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Evaluation of pharmacological activities of traditional herbal drug *Ocimum* sanctum in rats

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ABSTRACT

Ocimum sanctum is a traditional herbal plant possessing numerous pharmacological and therapeutic properties. The present study was carried out to evaluate antipyretic, analgesic and anti-ulcerogenic properties of Ocimum sanctum (500 and 1000mg kg⁻¹b.wt⁻¹) in model system of rats. Drug Indomethacin (10mg kg⁻¹b.wt⁻¹) was used as a reference drug. Animals were randomly allocated in four groups consisting of six animals each. Group I was control group which received normal saline 0.09%; Group II received O.sanctum (500mg kg⁻¹b.wt⁻¹) and Group III was administered with 1000mg kg⁻¹b.wt⁻¹ O.sanctum; Group IV received Indomethacin (10mg kg⁻¹b.wt⁻¹). Hot plate reaction test was used for determining the analgesic activity, acetic acid test was used to evaluate the writhing response, yeast induced pyrexia was used for determining the antipyretic activity test in control and experimental rats. Statistical analysis was performed using ANOVA to determine significant differences between groups followed by student's Newman-keul's test *p<0.05 implied significance. Rats were fasted to induce ulcer and then effect of O.sanctum was evaluated. Ocimum sanctum at both the doses was found to possess analgesic, antipyretic, anti- ulcerogenic activities experimental rats and O.sanctum at a dose of 1000mg kg⁻¹b.wt⁻¹ was found to be significantly effective than the other dose.

Keywords: Ocimum sanctum; antipyretic; analgesic; ulcer; indomethacin

INTRODUCTION

Ocimum sanctum (Tulsi), which belongs to the family Lamiaceae, is a principal medicinal and most sacred herb of India (Samson et al, 2007). Ocimum sanctum is 30-100 cm tall and is found in ample in semitropical and tropical parts of India. It has been in use in India for over 5000 years for rejuvenating body and mind.

Ocimum sanctum has many reported pharmacological effects like ulcer ameliorative, antioxidant, anticarcinogenic, antihelmintic, anti-septic, anti-rheumatic and anti-stress (Dharmani et al, 2004; Samson et al, 2007; Godhwani et al, 1987, 1988; Bhargava and Singh, 1981; Singh and Majumdar, 1999). However, the antipyretic and analgesic activities of this plant has not been reported up to best of our knowledge. The present study was carried out to evaluate analgesic, antipyretic, anti-ulcerogenic activity of the extract of Ocimum sanctum using the rats as model.

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MATERIALS AND METHODS

Test Drug

In this study, commercially available *Ocimum sanctum* and Indomethacin procured from Himalaya products Ltd., Bangalore, India was used. The other laboratory reagents were of analytical grade.

Animals

Rats of either sex, weighing 200-220gm, were used in this study. The animals were well treated and cared for in accordance of the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Culture, Govt. of India, Chennai, India. Animals were fed with commercially available pelleted feed and water.

Analgesic Activity

Hot plate reaction test

Hot plate test was done according to the method of Williamson et al (1996). Individually animals were kept in a beaker and placed on a temperature-controlled hot plate which was maintained at 50° C and it is speculated as pain threshold when animals lift and lick their paws or try to leap out of the beaker. The reaction of the rat response to the heat was noted using stopwatch.

The animals used in the experiment were first tested for paw licks or jump response and those which reacted after 4 sec were used. Animals were tested after 30 minutes of administration of Indomethacin (10mg kg⁻¹b.wt⁻¹) and *Ocimum sanctum* (500 and 1000mg kg⁻¹b.wt⁻¹), which were suspended in 0.09% saline solution prior of use. Control animal were given equal volume of normal saline and the experiment was repeated. The difference between the control and drug treated animals among different groups were compared for statistical significance.

Acetic acid test

Acetic acid is used to instigate writhing response in rat. This test was done using the method by Witkin et al (1961) and intra peritoneal injection of 0.6% solution of acetic acid was administered in rats by muscular contraction. Animals were kept in glass cages and number of stretching per animals was noted for next 30 minutes. *Ocimum sanctum* (500 /1000 mg kg⁻¹b.wt⁻¹) and Indomethacin (10mg kg⁻¹b.wt⁻¹) were suspended in 0.09% saline solution and were administrated 30 minutes before acetic acid injection.

Antipyretic Test

The rats were fasted overnight with water ad libitum before the experiments. This test was performed in rats by administering subcutaneously 20% aqueous suspension of baker's yeast to induce pyrexia and after 18 hours the rectal temperature of the animals were noted. Animals were given orally *Ocimum sanctum* (500 and 1000 mg kg⁻¹b.wt⁻¹) and Indomethacin (10mg kg-1b.wt⁻¹) and rectal temperature was noted at the interval of 1 hour (Mukerjee et al, 1996).

Ulcerogenic Test

The animals were kept fasting for 16 hours and then *Ocimum sanctum* (500 and 1000 mg kg⁻¹b.wt⁻¹) and Indomethacin (10mg kg-1b.wt⁻¹) orally. The animals were sacrificed after 6 hours of the last dose and the stomach was removed, opened along the great curvature and the severity of the ulcer index was measured using the arbitrary scale .0: no lesions, 0.5: hyperaemia, 1: one or two lesions, 2: severe lesions, 4: mucosa full of lesion (Cashin et al, 1997).

STATISTICAL ANALYSIS

Results were expressed as mean ±SD and statistical analysis was performed using ANOVA to determine significant differences between groups followed by student's Newman-keul's test *p<0.05 implied significance.

RESULTS

Pharmacological activities like analgesic, antipyretic, antiulcer activity of *Ocimum sanctum* were determined in rats.

Analgesic activity

After the treatment of animals with *Ocimum sanctum* (500 and 1000 mg kg⁻¹b.wt⁻¹) there was a significant inhibition in the abdominal writhes. Similar results were noted in case of treatment with Indomethacin (10 mg kg⁻¹b.wt⁻¹)(Fig 1).In hot plate method, the rats treated with *Ocimum sanctum* (500 and 1000 mg kg⁻¹b.wt⁻¹) withstood on hot plate reactions for longer period compared to the reference drug Indomethacin (10mg kg⁻¹b.wt⁻¹) (Fig. 2).

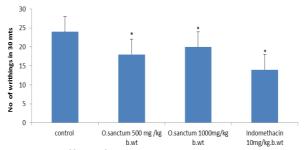


Figure 1: Effect of Ocimum sanctum and Indomethacin on acetic acid induced writhing response in rat.

Results are compared with control groups. Values are expressed as mean \pm S.D. (n=6). Symbols represent statistical significance at * p<0.05.

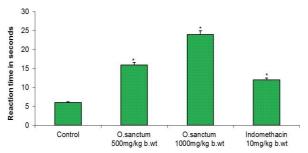


Figure 2: Effect of Ocimum sanctum and Indomethacin on hot plate reaction time in rats.

Results are compared with control groups. Values are expressed as mean \pm S.D. (n=6). Symbols represent statistical significance at * p<0.05.

Antipyretic activity

The aqueous suspension of *Ocimum sanctum* (500 and 1000 mg kg⁻¹b.wt⁻¹) was administered in rats and as compared to standard drug indomethacin (10 mg kg⁻¹b.wt⁻¹), *Ocimum sanctum* shows significant reduction in reaction in rectal temperature (Fig 3).

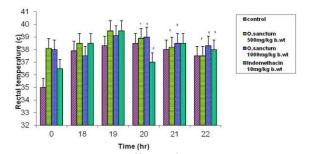


Figure 3: Antipyretic effects of *Ocimum sanctum* and Indomethacin

Results are compared with control groups. Values are expressed as mean \pm S.D. (n=6). Symbols represent statistical significance at * p<0.05

Antiulcer activity

After administration of *Ocimum sanctum* (500/1000 mg kg⁻¹b.wt⁻¹) in rats, there was significant reduction in the ulcer index as seen in Fig.4.

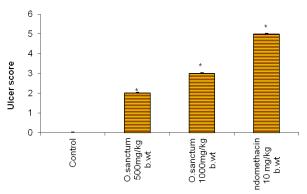


Figure 4: Ulcerogenic effects of Ocimum sanctum and Indomethacin in rats.

Results are compared with control groups. Values are expressed as mean \pm S.D. (n=6). Symbols represent statistical significance at * p<0.05

DISCUSSION

In the present scenario, some diseases like arthritis are treated with a number of anti-inflammatory drugs available in modern medicine. These drugs are always associated with various pyretic activities resulting in gastric damage. Effects of Ocimum sanctum in analgesic activity were evaluated by acetic acid test and hotplate method. The acetic acid writhing test is known as a non-selective antinociceptive model. After intraperitoneal injection of acetic acid in rats, a painful response and acute inflammation develop in the peritoneal area due to which nerve endings are excited (Gyires and Torna, 1984) and an elevation of prostaglandin levels takes place in peritoneal fluid (Daud and Habib, 2006). Thus the analgesic effect of Ocimum sanctum may be due to prevention of the local level of prostaglandins. However, the interpretation of this writhing test alone does not confirm that this effect is related with central analgesic substances.

The hot plate test is extensively applied method in the analgesic investigations for several decades. This test along with the writhing test, usually differentiates between central and peripheral effects (Srinivasan et al, 2003). A significant analgesic action was shown by *Ocimum sanctum* (500 and 1000 mg kg⁻¹b.wt⁻¹) in hot plate method after 30 minutes administration. The results showed significant analgesic effect in acetic acid writhing response and hot plate reaction test by *Ocimum sanctum*. This confirms *Ocimum sanctum* has been acting through both peripheral and central mechanism in both models (Fig 2).

Antipyretic activity is usually implicated as an aspect of drugs or compounds that have a restraining effect on prostaglandin-formation (Panthong et al, 2007; Vane, 1987). The anti-pyretic effort is investigated by yeast inducing pyrexia test in rat .Subcutaneous injection of yeast induces pyrexia by causing an increment in production of prostaglandin that sequentially elevates the body temperature since the region in the hypothalamus controls body temperature is stimulated by pyretic activity (Ghamdi, 2001; Zacaria et al, 2008; Shukla et al , 2010). After 15-18 hours of yeast injection and the administration of antipyretic drugs is a method followed by many researchers. Results obtained using Ocimum sanctum showed a significant(p<0.05) reduction in rectal pyrexia, similar to standard drug Indomethacin (fig3).

Production of gastric lesions and thus ulcers is a common side effect associated with nonsteroidal anti inflammatory compounds (Pegalla et al, 1983). For Indomethacin, already it has been seen to have ulcerogenic action in an empty stomach (Rasool et al, 2008). In this study it was found that *Ocimum sanctum* possesses significant (p<0.05) anti-ulcerogenic activity but gastric lesions were seen in Indomethacin treated rat.

CONCLUSION

Numerous analgesic compounds are available in market having several side effects so there is a need to evaluate the therapeutic potential of natural compounds. The result of the study shows *Ocimum sanctum* has antipyretic, analgesic and anti ulcerogenic properties, however further studies are required to study the mechanism of *Ocimum sanctum* to confirm these activities. Hence, our research contributes towards traditional use of *Ocimum sanctum* with scientific support.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest between the authors

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