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Research Article

PSYCHOPHARMACOLOGICAL INVESTIGATION OF THE NOOTROPIC POTENTIAL OF *TRIGONELLA FOENUM* LINN. IN MICE

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ABSTRACT

Aim of the study: *Trigonella foenum-graceum* L. (Leguminosae) was traditionally used in inflammation, diabetes, burns, eczema, gout, skin ulcers and oral insulin substitute. In this study, Psychopharmacological potential of *Trigonella foenum* seed powder (TFSP) for its nootropic activity was evaluated.

Materials and methods: Various concentrations of *Trigonella foenum* seed powder (TFSP) were assayed by using both interoceptive (Elevated Plus maze, Passive Avoidance Apparatus) and exteroceptive model (Diazepam or scopolamine induced amnesia) of amnesia.

Results: *Trigonella foenum* seed powder (TFSP: 5, 10 and 15%) shows the significant increased in memory by reduction of increased cholinergic transmission. Moreover, it also reduces cholesterol, glucose level and lipid peroxidation (MDA level) as compared to standard drug (Simvastatin, 5mg/kg, o.p.) in young mice which mainly trigger the process of deposition of amyloid plaques or neurofibrillary tangles in brain that leads to alzheimer's disease.

Conclusion: TFSP leads a significant decreased in cholinergic transmission, lipid peroxidation level brain and lowered the serum cholesterol, glucose level in mice accounts for its multifarious beneficial effects such as memory improving property, cholesterol lowering, anticholinesterase and antioxidant property.

Keywords: *Trigonella foenum*, Alzheimer's disease, Elevated plus maze, Nootropic, Anticholinergic, Hypolipidemic

INTRODUCTION

Alzheimer's is an irreversible, progressive, neurodegenerative disorder with a complex etiology & pathogenesis[1, 2]. It is characterized by the development of senile plaques and neurofibrillary tangles, which are associated with neuronal loss affecting to a greater extent cholinergic neurons [2]. Around 35 million patients suffered from alzheimer's disease all over the world[3]. An epidemiological study reveals that dementia is largely a hidden problem, especially in rapidly developing and heavily populated regions such as India, China and Latin America[4]. Dementia associated with alzheimer's disease is the most common cause of memory impairment or cognitive disability in elderly people[5, 6, 7]. Due to increase incidence of side effects of allopathic medicine (both nootropic and cholinesterase inhibitors) more research will be manifested towards the use of natural resources, e.g. medicinal plants for the management of various cognitive disorders. The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence: *Bacopa monniera*[8], *Withania somnifera*[9], *Centella asiatica*[10], *Convolvulus pluricaulis*[11], *Zingiber officinale*[12], *Pongomia pinnata*[13], *Nardostachys jatamansi*[14], *Papaver somniferum*[15], *Trikatu churna*[16], *Glycyrrhiza glabra*[17], *Myristica fragrans*[18], *Dactus carota*[19] as well as *Ocimum sanctum*[20].

Trigonella foenum-graceum is an annual plant form the family of Leguminosae. From thousand of year *Trigonella foenum* seeds have been used traditionally as a remedy for diabetes, gastric ulcer, hypercholesterolemia, diarrhoea & dysentery. Further research envagised proves to be used as immunomodulator[21], antihyperglycemic[22], anti-fertility[23], anti-inflammatory[24], antipyretic[25], galactogogue[26], antioxidant[27, 28], anticancer[29], antiplatelet[30], antihypertensive[31], cardio tonic[32], antibacterial[33, 34], antihistamanic[35], analgesic[36], anti-ulcer[37]. Various phyto-chemical investigation on the seeds reveals that presence of saponins, flavonol glycosides, amino acids and alkaloids may contribute, for its anti-diabetic, cardiotonic, antioxidant and anti-inflammatory activity[34, 38]. The present study was undertaken to investigate the nootropic potential of *Trigonella foenum* seed powder (TFSP) on mice.

MATERIALS AND METHODS

Plant material

The dried seeds of *Trigonella foenum* (Fenugreek) were purchased in the month of July from local market of Hisar, Haryana (India). The plant material was shade dried and ground into a fine paste using an electric grinder. Different concentrations of TFSP (5, 10, 15% w/w) were fed to separate groups of mice through a specially prepared diet. This special diet comprised of a mixture of TFSP, wheat flour kneaded with water, a small amount of refined vegetable oil and a pinch of salt (sodium chloride), to impart taste. Each animal consumed around 3gm/day of this specially prepared diet. Control animals received the normal diet consisting of wheat flour, kneaded with water, small amount of refined vegetable oil and a pinch of salt but without TFSP.

Animals

All the experiments were carried out using male, swiss albino mice procured from the disease-free small animal house of CCS Haryana Agricultural University, Hisar (Haryana), India. Adult (4-6 months old) mice weighing around 25 g and aged (12-15 months old) mice weighing around 35 g were used in the present study. The animals had free access to food and water, and they were housed in a natural (12 h each) light-dark cycle. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. Experiments were carried out between 0900 h and 1800 h. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

Drugs and Chemicals

The chemicals used in this study were obtained from following drug houses. Scopolamine hydrobromide (Sigma-Aldrich, U.S.A.), Diazepam injection (Calmpose, Ranbaxy, India), Donepezil (Sun Pharm, Gujrat) 5,5-dithiobis-2-nitrobenzoic acid (DTNB), Acetylcholine iodide, Eserine salicylate, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium lauryl sulphate, tris buffer hydrochloride, Thio-barbituric acid (Hi-Media, India),

Erba glucose diagnostic kit and Erba cholesterol diagnostic kit (Transasia Biomedicals Limited, Mumbai, India).

Vehicle

Scopolamine hydrobromide, diazepam, piracetam and donepezil were dissolved separately in normal saline and injected i.p. However, simvastatin was suspended with 0.5% carboxy-methyl cellulose sodium and given orally. Volume of oral administration and i.p. injection was 1ml/100g of mouse.

Acute Toxicity Studies

TFSP was administered orally at different doses (5-25% w/w) to mice with a specially prepared diet. TFSP was administered at the same time on each day (i.e. 8 AM- 9 AM). During the first four hours after the drug administration, the animals were observed for gross behavioral changes if any, for 7 days. Parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia and mortality were observed. The doses selected were 5, 10 and 15% w/w/day.

Exteroceptive Behavioural Models

Elevated Plus-Maze

Elevated Plus-Maze served as the exteroceptive behavioral model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory was followed as per the parameters described by the investigators working in the area of psychopharmacology[39]. The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (training) for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h after the first day trial[40, 41].

Passive Avoidance Paradigm

Passive Avoidance Behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of plexiglas**s**, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V, A.C.) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down placing all its paws on the grid floor, shocks were delivered for 15 seconds and the step-down-latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2-15 seconds during the first test were used for the second session and the retention test. The second session was carried out 90 minutes after the first test. During second session, if the animals stepped down before 60 seconds, electric shocks were delivered once again for 15 seconds. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 seconds and were subjected to retention test. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300 seconds[41, 42].

Biochemical Estimations

Collection of Blood and Brain samples

The animals were sacrificed by cervical decapitation under light anesthesia on the 15th day, 90 minutes after diet. Immediately after decapitation, the trunk blood was collected. Then whole brain was carefully removed from the skull. The collected blood was centrifuged at 3000 rpm for 15 minutes so as to separate the serum. The serum was used for estimation of total cholesterol and glucose levels. For the preparation of brain homogenate, fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9 % sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 minutes and the resultant cloudy supernatant liquid was used for estimation of brain acetyl cholinesterase (AChE) activity, malionaldehyde (MDA) and reduced glutathione (GSH) level.

Estimation of Brain Acetyl Cholinesterase

Brain acetyl cholinesterase activity (AChE) was measured by the method of colorimetric measurement[43]. 0.5 ml of the cloudy supernatant liquid was pipette out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB (5,5-dithiobis-2-nitrobenzoic acid) solution (10 mg DTNB in 100 ml of sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4ml portions were pipette out into two test tubes. Into one of the test tubes, 2 drops of serine solution was added. 1 ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipette out into both the tubes and incubated for 10 minutes at 30° C. The solution in the tube containing eserine was used for zeroing the colorimeter. The resulting yellow color was due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After calibrating the instrument, change in absorbance per minute of the sample was read at 420 nm[44].

Estimation of Brain Mailonaldehyde (MDA)

Malionaldehyde, indicator of lipid peroxidation was determined as described by Ohkawa *et al*, with slight modification. The reaction mixture consisted of 0.2 ml of 8.1 % sodium lauryl sulphate, 1.5 ml of 20 % acetic acid (pH-3.5) and 1.5 ml of 0.8 % aqueous solution of thio-barbituric acid was added to the 0.2 ml of processed brain homogenate. The mixture was made up to 4.0 ml with distill water and heated at 95°C for 60 minutes. After cooling with tap water, 5 ml of n-butanol and pyridine (15:1 v/v) and 1 ml of distill water was added and centrifuged. The organic layer was separated out and its absorbance was measured at 532 nm using a UV-Visible spectrophotometer and MDA content was expressed as mole/mg of protein[45, 46]. Tissue protein was estimated using Lowry method of protein assay.

Estimation of Brain Reduced Glutathiuone (GSH)

GSH estimation in brain homogenate was measured according to the Ellman method. This method is based on the development of a yellow color when 5,5 -dithio-bis-2-nitrobenzoic acid (DTNB) is added to the compound containing the sulfhydryl groups. To the 0.5 ml of brain homogenate was mixed with 1.5 ml of 0.2 M Tris buffer (pH-8.2) and 0.1 ml of 0.01 M DTNB and this mixture was brought to 10.0 ml with 7.9 ml of absolute methanol. The above reaction mixture is centrifuged at approximately 300 g at room temperature for 15 minutes. The absorbance of supernatant was read in a spectrophotometer against reagent blank (without sample) at 412 nm. Tissue protein was estimated using Lowry method of protein assay[45].

Estimation of Serum Total Cholesterol Level

CHOD-PAP method was used for the estimation of serum total cholesterol. In this method, the blank sample, standard sample and test sample were pipette into the respective reaction vessels using a micro pipette[47, 48, 49]. For the blank sample, 20 µl of distilled water and 1000 µl of working reagent were mixed. For the standard sample, 20 µl of standard cholesterol and 1000 µl of working reagent, while for the test sample, 20 µl of serum and 1000 µl of working reagent were mixed. These mixtures were incubated for 10 minutes at 37° C. The absorbance was read at 510 nm and 630 nm (Filter 1 and Filter 2) against the blank sample by using Auto-analyzer (Erba Mannheim Chem-5 plus V_2).

Estimation of Blood Glucose Level

GOD-POD method was used for the estimation of blood glucose using Auto-analyzer. In this method, the blank sample, standard sample

and test sample was pipette into the respective reaction vessels using a micropipette[50]. For the blank sample, 10 µl of distilled water and 1000 µl of working reagent were mixed. For the standard sample, 10 µl of standard glucose and 1000 µl of working reagent, while for the test sample, 10 µl of serum and 1000 µl of working reagent were mixed. These mixtures were incubated for 15 minutes at 37° C. The absorbance was read at 510 nm and 630 nm (Filter 1 and Filter 2) against the blank sample by using Auto-analyzer (Erba Mannheim Chem-5 plus V_2)

Statistical Analysis

All the results were expressed as Mean ± Standard Error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. Values of $P < 0.05$ were considered as significant change.

RESULTS

Acute Toxicity Study

No mortality was observed following oral administration of TFSP even with the highest dose 25 % w/w. However at doses more than 20 % w/w produced profuse Black watery stools in animals. The above selected doses of TFSP had no toxic effect on the normal behavior of the rats.

Effect on TL (Using Elevated plus maze)

Mice readily accepted the specially prepared diet containing TFSP, when compared to their normal diet. Transfer latency (TL) on second day reflected retention of learned task or memory. The young mice fed with specially prepared diet containing 5, 10 and 15 % w/w of TFSP showed a dose dependent ($p<0.01$) reduction in TL of 15th day indicating significant improvement of memory, when compared with control groups (Fig 1). These concentrations of TFSP 5, 10 and 15 % w/w of diet also produced significant memory improvement ($p<0.01$) in aged mice (Fig 2). The acute single dose pretreatment of scopolamine (0.4 mg/kg i.p.) and diazepam (1 mg/kg, i.p.) before training significantly increased ($p<0.01$) the TL on 15th day indicating impairment of memory. The mice treated chronically for 15 successive days with TFSP (5, 10 and 15 % w/w of diet) revised successfully scopolamine (Fig 3) as well as diazepam induced amnesia (Fig 4). Piracetam (used as positive control) at the dose of 400 mg/kg, i.p. also improved memory ($p<0.01$) of both young and aged mice and reversed the amnesia induced by scopolamine and diazepam as expected.

Effect on SDL (Using Passive Avoidance Paradigm)

Step Down Latency (SDL) (15th day of drug treatment) reflected long term memory of animals. The young mice fed with diet containing 5, 10 and 15 % w/w of TFSP for 15 days, showed a dose dependent ($p<0.01$) increase in SDL of 15th day when compared with control group (Fig 5). The acute single dose pretreatment of scopolamine (0.4 mg/kg i.p.) and diazepam (1 mg/kg, i.p.) before training significantly decreased ($p<0.01$) the SDL of 15th day indicating impairment of memory. The mice treated chronically for 15 successive days with TFSP (5, 10 and 15 % w/w of diet) countered successfully scopolamine (Fig 6) as well as diazepam induced amnesia (Fig 6). Piracetam (used as positive control) at the dose of 400 mg/kg i.p. also improved memory ($p<0.01$) of both young and aged mice and reversed the amnesia induced by scopolamine and diazepam as expected.

Effect on Brain Cholinesterase Activity

TFSP (5, 10 and 15 % w/w of diet for 15 days) showed profound reduction in brain cholinesterase level in young and aged mice, as compared to respective control groups by using Ellman's kinetic colorimetric method (Fig. 7). The percentage reductions in cholinesterase activity in young mice were 19.85 % ($p<0.05$), 23.09 % ($p<0.01$) and 28.24 % ($p<0.01$) at various concentrations of TFSP (5, 10 and 15 % w/w) of diet. Donepezil (0.1 mg/kg i.p.), a standard AChE inhibitor produced 33.7 % inhibition of AChE enzyme activity where as the percentage inhibition of reductions of cholinesterase activity were 15.55 %, 23.79 % ($p<0.01$) and 39.26 % ($p<0.01$) at respective concentrations of TFSP 5, 10 and 15 % w/w of diet in aged mice.

Effect on Total Cholesterol Level

The animals receiving TFSP (5, 10 and 15 % w/w of diet p.o.) for 15 days consecutively showed significant reduction in total cholesterol levels in young and as well as aged mice (Fig. 8). The extent of reduction of cholesterol in young mice were found to be 8.43 %, 16.35 % ($p<0.01$) and 30.93 % ($p<0.001$) at doses of TFSP 5, 10 and 15 % of diet respectively. Simavastatin, a standard cholesterol lowering agent evoked reduction in cholesterol levels 45.78 % ($p<0.001$). The extent of reduction in total serum cholesterol levels of aged mice were 9.07 %, 17.76 % ($p<0.001$) and 25.52 % ($p<0.001$) at doses of TFSP (5, 10 & 15 %) of diet respectively ($p<0.001$).

Effect on Serum Glucose Level

The animals receiving TFSP (5, 10 and 15 % w/w of diet) for 15 days consecutively showed significant ($p<0.01$) reduction in blood glucose levels of young and aged mice, when compared to the respective control groups (Fig. 9). The extent of reduction in serum glucose levels of young mice were 8.12 %, 19.47 % ($p<0.01$) and 28.34 % ($p<0.01$) at the concentrations of 5, 10 and 15 % w/w of TFSP respectively. Similarly the reduction in blood glucose level of aged mice were 11.27 % ($p<0.05$), 15.92 % ($p<0.01$) and 28.84 % ($p<0.01$) at the concentrations of 5, 10 & 15 % w/w of TFSP, when compared to control group of aged mice.

Effect on Brain Malionaldehyde Level

The animal receiving the TFSP (5, 10 and 15 % w/w of diet) showed the significant decreased in the brain malionaldehyde level of both young and aged mice as compared to control group. The extent of reduction were 9.29 %, 24.12 % ($p<0.01$) and 15.69 % ($p<0.01$) of young mice at the concentration of 5, 10 & 15 % w/w of TFSP when compared to control group. In the aged mice, reduction were 15.95 % ($p<0.05$), 20.75 % ($p<0.01$) and 31.90 % ($p<0.01$) at 10, 20 & 30 % w/w respectively (Fig. 10).

Effect on Brain Reduced Glutathione Level

TFSP show a remarkable increase in brain reduced glutathione level in the young and aged mice. The percent decline in the reduced glutathione level were 19.56 % ($p<0.01$) at TFSP concentration of 5 % w/w of diet, 36.26 % ($p<0.01$) and 43.93 % ($p<0.01$) at TFSP concentration of 10 & 15 % w/w in young mice. In the aged mice percent reduction were 22.78 % ($p<0.05$), 36.70 % ($p<0.01$) & 46.83 % ($p<0.01$) at 5, 10 and 15% w/w respectively (Fig. 11).

DISCUSSION

In the present study *Trigonella foenum* seeds (5-15 % w/w), when fed with normal diet for 15 day improve the memory of mice reflected by diminished TL as well as enhanced SDL valves when compared to control group. Furthermore TFSP administration protected the mice from the development of memory deficits observed after scopolamine/diazepam treatment.

Biochemical estimation of different parameter as mentioned above show the elevation of acetylcholine level by significant reduction of acetyl cholinesterase activity in brain and decreased level of serum cholesterol and glucose level of young and aged mice. Furthermore TFSP administration decreased the increase potential of MDA level, an indicator of lipid per oxidation index and increased level of reduced glutathione a potential element of free radical scavenging cycle in the brain as compared to control group of young and aged mice. Therefore, it appears that *Trigonella foenum* seeds may possesses the memory improving capacity or useful in the treatment of the disorder related to memory deficits specially Alzheimer's disease, in the view of its (i) AChE inhibitory activity (ii) cholesterol and glucose lowering activity (iii) on the basis of its antioxidant property a significant decreased in MDA level and sharp increase in antioxidant process by increase in reduced glutathione level in mice brain.

Drug protocol

A total of 312 mice divided in 52 different groups were employed in the present investigation. Each group comprised of a minimum of 6 animals.

Group I	Control group for young mice. Normal specially prepared diet (without TFSP) was fed for 15 successive days. TL was recorded 90 minutes after the specially prepared diet of day 15th and retention was examined after 24 h (i.e. on 16th day).
Group II	Positive control for young mice. Piracetam (400mg/kg) was injected to young mice for 7 successive days. TL was recorded after 60 minutes of i.p. Injection on seventh and retention was recorded after 24 h (i.e. on 8th day).
Groups III, IV and V	TFSP (5, 10 and 15 % w/w, respectively) mixed in specially prepared diet was fed for 15th successive days to young mice. TL was noted 90 minutes after the specially prepared diet of day 15th and after 24 h (i.e. on 16th day).
Group VI	Scopolamine alone group. Normal specially prepared diet (without TFSP) was fed for 15th successive days to young mice. Scopolamine (0.4 mg/kg) was injected i.p. at 90 minutes after the specially prepared diet of day 15th and TL was recorded 45 minutes after the injection. Retention was examined after 24 h (i.e. on 16th day).
Group VII	Piracetam (400 mg/kg) was injected to young mice for 7 successive days. At 60 minutes after the injection of piracetam on the seventh day, Scopolamine (0.4 mg/kg) was injected i.p. TL was recorded 45 minutes after the injection of Scopolamine. Retention was examined after 24 h (i.e. on 8th day).
Group VIII,IX and X	TFSP (5, 10 and 15 % w/w, respectively) mixed in specially prepared diet was fed for 15th successive days. Scopolamine (0.4 mg/kg) was injected intraperitoneally to young mice at 90 minutes after the specially prepared diet of day 15th. TL was recorded 45 minutes after the injection and after 24 h (i.e. on 16th day).
Group XI	Diazepam alone group. Normal specially prepared diet (without TFSP) was fed for 15th successive days to young mice. Diazepam (1 mg/kg) was injected i.p. at 90 minutes after the specially prepared diet of day 15th and TL was recorded 45 minutes after the injection. Retention was examined after 24 h (i.e. on 16th day).
Group XII	Piracetam (400 mg/kg) was injected to young mice for 7th successive days. At 60 minutes after the injection of piracetam on the seventh day, Diazepam (1 mg/kg) was injected i.p. TL was recorded 45 minutes after the injection of Diazepam (1 mg/kg). Retention was examined after 24 h (i.e. on 8th day).
Groups XIII, XIV and XV	TFSP (5, 10 and 15 % w/w, respectively) mixed in specially prepared diet was fed for 15th successive days. Diazepam (1 mg/kg) was injected i.p. 90 minutes after the specially prepared diet of day 15th. TL was recorded 45 minutes after the injection and after 24 h (i.e. on 16th day).
Group XVI	Control group for aged mice. Normal specially prepared diet (without TFSP) was fed for 15th successive days. TL was recorded 90 minutes after the specially prepared diet of day 15th and retention was examined after 24 h (i.e. on 16th day).
Group XVII	Positive Control for aged mice. Piracetam (400 mg/kg) was injected to aged mice for 7 successive days. TL was recorded after 60 minutes of i.p. Injection on seventh day and retention was recorded after 24 h (i.e. on 8th day).
Groups XVIII, XIX and XX	TFSP (5, 10 and 15 % w/w, respectively) mixed in specially prepared diet was fed for 15th successive days to aged mice. TL was noted 90 minutes after the specially prepared diet of day 15th and after 24 h (i.e. on 16th day).
Group XXI to XL	Separate groups were assigned for observations using passive avoidance apparatus on the similar lines of elevated plus maze.
Group IXL	Control group for young mice. Normal specially prepared diet (without TFSP) was fed for 15th successive days. The animals were sacrificed 90 minutes after the specially prepared diet of day 15th. The blood and brain samples were obtained for estimation of brain cholinesterase, malionaldehyde, reduced glutathione and blood glucose & total cholesterol levels.
Group VIIIL	Control group for aged mice. Normal specially prepared diet (without TFSP) was fed for 15th successive days. The animals were sacrificed 90 minutes after the specially prepared diet of day 15th. The blood and brain samples were obtained for estimation of brain cholinesterase, blood glucose and total cholesterol levels.
Group VIIL	Donepezil (0.1 mg/kg i.p.), an anti-cholinesterase agent (standard drug) was injected to young mice, 60 minutes before dissecting the animals for estimation of brain cholinesterase levels.
Group VIL	Donepezil (0.1 mg/kg i.p.), was injected to aged mice, 60 minutes before dissecting the animals for estimation of brain cholinesterase levels.
Group VL	Simvastatin (5 mg/kg), a cholesterol-lowering agent (standard drug) was given orally to young mice for 7 successive days. The animals were dissected for estimation of total cholesterol levels after 90 min of drug administration i.e. on seventh day.
Group IVL	Simvastatin (5 mg/kg), was given orally to aged mice for 7 successive days. The animals were dissected for estimation of total cholesterol levels after 90 minutes of drug administration i.e. on seventh day.
Group IIIL,IIL and IL	TFSP (5, 10 and 15 % w/w, respectively) mixed in specially prepared diet was fed to young mice for 15th successive days. The animals were sacrificed 90 minutes after the specially prepared diet of day 15th. The blood and brain samples were obtained for estimation of brain cholinesterase, blood glucose and total cholesterol levels.
Group L, LI and LII	TFSP (5, 10 and 15 % w/w, respectively) mixed in specially prepared diet was fed to aged mice for 15th successive days. The animals were sacrificed 90 minutes after the specially prepared diet of day 15th. The blood and brain samples were obtained for estimation of brain cholinesterase blood glucose and total cholesterol levels.

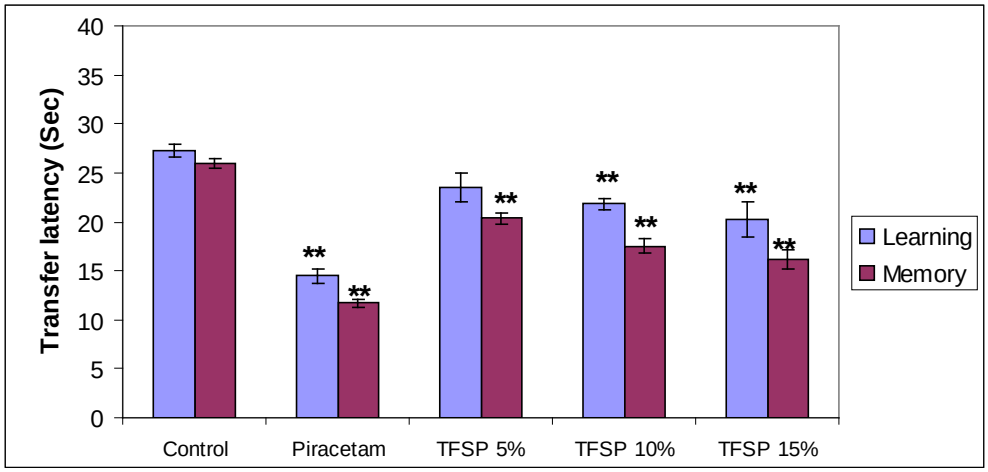

Fig. 1: Effect of TFSP (5, 10 and 15 % w/w) on the transfer latency of young mice using elevated plus maze

Valves are in mean ± SEM (n=6): **denotes p < 0.01 as compared to control group of young mice. (One-way ANOVA followed by Dunnett's t-test).

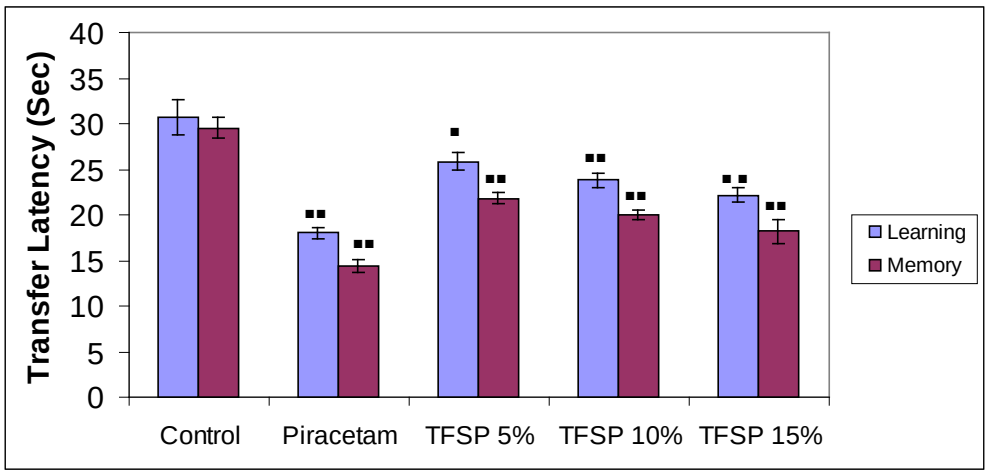

Fig. 2: Effect of TFSP (5, 10 and 15 % w/w) on the transfer latency of aged mice using elevated plus maze

Valves are in mean ± SEM (n=6): ▪denotes p < 0.05 as compared to control group of aged mice. ▬denotes p < 0.01 as compared to control group of aged mice. (One-way ANOVA followed by Dunnett's t-test).

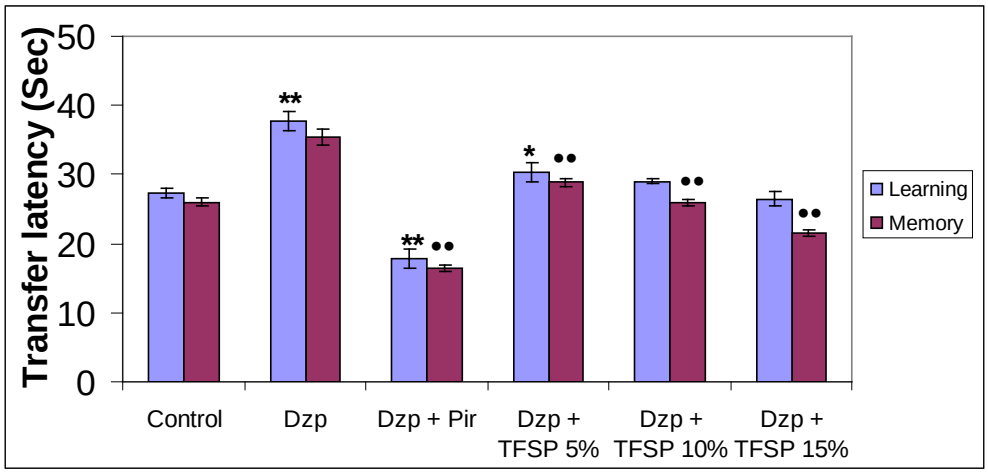

Fig. 3: Effect of TFSP (5, 10 and 15 % w/w) on diazepam induced amnesia in young mice using elevated plus maze.

Valves are in mean ± SEM (n=6): *denotes p < 0.05 as compared to control group of young mice. **denotes p < 0.01 as compared to control group of young mice. ●●denotes p < 0.01 as compared to Diazepam group of young mice. (One-way ANOVA followed by Dunnett's t-test).

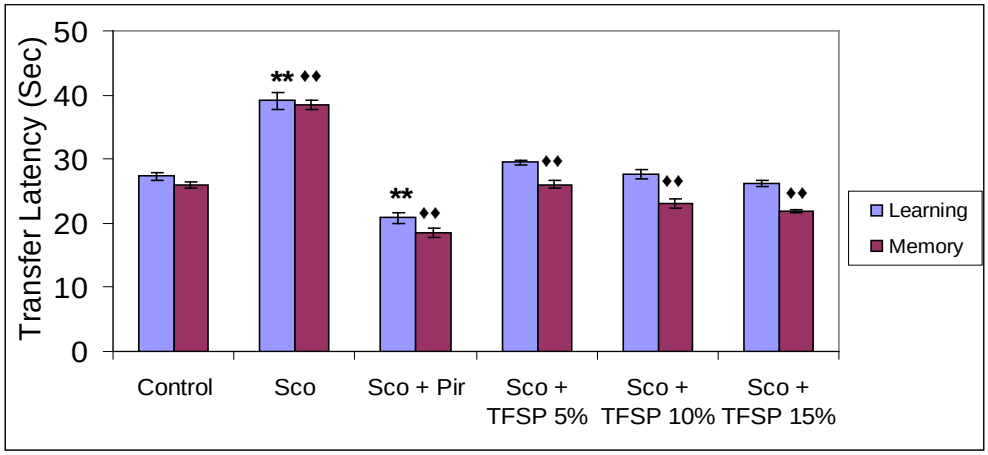

Fig. 4: Effect of TFSP (5, 10 and 15 % w/w) on scopolamine induced amnesia in young mice using elevated plus maze

Valves are in mean ± SEM (n=6): **denotes p < 0.01 as compared to control group of young mice. ♦♦denotes p < 0.01 as compared to Scopolamine group of young mice. (One-way ANOVA followed by Dunnett's t-test).

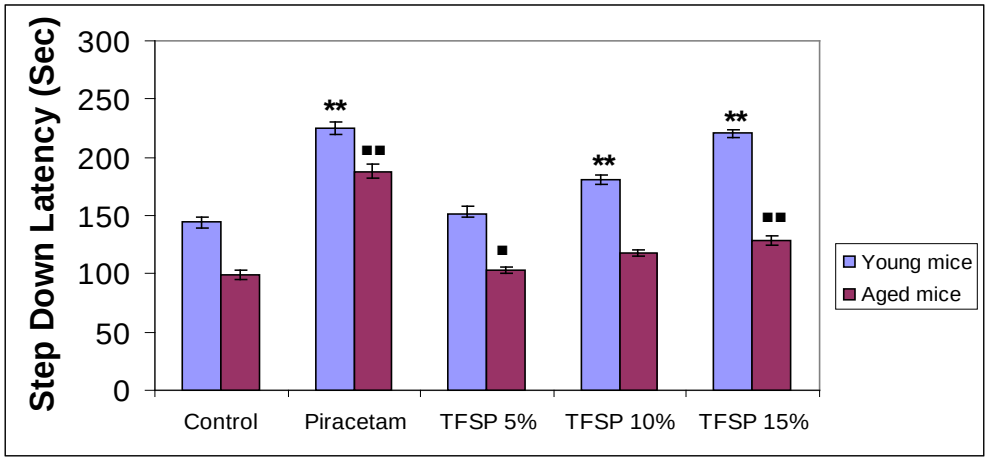

Fig. 5: Effect of TFSP (5, 10 and 15 % w/w) on the step down latency of young & aged mice using passive avoidance apparatus

Valves are in mean ± SEM (n=6): * & ■denotes p < 0.05 as compared to control group of young & aged mice. ** & ■■ denotes p < 0.01 as compared to control group of young & aged mice. (One-way ANOVA followed by Dunnett's t-test).

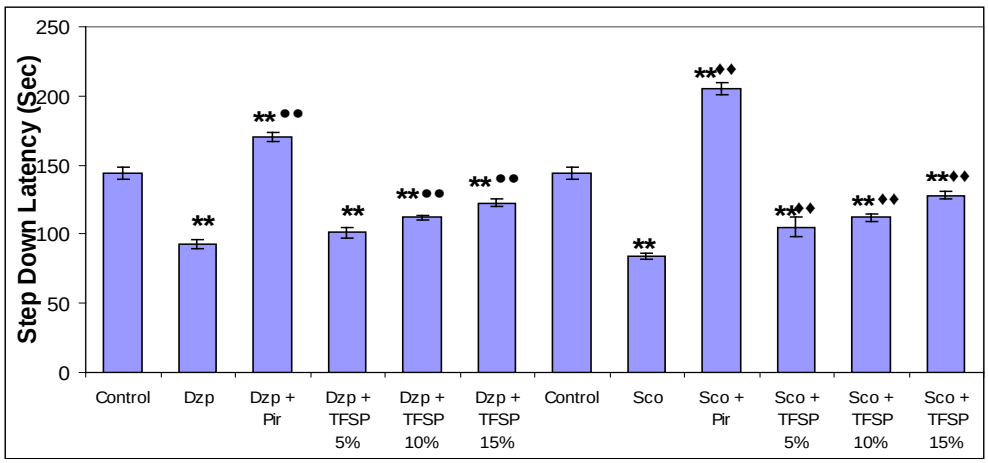

Fig. 6: Effect of TFSP (5, 10 and 15 % w/w) on the diazepam or scopolamine induced amnesia in young mice using passive avoidance apparatus

Valves are in mean ± SEM (n=6): * denotes p <0.05 as compared to control group of young mice. ** denotes p < 0.01 as compared to control group of young mice. ●●denotes p < 0.01 as compared to Diazepam group of young mice. ♦♦denotes p < 0.01 as compared to Scopolamine group of young mice. (One-way ANOVA followed by Dunnett's t-test).

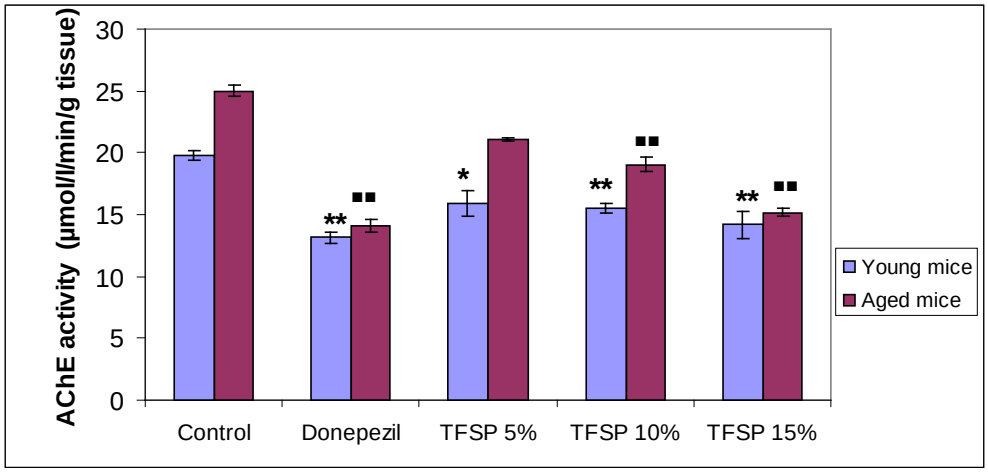

Fig. 7: Effect of TFSP (5, 10 and 15 % w/w) on brain acetyl cholinesterase activity of young and aged mice

Valve are expressed as Mean ± SEM, (n=6). * & ■denotes $p < 0.05$ when compared to control group of Young & aged mice. ** & ■denotes $p < 0.01$ when compared to control group of Young & aged mice. (One-way ANOVA followed by Dunnett's t-test).

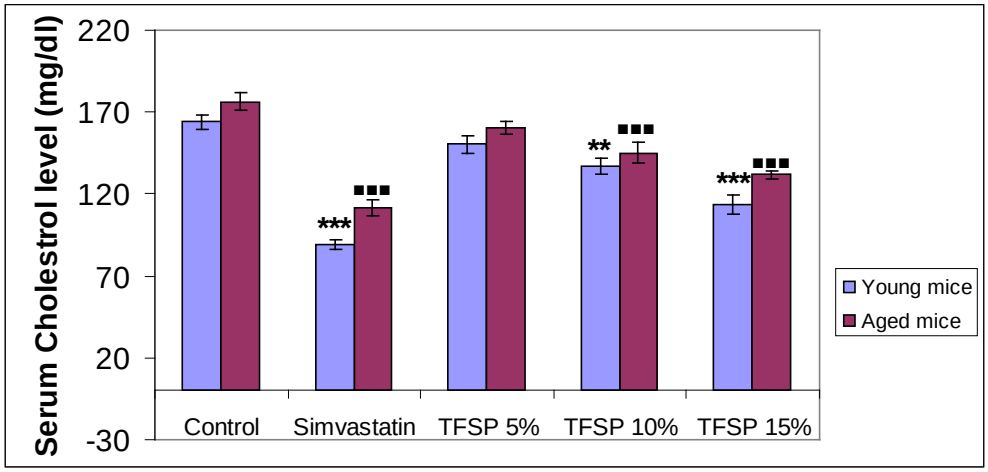

Fig. 8: Effect of TFSP (5, 10 and 15 % w/w) on serum cholesterol level of young and aged mice

Valve are expressed as Mean ± SEM, (n=6). * & ■denotes $p < 0.05$ when compared to control group of Young & aged mice. ** & ■denotes $p < 0.01$ when compared to control group of Young & aged mice. (One-way ANOVA followed by Dunnett's t-test).

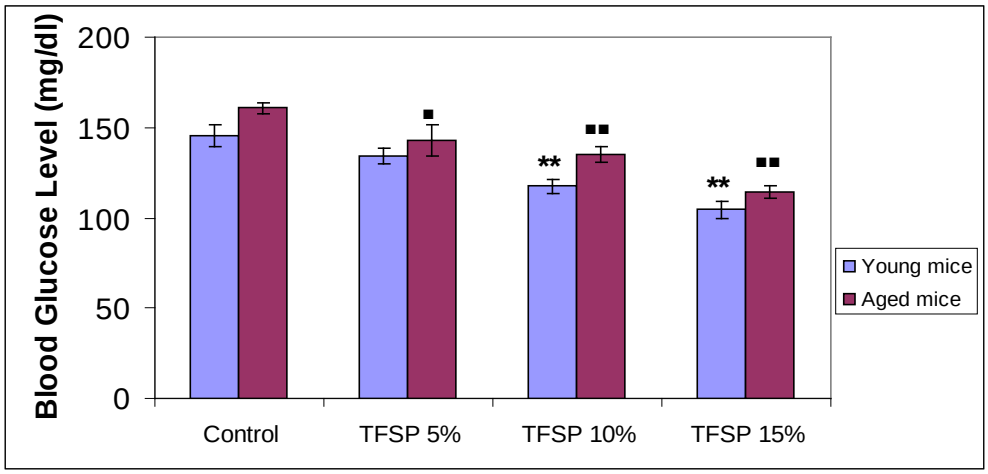

Fig. 9: Effect of TFSP (5, 10 and 15 % w/w) on blood glucose level of young and aged mice

Valve are expressed as Mean ± SEM, (n=6). * & ■denotes $p < 0.05$ when compared to control group of Young & aged mice. ** & ■denotes $p < 0.01$ when compared to control group of Young & aged mice. (One-way ANOVA followed by Dunnett's t-test).

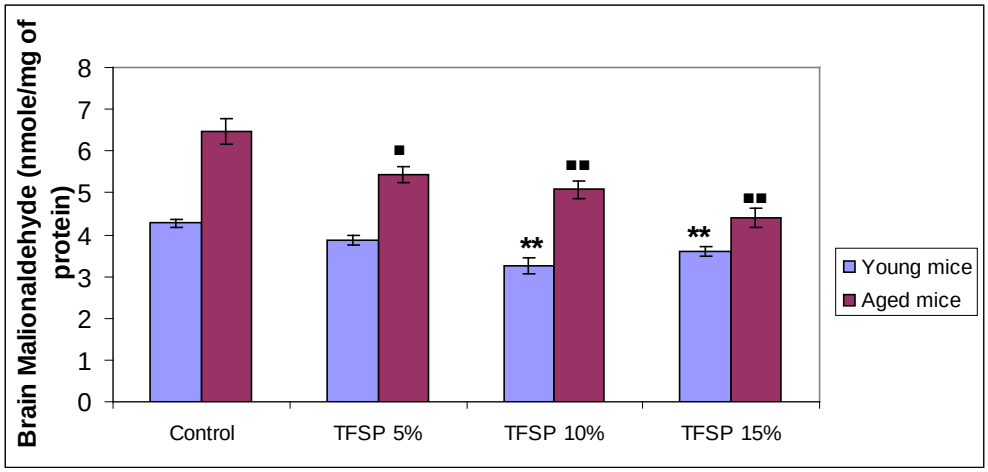

Fig. 10: Effect of TFSP (5, 10 and 15 % w/w) on brain malionaldehyde level of young and aged mice

Valve are expressed as Mean ± SEM, (n=6). * & ■denotes $p < 0.05$ when compared to control group of Young & aged mice. ** & ■denotes $p < 0.01$ when compared to control group of Young & aged mice. (One-way ANOVA followed by Dunnett's t-test).

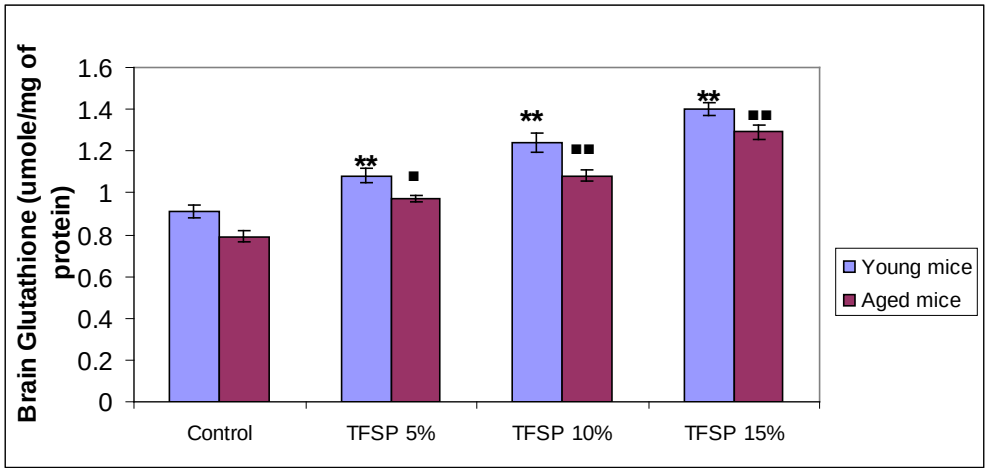

Fig. 11: Effect of TFSP (5, 10 and 15 % w/w) on brain glutathione level of young and aged mice

Valve are expressed as Mean ± SEM, (n=6).* & ■denotes $p < 0.05$ when compared to control group of Young & aged mice.** & ■denotes $p < 0.01$ when compared to control group of Young & aged mice. (One-way ANOVA followed by Dunnett's t-test.

CONCLUSION

TFSP (5, 10 & 15 % w/w) were fed orally with a specially prepared diet for 15 days consecutively to mice showed a dose dependent improvement in memory of young as well as aged mice. TFSP also successfully reversed the memory deficits induced by scopolamine and diazepam. Furthermore TFSP leads a significant decreased in cholinergic transmission, lipid peroxidation level brain and lowered the serum cholesterol, glucose level in mice accounts for its multifarious beneficial effects such as memory improving property, cholesterol lowering, anticholinesterase and antioxidant property.

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