

## An Experimental Study of Analgesic Effect of Medicinal Plant *Tulsi (Ocimum sanctum)*

Reema Rathore\* and Shashi Jain\*\*

\*Department of Food & Nutrition, College of Home Science, SKRAU, Bikaner, Rajasthan, India

\*\*Department of Food & Nutrition, College of Home Science, MPAUT, Udaipur, Rajasthan, India

**KEYWORDS** Analgesic Effect. Intraperitoneally. Doses. Concentration

**ABSTRACT** An experimental study was conducted to evaluate the analgesic effect of *Tulsi*. After pre-preparation in one part of plant material (50 gm) eight parts water (400 ml) was added and boiled till it reduced to 100 ml. Three doses mild (0.3 ml), moderate (0.6 ml) and maximum (0.9 ml) were prepared. Zero dose was used for control group as placebo, which was of distilled water. A total of 24 adult albino rats were used for study. Four groups were made for different doses of the plant. Each of the group was containing six rats. The dose was injected intraperitoneally to rats. The 'rat tail method' was used to find out the analgesic effect. *Tulsi* showed an increase of 20.34 per cent from mild dose, 43.80 per cent from moderate dose and of 51.47 per cent from maximum dose. The effect would remain up to 3 hours with all the three doses. The analgesic activity showed by *Tulsi* was statistically significant with all the three doses [ $P < 0.01$ ]. The higher concentration of dose showed better efficiency. The results depict that *Tulsi* had a long lasting analgesic effect so can be effective in chronic pains.

### INTRODUCTION

In the Indian system of medicine, plants occupy a predominant place in the therapeutic field. Easy procurability together with effectiveness and safety are the prime reasons for the choice of plants in the field of treatment. *Ocimum sanctum* natively known as *tulsi* is mentioned in the Charaka Sanhita, an ancient Ayurvedic text by Charaka for its medicinal importance. Its extracts are used in ayurvedic remedies for common colds, headaches, stomach disorders, heart disease, inflammatory and allergic disorders (Kalabharti et al. 2011). Several medicinal properties have been attributed to this plant in ancient Indian and other systems of medicine like Ayurveda, Siddha, Greek, Roman and Unani. The leaves have been used as expectorant, diaphoretic, antiemetic, anti-rheumatic, anticancer, anthelmintic, antiseptic, antipyretic and in relieving various gastric disorders. The importance of *Tulsi* as a remedy described out by Kirtikar and Basu (1935) that *Tulsi (Ocimum sanctum)* is an erect herbaceous plant found throughout India. The plant is held sacred and is grown in temples. About chemical composition, the leaves on steam distillation yield a bright yellow oil possessing a pleasant colour. It has therapeutical uses as the juice from the leaves possess diaphoretic and expectorant properties. Along with this, Krishnamurthy (1959) reported that *tulsi* leaves possess

anabolic, hypotensive, cardiac, depressant, smooth muscle relaxant, hypoglycemic, sedative, antispermatic and antifertility actions etc. In the absence of scientific proof of their efficacy and official standards, the reliability of such preparations at the best can be rated as empirical. The effect of some indigenous drugs on pain models in mice was studied by Khanna and Sen (1995). This study was conducted to find out the effect of *Tulsi* (OS) (*Ocimum Sanctum*), and *Al neem* (*Azadirachta Indica*) on tail flick latencies and GAA induced writhing responses in mice. These drugs produced differential degrees of analgesia in both tests of nociception. The results suggest the differential degrees of both opioid and non-opioid mechanisms are involved in the analgesic effect of these indigenous drugs. Hence the present study was undertaken on animals to find out the analgesic effect of medicinal plant *Tulsi* with the following objectives :

1. To evaluate the analgesic effect of *Tulsi* plant.
2. To find out the effective dose of *Tulsi* to relieve the pain threshold.

### METHODOLOGY

The study was performed in Udaipur, Rajasthan. The experiment was conducted at R.N.T. Medical College, Udaipur. Facilities available at Home Science College and Medi-

cal College were utilised to conduct the experiment. A total of 24 healthy albino rats were selected for the study on the basis of their age and weight. The albino rats were procured from Choudhry Charan Singh, Haryana Agriculture University, Hissar. The rats aged more than 9 week were taken for the purpose. The weights of these animals ranged between 100 to 200 g. The experimental group consisted of rats of both sexes equally. All the animals were physically examined for not having any disease or injury before experiment. The plants of *Tulsi* were collected from Department of Horticulture, R.C.A., Udaipur. Fresh leaves of *Tulsi* were taken and cleaned. These leaves were then crushed (ground) in a mortar to prepare a paste. The animals were housed in galvanised iron, screen bottom suspended cages. For first 6 days, all the 24 animals were fed on standard laboratory diet and acclimatized to the conditions of the animal house. Rats were divided into 4 groups consisted of six rats in each of the group.

#### (i) Preparation of Doses

The extract from *Tulsi* was prepared using a standardised technique suggested by Khandal (1992). For the preparation of doses in one part of plant material, eight parts water was added and boiled till it reduced to one-fourth of its content. The boiled mixture was then strained through a linen cloth to get the extract which was cooled at room temperature. From this extract three doses, that is, mild, moderate and maximum were prepared. The details of doses prepared are as follows:

The test dose was given to rats intraperitoneally in form of aqueous suspension through tuberculine syringe (Table 1).

**Table 1: Dose description**

Dose (O):	Control group 0.9 ml of distilled water as placebo.
Dose (I):	Mild dose 0.3 ml extract ± 0.6 ml distilled water
Dose (II):	Moderate dose 0.6 ml extract ± 0.3 ml distilled water
Dose (III):	Maximum dose 0.9 ml extract ± 0.0 ml distilled water.

#### (ii) Experiment

Artificial pain was induced using 'rat tail method' (Davies et al. 1946). Analgesia is re-

flected in a prolongation of the reaction time, the increase over normal in the mean reaction time of the treated animals was taken as a measure of analgesic effect. The reaction time before and after the drug treatment was noted up to two hours with an interval of 15 minute up to 1 hour and 30 minutes in next one hour.

Statistical analysis was carried out for the significance of the test.

## RESULTS AND DISCUSSION

### Dose Prepration

Fifty gram of fresh leaves of *Tulsi* were taken. These leaves were crushed in a mortar to prepare a paste. Then 400 ml water was added into it and boiled till it reduced to 100 ml. After straining 70 ml of extract was obtained. Three doses were prepared from this extract. The 0 dose and dose I, II and III were given to each groups of rats.

### The Effective Dose of *Tulsi* to Relieve the Pain Threshold

Dose 0 showed the mean reaction time varying from 6.16 sec. to 6.38 sec. which was rat's own response to pain (Table 2).

The mild dose with 0.3 ml extract showed the mean reaction time at its peak, that is, 6.98 sec. showing an increase of 20.34 per cent from the initial value. The multiple regression equation revealed effect of dose upto 150 min. (2 hours and 30 minutes).

The time noted before injecting the moderate dose (0.6 ml) was 3.95 sec, which increased to 6.98 sec. at its peak at 90 min. At two hours also there was an increase of 43.80 per cent from its initial value. This indicates a long lasting effect of *Tulsi* in relieving pain. Ghosh (1984) suggests the time until these responses occur is prolonged after administration of centrally acting analgesics. The multiple regression analysis showed that this dose would be effective at three hours also. The time noted before injecting the 0.9 ml of drug was 5.46 sec. which increased to 8.27 sec. at 90 min. There was highest increase of 51.47 per cent from initial value at 2 hours (Tables 2 and 3).

The analgesic activity showed by *Tulsi* was statistically significant with all the three doses (P £ 0.01) (Table 3). The multiple regression

**Table 2: Mean  $\pm$  S.E. values of tail withdrawal reaction time (sec.) observed at various time-intervals using *Tulsi* [*Ocimum sanctum*]**

Dose	Observation time (minutes)						
	Before injecting the drugs	15	30	45	60	90	120
Zero	6.23 $\pm 0.56$	6.35 $\pm 0.57$	6.16 $\pm 0.47$	6.40 $\pm 0.61$	6.36 $\pm 0.67$	6.38 $\pm 0.47$	6.18 $\pm 0.63$
Mild	5.80 $\pm 0.60$	6.16 $\pm 0.61$	6.48 $\pm 0.61$	6.66 $\pm 0.63$	6.85 $\pm 0.62$	6.98 $\pm 0.66$	6.56 $\pm 0.51$
Moderate	3.95 $\pm 0.29$	4.30 $\pm 0.25$	4.88 $\pm 0.38$	5.60 $\pm 0.38$	6.16 $\pm 0.38$	6.46 $\pm 0.38$	5.68 $\pm 0.32$
Maximum	5.46 $\pm 0.75$	5.86 $\pm 0.74$	6.32 $\pm 0.74$	7.17 $\pm 0.65$	7.96 $\pm 0.59$	8.27 $\pm 0.58$	8.03 $\pm 0.61$
Level of significance		**	**	**	**	**	**

\*\* -  $p \leq 0.01$

equation indicated the effect of 0.9 ml of dose upto 3 hours. A similar study by Hannan et al. (2011) showed that extract of leaves of *ocimum sanctum* 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. The correlation coefficient values of 3 doses were 0.99, 0.97 and 0.97 respectively indicating that the line is best fit to find out the further effect of selected doses. These results revealed that *Tulsi* showed analgesic effect although to a lesser extent, but for a longer period of time. The higher concentration of dose showed better efficiency. The hot plate test with similar results employed for measurement of analgesic activity as previously described by Fleurentin et al. (1992) and modified by Mahomed and Ojewole (2004). Similar results on analgesic effect of *Tulsi* are reported (Chopra 1958; Nadkarni 1976; Godhwani 1983) described as this may be due to the presence of sterols and amino acid resembling creatine and isoleucine which were analysed phytochemically

and identified chromatographically. These are salicylates, pyrazolones, para aminophenols, indoles, fenamates, propionic acid derivatives and many other new analgesic antipyretic and anti inflammatory agents. So the leaves are believed to possess ailment relieving properties. A study conducted by Singh et al. (1996) also highlights the constituents of *Tulsi* which aids in pharmacological properties of *Tulsi* which confirms results of present study.

#### Comparison of Analgesic Effect of Different Doses

The maximum dose of 0.9 ml showed significantly better effect than mild and moderate doses in relieving pain. *Tulsi* showed its peak effect at 90 min. and retained the analgesic activity up to 3 hours.

#### CONCLUSION

The study was conducted to evaluate the analgesic effect of *Tulsi* on the albino rats in the laboratory. The plants were cleaned for any im-

**Table 3: Percentage increase in mean reaction time from initial value\* of *Tulsi* [*Ocimum sanctum*]**

Dose	Observation time (minutes)					
	15	30	45	60	90	120
Mild	6.12% (0.36)	11.72% (0.68)	14.83% (0.86)	18.10% (1.05)	20.34% (1.18)	13.10% (0.76)
Moderate	8.86% (0.35)	23.54% (0.90)	41.77% (1.65)	55.95% (2.21)	63.54% (2.51)	43.80% (1.90)
Maximum	7.33 (0.40)	15.75 (0.85)	31.31 (1.70)	45.79 (2.50)	51.47 (2.80)	47.07 (2.56)

Figure in parenthesis is increase in reaction time in seconds.

\* - Initial value is the observation before injecting the drug.

purities. Fresh leaves of Tulsi were ground. Further in one part of plant material (50 g), eight parts water (400 ml) was added and boiled till it reduced to one-fourth of its content, that is, 100 ml. The boiled content was strained with muslin cloth and cooled. The extract obtained from *Tulsi* was 70 ml. This extract was used in three concentration to find out their analgesic effect. The details of dose concentration include 0 dose - 0.9 ml distilled water, mild dose - 0.3 ml extract  $\pm$  0.6 ml distilled water moderate dose - 0.6 ml extract  $\pm$  0.3 ml distilled water and maximum dose - 0.9 ml extract. A total of 24 healthy albino rats were taken for the study. Animals were physically examined for not having any disease or injury prior to experiment. There were 6 rats in each of the group. The first group served as placebo and given 0 dose of 0.9 ml distilled water. Three doses, that is, mild moderate and maximum were given to three groups of rats respectively. The dose was injected intraperitoneally. The pain was induced using 'rat tail method'. The increase over normal in the mean reaction time of the treated animals was taken as a measure of analgesic effect. The reaction time before and after the drug treatment was noted up to two hours with an interval was 15 min upto one hour, and with half an hour in next one hour. *Tulsi* showed an increase of 20.34 per cent with mild dose, 43.80 per cent with moderate dose and of 51.47 per cent with maximum dose at 90 min. after injection. The regression line indicated that the analgesic effect remain upto 3 hours irrespective of dose concentration. Analysis of variance revealed that the analgesic activity of Tulsi was statistically significant with all the three doses [ $p \leq 0.01$ ].

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