Analgesic and Anti Inflammatory Effects Of *Ocimum americanum* (Linn) In Laboratory Animals

Sripriya D, Venkanna L, Estari Mamidala

Abstract— *Ocimum americanum*(L.) is a one type of weed, but some tribes are used as a herbal remedy for various ailments. But the scientific bases for its medicinal use especially in pain and inflammation remains unknown. Therefore, the present study was aimed to investigate the analgesic and anti-inflammatory effects of the leaves of *Ocimum americanum* in Laboratory animals. The methanol extracts of the leaves of the *Ocimum americanum* was used to investigate the acute effect on analgesia by hot-plate test in spargue dawley mice (Sri Raghavendra Enterprises, Bangalore) and on inflammation in mice using carrageenan-induced mice paw edema model. The extract showed a significant ($p<0.05$) dose dependent increase in reaction time in mice in the hot-plate test at the doses of 250 and 500 mg/kg body weight. The extract also exhibited promising anti-inflammatory effect as demonstrated by statistically significant ($p<0.05$) inhibition of paw value by 45.66% at the dose of 500 mg/kg body weight at the fourth hour of study. This study suggests that the methanol extract of *Ocimum americanum* have both analgesic and anti-inflammatory activity in a dose dependent manner which supported its use an analgesic and anti-inflammatory drug in folk medicine. This may be useful source of lead components in the treatment of pain and inflammation.

Index Terms— Analgasic, Anti-Inflammatory, Laboratory animals, Ociumum americanum, Methanol extraction and Tribes.

1 INTRODUCTION

The development of traditional medicinal systems incorporating plants as means of therapy can be traced back to the middle Paleolithic age some 60,000 years as found from fossil studies (Solecki and Shanidar, 1975). Medicinal plants were very commonly available in abundance especially in the tropical regions.

Apart from the use in the treatment of illness through self-medication, these medicinal plants are valuable for modern medicine in other ways. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996).

Ocimum americanum (Linn) commonly called as *Ocimum canum* belongs to the Family: Lamiaceae (Labiatae) (Wealth of India, 2001). It is generally distributed throughout India, in fields of waste lands, Plains and lower hills of India (Ram Rastogi and Mehrotra, 1993). It is common in wastelands, by arable lands; and pleotropic. The plant is a pubescent erect much branched herb, 15-60 cm high with sub-quadrangular striate brances(Ram Rastogi and Mehrotra, 1993). Leaves are elliptic-lanceolate, entire, glabrous and gland dotted strongly aromatic herb; branchlets puberulous, tetra or four-angular (Joshi, 2000). Leaves are elliptic–lanceolate, entire or faintly toothed almost glabrous, gland-dotted (Parrotta, 2001). The main chemical constituents are volatile oils include methyl cinnamate, methylheptenone, methylnonylketone, d-camphor, citral, ocimin, methylchavicol, linalook, nevadensin, salvigenin, beta-sitoseterol, betulinic, ursolic, oleanolic acids, falvanoids, pectolinarigenin-7 –methylenther and nevadensin. Polysaccharides composed of xylose, arabinose, rhamnose and galacturonic acids(Chopra, Nayar and Chopra, 1956). The main uses of *Ocimum americanum* are antimicrobial, antioxidant, anthelmintic and anti diabetic (Khare, 2007).

Ocimum americanum(Linn) family belongs to Labiatae locally known as "Kukkatulasi" and in Sanskrit "Vanabarbarika" is an erect softy aromatic herb. Tribes are commonly used for curing skin diseases, cold, cough, malarial fever and soon. *Ocimum americanum* also possesses anti-fungal activity against *Asperigillus niger* and aqueous extract of the plant is found effective in patients suffering from viral diseases.

This plant contains many aromatic and volatile oils and the main constituents are Eugenol and Thymol the active principals of *Ocimum americanum* comprising of phenols and flavonoids have been shown to have significant anti-inflammatory activity both *in vivo* and *in vitro*.

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This plant contains many aromatic and volatile oils and the main constituents are Eugenol and Thymol the active principals of *Ocimum americanum* comprising of phenols and flavono -lus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat induced pain stimulus.

2 Materials And Methods

2.1 Collection and Identification of Plants

The leaves of *Ocimum americanum* were collected from the sri venkateswara Botanical garden, Sri Venkateswara University, Thirupati, Chittoor Dt., Andhra Pradesh, India on 25th December 2012. When the plant is fully flowered the plant was identified by the senior taxonomists, Department of Botany, Sri Venkateswara University. The specimen was preserved in as (SVUBH/2010/L-675), Herbarium house, Department of Botany, Sri Venkateswara University, Thirupati.

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2.2 Preperation of Plant Meterials

The collected plant leaves were washed with water and separated from in desirable materials or plants or plant parts. They were aerated by fan aeration to be partially dried and were next heated in an oven at below 40ºC for two days to be fully dried. The fully dried leaves are then grinded to make them powder by the help of a piston and mortar. Then the powders were dissolved in methanol (80%) and kept for a period of 2 days accompanying occasional shaking and stirring. The whole mixture then underwent a filtration by a piece of clean white cotton material followed by a second filtration through what man filter paper. The filtrate (methanol extract) obtained was evaporated by rotary evaporator (EV 311 Plus, H Biomedical LTD, Bangalore) at 5 to 6 rpm and at 65ºC. It rendered a gummy concentrate of chocolate brown colour that was designated as crude extract or methanol extract was finally dried by freeze drier and preserved.

2.3 Animals

Young Spargue Dawley mice aged about 5 - 6 weeks with average weight of 30 – 35 gm and Adult Albino rats (wistar strain) having average weight of 200 – 250 gm were used for this study. They were kept in standard environmental condition for one week in the animal house of the Department of Zoology, Kakatiya University, Warangal, Andhra Pradesh, India for adoption after their purchase. The animals were provided with standard laboratory food and tap water ad libitum and maintained at natural day night cycle.

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2.4 Analgesic activity by Hot Plate Test in Mice

The hot plate text (Hot Plate, Inlabs, Bangalore) was employed for measurement of analgesic activity as previously described by Lanhers et al. and modified by Mohamed and ojewole(Lanhers et al, 1992 and Mohamed, 2001) . The temperature was regulated at 50° ± 1°. Mice were divided into four groups consisting of five animals in each group. The mice of each group were placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced pain stimul

The time for each mice to lick its paws or jump out of the beaker was taken as reaction time (in second). Before treatment, the reaction time taken once. The mean of this determination constituted initial reaction time before treatment of each group of mice. Each of the test mice was there after treated with either distilled water (DW), Ketorolac (2.5 mg/kg of body weight) (Ketorolac issued to provide short-term relief of moderate to severe pain) (Excipientes, Celulosa microcristalina, Croscarmelosa sodica, hidroxipropilmeticelulosa/ Polietilenglicorl, esterato de magmesio, hidrosipilmetilcelulosa/ dioxide de titanio / triacetima/ lactose celactosa) or methanol extract of *Osimum americanum* at the doses of 250 and 500 mg/kg body weight orally. After thirty minutes treatment, the reaction time of each group mice, were again evaluated five times individually in one hour interval on this occasion. Present analgesic score was calculated as

$$. \text{PAS} = (T_a - T_b) / T_a * 100$$

Where T_a – Reaction time (in seconds) before drug administration;

T_b – Reaction time (in seconds) after drug administration

2.5 Anti-inflammatory activity

The anti-inflammatory activities of the methanol extract was investigated on carrageenan (the carrageenan are a complex group of polysaccharides made up of repeating galactose related monomers) included inflammation in mice paw following the method of winter et al with minor modification[12]. Mice were randomly divided into two groups, each consisting of 3 animals, of which group-I was kept as control giving only water. Group-II was given the test material at a dose of 500 mg/kg body weight white Diclofenac sodium was used at a dose of 10 mg/kg body weight as the reference standard for comparison.

After half an hour the oral administration of the test materials, 1% (carrageenan was injected to the left hind paw of each animal. The volume of the paw edema was measured at 0, 1, 2, 3 & 4 hours using plethysmometer after administration of carrageenan. The right hind paw served as a reference non informed paw for comparison.

The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula.

$$\% \text{ inhibition of paw edema} = (V_c - V_t) / V_c * 100;$$

Where V_c and V_t represent average paw volume of control and treated animal respectively.

2.6 Statistical Analysis

The data are expressed as the mean + SEM analyzed by one-way analysis of variance (ANOVA) and t-test was used as the test of significance. P-value <0.05> was considered as the min-

imum level of significance. All statistical tests were carried out using SPSS statistical software.

3. RESULTS

3.1 Acute toxicity

Oral administration of graded doses (250 and 500 mg/kg body weight) of the methanol extract of *ocimum americanum* to mice and rats did not produce any significant changes in behavior, breathing, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality was recorded in any group after 24 hours of administrating the extract to the animals.

3.2 Analgesic activity:

The methanol extract of *Ocimum americanum* exhibited statistically significant (P<0.06) analgesic effect in hot plate test of Spargue Dowley mice. The results presented in Table -1 & Table -2 shows that the extract significantly increased the reaction time of mice in a dose-dependent manner. The maximum analgesic effect was observed at 3 hour post administration of the test material which was comparable to that of the standard drug ketorolac.

3.3 Anti-inflammatory activity:

Result of the anti-inflammatory activity experiment is shows in Table -1 and Table 2. The increase or decrease in paw volume indifferent hours of study with test material was compared to control for the evaluation of percent inhibition of paw edema.

In control animals, the sub plantar injection of carrageenan produced a local edema that increased progressively to reach a maximal intensity at fourth hour after the injection of the phlogisticagent. Methanol extract of *Ocimum americanum* showed a significant dose dependent reduction of paw edema at both the doses of 250 and 500 mg/kg body weight. Although the anti-inflammatory response of the extract was less than that of diclofenac sodium over a period of 4th hour in carrageenan-induced inflammation, the duration of action was found to be comparable to that of the standard drug.

4. DISCUSSION:

Hot plate method is one of the most common tests for evaluating the analgesic efficacy of drugs/compounds. The paws of mice and rats are very sensitive to heat at temperature which are not damaging to the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics (Ghosh, 1984). *Ocimun americanum* extract at the dose of 250 and 500 mg/kg. body weight showed the significant (P<0.05) increase in latency time as compared to control. Positive control ketorolac also showed significant (P<0.05) analgesic activity at the dost of 2.5 mg / kg body weight.

TABLE–1 : EFFECT OF OCIMUM AMERICANUM (L) METHANOL EXTRACTION ON LATENCY TO HOT PLATE TEST

Treatment Group	Post drug reaction time in seconds				
	0 hour	1 hour	2 hour	3 hour	4 hour
Control	9.11 ± 1.076	8.12 ± 0.94	7.52 ± 0.30	6.08 ± 0.38	5.70 ± 0.25
Standard (ketrolac 2.5mg / kg i.p.)	9.21 ± 0.51	13.88 ± 1.17	14.42 ± 0.72	15.20 ± 0.72	12.38 ± 1.04
250 mg / kg. Methanol extract (p.o)	9.04 ± 0.55	11.00 ± 0.57	12.12 ± 0.51	13.41 ± 0.54	11.00 ± 0.31
500 mg / kg. Methanol extract (p.o)	9.05 ± 0.48	13.40 ± 0.56	15.00 ± 0.48	16.02 ± 0.32	14.22 ± 1.18

Values are Mean ± SEM, n=5. One way analysis of variance (ANOVA) followed by t-test was performed as the test of significance.
The minimum value of P<0.05 was considered significant, as compared with control

TABLE–1 : ANTI-INFLAMMATORY EFFECT OF METHANOLIC OF OCIMUM AMERICANUM (L) ON CARRAGEENAN-INDUCED MICE PAW INFLAMMATION

Treatment Group	Volume of Paw edema (ml)					Inhibition of Paw edema (%)
	0 hour	1 hour	2 hour	3 hour	4 hour	
Control	2.44 ± 0.13	4.92 ± 0.2	5.46 ± 0.38	6.31 ± 0.54	6.95 ± 0.55	------
Diclofinac 10mg / kg.	2.65 ± 0.26	2.60 ± 0.18	2.55 ± 0.22	2.46 ± 0.33	2.30 ± 0.30	67.59
250 mg / kg Methanol extract	2.75 ± 0.19	4.06 ± 0.17	4.28 ± 0.30	4.30 ± 0.37	4.20 ± 0.39	29.85
500 mg / kg Methanol extract	2.81 ± 0.23	3.77 ± 0.15	4.09 ± 0.35	4.17 ± 0.41	3.85 ± 0.41	30.50

Values are Mean ± SEM, n=5. One way analysis of variance (ANOVA) followed by t-test was performed as the test of significance.
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Carrageenan – induced edema involves the synthesis or release of mediators at the injured site correlated with early exudative stage of inflammation (Silva et al, 2005). These mediators include prostaglandins, especially the Eseries, histamine, bradykinins, leucotriences and serotonin all of which also cause pain and fever(Asongalem et al 2004).

The first occurs within an hour of carrageenan injection and in partly due to the trauma of injection and also to histamine and serotomin component. The second phage (over 1 hour) is mediated by prostaglandins, the cyclooxygenase products and the continuity between the two phases is provided by kinins (Perianayagam et al, 2004).

The presence of PGE_2 in the inflammatory exudates from the injected foot can be demonstrated at third hour and period there after. Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorates inflammation and other symptoms.

Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (Sawadogo et al, 2006). The result of our current study is an indication that *Ocimum americanum* can be effective in acute inflammatory disorders.

Since the methanol extract exhibited significant inhibition of edema volume at fourth hour after administration of carrageenan in comparison to control, the possible mechanism of anti-inflammatory activity of the extract may be its ability to inhibit the biosynthesis and or release of prostaglandin-like substances.

However the inhibitory effect on the release of histamine or serotonin like substances cannot be ruled out, because the extract showed significant inhibition of mice paw edema during first hour of carrageenan administration as well. Thus the extract may possess chemical constituents that may cause inhibition of the enzyme "cyclooxygenase".

4 Conclusion

This study revealed the analgesic and anti-inflammatory activity of methanol extract of *Ocimum americanum* in a dose-dependent manner. Further investigations are required to isolate the active component of the extract and to confirm the mechanism of action in the development of potent analgesic and anti-inflammatory compound.

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