Original article

Effect of *Ocimum sanctum* extract on sodium nitrite-induced experimental amnesia in mice

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Abstract:

The present study investigated the antiamnesic effect of the extract of *Ocimum sanctum* (OS) on time induced amnesia and sodium nitrite-induced amnesia in mice by using two different rodent models of memory. The aqueous extract of OS was administered to mice at dose of 100 and 300 mg/kg intraperitoneally for 3 days prior to experiment. The effect of the extract on time induced amnesia and sodium nitrite-induced amnesia in mice was evaluated using object discrimination task and elevated plus maze task. Piracetam was used as the standard drug. When given before training trial OS treatment significantly enhanced the exploration time of the novel object memory in mice at both 100 and 300 mg/kg doses. The memory enhancing effect was also observed when the extract was administered post training trial. Sodium nitrite at a dose of 75 mg/kg, i.p. produced amnesia in mice. Administration of OS pre and post training also significantly reversed the amnesic effect of sodium nitrite in mice. The results reveal that OS has a potential memory enhancing effect at least in mice.

Keywords: *Ocimum sanctum;* Object discrimination task; Elevated plus maze; Sodium nitrite; Memory; Mice

Introduction

Ocimum sanctum Linn. (Labiateae), popularly known as "Tulsi" in India and holybasil in English. It is one of the sacred herbs for Hindus in Indian sub-continent. The genus *Ocimum* contains 50-150 species of herbs and shrubs found in tropical regions of Asia, Africa and America. The royal fragrance of the herb make it "king" of the smell the major constituents of *Ocimum sanctum* (OS) are eugenol [1], flavonoids [2] and traces of zinc, manganese and sodium [3]. OS has been used in Ayurveda & siddha systems for the treatment of diverse ailments like infections, hepatic disorders, common cold and cough, malarial fever, vomiting, cutaneous disease, hypertension, stressful condition pain and inflammation, postnatal disorder, cholera, constipation, septicemia, ulcer, arthritis, stomachic, leucoderma, disease of heart and blood. The aqueous or alcoholic extract of OS also possesses numerous pharmacological activities such as insecticidal, antibacterial, antiviral, anticryptococcus, antimycotic, anthelmintic and nematicidal. OS acts as antidote in bees, warms, leeches, mosquitoes, snake, and scorpion sting bite. It also exhibits adaptogenic, antifertility, anticarcinogenic, hepetoprotective, immunomodulatory, analgesic, anti-inflammatory [4], diaphoretic, and antitoxic in copper sulphate toxicity. It regulates thyroid functions, chromosomal anomalies and carbohydrate metabolism in diabetes mellitus [5]. It is effective in the treatment of leucoderma, asthma, bronchitis, hiccough, cataract, earache, diarrhea [6], and gout [7]. OS also has potent psychopharmacological activities such as hypnosis [8], anticonvulsant [9], antistress [10], anti despair [9] and antiamnestic effects [11]. OS possesses antioxidant properties which were evaluated by using several paradigms of oxidative stress induced by various techniques and comparable to flavanoids and vitamin E. The preparations of *OS* have been also reported to be beneficial in the treatment of cognitive disorders. The extract of the herb reversed the age related and scopolamine induced amnesia in mice [12]. Administration of sodium nitrite in rodents produces lasting effects as hypoxia, neuron damage and impaired behavior [13]. It has been reported to impair acquisition of an inhibitory avoidance response in rats and mice [14,15]. Nitrite ions or free radicals cause organic damage to the living system that may be responsible for development of amnesia and serve as a model for aging brain. As stated earlier OS reversed age related and scopolamine-induced amnesia in mice but till now there are no reports related to reversal of hypoxia-induced and delay amnesia in mice. Elevated plus maze is a model for the evaluation of spatial learning and memory. The shortening of the time in which animal moves from the open arm to closed arm in the test trial indicates that animal remembers the configuration of the arms of the maze. Object discrimination task is especially suited to test the effects of pharmacological interventions on learning and memory. Rodents have an instinct to explore the novel object and such novelty preference paradigm yields results that can be safely related to changes in learning and memory. Thus, the aim of the present study was to investigate the anti-amnesic effect of OS on time induced and sodium nitrite-induced amnesia in mice by using two different rodent models of memory.

Materials and Methods

Collection and processing of plant material

Plant extract: Leaves of *OS* were collected from the garden of Institute of Pharmacy, B.U, Jhansi and authentification was done from National Botanical Research Institute (NBRI), Lucknow by Dr. Anil Kumar Goel, Scientist Botanical Garden. Voucher of OS was preserved for reference at Institute of Pharmacy, B.U, Jhansi. Leaves were dried in normal environmental conditions under shade and then reduced the size by pulverization. A hundred g of dried leaves of OS was powdered and extraction was performed by refluxing with distilled water at 80°C, in soxhlet apparatus. Extract was concentrated under vacuum [16]. The yield of the extract after extraction was 11.5% of the air-dried powder. The dose of the drug has been expressed considering the dry weight of the extract.

Animals

Male Swiss albino mice (20-30 g), supplied by the Defense Research and Development Organization

(DRDO), Gwalior, and provided by the Institute of Pharmacy, B.U, Jhansi were used. Animals were housed in a group of four in hanging polycarbonate cages. Animals were introduced to the experimental holding rooms at least 7 days prior to the commencement of the study and maintained at 22-24°C and 55 ± 5% humidity on a standard 12 h light/dark cycle, with food and water available *ad libitum.* All the procedures were performed in accordance with the Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (registration no-453). All protocol was performed between 9 a.m. to 3 p.m.

Drugs and chemicals

Sodium nitrite (Qualigens fine chemicals, Glaxo Smithcline Pharmaceuticals Ltd., Mumbai) was provided. Piracetam (Neurocetam, Brown and Burk Pharmaceuticals Ltd., Bangalore) injection was purchased from the market and served as a standard drug.

Selection of doses

The LD_{50} of OS leaf extract was reported to be 4.5 g/kg by the oral route [17] indicating the low toxicity of the plant material. Devi and Ganasundari [16] reported that a dose up to 5 g/kg did not produce any acute toxic symptoms and they indicated an LD_{50} value of 6.2 g/kg (for 30 days). In the light of above references we selected the extract at doses of 100 and 300 mg/kg of body weight and administered them intraperitoneally (i.p.) [12]. Sodium nitrite and piracetam were also administered intraperitoneally at a dose of 75 mg/kg and 150 mg/kg respectively.

Apparatus

Object discrimination task (ODT) was performed in black open field box (46 x 57 x 30 cm) [18]. The light intensity in the box was about 30 lux and there was no shadow area within the box. Elevated plus maze apparatus consisted of two open (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) for mice and an open roof with the entire maze elevated (25 cm) from the floor the animals were placed individually at the end of one arm facing away from the central platform [19].

Experiment design

The experiments in both the memory models were carried out in two groups. In Group 1 (prior to trial) animals were administered with saline (5 ml/kg, i.p.) or extract (100 and 300 mg/kg, i.p.) or piracetam (150 mg/kg, i.p.) followed by sodium nitrite (75 mg/kg, i.p.) and after 30 min they were subjected to acquisition trial while in Group 2 the animals were treated immediately after the trial. The treatment with OS was started 3 days prior to the experiment.

Object discrimination task (ODT)

Experiment was conducted in three sessions (habituation, training and test) with small modifications as previously described by Walf *et al.,* [18]. Before starting each session the animals were handled. In the habituation session on day 1 the animals were allowed to accustom with the box for 30 min. On day 2 in the training session, two similar objects (white plastic bottles filled with sand, diameter of 4.2 cm and height of 6.8 cm) were placed inside the box at a symmetrical position about 5 cm away from the wall of the box. The animal was allowed to explore the box for 3 min. Exploration of the object by the animal was considered when it approached the object through its nose within 2 cm of the object. The time spent by the animal in exploring both the objects was recorded by stopwatches. Test session was conducted 24 h after the training session for a time period of 3 min. During the test session, one of the objects was replaced with a novel object (an aluminum cube with flat top, dimension of 4.5 x 4.5 x 8.5 cm). The time spent by the animal in exploring the novel and familiar objects was recorded. The animals with total exploration less that 7 sec in the test session were excluded from the study. Throughout the experiment, the presentation order and position of the objects was counterbalanced to prevent bias from order or place preference. Discriminative index, ratio of the time spent exploring the novel object to the total time spent exploring both objects in the test session, was used to measure

memory performance. The discriminative index (D) was calculated from the formula: D = Time spent with novel object/Time spent with novel and familiar object. After completion of each trial, the arena and objects were wiped with 30% alcohol.

Elevated plus maze test (EPM)

The protocol consisted of 2 trials. The first trial was the acquisition trial and the second trial was the retrieval trial, which was performed 24 h after the first trial. The mice were placed individually at the end of open arm facing away from the central platform. The time taken by the animals to move from the open arm to either of two sides of enclosed arms was recorded as transfer latency. After 24 h the mice were again placed on the elevated plus-maze and the transfer latency was recorded again. If they did not enter the enclosed arm within 90 sec it was removed from the maze and transfer latency was assigned as 90 sec.

Data analysis

Data from the object discrimination task and elevated plus maze test were calculated as mean ± SEM. The presence of significant difference ($p < 0.05$) in the time spent with the novel and familiar object was determined by paired Student's *t*-test. The discriminative index was statistically analyzed using the Kruskal-Wallis followed by Dunn's post hoc test. Differences in the transfer latency in elevated plus maze between the treatment groups and vehicle were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's pos hoc test. All statistical analysis was carried out using Graph Pad Prism (Version 4).

Results

Effect of OS on time induced amnesia in ODT

In the test trial, the mean exploration time for familiar and novel object in the saline treated animals was 6.22 ± 0.66 sec and 6.45 ± 0.99 sec, respectively. Treatment with the extract at doses of 100 and 300 mg/kg, i.p. before the acquisition trial increased novel object exploration time to 7.32 ± 0.50 sec ($p < 0.05$) and 7.17 ± 0.62 sec ($p < 0.05$) respectively, while the exploration time for familiar object was 5.81 ± 0.58 sec and 4.82 ± 0.24 sec respectively (Fig. 1). The post trial treatment with OS also increased the exploration time for the novel object but OS did not reach the significance level (Fig. 2). Piracetam treatment produced significant increase in the exploration time for the novel object when administered pre and post trial (Fig. 1 and Fig. 2). The discriminative index in the treatment groups was more than the saline treatment groups (Table 1).

Effect of OS on sodium nitrite induced amnesia in EPM

Sodium nitrite when administered before the acquisition trial produced marked amnesia, which was evident through an increase in transfer latency of mice in the test phase as compared to the saline treated mice. The transfer latency when recorded after 24 h of the acquisition phase for sodium nitrite treated group was 18.89 ± 0.80 sec while for saline treated group was

Table 1 Effect of extract of *Ocimum sanctum* (OS) at doses of 100 and 300 mg/kg, i.p. on discriminative index in object discrimination task (ODT) when administered before and post training trial. Values represent mean ± S.E.M. of 8-10 animals in each group

Group	Treatment	Dose	Discriminative index
Pre training	Saline	5 ml/kg, i.p.	0.49 ± 0.04
	Ocimum sanctum	100 mg/kg, i.p.	0.56 ± 0.03
	Ocimum sanctum	300 mg/kg, i.p.	0.59 ± 0.03
	Piracetam	150 mg/kg, i.p.	0.66 ± 0.05
Post training	Saline	5 ml/kg, i.p.	0.55 ± 0.05
	Ocimum sanctum	100 mg/kg, i.p.	0.54 ± 0.05
	Ocimum sanctum	300 mg/kg, i.p.	0.57 ± 0.04
	Piracetam	150 mg/kg, i.p	0.64 ± 0.04

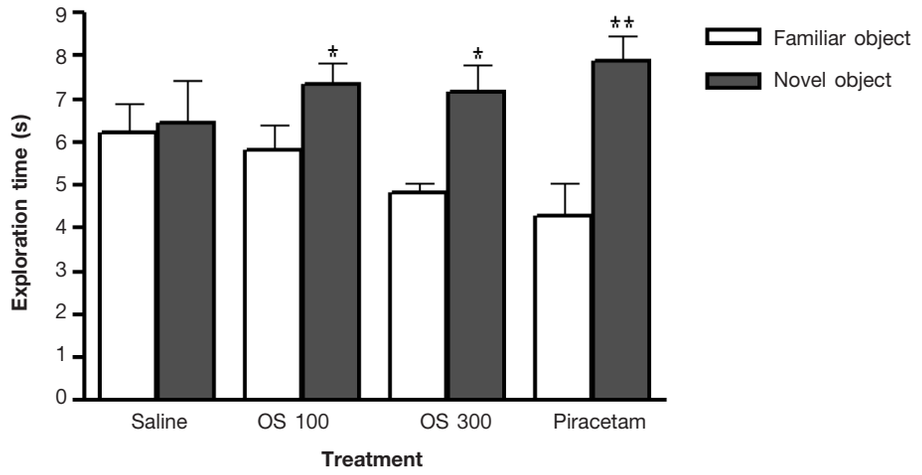

Figure 1 Effect of extract of OS at doses of 100 and 300 mg/kg, i.p. on time induced memory deficits in object discrimination task (ODT) when administered before the training trial. Each bar represents mean ± S.E.M exploration time in seconds of 8-10 animals in each group. Values differing significantly are marked by asterisk. *P < 0.05, ** P < 0.01, Paired Student's *t*-test

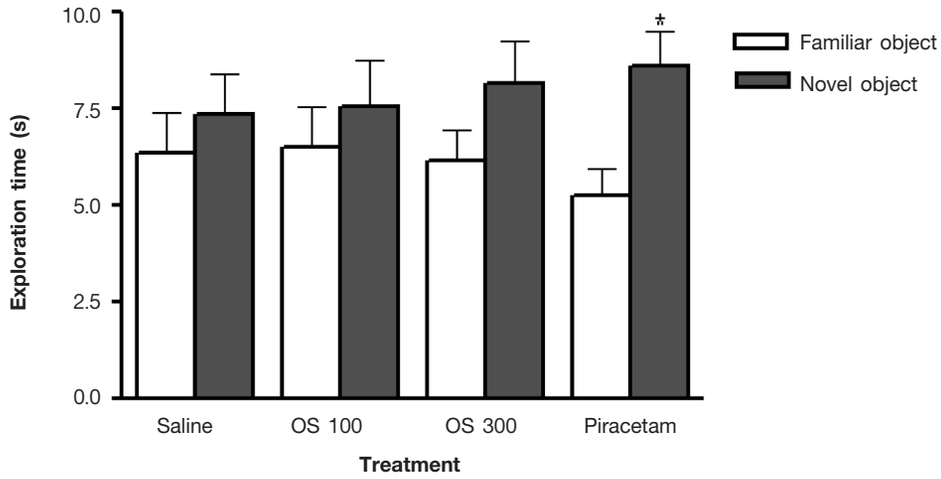

Figure 2 Effect of extract of OS at doses of 100 and 300 mg/kg, i.p. on time induced memory deficits in object discrimination task (ODT) when administered post training trial. Each bar represents mean ± S.E.M exploration time in seconds of 8-10 animals in each group. Values differing significantly are marked by asterisk. *P < 0.05, Paired Student's *t*-test

12.63 ± 0.52 sec ($p < 0.01$). Treatment with extract of OS at doses of 100 and 300 mg/kg reversed the amnesia produced by sodium nitrite. The transfer latency was 14.24 ± 1.34 sec ($p < 0.05$) and. 14.11 ± 1.63 sec ($p < 0.05$) at doses of 100 and 300 mg/kg, respectively (Fig. 3). Similar effect on transfer latency of mice was observed when the treatments were given immediately after the trial. Sodium nitrite treatment increased the transfer latency to 15.33 ± 1.02 sec while the transfer latency for saline treated group was 11.61 ± 0.69 sec ($p < 0.05$). Treatment with extract of OS at doses of 100 and 300 mg/kg, i.p. produced significant decrease in transfer latency, which was recorded as 11.67 ± 0.91 sec ($p < 0.05$) and 11.80 ± 1.38 sec ($p < 0.05$) respectively (Fig. 4). Piracetam treatment also produced the significant reversal of sodium nitrite induced amnesia (Fig. 3 and Fig. 4).

Discussion

Alzheimer's disease (AD) is a degenerative dementia that destroys the higher structures of the brain. There are several hypotheses regarding the

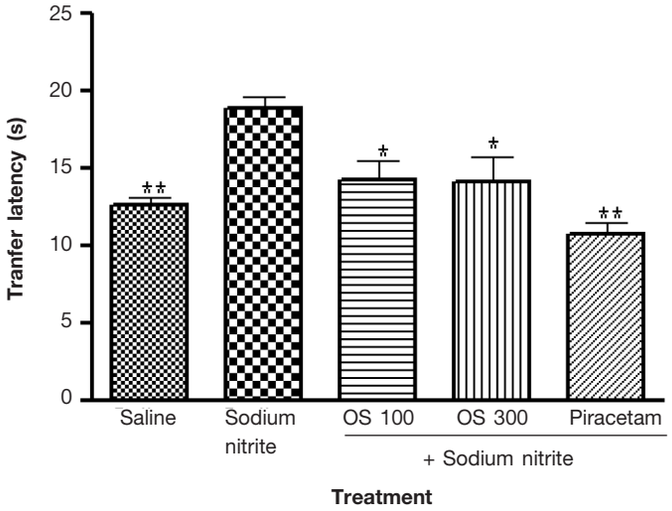

Figure 3 Effect of extract of *OS* at doses of 100 and 300 mg/kg, i.p. on sodium nitrite-induced memory deficits in elevated plus maze test (EPM) when administered before the training trial. Each bar represents mean ± S.E.M transfer latency in seconds of 8 animals in each group. Values differing significantly are marked by asterisk. *P < 0.05, **P < 0.01, One-way ANOVA followed by Dunnett's test

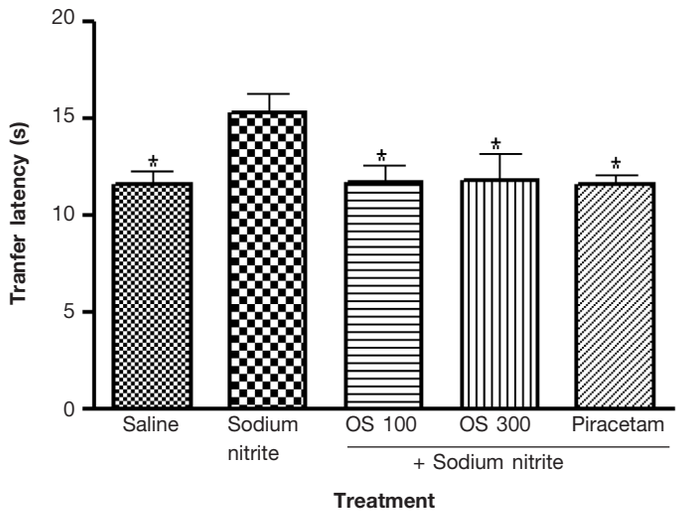

Figure 4 Effect of extract of *OS* at doses of 100 and 300 mg/kg, i.p. on sodium nitrite induced memory deficits in elevated plus maze test (EPM) when administered post training trial. Each bar represents mean ± S.E.M transfer latency in seconds of 8 animals in each group. Values differing significantly are marked by asterisk. *P < 0.05, One-way ANOVA followed by Dunnett's test

etiology of AD, but none has been confirmed. Establishing a valid animal model of the memory impairment in AD is an important step in understanding the disease and exploring new treatments. Recognition measures are beneficial for animal models as well, because animals learn recognition tasks faster than they learn recall tasks. Rodents exhibit a natural exploratory behaviour when exposed to a novel object compared to a familiar one [20]. Many memory-enhancing drugs have been found to improve 24-h forgetting in rats [21]. Consistent to the above report, after 24 h of delay mice did not discriminate between the familiar and novel object. It seemed that OS treatment enhanced the memory in mice in object discrimination task. It was found to increase memory in the both the acquisition and consolidation phases, although the effect in the acquisition phase

was more pronounced than the consolidation phase.

Normal aging is associated with the impairments in learning and memory. Oxygen free radicals are implicated in the process of aging and may be responsible for development of AD. Using sodium nitrite hypoxia, a model of aging brain, Bhattacharya [22] studied the effects of an herbal formulation (Mentat) on learning acquisition by the elevated plus maze and step-down tests and showed facilitating effect. In the present investigation, the sodium nitrite treatment induced marked amnesia in mice. OS administration reversed the amnesia produced by sodium nitrite. Oxygen free radicals, the harmful by-products of oxidative metabolism, cause organic damage to the living cells. OS has been reported to have antioxidant properties [1] and thus provides a neuroprotective role against the hypoxia caused due to sodium nitrite treatment. Pretreatment with OS reversed the cerebral reperfusion injury and chronic cerebral hypoperfusion which were due to free radicals in rats providing antioxidant and neuroprotective effect [11]. Furthermore, OS has been shown to have strong free radical scavenging activity [23]. The drugs having anti-inflammatory action may inhibit the onset and progression of AD [24]. Hence, OS fixed oil has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity [8]. Moreover, 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 antistress potential of this plant [25]. The neuroprotective, antistress and anti-inflammatory activities might contribute to the memory enhancing property of OS in time and hypoxia induced amnesia in mice. Conclusively, OS has nootropic effect and thus can be used in treatment of various cognitive disorders.

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