



## EVALUATION OF ANXIOLYTIC ACTIVITY OF ETHANOLIC EXTRACT OF OCIMUM SANCTUM LEAVES IN ALBINO MICE

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**Abstract :** OBJECTIVES To evaluate the anxiolytic property of ethanolic extract of Ocimum sanctum leaves in albino mice. MATERIALS AND METHODS Thirty six inbred male albino mice weighing 20-25 grams were selected from central animal house. For elevated plus maze test 18 animals were divided into three groups and for open field test 18 animals were divided in to three groups. Each group consist of six animals. The control group received vehicle propylene glycol (2ml/kg) orally, standard group received Diazepam (1mg/kg, ip) 15 minutes before experimentation and test group received extract of Ocimum sanctum leaves (200mg/kg) orally once daily for seven days. Anxiolytic activity assessed by using elevated plus maze and open field test. The results were tabulated and analysed with suitable statistical method. RESULTS Ocimum sanctum leaves showed statistically significant anxiolytic activity at the dose of 200mg/kg after seven days of daily administration (P 0.0001). The results were comparable to that produced by standard drug diazepam. CONCLUSION Ocimum sanctum leaves have anxiolytic activity which is comparable to diazepam. Further studies are essential to prove the anti-anxiety activity of Ocimum sanctum in human.

**Keyword :** Anxiolytic, Ocimum sanctum, Diazepam

### INTRODUCTION

Anxiety is a normal emotional behaviour<sup>1</sup>. When it is severe or chronic, it becomes pathological and can precipitate or aggravate cardiovascular and psychiatric disorders<sup>2</sup>. Benzodiazepine are the major class of compounds used in anxiety and they have remained the most commonly prescribed drug for anxiety<sup>3,4</sup>, despite the important unwanted side effects that they produce such as sedation, muscle relaxation, ataxia, amnesia, ethanol and barbiturate potentiation and tolerance<sup>5</sup>. Therefore the development of new pharmacological agents from plant sources claimed to be free from side effects and less toxic than synthetic drugs are tried for treatment of anxiety. Ocimum sanctum Linn. belongs to the family Lamiaceae, commonly known as Tulsi<sup>6</sup>, the sacred plant of India and is also known by various names as Tulassi, Manjari, Krishna Tulsi, Trittavu, Thulsi. The plant is known in English as Holy Basil. The entire plant body including its leaves, stem, root and seed are proved to be of significant medicinal value and hence it is one among the inevitable plants used in the preparation of various ayurvedic pharmacological products. The plant possess diverse functions like anti- diabetic<sup>7</sup>, anti- fungal, anti- microbial<sup>8</sup>, anti- fertility,

anti- cancer<sup>9</sup>, cardioprotective, hepatoprotective, expectorant<sup>10</sup>, anti- spasmodic, adaptogenic<sup>11</sup>, anti- helminthic, antiseptic, anti-inflammatory and analgesic properties. The unique aromatic odour of Ocimum sanctum is due to the presence of essential or volatile oils<sup>12</sup>. The aromatic volatile oil mainly constitutes phenols, terpenes and aldehydes. Besides oil, the plant also contains alkaloids, glycosides, saponines and tannins. The volatile oils are mainly concentrated in the leaf.

### AIM AND OBJECTIVES:

The present study was aimed to evaluate the anxiolytic activity of ethanolic extract of Ocimum sanctum leaves. The anxiolytic activity was evaluated by elevated plus maze and open field test.

### MATERIALS AND METHODS:

#### Study centre:

This study was carried out in the Institute of Pharmacology, Madurai Medical College, Madurai, after getting clearance from the Institutional Animal Ethics Committee. Ref. No: Roc No 12677/E1/5/2012

#### PREPARATION OF EXTRACTS:

The leaves of Ocimum sanctum were collected from the local garden of Namakkal, Tamilnadu. The leaves were identified and authenticated by Professor of Botany, American college, Madurai. The leaves were shade dried and subjected to ethanolic extraction in the soxhlet apparatus. The extract was stored in a closed container and kept in a refrigerator at temperature below 4 °C.

#### STUDY DESIGN:

Thirty six inbred male albino mice were divided into six groups of six animals each. Three groups were used for elevated plus maze test and another three groups utilised for open field test. The control group received propylene glycol (2ml/kg) orally, standard group received Diazepam (1mg/kg, ip) 15 minutes before the experiment and test group received the extract of Ocimum sanctum 200mg/kg orally once daily for seven days.

#### METHODOLOGY:

##### ELEVATED PLUS MAZE TEST IN MICE:

The plus maze apparatus consisted of two open arms, measuring 16 x 5 cm and two closed arms, measuring 16 x 5 x 12 cm, connected to a central platform 5 x 5 cm. The maze was elevated to a height of 25 cm above the floor. Each mouse pre-treated with drug was placed individually at the centre of

elevated plus maze with its head facing toward an open arm and observed for 5 minutes to record the number of entries into open arm, closed arm and time spent in each arm. An arm entry was defined as all four feet in the arm. The apparatus was cleaned after each experiment<sup>13</sup>.

#### OPEN FIELD TEST IN MICE:

The floor was divided into squares with surrounding walls. Each mouse pre-treated with drug were placed individually at one corner of the open field for a period of 3 minutes to observe the behaviour patterns, number of squares crossed, period of immobility, number of rearing, faecal pellets and urination. All animals are tested individually, always being placed in the same position and cleaned after each use<sup>14</sup>.

#### STATISTICAL ANALYSIS:

The results were expressed as mean  $\pm$  SD. The data were analysed by one-way ANOVA (F) followed by post hoc comparisons using the Turkey's HSD test for multiple comparison.

#### RESULTS:

##### OPEN FIELD TEST

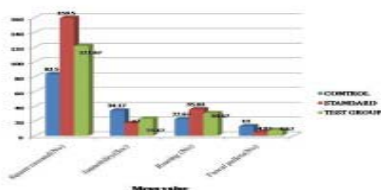
Table 1 – Effect of Ocimum sanctum leaf extract on open field behaviour test:

Treatment	Squares crossed (No)	Immobility (Sec)	Rearing (No)	Faecal pellets (No)
Control group (Mean $\pm$ SD) N=6	83.50 $\pm$ 3.450	34.17 $\pm$ 2.317	22.67 $\pm$ 2.160	13.00 $\pm$ 0.894
Standard Group (Mean $\pm$ SD) N=6	159.50 $\pm$ 2.345*	17.00 $\pm$ 1.549*	35.83 $\pm$ 1.329*	4.83 $\pm$ 0.983*
Test Group (Mean $\pm$ SD) N=6	121.67 $\pm$ 3.502*	23.17 $\pm$ 2.483*	30.67 $\pm$ 1.751*	8.17 $\pm$ 1.169*
ANOVA (F)	876.140	97.691	83.386	96.862

\*P < 0.0001

[Table 1] One-way ANOVA shows that there was a statistically significant ( $p < 0.0001$ ) effect in the number of squares crossed, period of immobility, numbers of rearing and numbers of faecal pellets by albino mice in the standard and test group compared to control group. Post hoc comparisons using the Turkey's HSD test indicated that there was a statistically significant ( $p < 0.0001$ ) increase in the numbers of square crossed by albino mice in the test group ( $M = 121.67$ ,  $SD = 3.502$ ), when compared to control group ( $M = 83.50$ ,  $SD = 3.450$ ) and decreased when compared to standard group ( $M = 159.50$ ,  $SD = 2.345$ ). There was a statistically significant ( $p < 0.0001$ ) reduction in the period of immobility in the test group when compared to control group and increased when compared to standard group. There was a statistically significant ( $p < 0.0001$ ) increase in rearing behaviour in the test group when compared to control group and decreased when compared to standard group. There was a statistically significant ( $p < 0.0001$ ) decrease in the number of faecal pellets in the test group when compared to control group and increased when compared to standard group. (Figure 1)

Figure 1 - Effect of Ocimum sanctum leaf extract on open field behaviour test



#### ELEVATED PLUS MAZE TEST:

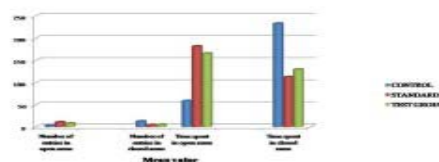
Table 2 – Effect of Ocimum sanctum leaf extract on Elevated plus maze

Treatment	Number of entries in open arms (No)	Number of entries in closed arms (No)	Time spent in open arms (Sec)	Time spent in closed arms (Sec)
Control group (Mean $\pm$ SD) N=6	3.33 $\pm$ 1.211	12.50 $\pm$ 1.049	58.50 $\pm$ 4.370	233.00 $\pm$ 3.688
Standard Group (Mean $\pm$ SD) N=6	10.50 $\pm$ 1.049*	4.50 $\pm$ 0.548*	181.50 $\pm$ 9.935*	112.67 $\pm$ 11.343*
Test Group (Mean $\pm$ SD) N=6	7.83 $\pm$ 0.753*	5.17 $\pm$ 0.753*	165.67 $\pm$ 12.612*	129.83 $\pm$ 9.475*
ANOVA (F)	75.372	180.339	291.090	328.636

\*P < 0.0001

[Table 2] One-way ANOVA shows that there was a statistically significant ( $p < 0.0001$ ) effect in the number of entries in the open arms and closed arms, time spent in open arms and closed arms by albino mice in the standard and test group when compared to control group. Post hoc Turkey's HSD test indicated that there was a statistically significant ( $p < 0.0001$ ) increase in number of entries in open arms by albino mice in test group ( $M = 7.83$ ,  $SD = 0.753$ ), when compared to control group ( $M = 3.33$ ,  $SD = 1.211$ ) and less number of entries when compared to standard group ( $M = 10.50$ ,  $SD = 1.049$ ). There was a statistically significant ( $p < 0.0001$ ) decrease in the number of entries in closed arms in test group when compared to control group and increased when compared to standard group. There was a statistically significant ( $p < 0.0001$ ) increase in time spent in open arms in test group when compared to control group and time spent is less when compared to standard group. There was a statistically significant ( $p < 0.0001$ ) reduction in time spent in closed arms in test group when compared to control group and time spent is more when compared to standard group. (Figure 2)

Figure 2 - Effect of Ocimum sanctum leaf extract on Elevated plus maze



#### DISCUSSION:

Anxiety occurs in the animals when placed on the elevated plus maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain in safer places. The reduction in entry, time spent, ratio of open arm to total arm entries and increased defecation are the indications of high level anxiety. Anti anxiety agents are expected to increase the motor activity, which is measured by increase in proportion of entries, time spent by the animal in the open arms<sup>15</sup>. In open field method placing an animal in an unknown environment to observe a number of behaviour changes like the tendency to stay on the periphery of the field without entering the centre, increased levels of defecation and urination are noticed. Higher levels of anxiety should mainly lead to decreases in the number of squares visited in centre. Treatment with anxiolytic agents increases the

number of square crossed, reduction in period of immobility, increase in rearing behaviour and decrease in number of faecal pellets. Benzodiazepines have been extensively used to treat several forms of anxiety, but due to their unwanted side effects, alternative treatment strategies with favourable side-effect profiles, credible benefits and moderate costs are being evaluated. Medicinal plants are a good source to find new remedies for these disorders. The test compound, *Ocimum sanctum* (200mg/kg) showed statistically significant ( $P < 0.0001$ ) anxiolytic activity in both elevated plus maze and open field test. All these behavioural changes in both paradigms are suggestive of decreased fear, decreased aversion and increased exploratory behaviour of the animal. These behavioural changes produced by the test compound *Ocimum sanctum* were comparable to those produced by Diazepam. The chemical constituents of plants containing flavonoids, saponins and tannins possess activity against many central nervous system disorders. The active principles of *Ocimum sanctum*, ursolic acid and eugenol have antistressor activity.

#### CONCLUSION:

The present study demonstrates that the ethanolic extract of *Ocimum sanctum* leaves can produce significant anxiolytic activity. Further studies on isolation and fractionation of the active components from the leaf of *Ocimum sanctum* and its mechanism of action are strongly recommended.

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