IJBPAS, July, 2020, 9(7): 1564-1581

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATING THE NOOTROPIC ACTIVITY OF *LEPIDIUM SATIVUM* AND *TRIGONELLA FOENUM-GRAECUM L.* COMBINED SEED EXTRACTS AND ITS HERBAL TABLET FORMULATION

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Received 29th Oct. 2019; Revised 28th Nov. 2019; Accepted 5th Jan. 2020; Available online 1st July 2020

https://doi.org/10.31032/IJBPAS/2020/9.7.5101

ABSTRACT

Neurodegenerative diseases became an encumbrance to the society, especially the geriatrics. Various plants are available in the literature having the protective, memory and cognitive enhancing properties. The current work has been taken to approach it through two of them. The study was aimed to screen the phytochemical, acute toxicity studies, nootropic activities (*In vivo*) of ethanolic extract of *Lepidium sativum* (LS) and *Trigonella foecum* (TF) and also to develop a solid dosage form using the combined ratio extracts (1: 1) with suitable excipients to get the synergistic/ complementary effects. Preliminary phytochemical investigation, acute toxicity study was evaluated and the therapeutic dose was fixed for the combined test ethanolic extracts. Combination (1:1) of extracts of ESLS and ESTF were screened for the effect on memory and cognition on mice both by discrimination learning and avoidance behaviour. The herbal tablets were formulated using the direct compression method and evaluated for pre and post compressional parameters. The results for the various

maze experiments showed statistically significant ($p<0.05$) improvement in both working and spatial memory in animals. The extract blend was formulated as tablets using suitable excipients are found to be within the range. From the results, it was found that the extracts are safe even at high doses (2000mg/kg body weight) and the behavioural studies results affirmed the nootropic effects of the extract combined mixture of ESLS and ESTF at a dose of 200 and 400mg/kg body weight.

Keywords: Nootropic, cognitive, memory, formulation, evaluation

1. INTRODUCTION

Though there are many advancements adopted in the healthcare systems, herbal medicine are of primary importance. Plant-based medicine is comparatively safe than the Allopathic based medicine.

Many species of *Lepidium* of family Brassicaceae have got the attention because of their white or pinkish flowers and seeds. Since they are having prominent pharmacological activities that can provide better health to humans to treat various ailments**[1]**. The phytochemical and pharmacological investigation on *Lepidium sativum* and related species indicate that the plant contains alkaloids (imidazole alkaloids like lepidine), glycosides (anthraquinone and cardiac glycosides), saponins, tannins (both hydrolysable and condensed tannins), flavonoids(both in free form and as glycosides), steroids etc **[2]**. The red-colored seeds of *Lepidium sativum* possess various properties such as are carminative, diuretic, aphrodisiac, galactagogue, emmenagogue, tonic, stimulant, mucilage is gastric protective and used to control diarrhea **[3]**. Some of the constituents present in the seeds like flavonoids and tannins are antioxidant, anticancer in nature. Presence of aminoacids, proteins, minerals, and carbohydrates in the seeds make it nutritive and so traditionally it is a functional food ingredient **[4]**.

Similarly, *Trigonella foenum-graecum L.* seeds are well known to Indians as a spice from centuries **[5]**. The dicotyledons of the seeds are reported to possess high proteins, saponins (like diosgenin and fenugrins), alkaloids (like trigonelline and gentianine), glycosides (rhaponticin), lipids, coumarins and polyphenolics like flavonoids (like isovitexin and rutin) **[6, 7]**.

As part of our continuing search for nootropic and neuro-protective plants, the phytochemical and pharmacological profile of *Lepidium sativum* (LS) and *Trigonella foenum* (TF) seeds initiated us to screen the synergistic effect on animals for their nootropic activity and formulation of herbal tablets followed by evaluation.

Herein, we describe the extraction, nootropic activity screening of ethanolic seed extracts of *Lepidium sativum* and *Trigonella foenum-graecum L.*, formulation and evaluation of herbal tablets.

2. MATERIALS AND METHODS

2.1. Plant material

Lepidium sativum (LS) and *Trigonella foenum* (TF) seeds were procured from the local suppliers and authenticated by Dr. K. Madhava Chetty, Plant taxonomist, Assistant Professor, Sri Venkateshwara University, Tirupathi and sample vouchers (0332 and 0453) were preserved at the herbarium for future references.

2.2. Extraction procedure

The seeds were dried in shade and powdered with a pulverizer. The powder was subjected to soxhlet extraction using ethanol (70%v/v). The resultant extract (LS and TF) solutions were evaporated to dryness using the rotary evaporator under vacuum and lyophilized in powdered form.

2.3. Preliminary phytochemical investigation

Phytochemical investigation of the ethanolic seed extracts for *Lepidium sativum* and *Trigonella foenum-graecum L.*, were done using standard protocols.

2.4. Treatment protocol

Swiss Albino Mice with about 25-35g were selected for this experiment. Mice were procured from Sainath agencies, Musheerabad. During the experiment, polypropylene cages were used for the maintenance of animals at the animal house. Animals were acclimatized to standard laboratory conditions for one week and freely allowed to feed on standard rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*. The temperature (25 ± 2^0C), relative humidity (60%±10%) and 12hr light/dark cycle were maintained throughout the experiment **[8]**. All the animals were grouped into five (control, negative control, standard, T1 and T2) having six animals in each group. Vehicle was served as control, Scopolamine hydrobromide 1 mg/kg, i.p. was used as the negative control to induce amnesia to the mice and the standard drug used was Piracetam 200mg/kg p.o. The combined ratio of ethanolic seed extracts (1:1) of *Lepidium sativum* and *Trigonella foenum-graecum* at 200 mg/kg, p.o (T1) and 400 mg/kg, p.o (T2) were taken as test groups.

2.4. Acute toxicity studies

Acute toxicity studies were carried out as per the OECD guidelines 423. All the standard laboratory conditions were maintained throughout the experiment. The study protocols were approved from the Institutional animal ethics committee (IAEC) before the commencement of

experimental studies (CPCSEA/ 1657/ IHECCMRCP/22/65).

2.5. Discrimination learning tasks

2.5.1. Radial arm maze

Radial arm maze is used to assess spatial memory and learning ability of animals **[9]**. It contains eight arms with 60×9 cm dimensions which are connected to a central platform having a diameter of 37 cm. the radial arms are colored with black and elevated 60 cm above the ground. Food cup is fixed at the end of each arm and arms were baited alternatively with food. The experiment chamber was arranged with extra maze cues like a clock, bench, chair, etc. The food was restricted to the animals before and during the experiment. Animals were trained by keeping them at the central area for five trials per day with five minutes interval time for five days. The entry of the animal is taken in consideration when all the limbs were completely entered into the arm.

The calculations for reference and working memories were recorded when the animal enters the non-baited arm (reference memory error-RME) and re-entering into the arm (working memory error-WME) and with food respectively. The animals that make not more than one trial or 2 errors in three trials during training were considered for the experiment.

2.5.2. Morris water maze test

Morris water is a convenient and simple method to evaluate the spatial memory in the animals. It is having a circular water pool (45×26 cm) with depth 20cm and the water temperature should be maintained at 26±1 ºC. The pool was labeled with four directions namely, N, E, S and W. Milk was added to the water to make the water non-transparent. An escape platform was arranged in the pool which is dipped 1 cm in the water in SW direction and kept unchanged during the experiment. The animals are allowed to find the platform only through stable distal spatial cues arranged at the testing room. Four different starting points (N, E, SE, NW) were made in the pool and all these starting were randomly used during the experiment.

In the experiment, the animal must find the platform within 2 minutes after placing on the starting point, facing towards the wall of pool. After reaching the platform, the animal is allowed for 30 seconds on the platform. Escape latency time (ELT) is to locate the hidden platform in the water maze was noted as an index of learning **[10]**.

2.5.3. Y-maze

Y-maze is a well-known model to evaluate the short-term memory in animals **[11]**. Since the arms are arranged in a way that it

looks like "Y". It is 40×10×12cm in dimensions with black color paint, elevated 200 cm above the ground and properly illuminated. The animals were placed at the end of one arm and observed for 8 minutes. The number entries (returning to the starting arm after visiting all other arms) attempted by the animal in the given interval time was recorded and percentage alternation was calculated.

2.5.4. Figure-8 maze

This model used to study the spatial ability of animals. The maze is having a square metallic body partitioned in a way that forms the shape "8" with a 12.7 cm corridor. The total system is located 750cm above the ground. The edges were fitted with mesh to avoid the escape of the animal from the maze. Four gates were also arranged to allow or prevent the entry of the animal and to get the reward like water. Animals are trained to make proper turn and will be rewarded with water by preventing their re-entry to the already visited side **[12]**.

2.5.5. T-maze

T-maze is used to assess the working memory in animals. It consists of three metallic arms in the shape "T". One long arm with dimensions 60x10x10cm joins two identical arms with dimensions 45x10x10cm. Animals were trained for 5 minutes for 5 days to explore the baited arm to get the reward. The animals that make the errors are not considered for the study. During the experiment, the number exploration burst was recorded with an interval of 10 minutes for six consecutive trials **[13]**.

2.6. Avoidance behavior tasks

2.6.1. Three-panel runway test

Three-panel runway apparatus is an advanced model to evaluate both reference and working memory. The apparatus is a wooden box consists of a star box, a goal box, and four successive choice points associated with a gate with three panels with 12 x 25 cm dimensions to allow or restrict the entry through the gates. Animals were trained to assess working memory by giving freedom to move in the apparatus and returning it to the home cage after every reward. But, in case of reference memory, the animals were restricted through gates to enter the already explored areas and forced to explore the baited area. The animals that fail to do so are considered as an error in on an average of six trials **[14]**.

2.6.2. Elevated plus-maze

The retention and memory can be assessed by the elevated plus-maze. The maze is made up of two open arms (50 × 10 cm) located opposite to each other and crossed with another two closed arms with the same length and width with a connected central

square (10 × 10 cm). The entire system was kept at 50cm height from the ground. On the first and second day, one animal at a time was placed on the edge of an open arm and time taken for (transfer latency) the mice to enter one of the closed arms was noted. The mice were allowed to stay in the open arm for 10-15 seconds and returned to the cage. On the 7th day, after the regular treatment with the dose again the transfer latency (TL) was noted **[15]**.

2.6.3. Two-way shuttle box

This is a familiar model to evaluate the learning by passive avoidance. The box (40×20×20) partitioned into two halves (dark and light compartments) with an opening at the middle to allow the free movement. The animals are trained by giving mild electric shock in the dark box if the animal is spending more than 300 seconds in that box and should return to the lightbox on its own. No shock is given to the control animals. The numbers of response latencies were measured and the second trial was done on the next day **[16]**.

2.7. Preparation of tablets

The extracts were blended with suitable excipients according to the composition mentioned in **Table 1**. All the ingredients were mixed in the increasing order of their weights in a mortar and pestle. The herbal tablets were prepared by direct compression method.

Table 1: Composition of tablets

Ingredients	Quantity (in mg) per one tablet (400mg)					
ESLS	150	150	150	150	150	150
ESTF	150	150	150	150	150	150
Ethyl cellulose	-	-	-	20	30	40
Carbapol	20	30	40	-	-	-
MCC	40	40	40	40	40	40
DCP	30	20	10	30	20	10
PEG 4000	10	10	10	10	10	10
Methyl Paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%

2.8. Evaluation of tablets

2.8.1. Pre-compressional studies

The powdered blend was studied for the following pre-compressional parameters to achieve better formulation with anticipated properties.

2.8.1.1. Angle of repose: It is measured by passing the powder through a funnel on a flat surface. The angle made by the powder pile was measured in θ.

Angle of repose (θ) = $\tan^{-1}$ 2h/d

2.8.1.2. Bulk density: Homogeneity of the powder blend can be measured by bulk density (ρb) values. It is the ratio of bulk mass (M) to bulk volume (Vb) of the powder.

ρb =M/Vb

2.8.1.3. Tapped density: It (ρt) is the ratio of the weight of the powder blend (M) to

the minimum volume occupied by the powder (Vt) in the measuring jar.

ρt =M/Vt

2.8.1.4. Compressibility indices

2.8.1.4.1. Carr's compressibility index: The percentage compressibility of the powdered blend can be measured by Carr's index (C) by using the formula

C= (Vt- Vb) ×100/Vt

where, Vb is the freely settled volume of a given mass of powder, and Vt is the tapped volume of the same mass of powder.

2.8.1.4.2. Hausner's ratio: The Hausner ratio is a number that is correlated to the flowability of a powder or granular material.

H=Vt/ Vb

where, Vb is the freely settled volume of a given mass of powder, and Vt is the tapped volume of the same mass of powder

2.8.2. Post-compressional studies

2.8.2.1. Physical appearance: Physical appearance of the prepared herbal tablets were studied organoleptically for their color, shape, odor, and texture.

2.8.2.2. Thickness: Average thickness (in mm) of the prepared tablets (6 tablets) were measured using Vernier calipers.

2.8.2.3. Weight variation: To check the uniformity of the prepared tablets, the average weight of 20 tablets were compared with the individual tablets.

2.8.2.4. Hardness: Monsanto hardness tester was used to check the crushing strength of the tablets by placing between the plungers and recorded in Kg/cm.

2.8.2.5. Friability: Roche friabilator was used to determine the physical strength and durability of the prepared tablets. The friabilator was rotated at 25 rpm for four minutes. Percentage of weight loss was calculated as

Percentage of friability= weight loss/ initial weight

2.8.2.6. Disintegration test: USP dissolution apparatus (paddle type, 8 stations) was used to measure the disintegration time in distilled water at 37ºC.

3. RESULTS AND DISCUSSIONS

3.1. Preliminary phytochemical investigation

Phytochemical investigation of the ethanolic seed extracts were screened using standard protocols and revealed the presence of various metabolites like alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrates, amino acids, proteins, lipids, and fatty acids.

3.2. Acute toxicity studies

Animals administered with acute doses of the combined extracts (1:1) of ESLS & ESTF did not develop any significant clinical signs of toxicity or mortality either immediately or during the post-treatment even at a high dose of 2000 mg/kg body

weight as per OECD-423 guidelines and doses were fixed as 200 and 400 mg/kg body weight.

3.3. Discrimination learning tasks

3.3.1. Radial arm maze

In the radial arm maze experiment, all the animals treated with *Lepidium sativum* (LS) and *Trigonella foenum* (TF) combined seed extract (200 and 400 mg/kg, p.o) and standard Piracetam exhibited significant cognitive property in a dose-dependent manner **(Table 2 and Figure 1)**. When compared to the standard control group (28.31±0.74) , the test group (43.56±0.57 and 48.69±1.03, $p<0.05$) showed decreased latency time to explore the baited arms and with fewer errors than the negative control group (68.14±0.8) and vehicle control groups (54.78±0.99) which support the memory-enhancing property of the given seed extract mixture.

3.3.2. Morris water maze (MWM) test

In the MWM test, the combined ethanolic seed extract of *Lepidium sativum* (LS) and *Trigonella foenum-graecum* (TF) have notable learning capability in mice in a dose-dependent manner **(Table 3 and Figure 2)** referenced with standard Piracetam The study reveals the combined extract is showing the decreased latency time in a dose dependent manner from 1st day to 14th day. At 200mg/kg, the chronic treatment was effective (67 sec) to improve the escape latency. Interestingly, the escape latency was statistically decreased significantly at higher concentration 400 mg/kg (62 sec) on 7th day only and showed escape latency time 42 seconds on 14th day which is almost near to the standard Piracetam (40 sec).

3.3.3. Y-maze

The spatial working memory was evaluated by spontaneous alternation behavior using Y-maze **(Table 4 and Figure 3** When compared to the control (89.18±0.75), the combined seed extracts (1:1) had shown a significant decrease in the percentage of spontaneous alternations in 200 and 400mg/kg as 52.24 ± 2.41and 55.32±3.32 respectively after 14 days treatment of the combined extract. The results reveal that the extracts had a significant enhancement of the retention of the memory from the first trial to the last day trial, suggesting the improved long term memory.

3.3.4. Figure-8 maze

From the figure-8 maze experiment data, **(Table 5 and Figure 4)** it is observed that the combined extracts (1:1) of *Lepidium sativum* (LS) and *Trigonella foenum-graecum* (TF) at a dose 200mg/kg (64.44 ± 3.47) showed decreased percentage of the correct response to explore the baited arms and with fewer errors which support the memory-enhancing property of the given

seed extract mixture. At higher concentration 400mg/kg, the combined ethanolic extract of LS and TF is showing 58.92±3.58 as percentage of the correct responses to explore the baited arms and significant to the decreased percentage of the correct response of Piracetam (reference standard) which is having 46.2± 9.57.

3.3.5. T-maze test

From the data **(Table 6 and Figure 5)**, it is apparent that the combined ethanolic extracts (1:1) of *Lepidium sativum* (LS) and *Trigonella foenum-graecum* (TF) have exhibited a significant ($P<0.05$) development in spatial memory of animals. When compared to the control (11.22±7.22), the combined extracts had shown a significant decrease in the number of spontaneous alternations in 200 mg/kg and 400 mg/kg as 13.47 ± 4.37 and 14.56±5.87 respectively after 7^{th} to 14^{th} days treatment of the extract The result suggests that the combined extracts had a significant enhancement of the retention of the memory from the first trial to the last day trial, suggesting the improved long term memory.

3.4. Avoidance behavior tasks

3.4.1. Three-panel runway test

In this test, the total number of working memory errors were significantly decreased ($p < 0.05$) by the administration of combined ethanolic seed extracts in a dose-dependent manner **(Table 7 and Figure 6)**. The extract blend decreased both the RME (4.01 at 200 and 4.87 at 400µg/mL) and WME (3.9 at 200 and 3.7 at 400µg/mL) which are less than the scopolamine (at 1 mg/kg, i.p.) treated group values WME (5.6) and RME (6.2). Whereas, the standard Piracetam showed WME (3.15) RME (5.01). Interestingly, the treatment is showing the decreased latency time in a dose dependent manner. At higher concentration 400 µg/mL, the extract blend is showing 28.2sec as latency time to explore the baited arms and significant to the decreased latency time of Piracetam which is having 27.9±9.3sec.

3.4.2. Elevated plus maze

In this study **(Table 8 and Figure 7)**, when compared to the control (21.13±0.65), the combined extracts (1:1) of ESLS & ESTF had shown a significant decrease in the latency time in 200 and 400mg/kg as 17.77±0.45 and 15.25±0.37 respectively after 14 days treatment of the combined seed extract. The results suggest that the extracts had a significant enhancement of the working memory errors and improvement in the latency from the first trial to the last day trial, indicating improvement in both learning ability and memory.

3.4.3. Two-way shuttle box test

From the two-way shuttle model on mice, the results **(Table 9 and Figure 8)** indicates that the combined extracts (1:1) of ESLS & ESTF have markedly increased the avoidance response (7.77±1.42 and 8.25±5.32) when compared to the negative control (2.13±2.65) after 7 days treatment of the extract. The improved avoidance response is statistically significant ($P<0.05$). From this data, it is apparent that the test combined seed extracts are having marked cognitive enhancing properties.

3.5. Evaluation of tablets

Herbal tablets were formulated by direct compression method **Table 1**. Various parameters have been evaluated for the prepared tablets of ESLS and ESTF extract.

3.5.1. Pre-compression studies

Pre-compression studies of the powdered blend were done and the results were depicted in **Table 10**. From the data it was found that angle of repose (θ) ranges between 29.3 to 32.8, bulk density 0.44 to 0.48 g/ml, tapped density 0.51 to 0.56 g/ml, Carr's index 7.98 to 17.91 and Hausner's ratio was found to be 1.07 to 1.29 respectively. It is also apparent from the above data that the blend was found to have better flow properties which helps to get content uniformity in the post-compression studies.

3.5.2. Post-compression studies

From the Post-compression studies, it is clear that the tablets that have been prepared by the direct compression method were uniform in their weight as well as in size, stable to mild abrasion and all the parameters were within the standard range. All the ingredients in the formulations helped to increase the bulkiness of the tablet to a satisfactory level. **(Table 11)**

Table 2: Effect of combined (1:1) of ESLS & ESTF on radial arm maze test

Group	Treatment	Latency to find food	WME	RME
1	Vehicle control	54.78±0.99	3	5
2	Negative control (Scopolamine 1 mg/kg, i.p.)	68.14±0.8#	5	9
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	28.31±0.74*	3	5
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	43.56±0.57*	4	7
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	48.69±1.03*	4	8

The results were expressed as mean ± SEM; the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
***p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test.**

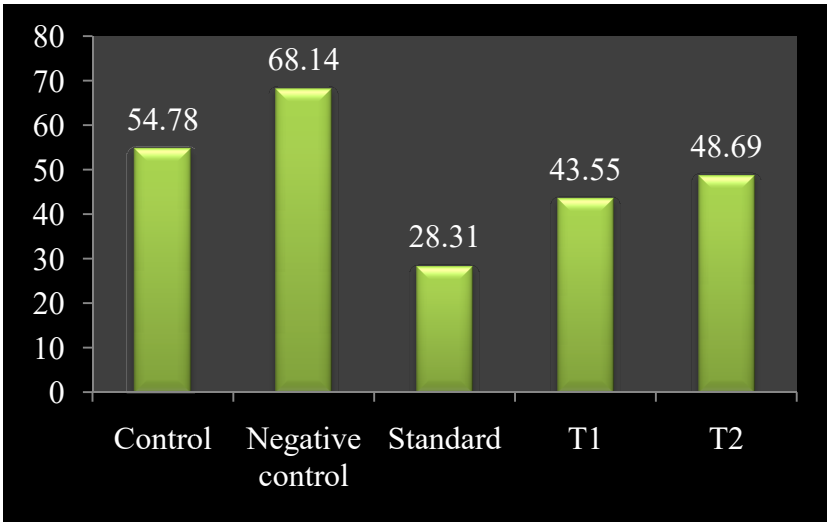

Figure 1: Effect of combined (1:1) of ESLS & ESTF on radial arm maze test

Table 3: Effect of combined (1:1) of ESLS & ESTF using Morris water maze test

Group	Treatment	Escape latency (sec)		
		1st day	7th day	14th day
1	Vehicle control	120	119	121
2	Negative control (Scopolamine 1 mg/kg, i.p.)	121	135	149#
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	115	50*	40*
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	118	84	67*
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	116	62*	42*

The results were expressed as mean ± SEM, the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
***p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test.**

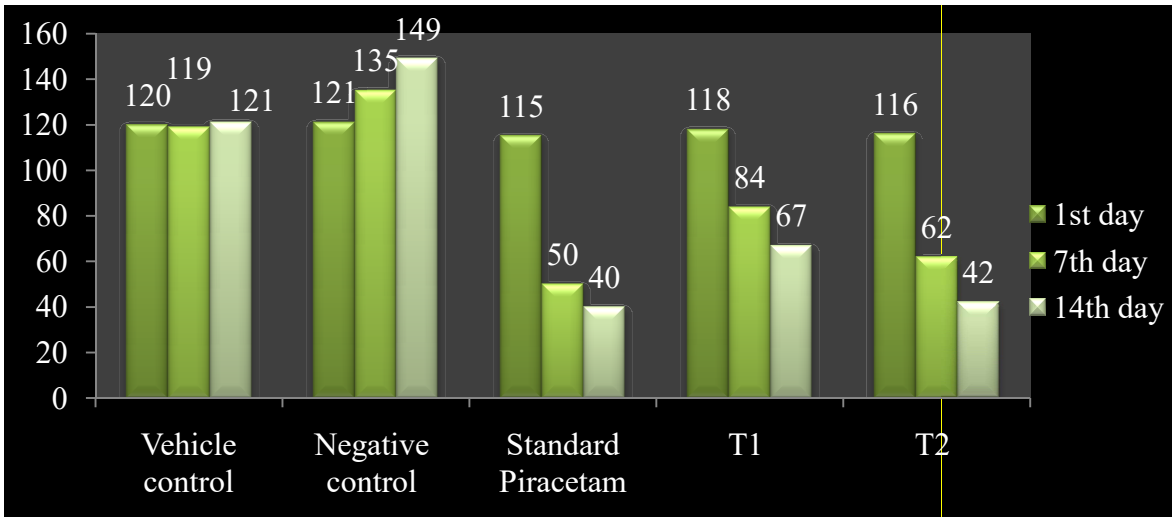

Figure 2: Effect of combined (1:1) of ESLS & ESTF using Morris water maze test

Table 4 Effect combined (1:1) of ESLS & ESTF in Y-maze test

Group	Treatment	Percentage of alternations	
		Before treatment (1st day)	After treatment (14th day)
1	Vehicle control	88.11±0.23	89.18±0.75
2	Negative control (Scopolamine 1 mg/kg, i.p.)	88.27±0.13	95.65±0.95#
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	87± 0.56	42± 0.75*
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	88.03±0.05	55.32±0.85*
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	86.34 ± 1.8	52.24 ± 0.54*

The results were expressed as mean ± SEM, the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
***p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test.**

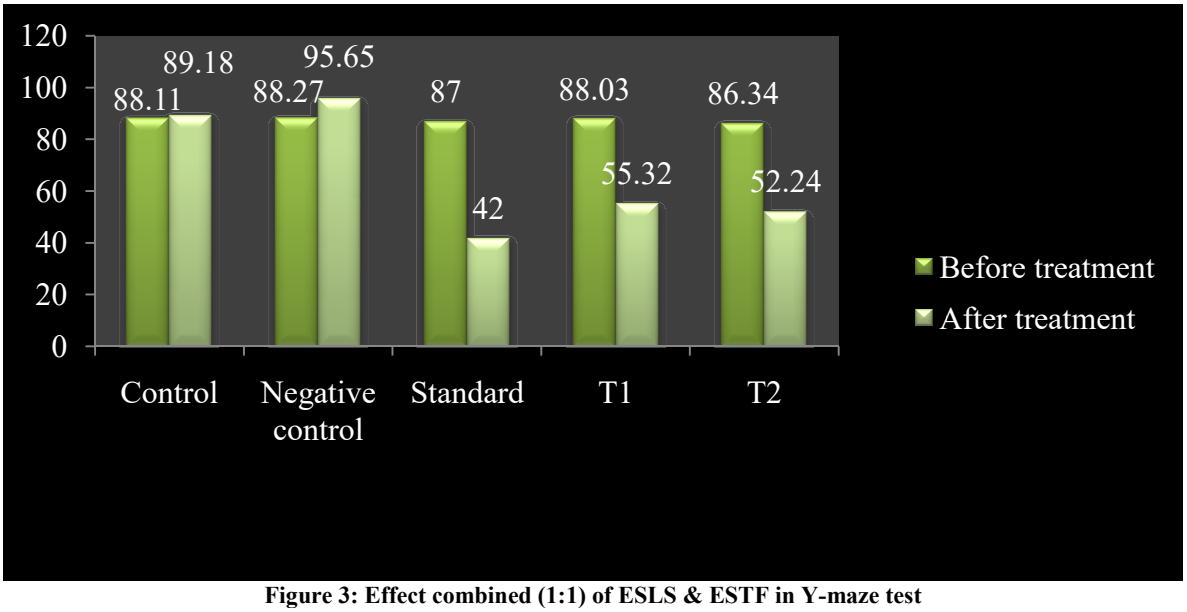

Figure 3: Effect combined (1:1) of ESLS & ESTF in Y-maze test

Table 5: Effect of combined (1:1) of ESLS & ESTF in Figure-8 maze test

Group	Treatment	Mean percentage of the correct response
1	Vehicle control	85.24±4.25
2	Negative control (Scopolamine 1 mg/kg, i.p.)	91.5±2.24#
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	46.2± 9.57*
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	64.44 ± 3.47*
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	58.92±3.58*

The results were expressed as mean ± SEM, the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
*** p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test.**

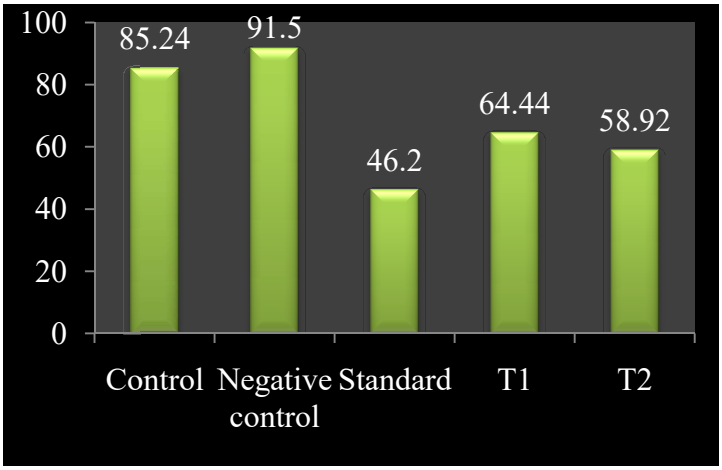

Figure 4: Effect of combined (1:1) of ESLS & ESTF in Figure-8 maze test

Table 6: Effect of combined (1:1) of ESLS & ESTF in T- maze

Group	Treatment	Total number of alternations		
		1st day	7th day	14th day
s1	Vehicle control	10.14±0.21	11.15±2.47	11.22±7.22
2	Negative control (Scopolamine 1 mg/kg, i.p.)	10.05±2.28	8.23±3.24#	6.35±1.37#
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	15.86± 10.29	16.02± 0.12*	16.86± 10.29*
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	9.15 ± 7.26	12.31 ± 0.89*	13.47 ± 4.37*
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	9.78±1.36	13.71±2.74*	14.56±5.87*

The results were expressed as mean ± SEM, the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
*** p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test.**

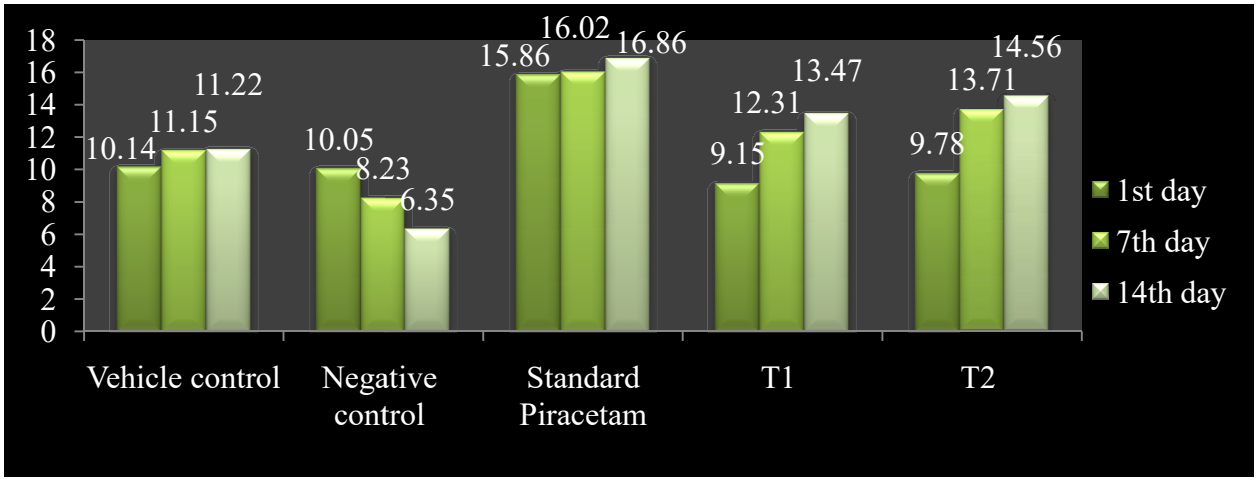

Figure 5: Effect of combined (1:1) of ESLS & ESTF in T- maze

Table 7: Effect of combined (1:1) of ESLS & ESTF in three-panel runway test

Group	Treatment	Latency	WME	RME
1	Vehicle control	28.3±7.3	4.8±1.3	6.2±0.5
2	Negative control (Scopolamine 1 mg/kg, i.p.)	29.8±2.8	5.6±0.35#	6.2±1.24#
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	27.9±9.3	3.15± 2.4*	5.01±0.67*
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	28.2±5.4	3.9±1.75*	4.01±1.97*
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	28.1±8.7	3.7 ± 2.5*	4.87±5.67*

The results were expressed as mean ± SEM, the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
***p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test**

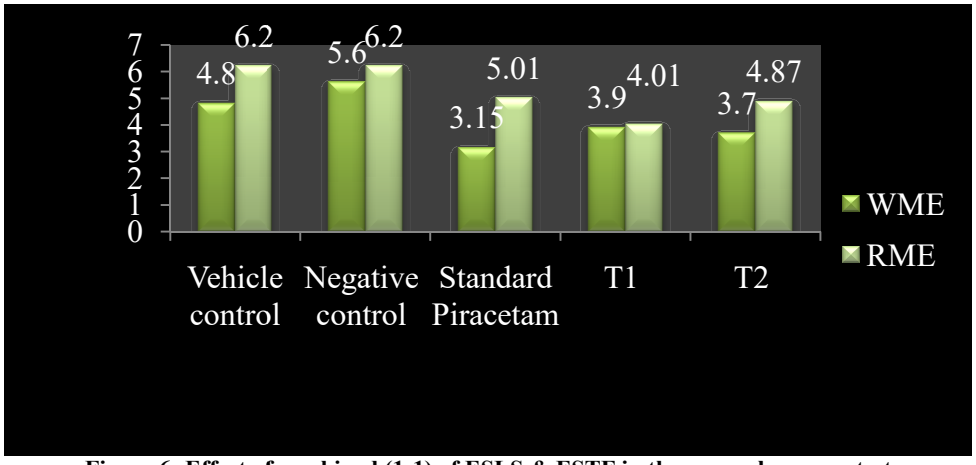

Figure 6: Effect of combined (1:1) of ESLS & ESTF in three-panel runway test

Table 8: Effect of combined (1:1) of ESLS & ESTF in elevated plus-maze.

Group	Treatment	Latency	
		Acquisition day (1^{st} day)	Retention day (14^{th} day)
1	Vehicle control	21.52±1.47	21.13±0.65
2	Negative control (Scopolamine 1 mg/kg, i.p.)	21.01±2.35	23.27±1.03#
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	18.02±1.42*	12.75±0.83*
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	20.21±0.22*	17.77±0.45*
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	19.36±0.12*	15.25±0.37*

The results were expressed as mean ± SEM, the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
***p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test.**

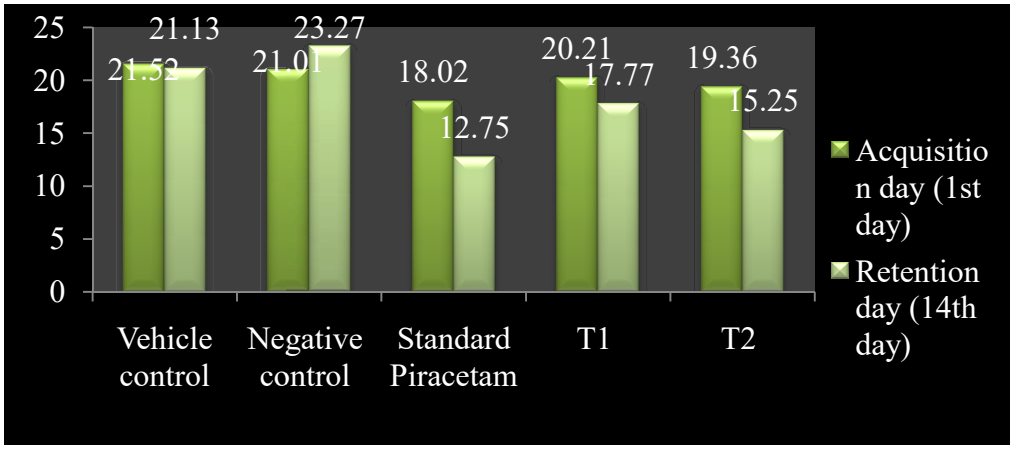

Figure 7: Effect of combined (1:1) of ESLS & ESTF in elevated plus-maze.

Table 9: Effect of combined (1:1) of ESLS & ESTF in Two-way shuttle box test.

Group	Treatment	Step-down latency	
		Acquisition day (1^{st} day)	Retention day (14^{th} day)
1	Vehicle control	4.61±0.22	4.94±0.69
2	Negative control (Scopolamine 1 mg/kg, i.p.)	3.87±1.76	2.13±2.65#
3	Scop (1 mg/kg, i.p.) + Standard Piracetam (200 mg/kg p.o)	3.97±6.34	10.54±1.53*
4	Scop (1 mg/kg, i.p.) + T1 (LS+ TF (200 mg/kg, p.o)	4.68±2.57	7.77±1.42*
5	Scop (1 mg/kg, i.p.) + T2 (LS+ TF (400 mg/kg, p.o)	4.86±0.67	8.25±5.32*

The results were expressed as mean ± SEM, the values were statistically analyzed by
 # p < 0.001 compared with the vehicle control
***p < 0.05 was compared with control group one way ANOVA followed by post hoc use of Boenferoni's multiple comparison test.**

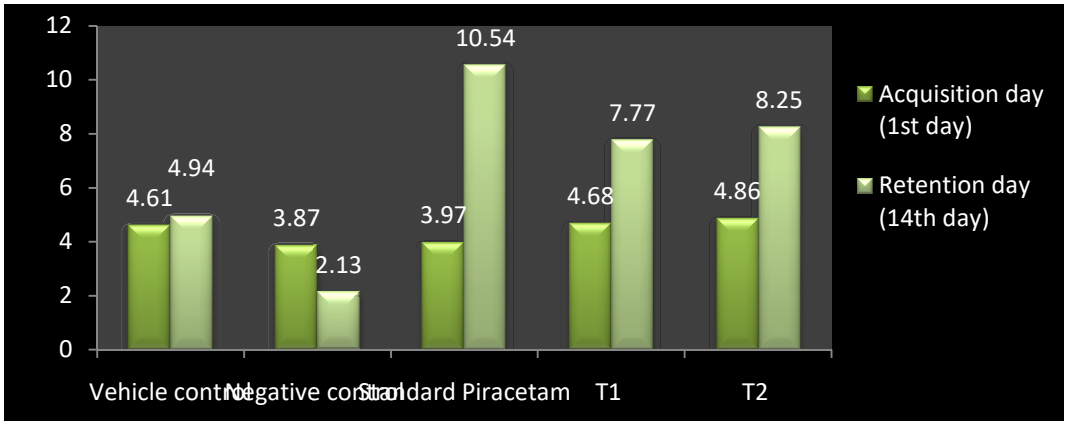

Figure 8: Effect of combined (1:1) of ESLS & ESTF in Two-way shuttle box test.

Table 10: Pre-compression studies of herbal tablet containing ESLF and ESTF extract

Pre-compression parameters	F1	F2	F3	F4	F5	F6
Angle of repose(θ)	31.24	30.89	30.45	32.8	29.3	30.14
Bulk density(g/ml)	0.48	0.46	0.47	0.46	0.44	0.47
Tapped density (g/ml)	0.52	0.55	0.56	0.55	0.51	0.55
Carr's index	7.98	17.21	17.02	17.91	14.97	12.98
Hausner's ratio	1.07	1.14	1.19	1.14	1.29	1.17

Table 11: Post-compression studies of herbal tablet containing ESLF and ESTF extract

Post-compression parameters	F1	F2	F3	F4	F5	F6
Thickness (mm)	4.01±0.013	3.86±0.01	3.96±0.001	4.00±0.005	3.79±0.005	3.66±0.023
Hardness (Kg/cm2)	5.6±0.7	4.8±0.4	4.9±0.2	5.1±0.1	4.78±0.1	5.1±0.3
Weight variation (%)	0.247±0.014	0.214±0.026	0.265±0.035	0.248±0.071	0.312±0.0432	0.288±0.021
Friability (%)	0.21±0.014	0.25±0.023	0.31±0.036	0.278±0.029	0.24±0.0285	0.21±0.045
Disintegration time	12.47± 3.4	13.14±0.5	12.58±0.8	15.34±0.74	12.32±5.1	12.84±0.15

4. SUMMARY AND CONCLUSION

This paper illustrates on the combined effects of extracts, ESLS and ESTF (1:1) for nootropic activity. The plant was collected, authenticated and extracted using Soxhlet apparatus using ethanol. The results of acute toxicity study suggested that the extract was safe up to 2000 mg/kg p.o. Pharmacological evaluations were conducted at 200 mg/kg and 400 mg/kg p.o.

In all the models, the combined seed extracts (1:1) of ESLS and ESTF improved learning and memory, with minimum errors that are statistically significant. Thus the present study reveals that the plant combined seed extracts (1:1) ESLS and ESTF have complementary or synergistic

effects by proving its potential therapeutic effects on improving memory in amnesia. Therefore, the seeds can be used to control the memory-related issues in neurodegenerative diseases like Amnesia and Alzheimer's diseases.

The lyophilized powdered extracts in equal quantities were blended with suitable excipients and tablets were prepared by direct compression method. The tablets were evaluated for various pre and post compressional studies and ended with satisfactory results. Further studies are recommended to find the mechanism behind this synergistic or additive effect.

5. ACKNOWLEDGEMENT

Authors are thankful to the Management of Nizam Institute of Pharmacy, Hyderabad for providing the facilities required to carry out the present work.

6. CONFLICT OF INTEREST

Authors declare no conflict of interest.

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