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MEMORY ENHANCING ACTIVITIES OF *FICUS RELIGIOSA* LEAVES IN RODENTS

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ABSTRACT

Ficus religiosa, a sacred tree to both Hindus and Buddhists, is recognized for its medicinal as well as religious purposes in India. The ethanolic extract prepared from the leaves of *Ficus religiosa* was studied for memory enhancing activities in Wistar albino rats and Swiss albino mice. The present study was carried out on five models such as Elevated-Plus Maze, Step through passive avoidance test, Sodium nitrite intoxication, Hebb-Williams Maze and Radial Arm Maze to evaluate learning and memory parameters. Scopolamine (1mg/kg, i.p) was used as inducing agent in Elevated-plus maze, Step through passive avoidance test and sodium nitrite (95mg/kg, s.c) was used as inducing agent in Sodium nitrite intoxication model. Piracetam (200mg/kg, i.p) was used as standard nootropic agent for all the models except for Sodium nitrite intoxication; Mentat was used as positive control for Sodium nitrite intoxication model. The ethanolic extract of *Ficus religiosa* leaves significantly improved memory and reversed the amnesia induced by scopolamine and hypoxia induced by sodium nitrite. The ethanolic extract of *Ficus religiosa* leaves (100 mg/kg) was comparable to that of piracetam (200 mg/kg) and Mentat (100mg/kg). From the results of the present study it is concluded that the leaf extract of *Ficus religiosa* might possess anti-amnesic as well as nootropic properties. Also the major active constituents present in its leaves such as amino acids may be responsible for these activities.

KEYWORDS: *Ficus religiosa*, anti-amnesic, nootropic, amino acids and piracetam

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INTRODUCTION

Memory is one of the complex functions of the brain. It ultimately involves multiple neuronal pathways and neurotransmitters.[1] Cognitive disorders like Alzheimer's disease, amnesia, delirium, depression and schizophrenia are the results of impairments in learning and memory. All these diseases have a huge burden on society and their prevalence is still growing[2]. *Ficus religiosa* linn. is a large evergreen tree found throughout India, wild as well as cultivated. It is a popular indigenous plant used in traditional system of medicine like ayurveda, siddha, unani and homeopathy. The plant has shown anti-inflammatory, antioxidant, anti-diabetic, analgesic, anticonvulsant, antimicrobial, wound healing, anti-acetylcholinestrase and proteolytic activities[3]. From the previous studies it is evident that the figs of *Ficus religiosa* possessed anti-amnesic activity. The main phytochemicals present in its figs include amino acids[4]. Also the pharmacological studies involving phytochemical analysis suggested the presence of sterols, glycosides, tannins, amino acids in the *Ficus religiosa* leaves extract[4,5]. Since no scientific evidence has been reported for the nootropic activity of the leaf extract of *Ficus religiosa,* the present study was undertaken to envisage the memory enhancing activities of its leaf extract.

MATERIALS AND METHODS

Chemicals

All standard chemicals used in this study were of analytical grade. Piracetam was obtained from UCB (Belgium), dimethylsulfoxide (DMSO) and sodium nitrite from Qualigens Fine Chemicals (Mumbai, India), carboxymethyl cellulose (CMC), scopolamine from Acrose (Belgium), Mentat from Himalaya Drug Company, Bangalore.

Plant Material

Leaves of *Ficus religiosa* were collected during the month of July 2010 from our college campus (Nandha College of Pharmacy), Erode, Tamilnadu. Authentication has been done by Prof. V. S. Kumar, Scientists (F) and

Head of the Office, Tamilnadu Agriculture University, Coimbatore (Tamilnadu). The voucher specimen (No.: BSI/ SRC/ 5/ 23/ 09- 10/ Tech. 816) has been deposited in the herbarium for future references.

Preparation of Extract

The leaves were washed with fresh water to remove adhering dirt and foreign particles. They were shade dried, crushed and grinded to get coarse powder. 500g of the coarse powder of the leaves of *Ficus religiosa* in 1.0 litre of 90% ethanolic solution were macerated in a round bottomed flask for 7 days. The mensturm was collected, concentrated by vacuum distillation and then air dried in an evaporating dish till constant weight was obtained[6].

Phytochemical Analysis

The phytochemical screening and TLC analysis of the leaf extract of *Ficus religiosa* was carried out and showed the presence of sterols, glycosides, tannins and amino acids.

Animals

Wistar Albino rats (150-200g) and Swiss Albino mice (20-25g) of either sex were employed in the present study. The animals were housed in standard cages and maintained at room temperature with natural day and night cycles. The animals were allowed free access to standard laboratory rodent's chow (Pranav Agro Industries Ltd., Bangalore) and water during the study. The animals were acclimatized to the laboratory conditions 5 days prior to the behavioral study. All procedures were conducted according to guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA) and were approved by the Institutional Animal Ethical Committee (Proposal Number: NCP/ IAEC/ PG/ 2010- 05).

Elevated-Plus Maze

The elevated plus- maze served as an exteroceptive behavioral model to evaluate learning and memory in mice. The elevated plus-maze for mice consisted of two open arms (16cm x 5cm) and two closed arms (16cm x 5cm x12 cm) and the maze was elevated to a height of 25cm from the floor. The animals were divided into 4 groups and were treated as shown below[3].

Group I received normal vehicle (0.5% carboxymethylcellulose, oral), Group II received scopolamine (1 mg/kg, i.p.), Group III received extract (100 mg/kg) orally and Group IV received positive control (piracetam, 200 mg/kg, i.p). Transfer latency was recorded after 30 min and 24 hr for the animals of all the groups.

On the first day each mouse was placed at the end of an open arm, facing away from the central platform, and transfer latency(TL) was recorded, which is defined as time taken by the animal to move from an open arm into one of the closed arms with all four legs. The mouse was allowed to explore the maze for another 2 min and then returned to the home cage. Retention of this learned task was examined 24hr after the first day trial. Scopolamine (1mg/kg, i.p.) was administered before retrieval for induction of retrograde amnesia, and TL was recorded. A significant decrease in TL compared to the vehicle control group was considered as an anti-amnesic effect[3].

Step through Passive Avoidance Test

The experimental apparatus for the step through passive avoidance test is an automated shuttle-box that is divided into an illuminated compartment and a dark compartment of the same size by a wall with a guillotine door. The experiment is carried on mice and the animals are divided into 4 groups and were treated as shown below:

Group I received normal vehicle (0.5% carboxymethylcellulose, oral), Group II received scopolamine (1 mg/kg, i.p.), Group III received extract (100 mg/kg) orally and Group IV received positive control (piracetam, 200 mg/kg, i.p). Step through latency was recorded after 24 hr for the animals of all the groups. Each mouse was put through the adaptation trial by placing it gently in the illuminated compartment, facing away from the dark compartment. After 10 sec the door was opened and the mouse moved into the dark compartment freely. When the latency to leave the illuminated compartment was less than 30 sec, the mouse was chosen for the training trial 2 hr later. The training trial is similar to the adaptation trial except that the door is closed as soon as the mouse steps to the dark compartment and an inescapable foot shock (0.6 mA, 2 sec) is delivered through the grid floor. The responses to the electric shock were observed and scored as follows: 0, no response; 1, flinch (movement of any part of the body); and 2, run (running or jumping) or vocalization. The retention test was performed 24 hr after the training trial in the similar manner without the electric shock and the step-through latency to the dark compartment was recorded. The maximal cut-off time for step-through latency was 300 sec[7].

Sodium Nitrite Intoxication

Mice were randomly divided into 4 groups of 5 animals each. Group I and II received normal vehicles (0.5% carboxymethylcellulose, oral) for 8 days. Group III received extract (100mg/kg, orally) and group IV received positive control (Mentat, 100 mg/kg, p.o) respectively for 8 days. Retention test was performed in all groups 1 hr after administration of last dose 8th day. $NaNO_2$ was administered (95mg/kg, s.c) after the 1st

retention test to all animals except group 1. 24 hr after $NaNO_2$ a 2nd test was performed.

Spatial two–chambered cage was used with the dimension 16 inches x11 inches x 5 inches (l x b x h). A partition placed at a distance of 6.5 inches from one of the end of the cage; divide the cage into a smaller and larger chamber. A water bottle was kept in the small chamber. The animal was placed in the larger chamber and allowed to explore the cage. Animals were water deprived for 24 hours. Once the water deprived animals locate the bottle, it was allowed to drink the water for 30 sec. The time taken to locate the water bottle was noted as 1st day reading, this was the first retention test. Each animal then administered $NaNO_2$ (95mg/kg, s.c) before being placed in home cage.24 hr later the animal was again placed in the larger chamber of two –chamber cage. The time required to locate the water bottle was noted as a day 2 reading. But this time the bottle was kept empty. This was the 2nd retention test. A cut of time 3 min was kept[8].

Hebb-Williams Maze

Hebb- Williams Maze is an incentive –based exteroceptive behavioral model useful for measuring spatial working memory of rodents. HWM consists of mainly three components, (i) animal (mice) chamber (or start box), which is attached to (ii) the middle chamber (or exploratory area) and (iii) a reward chamber at the other end of the maze in which reward (food) is kept. All the three components were provided with guillotine removable doors. The animals were divided into 3 groups and were treated as below:

Group I received normal vehicle (0.5% carboxymethylcellulose, oral), Group II received extract (100 mg/kg, oral) and Group III received positive control (piracetam, 200 mg/kg, i.p). Time taken by the animal to reach reward chamber (TRC) was recorded after 24 hr for the animals of all the groups.

On the first day (i.e. 15th day of extract administration), the mouse was placed in the animal chamber and the door was opened to facilitate the entry of animal into the next chamber. The door of the start box was closed immediately after the animal moved into the next chamber so as to prevent back entry. Time taken by the animal to reach reward chamber (TRC) from start box on 1st day reflected the learning index. Each animal was allowed to explore the maze for 3 min with all the doors opened before returning to home cage .Retention (memory score) of this learned task was examined 24 hr after the first- day trial. Significant reduction in TRC value indicated improvement of memory[9].

Radial Arm Maze

The animals (rats) were divided into 3 groups and were treated as follows:

Group I received normal vehicle (0.5% carboxymethylcellulose, oral), Group II received extract (100 mg/kg, oral) and Group III received positive control (piracetam, 200 mg/kg, i.p). Time taken by the animal to reach reward arm was recorded after 24 hr for the animals of all the groups.

Locally fabricated wooden radial arm maze elevated 50 cm above the floor consisted of an octagonal central hub 36 cm in diameter with eight radial arms. Each arm was 46 cm long, 15 cm wide with 12 cm sides, and had small black plastic cups mounted at 30 cm from the central hub. Each rat maintained at 85% of its total diet, was exposed to the maze daily with the food pellet in a fixed arm followed by respective drug treatment for the period of 7 days. The apparatus was cleaned with damp cloth after each trial to avoid place preference and the influence of olfactory stimuli.

The evaluation was carried out on 7th day 24 hr after the respective drug treatment wherein a food pellets was placed in a variable arm for evaluation of working memory. Each rat placed on the central hub was allowed to choose any of the arms freely to get the food. Latency to find food was recorded as a measure of working memory evaluation. The comparison was made against the vehicle treated control and the data was expressed as mean ± SEM[10].

Statistical Analysis

The data were analysed using ANOVA and Student's (unpaired) t-test. Kruskal-Wallis one-way ANOVA followed by multiple range tests was used for the analysis of non-normally distributed data. $p<0.05$, $p<0.01$, $p<0.001$ were considered as significant, more significant, most significant respectively.

RESULTS AND DISCUSSION

At present, cognitive disorders emerged as major public health problems so the role of brain neurochemical systems in cognitive functioning is a subject of increasing research interest. Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed. In today's stressful and competitive world, poor memory and lower retention are common. Age, stress, emotions are conditions that may lead to memory loss, amnesia, anxiety, high blood pressure, dementia, or to more ominous threats like schizophrenia and Alzheimer's disease. Since the allopathic system of medicine has so far to offer a fundamental treat for memory damage, it is

meaningful to look for new guidelines, which would diminish the memory loss of patients with neuropsychiatric disorders.

Since olden period, plants have been an ideal basis of medication. Ayurveda and other Indian journalism reveal the use of plants in dealing various human diseases. *Ficus religiosa,* which is planted all over India, is a sacred tree and it is well known for its therapeutic purposes. From the previous findings and also from the phytochemical analysis of *Ficus religiosa*, it may be stated that the plant might have possessed nootropic activity and so the study was carried out in its leaves. The preliminary phytochemical evaluation and TLC analysis of the ethanolic extract of the leaves of *Ficus religiosa* showed prominent presence of sterols, glycosides, tannins, amino acids indicating the role of these phytochemicals in the observed effect. Based upon this, the present study was designed.

Table.1 shows the Transfer latency (TL) of *Ficus religiosa* leaves on Elevated-Plus Maze by scopolamine-induced amnesia in mice. Scopolamine (Sco) administered before retrieval significantly ($p<0.001$) increased the TL in scopolamine control compared to vehicle control on the first day (after 30 min) indicating the induction of retrograde amnesia. On the other hand, the animals pretreated with *Ficus religiosa* extract showed a reduction in TL compared to scopolamine control, indicating a significant ($p<0.01$) attenuation of retrograde amnesia. Piracetam control significantly ($p<0.01$) improved memory and reversed the retrograde amnesia induced by scopolamine as compared to scopolamine control.

Similarly, on the second day (after 24 hr), the TL in scopolamine control significantly ($p<0.001$) increased as compared to the normal control when scopolamine is administered before retrieval. Also the animals pretreated with *Ficus religiosa* extract showed a reduction in TL compared to scopolamine control indicating more significant ($p<0.001$) attenuation of retrograde amnesia. Piracetam control significantly ($p<0.001$) improved memory and reversed the retrograde amnesia induced by scopolamine as compared to scopolamine control. The result is more significant on the second day than that on the first day.

Table.2 shows the Step through latency of *Ficus religiosa* leaves by scopolamine induced amnesia in mice. Scopolamine administered before retrieval significantly ($p<0.001$) decreased the step through latency in scopolamine control as compared to control vehicle on the second day (after 24 hr), indicating the induction of retrograde amnesia. On the other hand, the animals pretreated with *Ficus religiosa* leaves extract showed an increased in step through latency on the second day compared to vehicle control, indicating a significant ($p<0.01$) attenuation of retrograde amnesia. Piracetam control significantly ($p<0.01$) improved memory and reversed the retrograde amnesia induced by scopolamine as compared to scopolamine control.

Table.3 shows the Retention test of *Ficus religiosa* leaves on Sodium nitrite intoxication by sodium nitrite induced hypoxic condition in mice. The result of the above study showed that the mean time required to locate the water bottle increased significantly ($p<0.001$) in sodium nitrite control as compared to control vehicle after 24 hr. While prior treatment with the extract and Mentat significantly ($p<0.05$) reversed the effect of sodium nitrite compared to sodium nitrite after 24 hr. Thus the study showed that the extract improved the learning capabilities of the animals in hypoxic condition as indicated by a better performance of animals in the learning task.

Table 4 showed the memory enhancing activities of *Ficus religiosa* leaves extract. The extract was found to produce significant ($p<0.001$) increased in memory at a dose of 100mg/kg when compared to the normal control.

In Radial Arm Maze (Table 5), the activities of *Ficus religiosa* leaves extract showed significant ($p<0.001$) memory enhancement when compared to the normal control. The leaf extract of *Ficus religiosa* at a dose of 100 mg/kg was comparable to that of piracetam (200 mg/kg).

The present study revealed that, the leaf extract of *Ficus religiosa* significantly possessed anti-amnesic and nootropic properties. The active constituents such as amino acids present in *Ficus religiosa* leaves may be involved in anti-amnesic and memory enhancing activities. It was previously found that the presence of phytochemicals likes sterols, glycosides, amino acids and tannins were responsible for nootropic activity[3, 11]. From the above, it was concluded that, the leaf extract of *Ficus religiosa* may be useful as a nootropic agent in the treatment of various cognitive disorders. However further isolation and identification of the active constituents present in the leaves of *Ficus religiosa* are still required to postulate the precise underlying mechanisms.

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Table 1: Transfer latency (TL) of *Ficus religiosa* leaves on Elevated-Plus Maze by scopolamine-induced amnesia in mice.

DRUG TREATMENT	TRANSFER LATENCY (sec)	
	After 30 min	After 24 hr
Group I Normal Control (0.5 %CMC)	12.0±1.22***	37.6±2.49***
Group II Scopolamine(1mg/kg, i.p)	50.0±3.53	220.0±7.89
Group III Scopolamine (1mg/kg, i.p) + *Ficus Religiosa* (100mg/kg, p.o)	17.6±2.50**	40.0±1.58***
Group IV Scopolamine (1mg/kg, i.p) + Piracetam (200mg/kg, i.p)	15.8±1.16**	38.0±2.54***

Values are expressed as mean ± SEM (n=5).
p<0.001*** and p<0.01** Vs scopolamine control

Table 2: The Step through latency of *Ficus religiosa* leaves on Step through passive avoidance test by scopolamine induced amnesia in mice.

DRUG TREATMENT	STEP THROUGH LATENCY (sec)
	After 24 hr
Group I Normal Control (0.5 %CMC)	296.0±1.86***
Group II Scopolamine(1mg/kg, i.p)	150.0±2.73
Group III Scopolamine(1mg/kg, i.p) + *Ficus Religiosa* (100mg/kg, p.o)	263.0±5.37**
Group IV Scopolamine(1mg/kg, i.p) + Piracetam(200mg/kg, i.p)	267.0±5.59**

Values are expressed as mean ± SEM (n=5).
p<0.001*** and p<0.01** Vs scopolamine control

Table 3: Retention test of *Ficus religiosa* leaves on Sodium nitrite intoxication by sodium nitrite induced hypoxic condition in mice.

DRUG TREATMENT	RETENTION TEST(sec)
	After 24 hr
Group I Normal Control (0.5 %CMC)	82.0±1.81***
Group II Sodium nitrite (95 mg/kg, s.c)	168.8±2.81
Group III Sodium nitrite (95mg/kg, s.c) + *Ficus Religiosa* (100mg/kg, p.o)	116.0±5.08*
Group IV Sodium nitrite (95 mg/kg, s.c) + Mentat (100mg/kg, p.o)	119.0±2.90*

Values are expressed as mean ± SEM (n=5).
p<0.001*** and p<0.05* Vs sodium nitrite control

Table 4: Time taken to reach reward chamber for *Ficus religiosa* leaves on Hebb-Williams Maze.

DRUG TREATMENT	TIME TAKEN TO REACH REWARD CHAMBER (sec)
	After 24 hr
Group I Normal Control (0.5 %CMC)	125.0±3.52
Group II *Ficus Religiosa* (100mg/kg, p.o)	82.0±1.81***
Group III Piracetam(200mg/kg, i.p)	77.0±2.01***

Values are expressed as mean ± SEM (n=5).
p<0.001*** Vs normal control

Table 5: Time taken to reach reward arm for *Ficus religiosa* leaves on Radial Arm Maze.

DRUG TREATMENT	TIME TAKEN TO REACH REWARD ARM (sec)
	After 24 hr
Group I Normal Control (0.5%CMC)	58.6±0.50
Group II *Ficus Religiosa* (100mg/kg, p.o)	42.4±0.58***
Group III Piracetam (200mg/kg, i.p)	39.2±0.41***

Values are expressed as mean ± SEM (n=5).
p<0.001*** Vs normal control