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ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF *OCIMUM SANCTUM* (LINN) IN LABORATORY ANIMALS

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ABSTRACT

Ocimum sanctum (L.) is popularly used as herbal remedy for various ailments. But the scientific basis 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 sanctum* in laboratory animals. The ethanol extract of the leaves of *O. sanctum* was used to investigate the acute effect on analgesia by Hot-plate test in mice and on inflammation in rats using carrageenan-induced rat 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 volume by 43.33% at the dose of 500 mg/kg body weight at the fourth hour of study. This study suggests that the ethanol extract of *Ocimum sanctum* have both analgesic and anti-inflammatory activity in a dose dependent manner which supported its use as an analgesic and anti-inflammatory drug in folk medicine. This plant may be a useful source of lead components in the treatment of pain and inflammation.

INTRODUCTION: The development of traditional medicinal systems incorporating plants as means of therapy can be traced back to the Middle Palaeolithic age some 60,000 years ago as found from fossil studies [1]. Medicinal plants were very commonly available in abundance especially in the tropics.

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 [2].

Ocimum sanctum Linn. (Family: Labiatae), locally known as 'Tulsi' in Hindi and 'Holy Basil' in English, is an erect softy hairy aromatic herb or under shrub found throughout Bangladesh. It has high traditional medicinal value as it is one of the important constituents of Ayurveda, Homeopathy and Siddha systems of medicine.

It is commonly used for curing skin diseases, hepatic disorders, cold, cough, malarial fever and so on. Several recent investigations using the extracts of *O. sanctum* have indicated that they possess significant anti-stress [3] and anti-carcinogenic properties [4].

Aqueous decoction of whole plant lowers the blood glucose level [5, 6]. The leaves of Tulsi plant have also been shown to possess good anti-stress (adaptogenic), anti-hyperlipidemic and antioxidant potentials in experimental animals [7]. Leaves and seeds of Tulsi plant have been reported to reduce blood and urinary uric acid level in albino rabbits and possess diuretic property [8]. *Ocimum sanctum* also possesses antifungal activity against *Asperigillus niger* and aqueous extract of the plant is found effective in patients suffering from viral encephalitis [9]. This plant contains many aromatic and volatile oils and the main constituent is Eugenol. The active principles of *O. sanctum* comprising of phenols and flavonoids have been shown to have significant anti-inflammatory activity both *in vivo* and *in vitro* [10].

Although numerous studies have shown the medicinal values of *O. sanctum*, there still remains ample scope for further in depth research. Accordingly, we disclose herein the analgesic and anti-inflammatory effects of the leaves of *O. sanctum* to further establish the scientific basis of the traditional uses of the plant.

MATERIALS AND METHODS:

Collection and identification of plants: The leaves of *Ocimum sanctum* were collected from the Mirpur National Botanical Garden, Dhaka, Bangladesh, on 12th September 2008 when the plant is fully flowered. The plant was identified by the experts of Bangladesh National Herbarium (BNH). The specimen was preserved in BNH and Department of Pharmacy, North South University, Bangladesh.

Preparation of plant materials: The collected plant leaves were washed with water and separated from undesirable materials or plants or plant parts. They were aerated by fan aeration to be partially dried and were next heated in an oven at bellow 40ºC for two days to be fully dried. The fully dried leaves are then grinded to make them powder by the help of a suitable grinder. Then the powders were dissolved in ethanol (80%) and kept for a period of 2 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman filter paper. The filtrate (ethanol extract) obtained was evaporated by rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68ºC temperature. It rendered a gummy concentrate of chocolate black colour that was designated as crude extract or ethanolic extract. The crude ethanolic extract was finally dried by freeze drier and preserved.

Animals: Young Swiss-Albino mice aged about 4-5 weeks with average weight of 25-30 gm and Adult Albino rats (Wistar strain) having average weight of 180-200 gm were used for this study. They were kept in standard environmental condition for one week in the animal house of the Department of Pharmacy, North south University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water ad libitum and maintained at natural day night cycle.

Analgesic activity by Hot-Plate Test in mice: The hot-plate test (Hot/Cold Plate Model-35100-001, UGO Basile, Italy) was employed for measurement of analgesic activity as previously described by Lanhers et al. and modified by Mahomed and Ojewole [11, 12]. The temperature was regulated at 55° ± 1°C. Mice were divided into four groups consisting of ten 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 stimulus. 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.

The time for each mouse to lick its paws or jump out of the beaker was taken as reaction time (in second). Before treatment, the reaction time was taken once. The mean of this determination constituted initial reaction time before treatment of each group of mice. Each of the test mice was thereafter treated with either distilled water (DW), Ketorolac (2.5 mg/kg of body weight) or ethanol extract of *O. sanctum* at the doses of 250 and 500 mg/kg body weight orally. Thirty minutes after treatment, the reaction time of each group mice were again evaluated five times individually in one hour interval on this occasion. Percent analgesic score was calculated as,

$$PAS = T_b\text{-}T_a/T_b \times 100$$

Where, T_b= Reaction time (in second) before drug administration; T_a = Reaction time (in seconds) after drug administration.

Anti-inflammatory activity by carrageenan- induced rat paw edema method: The anti-inflammatory activity of the ethanol extract was investigated on carrageenan induced inflammation in rat paw following the method of Winter et al with minor modifications [13]. Rats were randomly divided into two groups, each consisting of six 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 while Diclofenac sodium was used at a dose of 10 mg/kg body weight as the reference standard for comparison.

Half an hour after the oral administration of the test materials, 1% carrageenan was injected to the left hind paw of each animal. The volume of paw edema was measured at 0, 1, 2, 3 and 4 hours using Plethysmometer (Model 7141, UGO Basile, Italy) after administration of carrageenan. The right hind paw served as a reference non-inflamed 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-

$$\% \text{ Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

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

Statistical analysis: The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Dennett's *t*-test was used as the test of significance. P-value <0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software.

RESULTS:

Acute toxicity: Oral administration of graded doses (250 and 500 mg/kg body weight) of the ethanol extract of *O. sanctum* to rats and mice did not produce any significant changes in behaviour, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality was recorded in any group after 24 hours of administering the extract to the animals.

Analgesic activity: The ethanol extract of *O. sanctum* exhibited statistically significant ($p < 0.05$) analgesic effect in hot plate test of white albino mice. The results presented in **Table 1** and **Figure 1** 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.

TABLE 1: EFFECT OF *OCIMUM SANCTUM* (L) ETHANOL EXTRACT ON LATENCY TO HOT PLATE TEST

Treatment group	Post drug reaction time in sec				
	0 h	1 h	2 h	3 h	4 h
Control	8.24 ± 1.066	7.12 ± 0.92	6.52 ± 0.20	5.08 ± 0.30	4.72 ± 0.28
Standard (Ketorolac 2.5 mg / kg i.p.)	8.56 ± 0.46	13.98 ± 1.15 *	14.48 ± 0.76 *	15.30 ± 0.84 *	12.42 ± 1.05 *
250 mg/kg ethanol extract (p.o.)	8.14 ± 0.64	11.07 ± 0.67 *	12.21 ± 0.57 *	13.52 ± 0.58 *	11.05 ± 0.33 *
500 mg/kg ethanol extract (p.o.)	8.17 ± 0.42	13.57 ± 0.66 *	15.04 ± 0.54 *	16.08 ± 0.44 *	14.28 ± 1.28 *

All values are Mean ± SEM, n = 10. One way Analysis of Variance (ANOVA) followed by Dennett's test was performed as the test of significance. The minimum value of $p < 0.05$ was considered significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ as compared with control group

Anti-inflammatory activity: Result of the anti-inflammatory activity experiment is shown in **Table 2** and **Figure 2**. The increase or decrease in paw volume in different 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 phlogistic agent. Ethanol extract of *O. sanctum* showed a significant dose dependent reduction of paw edema at both the doses of 250 and 500 mg/kg body weight.

However, maximum (43.33%) and statistically significant ($p<0.05$) inhibition of paw volume was found to be at four hour of study at a dose of 500 mg/kg body weight (Table-3). Although the anti-inflammatory response of the extract was less than that of diclofenac sodium over a period of 4 hour in carrageenan-induced inflammation, the duration of action was found to be comparable to that of the standard drug.

TABLE 2: ANTI-INFLAMMATORY EFFECT OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM (*L) ON CARRAGENAN- INDUCED RAT PAW INFLAMMATION

Treatment group	Volume of paw oedema (ml)					Inhibition of paw oedema (%)
	0 h	1h	2h	3h	4h	
Control	2.45 ± 0.12	4.82 ± 0.3	5.43 ± 0.39	6.30 ± 0.56	6.97 ± 0.55	__
Standard Diclofenac 10 mg /kg	2.62 ± 0.25	2.59 ± 0.17*	2.53 ± 0.21*	2.45 ± 0.31*	2.33 ± 0.32*	66.57
250 mg/kg ethanol extract	2.71± 0.20	4.03 ± 0.19	4.25 ± 0.29*	4.28 ± 0.34 *	4.07 ± 0.37*	27.95
500 mg/kg ethanol extract	2.78 ± 0.22	3.88 ± 0.17*	4.11 ± 0.34*	4.15 ± 0.40*	3.95± 0.38*	30.32

Values are Mean ± SEM, n = 10. One way Analysis of Variance (ANOVA) followed by Dennett's test was performed as the test of significance. The minimum value of $p < 0.05$ was considered significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ as compared with control group

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 temperatures 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 [14]. *O. sanctum* 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 dose of 2.5 mg/kg body weight.

Carrageenan-induced edema involves the synthesis or release of mediators at the injured site correlated with early exudative stage of inflammation [15]. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin all of which also cause pain and fever [16]. The time course of edema development in carrageenan induced paw edema model in rats is generally represented by a biphasic curve.

The first phase occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also to histamine and serotonin component. The second phase (over 1h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins [17].

The presence of PGE_2 in the inflammatory exudates from the injected foot can be demonstrated at third hour and period thereafter. Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorates the 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 [18], the result of our current study is an indication that *O. sanctum* can be effective in acute inflammatory disorders.

Since the ethanol 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 can not be ruled out, because the extract showed significant inhibition of rat 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'.

CONCLUSION: This study revealed the analgesic and anti-inflammatory activity of ethanol extract of *Ocimum sanctum* 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 a potent analgesic and anti-inflammatory compound.

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