

## EFFECT OF *OCIMUM SANCTUM* (LINN) EXTRACT ON RESTRAINT STRESS INDUCED BEHAVIORAL DEFICITS IN MALE WISTAR RATS

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### Summary

Ocimum sanctum plant extract was screened for its effects on restraint stress induced behavioral changes in Morris water maze and passive avoidance task. Restraint stress for 21 days caused memory impairment in rats. Animals treated with 100mg/kg body weight of Ocimum sanctum plant extract, by oral feeding during stress, showed a decreased latency to enter the target quadrant in Morris water maze test compared to the stressed animals. Ocimum sanctum treated animals also showed increase in latency to enter the dark compartment during retention test in passive avoidance tests both after 24hours and 48hours. These results reveal the memory enhancing effects of Ocimum Sanctum plant in restraint stress induced memory impaired rats.

**Key words:** Ocimum sanctum; spatial memory, Morris water maze, restraint stress

Ocimum sanctum (Linn) (OS) is an herb belonging to the family Lamiaceae. This herbal plant is known for its medicinal value in various traditional medicines in India and other Asian nations, particularly Ayurveda and Unani type of medicine (1). The leaves of OS are commonly used in cough, cold, fever, respiratory disorders, non-healing ulcers etc. The important bioactive constituents of Ocimum sanctum are ursolic acid, a triterpenoid and rosmarinic acid a phenylpropanoid. It contains volatile oil comprising mainly of eugenol and  $\beta$ -caryophyllene with minor terpenes like bornyl acetate,  $\beta$ -elemene, methyl eugenol, neral,  $\beta$ -pinene etc (2, 3, 4, 5).

Ocimum sanctum has been evaluated for its various pharmacological activities. It has been reported that Ocimum sanctum has antistress (6, 7), anti-ulcerogenic (8, 9), radio protective (10), anti-inflammatory effects (11) and nootropic potential (12) The ethanol extract of OS leaves was found to prevent the reduction in adrenergic neurotransmitters in brain of rats

exposed to swimming stress and gravitational stress (13). Essential oil from leaves and seeds of OS showed anti-stressor effects in rats exposed to restrained stress (14)

Though various properties of *Ocimum sanctum* leaves extract are addressed in the literature, its role in memory enhancing in stress conditions is not addressed. It is well known that chronic restraint stress impairs acquisition and retention of spatial memory task in rats (15). Question whether such memory impairment can be prevented by pretreatment with *Ocimum sanctum* leaves extract is not answered. Accordingly aim of the present study is to evaluate the effects of *Ocimum sanctum* leaves extract in preventing the restraint stress induced memory deficit in passive avoidance and spatial learning in Morris water maze.

## Methods

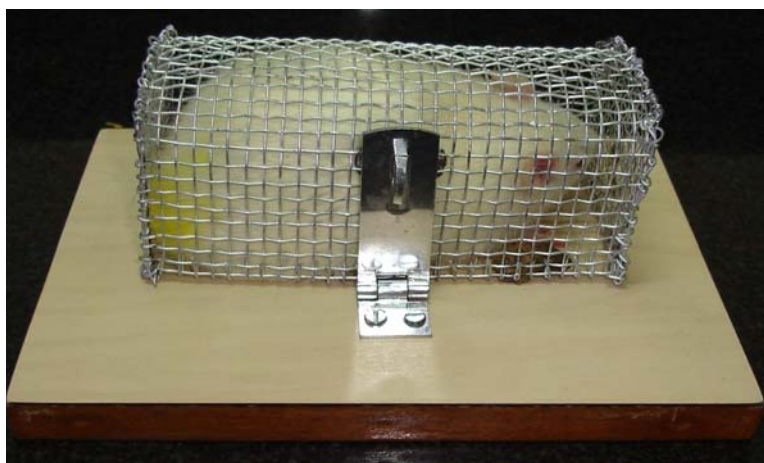
### Animals:

Adult male Wistar rats (3 months old), weighing 200-250g was obtained from Central Animal house, Manipal University, Manipal. Rats were housed in polypropylene cages (22.5 x 35.5 x 15cm), three rats per cage. All animals were maintained in 12:12hr Light: Dark environment, in an air-conditioned room in the central animal house. All rats were fed with water and food *ad libitum*. Institutional animal ethical committee (I.A.E.C) approval (IAEC/KMC.06/2006-2007) was obtained before the experiment and care was taken to handle the rats in humane manner.

### Experimental Design:

The adult animals (3months old) were divided into 4 groups (n = 6 in each group) as follows:(i)Normal control group (NC) – remain undisturbed in the home cage throughout the experimental period,(ii)Vehicle control(V) – these rats were fed with equivolume of vehicle solution (1% Sodium carboxy methyl cellulose solution ) orally for 21 days,(iii) Restrained stress group (S) – these rats were stressed in a wire mesh restrainer(Fig.1), for 6 hrs/ day, for 21 days.(iv) Restrained stress + *Ocimum sanctum* treatment group (S+ OS) – these rats were fed with 100 mg /kg of *Ocimum sanctum* plant leaves extract daily and stressed for 6 hrs/ day, for 21 days. After 21 days treatment period rats in all groups were subjected to Passive avoidance and Morris water maze behavioral tests as described below.

### Stress Procedure:



**Fig.1:** A rat in a wire mesh restrainer

Rats were subjected to restrained stress in a wire mesh restrainer (16, Fig. 1.) for 6 hours per day for 21 days. The wire mesh restrainer had a wooden base and stainless steel wire mesh restrainer hinged to the base. The restrainer having the dimensions of 8cm (L) x 4cm (B) x 4cm (H) was used for the experiments. A pad lock and latch helped to secure the rat in the restrainer.

#### **Ocimum Sanctum leaves extract:**

Ocimum Sanctum leaves extract was obtained from *Natural Remedies pvt. Limited*; Bangalore, India. Ocimum sanctum extract was dissolved in 1% Sodium Carboxy methyl cellulose solution (Merk, India) to get the desired concentration. Ocimum sactum was administered orally at a dose of 100mg/kg body weight/day for 21 days. It was given using a gastric gavage needle (*Popper and Sons*, USA)

#### **Behavioral Tests**

##### **a) Passive avoidance test**

To test the memory retention rats were subjected to passive avoidance test (17). The apparatus had two compartments, a rectangular larger compartment with 50x50cm grid floor and wooden walls of 35cm height. It had a roof, which could be opened, or closed One of the walls had a 6x6cm opening connecting the larger compartment to a dark smaller compartment. The smaller compartment had 15x15cm electrifiable grid connected to a constant current stimulator, wooden walls of 15 cm height and ceiling, which could be opened or closed. The connection between the two compartments could be closed with a sliding door made of plexi glass. The larger compartment was illuminated with a 100 W bulb placed 150 cm above the center. The experiment included 3 parts (i) Exploration test (ii) An aversive stimulation and learning (iii) Retention test. During exploration test, each rat was kept in the center of the larger compartment facing away from the entrance to the dark compartment. The door between two compartments was kept open. The rat was allowed to explore the apparatus (both larger and smaller compartments) for a period of three minutes. Each rat had three trial sessions. After the last exploration trial, as soon as the rat entered the dark compartment the sliding door between the two compartments was closed. Three strong foot shocks (50Hz, 1.5mA, and 1sec duration) were given at five-second intervals. The ceiling was then opened and the rat was returned to its home cage. Retention test was carried out after 24 and 48 hours. Each animal was placed in the larger compartment and a maximum of three minutes were given. The time taken by each rat to enter the dark compartment was measured using a stopwatch. Animals not entering the dark compartment within this given period received a latency time of three minutes. Absence of entry to the dark compartment or a longer duration in the bright compartment indicated a positive reaction.

##### **a) Morris-water Maze**

Spatial memory, of the rats was tested using Morris water maze test (18, Fig.2) Recordings were done using a video camera (*Sony color camera with F1.2 Lens, and 0.7Lux*). The water maze apparatus consists of a water tank of 1.83 meters in diameter, divided into 4 quadrants. There was a 4"x4" size platform submerged in one of the fixed quadrant, the target quadrant. Nontoxic white paint powder was added to the water just before the experiment to make the

water opaque. Visual cues were placed at fixed places for facilitating spatial orientation of the animal. Positions of the cues were kept unchanged through out the period of training. The rats were trained in the water maze in 11 sessions on 6 consecutive days, two sessions on each day except on first day where only one session was given. During each training session the rat was placed in water so that it faced the wall of the pool. Each session consisted of 4 trials. In each training session, the latency (time) to escape onto the hidden platform was



**Fig.2:** Water maze

recorded. If the rat was unable to find the platform within three minutes, the training session was terminated and a maximum score of three minutes was assigned. Twenty-four hours after the last session, rats were subjected to memory retention. This session was of 30sec duration. The hidden platform was removed and the rat was placed into the water. Here time taken to reach the target quadrant and time spent in the target quadrant were measured. Data were analyzed using *Panlab Smart Version2.5 video tracking software, Barcelona, Spain*. Greater latency to reach the target quadrant and less time spent in the target quadrant suggest the memory impairment.

### **Data Analysis**

Data was analyzed using One way analysis of variance (ANOVA) followed by Bonferroni's test (post-hoc) using Graph pad in stat software.

### **Results**

The rats treated with vehicle, Stressed and treated with *Ocimum sanctum* leaves extract, or Stressed rats remained healthy throughout the experimental period. There are no noticeable behavioral changes in rats belonging to any of the groups.

## Passive avoidance Test

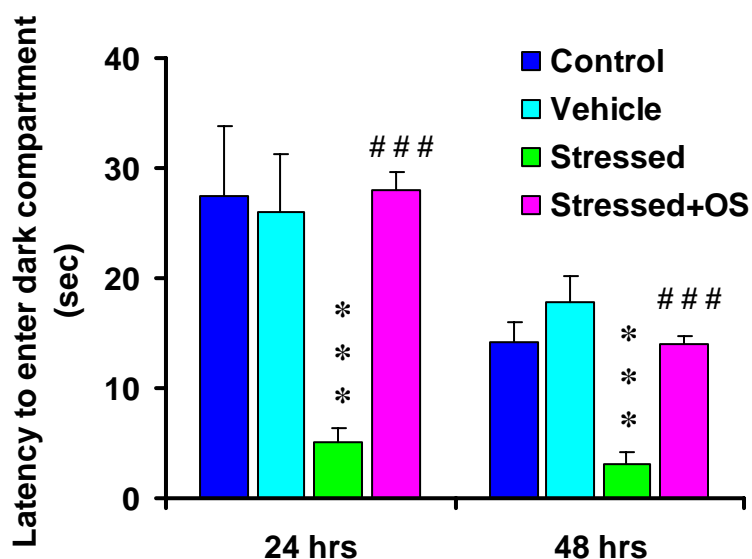


Fig.3: Latency to enter the dark compartment of passive avoidance apparatus during memory retention test at 24 and 48hr of learning. Control/Vehicle vs Stressed, \*\*\* P<0.001; Stressed vs Stressed + Ocimum sanctum treated (OS) ### P<0.001, One way Anova, Bonferroni's test. Note the very short latency to enter the dark compartment in the stressed rats and its reversal in stressed + ocimum sanctum treated group at both time points of tests

The memory retention tested in the passive avoidance test revealed a deficit in memory retention in the stressed rats and there was no such deficit in the stressed and treated with Ocimum sanctum leaves extract. The results of retention test performed 24 hrs and 48 hr learning session (after the Noxious Stimulus) is shown in the figure 3.

The stressed rats entered the dark compartment of the passive avoidance apparatus within  $5.16 \pm 1.25$  seconds during 24hr retention test and  $3.15 \pm 1.4$  seconds during 48hrs retention test. Rats which were treated with Ocimum sanctum leaves extract prior to stress on each day, took  $28.00 \pm 2.92$  sec. and  $13.91 \pm 2.1$  sec. during 24hr. and 48hr. retention test respectively. (S vs S+ OS, P<0.001 at both retention tests, (One way Anova, Bonferroni's test, P<0.0001, F= 22.53 in 24hr. and P<0.0001, F= 40.52 in 48 hrs.). These results suggest impairment in memory retention in stressed rats and its prevention by treatment with Ocimum sanctum leaves extract.

## Morris-water maze test

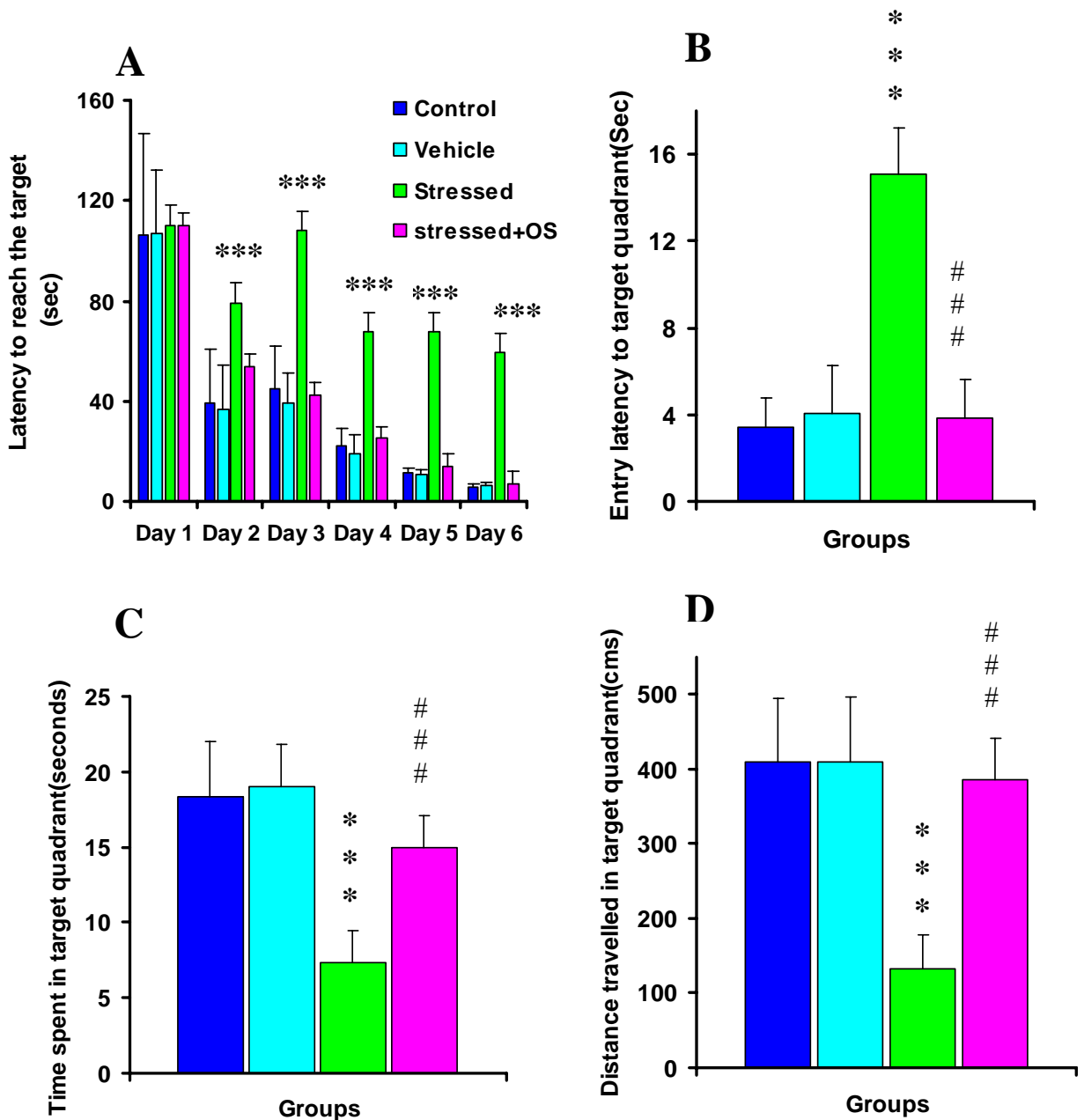
## Water maze test performance in the trail session

Rats in all the groups learnt reaching the target quadrant and identifying the hidden platform there and escape from swimming except the rats in stressed group (Fig.4 A). Except stressed group of rats all other group of rats begin to identify the platform from second day, and continued to retain the learnt behavior. They took about 2-3 seconds to reach the target and

there was progressive learning in them. The stressed rats took 60-70 seconds to reach the target, and there was no significant progressive learning over days in them.

**Water maze test performance in retention test**

Water maze retention test was done 24hr after last learning session. During this session hidden platform was removed and each rat was tested in a single trail of 30 second. Time taken to reach the target quadrant, time spent in the target quadrant and distance traveled in the target quadrant are measured (using PanLab smart version 2.5 video tracking software).



**Fig.4:** Performance of rats in different groups during learning (A) and retention test (B,C,D).A. Latency to reach the target quadrant on different days of learning, B-Latency to enter the target quadrant, C-Time spent in the target quadrant, D-Distance travelled in the target quadrant during memory retention test. Control/Vehicle vs Stressed, \*\*\* P<0.001; Stressed vs. Stressed + Ocimum sanctum treated (OS) ### P<0.001, One way Anova, Bonferroni's test.

**Latency to enter the target quadrant**

The stressed rats took longer time to reach the target quadrant, where as *Ocimum sanctum* treated and stressed rats and other groups of rats took less time to reach the target quadrant. Stressed rats took  $15.07 \pm 2.17$  second to reach the target quadrant where as control, vehicle treated, treated with *Ocimum sanctum* and stresses group of rats took  $3.44 \pm 1.35$ ,  $4.05 \pm 2.2$ ,  $3.84 \pm 1.78$  seconds to reach the target quadrant. (n=6 in all groups, NC vs S,  $P < 0.001$ ; S vs S+OS,  $P < 0.001$ ; V vs S,  $P < 0.001$ ; One way Anova, Bonferroni's test  $P < 0.0001$ ,  $F = 22.00$ ; Fig.4B).

**Time spent in the target quadrant**

The stressed rats spent lesser time to in the target quadrant, where as *Ocimum sanctum* treated + stressed rats and other groups of rats spent more time to in the target quadrant. Stressed rats spent only  $7.36 \pm 2.13$  seconds in the target quadrant where as control, vehicle treated, treated with *Ocimum sanctum* and stresses group of rats spent  $18.34 \pm 3.64$ ,  $14.92 \pm 1.87$ ,  $19.05 \pm 2.78$  seconds (Mean  $\pm$  SD) in the target quadrant. (n=6 in all groups, NC vs S,  $P < 0.001$ ; S vs S+OS,  $P < 0.001$ ; V vs S,  $P < 0.001$ ; One way Anova, Bonferroni's test  $P < 0.0001$ ,  $F = 23.62$ ; Fig.4C).

**Distance travelled in the target quadrant**

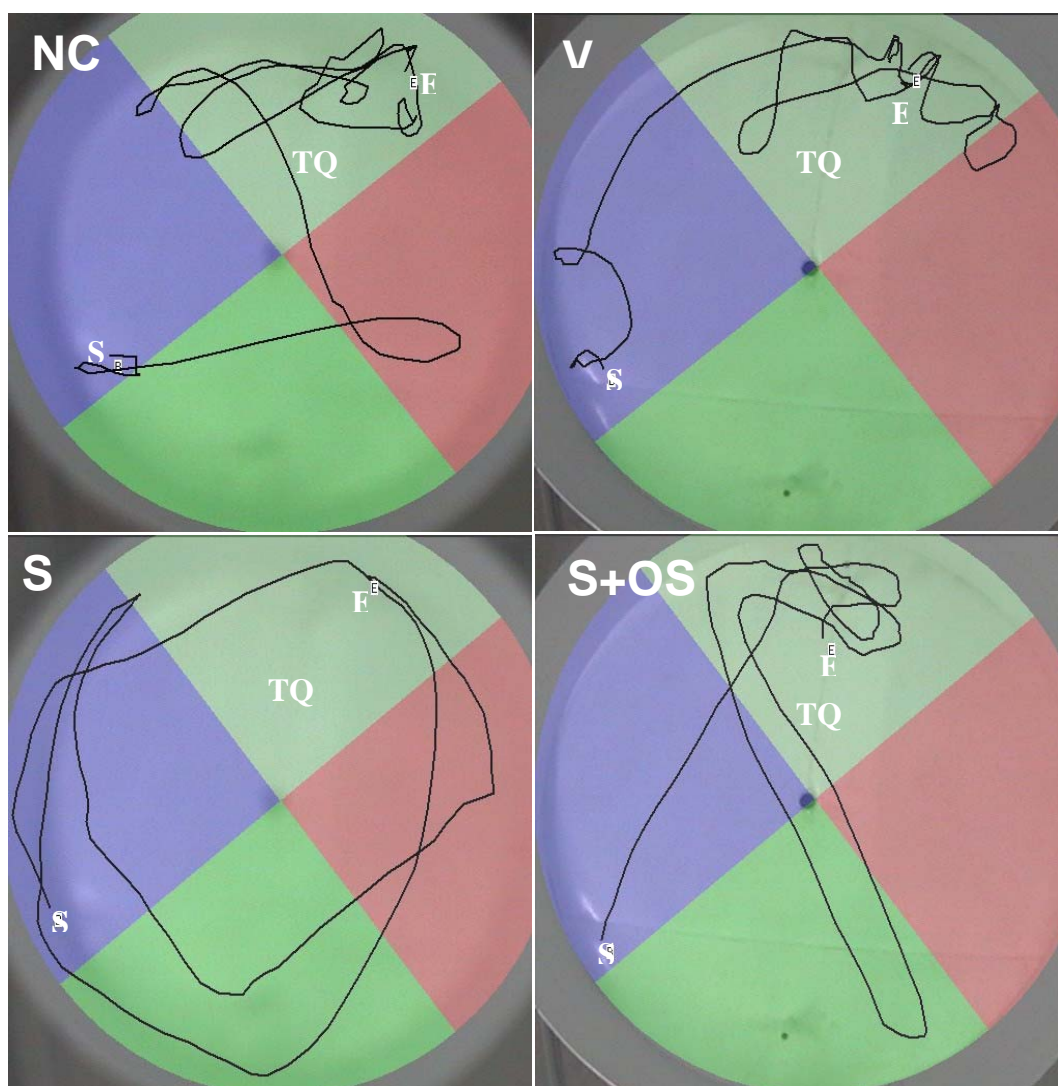
The stressed rats traveled shorter distance in the target quadrant, where as *Ocimum sanctum* treated + stressed rats and other groups of rats traveled longer distance in the target quadrant. Stressed rats travelled  $132.24 \pm 45.05$  cm in the target quadrant where as control, vehicle treated, treated with *Ocimum sanctum* and stresses group of travelled  $409.95 \pm 83.87$ ,  $384.9 \pm 55.64$ ,  $409.27 \pm 87.97$  cm (Mean  $\pm$  SD) in the target quadrant. (n=6 in all groups, NC vs S,  $P < 0.001$ ; S vs S+OS,  $P < 0.001$ ; V vs S,  $P < 0.001$ ; One way Anova, Bonferroni's test  $P < 0.0001$ ,  $F = 22.0$ ; Fig.4D).

Video tracking of performance of representative rats in each group is shown in the fig.5.

**Discussion**

In the present study we have evaluated the efficacy of extract of *Ocimum sanctum* in prevention of stress induced memory deficits in rats. Restraint stress is known to impair the spatial learning and memory (15). In the present study the restraint stressed animals showed memory impairment, which was evident in passive avoidance task as well in the Morris water maze test. Such memory impairment was attenuated by *Ocimum sanctum* leaves extract treatment.





**Fig.5:** Video tracking of movements of representative normal control (NC), Vehicle control (V), Stressed (S), Stressed and *Ocimum sanctum* treated (S+OS) rats during memory retention test in the water maze. Note that Stressed rat took a long route, spent less time, and traveled a short distance in the target quadrant (TQ), unlike Stressed and *Ocimum sanctum* treated rat and other control rats which reached the target quadrant in a shortest route and spent most of the time there. S (lower case)-start, E- end of the track

### Stress and memory

Chronic stress is known to cause memory impairments (15). Neural basis for such impairment is the neuronal injury in the hippocampal region due to excitotoxicity (19), alterations in the neurotransmitter levels in the brain regions. Alternatively, neuronal damage in this region may be mediated through increased glucocorticoids (20). Stress results in increased oxidative stress and decreases antioxidant defense status in brain (21), which may form the basis for decreased memory (21). Antioxidant treatment has shown modulating effects on brain free radicals in restrained rats (22). Thus in the present study also the observed memory deficit may be attributed to excitotoxicity, alteration in the neurotransmitters or glucocorticoids.



### **Anti-stress role of *Ocimum sanctum* and Neuroprotection**

Stress causes an increase in the corticosterone level in the blood. Treatment of stressed animals with ethanol extract of *Ocimum sanctum* has been shown to prevent the changes in plasma level of corticosterone induced by exposure to both acute and chronic noise stress, indicating the antistressor property of the plant against noise (23). Studies with ursolic acid, a major constituent in *Ocimum sanctum*, it has been shown that it protects hippocampal neurons from kainic acid induced injury (24).

### **Mechanisms of *Ocimum sanctum* induced memory enhancement**

#### **i. *Ocimum sanctum* and Neurotransmitters**

Noise stress has detrimental effect on brain and has been shown to decrease the amount of Acetyl choline contents and increases the Acetyl choline esterase activity in different brain regions such as hippocampus, hypothalamus, cerebral cortex, corpus striatum. Pretreatment of the animals with ethanol extract of *Ocimum sanctum* leaves for 7 days prevented the noise induced changes in these two cholinergic parameters in all the four areas of brain. The results of the study indicate the protective nature of the plant material on the brain tissues against the detrimental effect of noise stress (25).

Ravidran et al (26) estimated of norepinephrine, epinephrine, dopamine, and serotonin in discrete regions of the rat brain in noise stress exposed and control mice and showed that noise stress can alter the brain biogenic amines after 15 days of stress exposure. Administration of the 70% ethanolic extract of OS had a normalizing action on discrete regions of brain and controlled the alteration in neurotransmitter levels due to noise stress, emphasizing the antistressor potential of this plant (26.) The intraperitoneal administration of 70% ethanolic extract of *Ocimum sanctum*(OS) at the dosage of 100 mg/kg body weight to animals subjected to noise exposure has prevented the noise induced increase in neurotransmitter levels(NA,DA, 5HT) without affecting the normal levels. This indicates that OS can be a probable herbal remedy for noise induced biogenic amine alterations (27). Treatment with *Ocimum sanctum* might have altered the neurotransmitter levels in the stressed and treated group in the present experiment

#### **ii. New compounds in *Ocimum Sanctum***

Gupta et al(28) recently isolated three new compounds, (1)ocimumoside A, (2) Ocimumoside B, and (3) ocimarin, from an extract of the leaves of holy basil (*Ocimum sanctum*), together with other known substances. (4) apigenin, (5) apigenin-7-O-beta- d-glucopyranoside, (6) apigenin-7-O-beta- d-glucuronic acid (7), apigenin-7-O-beta- d-glucuronic acid 6"-methyl ester, (8) luteolin-7- O-beta- d-glucuronic acid 6"-methyl ester,(9)luteolin-7-O-beta-d-glucopyranoside,(10) luteolin-5-O-beta-d-glucopyranoside, (11)4-allyl-1-O-beta-d-glucopyranosyl-2-hydroxybenzene and two known cerebrosides. The new compounds displayed promising antistress effects by normalizing hyperglycemia, plasma corticosterone, plasma creatine kinase, and adrenal hypertrophy (28).

#### **iii. Anti amnesic role of *Ocimum sanctum***

Joshi et al (12) under taken experiments to assess the potential of *O. sanctum* extract as a nootropic and anti-amnesic agent in mice. Aqueous extract of dried whole plant of *O. sanctum* ameliorated the amnesic effect of scopolamine (0.4 mg/kg), diazepam (1 mg/kg) and

aging induced memory deficits in mice (12). In the present study we have used hydro-alcoholic extract of leaves alone to study its nootropic efficiency and demonstrated memory enhancing property in the stressed rats.

*O. sanctum* leaves extract could be beneficial in the treatment of cognitive disorders such as dementia, and Alzheimer's disease.

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