± 0.12	2.55 ± 0.17	2.60	17
V	Carrageenan + <i>Vitex Negundo</i> : <i>Murraya Koenigii</i> (100:100 mg/kg, p.o.)	2.53 ± 0.09	2.30 ± 0.19	2.27 ± 0.13	2.37	24

Table 1: Observation Table.

Animals

Albino rats of either sex weighing 180-200 gm were procured from Animal House of Kota College of Pharmacy, Kota, Rajasthan, and were selected in the study. The animals were kept in polypropylene cages and fed with standard pellet diet and water ad libitum, maintained at an ambient temperature of 25°C ± 2°C and relative humidity 50% ± 15%. The study was conducted after approval of Institutional Animal Ethical Committee.

Acute Toxicity Study

Albino mice of either sex, weighing 20-25 gm were used to determine the lethal dose. The animals were fasted overnight prior to the actual experimental procedure. Five groups of 6 mice each were administered with aqueous extract at 1.0-5.0 gm/kg p.o respectively. Animals were observed for clinical signs and mortality, continuously for first 2 hr. and then for every forth hr. The no. of dead or survived mice after 24 hr. was recorded and LD₅₀ was calculated according to Miller and Tainter. The parameter observed and recorded was changed in behavioral, neurologic and autonomic responses. 1/10th of lethal dose was taken as a screening dose [11].

Anti Inflammatory Activity

The activity was evaluated by using carrageenan induced paw edema in rats. Albino rats of either sex were divided into five groups of six animals each. Group I served as toxicant control, received carrageenan (0.1ml of 1% solution), group II served as positive control, received Aspirin (10 mg/kg, p.o), group III and IV were treated with hydroalcoholic leaves extract of *Vitex Negundo* and *Murraya Koenigii* (100 mg / kg, p.o.) respectively, and group V were treated with hydroalcoholic leaves extract of combination of *Vitex Negundo*: *Murraya Koenigii* (100 mg/kg : 100 mg/kg, p.o). All the respective grouped animals were injected with carrageenan (0.1ml, 1% solution).

Procedure

Thirty minutes after drug or test compound (extracts) administration, 0.1 ml. of 1% carrageenan in distilled water was injected into the sub plantar region of right hind paws of all groups. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema volume was measured with the help of plethysmometer at zero hr. (Immediately after injecting carrageenan). The same procedure was repeated at 30 minutes 1, 2, 3 hours. The difference between 1 hours and subsequent hours reading was taken as actual edema volume [12].

Results

Statistical analysis

The results were expressed as mean ± S.E.M. and statistically

analyzed by one-way ANOVA followed by Dunnett's test. P<0.05 was regarded as statistically significant (Table 1).

It was observed that Hydroalcoholic leaves extract of polyherbal combination of *Vitex Negundo* and *Murraya Koenigii* (100 mg/kg, p.o.) significantly reduced paw edema as compared to individual leaves extract.

Discussion

Carrageenan-induced paw edema being an *in-vivo* investigational model for acuteinflammation which been extensively used to determine the anti-inflammatory effect of new investigational agents. Due to increase in frequency of intake of synthetic drug and their side effect, there is need to focus on herbal drug having fewer side effect. The result obtained in this study suggested that hydroalcoholic leaves extract of *Vitex Negundo* and *Murraya Koenigii* possess significant anti-inflammatory activity against Carrageenan induced paw edema in rats. The carrageenan-induced paw edema in rats is believed to be biphasic. The first phase is due to the release of histamine or serotonin, and the second phase is caused by the release of bradykinin, protease, prostaglandin, and lysosome. Therefore, it can be assumed that the inhibitory effect of the polyherbal extract of *Vitex Negundo* and *Murraya Koenigii* on carrageenan-induced inflammation could be due to the inhibition of the enzyme cyclooxygenase, leading to the inhibition of prostaglandin synthesis.

The present study on extract of *Vitex Negundo* and *Murraya Koenigii* has demonstrated that this plant has significant anti-inflammatory properties, and it justifies the traditional use of this plant in the treatment of various types of pains and inflammation.

Conclusion

On the basis of these findings, it may be inferred that hydroalcoholic leaves extract of *Vitex Negundo* and *Murraya Koenigii* is an effective for anti inflammatory activity. This activity may be due to presence of active constituents like flavonoids, triterpenoids and alkaloids.

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