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Research Article

EVALUATION OF NOOTROPIC ACTIVITY OF *Trigonella foenum* LEAVES IN MICE

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ABSTRACT

Alzheimer's disease is a chronic, progressive, neurodegenerative brain disorder that occurs gradually and characterized by disturbance of multiple cortical functions including memory, judgment, orientation, comprehension, learning capacity and language. The present study was undertaken to evaluate the nootropic potential of leaves of *Trigonella foenum* Linn. Various concentrations of *Trigonella foenum* leaf powder (TFLP) was administered along with the specially prepared diet for 15 successive days to different groups of young and aged mice. The learning and memory scores were assessed by using both interoceptive and exteroceptive model of amnesia. *Trigonella foenum* leaf powder produced a significant dose dependent improvement in memory of young and aged mice. The feeding of TFLP for 15 successive days showed a marked decrease in brain acetyl cholinesterase, malondialdehyde and increase in reduced glutathione level. Apart from this, it also decreases the serum cholesterol and glucose levels in mice. Moreover, it also reversed the amnesia produced by scopolamine, diazepam and natural ageing process. Thus, the nootropic potential of *Trigonella foenum* may be attributed to (i) lowered serum cholesterol and glucose levels (ii) increased cholinergic transmission in mouse brain (iii) decreased lipid per-oxidation and elevated the reduce glutathione level in mouse brain and therefore ultimately improved memory of both young and aged mice. Therefore, it can be of enormous use in the management of mental disorders like Alzheimer's disease.

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

INTRODUCTION

Stressful lifestyle in this competitive world may be the root cause of mental illness such as alzheimer and other memory related disorders. Dementia associated with alzheimer has gripped human population world over and it has become an important area of research interest. Alzheimer's disease is an irreversible, chronic, progressive neurodegenerative brain disorder characterized by the development of senile plaques and neurofibrillary tangles, which are associated with neuronal loss affecting cholinergic neurons to a greater extent and results in memory loss, unusual behavior, personality changes and ultimately death[1-2]. Nootropics are a class of psychotropic drugs with selective facilitatory effect on integrative functions of the central nervous system, mainly on intellectual performance, learning capability and memory. Nootropic agents such as piracetam[3], pramiracetam, aniracetam[4] and choline esterase inhibitors like Donepezil are presently used for improving memory, mood and behavior. However, 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 for the management of various cognitive disorders.

Trigonella foenum-graceum (L) is commonly known as fenugreek, alholva, menthi, trigonella and methi in India. The plant has traditionally been used as carminative, demulcent, expectorant, laxative, and stomachic. It has also been used topically for abscesses, boils, burns, eczema, gout and ulceration of the skin. Moreover, further research envagised that the plant also possess immunomodulatory[5], anti-fertility[6] antihyperglycemic[7], antipyretic[8], anti-inflammatory[9], galactogogue[10], antioxidant[11-12], anticancer[13], antiplatelet[14], antihypertensive[15], Cardio-tonic[16], antibacterial[17-18], antihistamanic[19], analgesic[20], anti-ulcer[21] activity. Therefore, the aim of present work was to investigate the nootropic potential of *Trigonella foenum* leaf powder in mice.

MATERIALS AND METHODS

Plant material

The dried leaves of *Trigonella foenum* 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 TFLP (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 TFLP, 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 3 gm/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 TFLP.

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 7 days to the laboratory conditions before behavioral experiments. Experiments were carried out between 0900h and 1800h. The experimental protocol (Table 1) 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-dithio-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. 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

TFLP was administered orally at different doses (5-25 % w/w) to mice with a specially prepared diet. TFLP was administered at the

same time on each day (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 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 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[22-24].

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 plexiglass, 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. 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[24-25].

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 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 Cholinesterase

Brain cholinesterase activity was measured by the method of colorimetric measurement[26]. The 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-dithio-2-nitrobenzoic acid) solution (10 mg DTNB in 100 ml of sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4 ml portions were pipette out into two test tubes. Into one of the test tubes, 2 drops of eserine solution was added. 1ml 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 min of the sample was read at 420 nm[27].

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 nmole/mg of protein[28-29]. Tissue protein was estimated using Lowry method of protein assay.

Estimation of Brain Reduced Glutathione (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-2-nitrobenzoic acid 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 0.01M 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[28].

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 pipetted into the respective reaction vessels using a micro pipette[30-32]. 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 autoanalyzer (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[33]. 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.

Table1: Experimental protocol of animals

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 TFLP) 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 min of i.p. Injection on seventh and retention was recorded after 24h (i.e. on 8th day).
Groups III, IV and V	TFLP (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 TFLP) 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 min 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	TFLP (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 TFLP) 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 min 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	TFLP (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 TFLP) 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 min of i.p. Injection on seventh day and retention was recorded after 24h (i.e. on 8th day).
Groups XVIII, XIX and XX	TFLP (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 TFLP) 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 TFLP) 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 min 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 min 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 90min of drug administration i.e on seventh day.
Group IIIL, IIL and IL	TFLP (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	TFLP (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.

RESULTS

Acute Toxicity Study

No mortality was observed following oral administration of TFLP 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 TFLP 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 TFLP, when compared to their normal diet. Transfer latency 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 TFLP 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 TFLP 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 TFLP (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.

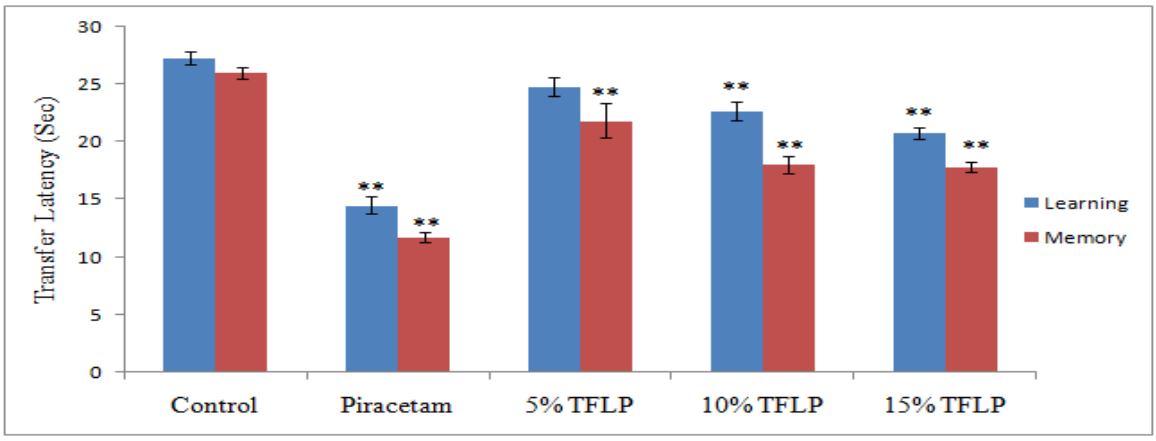

Fig. 1: Effect of TFLP (5, 10 and 15 % w/w) on the transfer latency of young mice using elevated plus maze

Values 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).

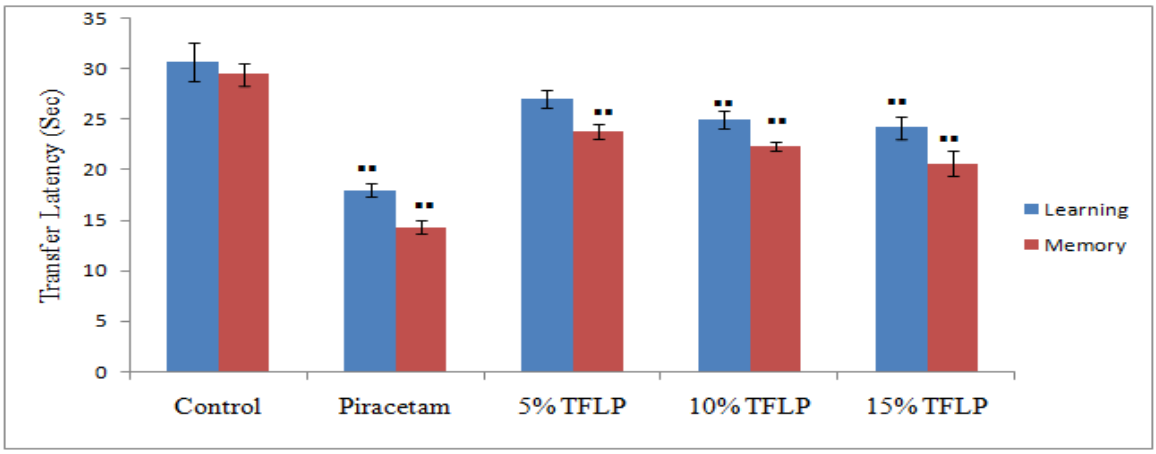

Fig. 2: Effect of TFLP (5, 10 and 15 % w/w) on the transfer latency of aged mice using elevated plus maze

Values 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).

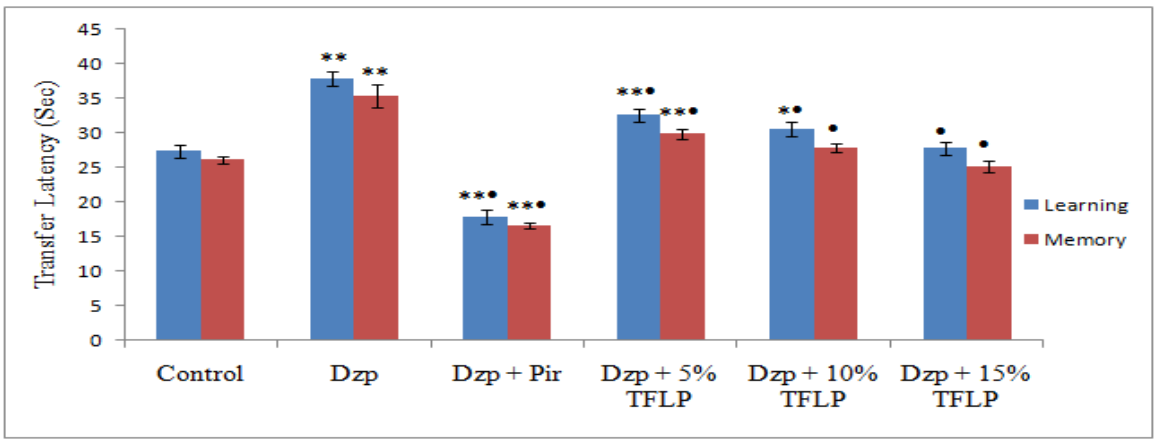

Fig. 3: Effect of TFLP (5, 10 and 15 % w/w) on diazepam induced amnesia in young mice using elevated plus maze.

Values 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).

Effect on SDL (Using Passive Avoidance Paradigm)

Step down latency (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 TFLP 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 TFLP (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.

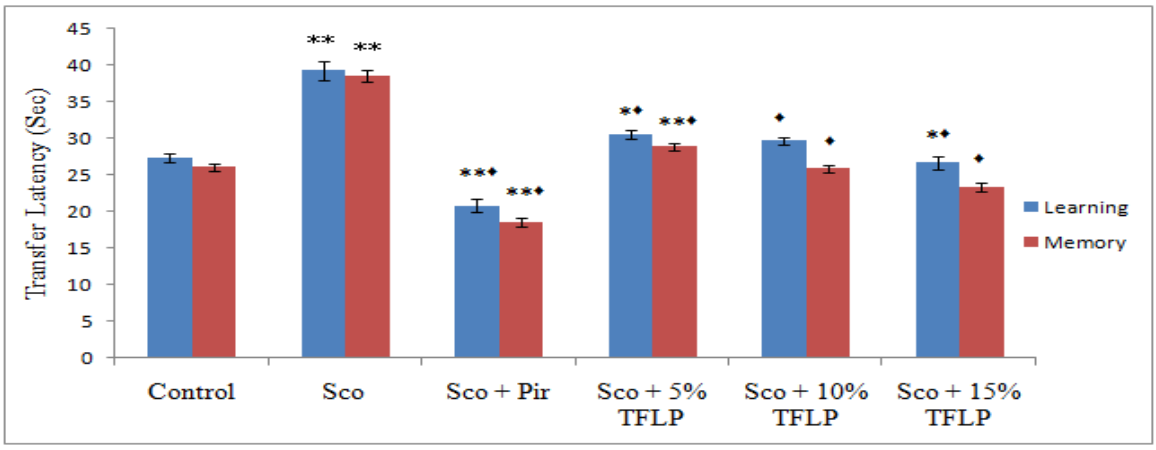

Fig. 4: Effect of TFLP (5, 10 and 15 % w/w) on scopolamine induced amnesia in young mice using elevated plus maze

Values 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).

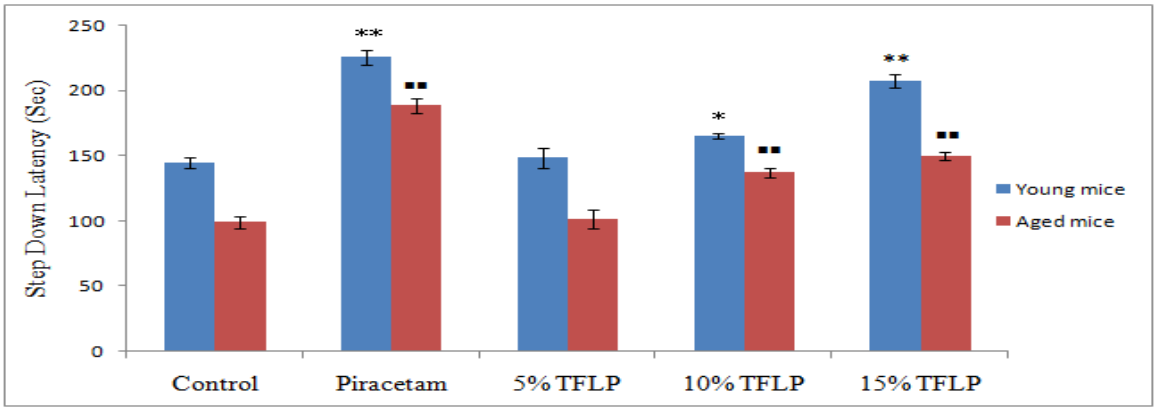

Fig. 5: Effect of TFLP (5, 10 and 15 % w/w) on the step down latency of young & aged mice using passive avoidance apparatus

Values 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).

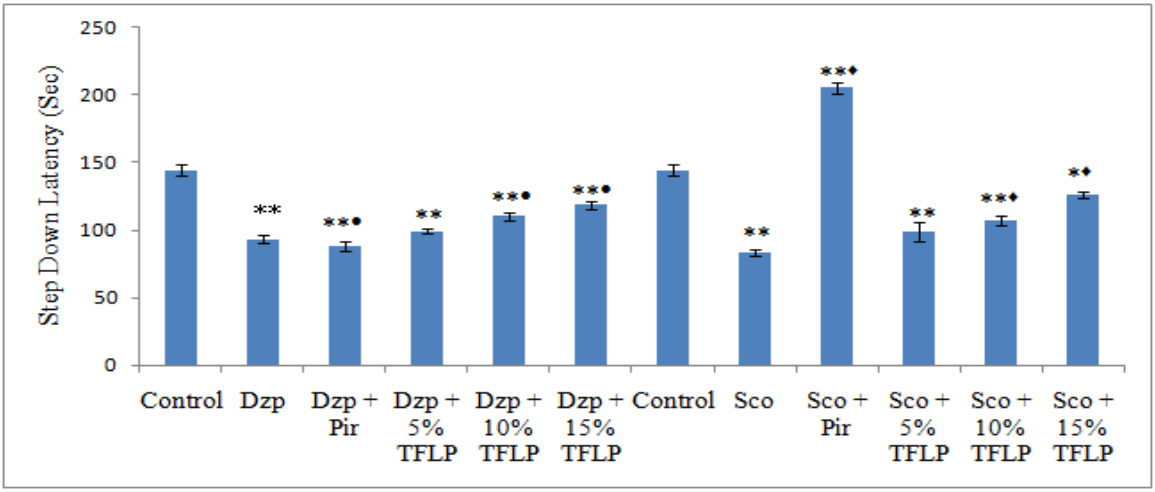

Fig. 6: Effect of TFLP (5, 10 and 15 % w/w) on the diazepam or scopolamine induced amnesia in young mice using passive avoidance apparatus

Values 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).

Effect on Brain Acetyl Cholinesterase Activity

TFLP (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 22.38 % ($p<0.01$), 26.68 % ($p<0.01$) and 30.26 % ($p<0.01$) at various concentrations of TFLP (5, 10 and 15 % w/w) of diet. Donepezil (0.1 mg/kg i.p.), a standard AChE inhibitor produced 33.7 % and 43.6 % inhibition of AChE enzyme activity in young and aged mice respectively where as the percentage inhibition of reductions of cholinesterase activity were 25.10 %, 30.8 % ($p<0.01$) and 34.97 % ($p<0.01$) at respective concentrations of TFLP 5, 10 and 15 % w/w of diet in aged mice.

Effect on Total Cholesterol Level

The animals receiving TFLP (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 5.20 %, 22.75 % ($p<0.001$) and 33.40 % ($p<0.001$) at doses of TFLP 5, 10 and 15 % of diet respectively. Simavastatin, a standard cholesterol lowering agent evoked reduction in cholesterol levels 45.78 % ($p<0.001$) and 36.76 % ($p<0.001$) in young and aged mice respectively. The extent of reduction in total serum cholesterol levels of aged mice were 9.68 %, 22.95 % ($p<0.001$) and 28.75 % ($p<0.001$) at doses of TFLP (5, 10 & 15 %) of diet respectively ($p<0.001$).

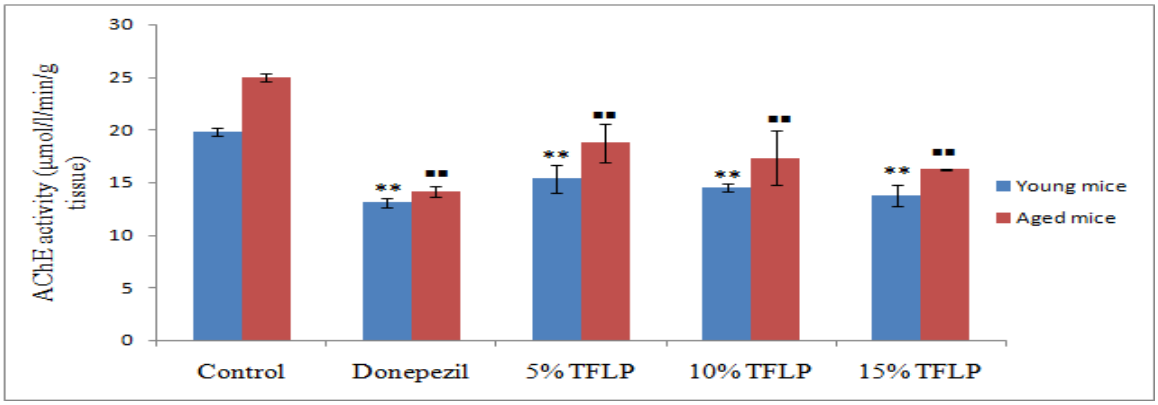

Fig. 7: Effect of TFLP (5, 10 and 15 % w/w) on brain acetyl cholinesterase activity of young and aged mice

Values 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).

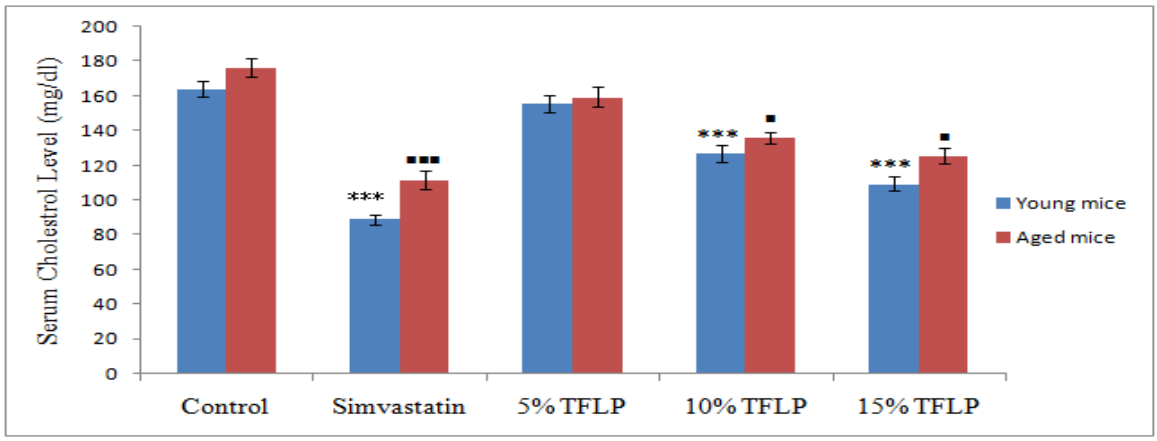

Fig. 8: Effect of TFLP (5, 10 and 15 % w/w) on serum cholesterol level of young and aged mice

Values 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).

Effect on Serum Glucose Level

The animals receiving TFLP (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 12.29 %, 19.45 % ($p<0.01$) and 30.31 % ($p<0.01$) at the concentrations of 5, 10 and 15 % w/w of TFLP respectively. Similarly the reduction in blood glucose level of aged mice were 8.58 % ($p<0.05$), 17.86 % ($p<0.01$) and 26.05 % ($p<0.01$) at the concentrations of 5, 10 & 15 % w/w of TFLP, when compared to control group of aged mice.

Effect on Brain Malionaldehyde Level

The animal receiving the TFLP (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 11.70 % ($P<0.05$), 33.45 % ($p<0.01$) and 46.46 % ($p<0.01$) of young mice at the concentration of 5, 10 & 15 % w/w of TFLP when compared to control group. In the aged mice, reduction were 13.77 % ($p<0.05$), 25.38 % ($p<0.01$) and 33.90 % ($p<0.01$) at 5, 10 & 15 % w/w respectively (Fig. 10).

Effect on Brain Reduced Glutathione Level

TFLP show a remarkable increase in brain reduced glutathione level in the young and aged mice. The percent decline in the reduced glutathione level were 9.89 % ($p<0.05$) at TFLP concentration of 5 % w/w of diet, 28.57 % ($p<0.01$) and 40.56 % ($p<0.01$) at TFLP concentration of 10 & 15 % w/w in young mice. In the aged mice percent reduction were 20.25 % ($p<0.05$), 37.97 % ($p<0.01$) & 40.50 % ($p<0.01$) at 5, 10 and 15 % w/w respectively (Fig. 11).

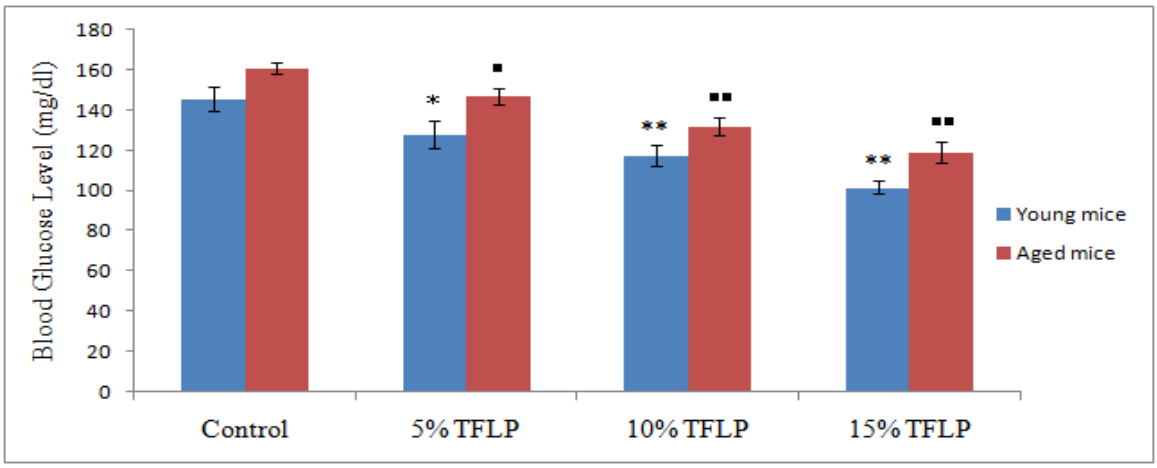

Fig. 9: Effect of TFLP (5, 10 and 15 % w/w) on blood glucose level of young and aged mice

Values 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).

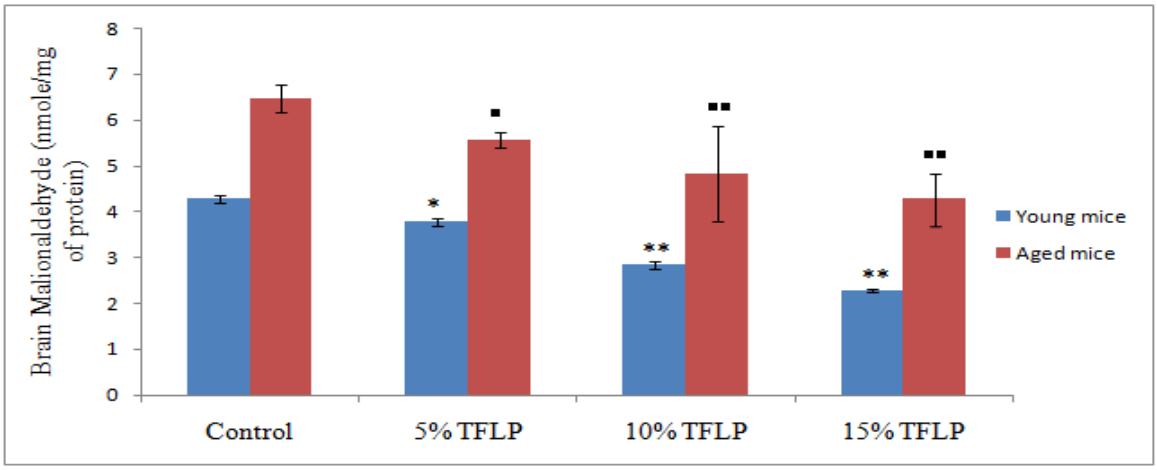

Fig. 10: Effect of TFLP (5, 10 and 15 % w/w) on brain malionaldehyde level of young and aged mice

Values 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).

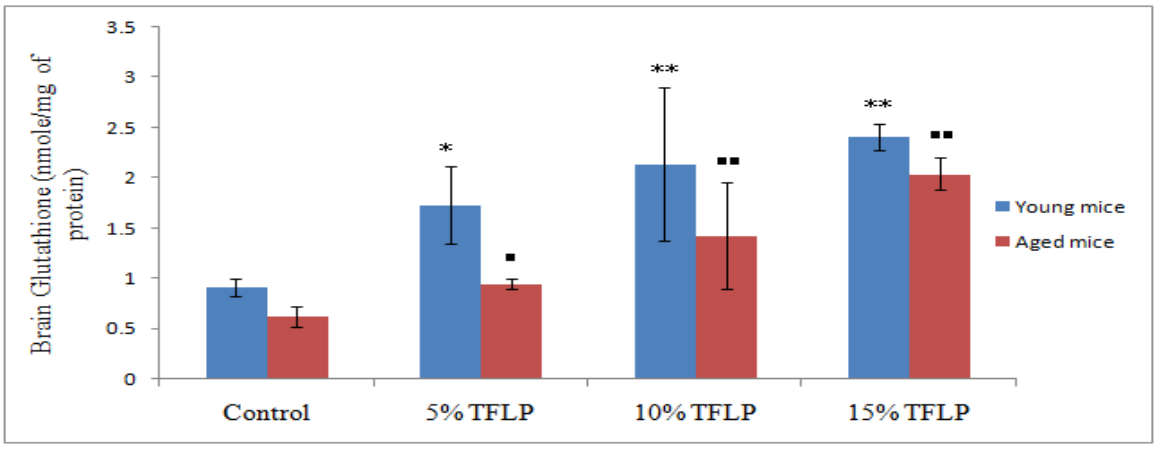

Fig. 11: Effect of TFLP (5, 10 and 15 % w/w) on brain glutathione level of young and aged mice

Values 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).

DISCUSSION

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

Biochemical estimation of different parameter 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, TFLP administration decreased the increase potential of MDA level an indicator of lipid peroxidation 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* leaves 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.

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