(Article)

Nootropic Effect of Fenugreek Seed Extract against Scopolamine Induced Cognitive Decline in Experimental Mice

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Abstract: Background: Alzheimer's disease affecting about 24 million people world-wide. The socio-economic burden on world-economies costing more than 172 billion US $ annually for the US alone. **Objectives**: To prepare aqueous extract of T. foenum graecum seeds (FSE) to explore the possible treatment for cognitive deficit in experimental animals. **Materials and methods:** FSE was subjected to preliminary phytochemical evaluation and antioxidant effect using free radical scavenging method (DPPH). All the animal behavior was video recorded with no human intervention during observation and animal groupings were blinded to avoid investigator bias**.** Different doses of FSE (5%, 10% and 20%), control, standard (Piracetam, 200 mg/kg, IP.) were given for male albino mice a period of 15 days followed by cognitive assessment in elevated plus maze and novel objection recognition tests. Ttransfer latencies and time exploring novel and familiar objects were recorded in respective tests. Retention of this learned-task was examined again 24 h later and inflexion ratio (IR) and discriminative index (DI) were calculated respectively. Next in the second set of experiment same groups and treatments were continued but scopolamine was administered to all the groups except normal control one hour after the last dose and examined similarly. **Results:** FSE showed potential antioxidant effect and a dose dependent increase in transfer latency and improved DI indicating a nootropic effect. FSE at 20% showed significant reversal of scopolamine induced dementia in the second set of experiment. **Conclusion:** FSE improved memory as well as reversed the chemically induced memory deficits in experimental mice.

Keywords: Fenugreek, Alzheimer's disease, nootropic, cognitive disorders, herbs, memory

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1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that it is the most common cause of dementia in the old age who are slowly deprived in memory and the ability to carry out the simple tasks. People with AD tend to lose their cognitive skills, including behavioral disabilities and loss of functional autonomy. Both genetic and environmental factors are known to be an AD risk factor. Free radicals, elevated oxidative stress and mitochondrial dysfunction, eventually triggering neuronal / synaptic and neurodegenerative dysfunction[1]. According to the National Institute of Health, some 18 million people worldwide have been affected and are estimated to rise by 33 to around 65.7 million by 2030 and 115.4 million by 34 by 2050[2]. The Alzheimer's Disease Association estimated that Alzheimer's disease accounts for 50 to 80 per cent of cases of dementia worldwide, with the largest identified risk factor rising the age of 65 and older and the prevalence rate of Alzheimer's disease was not documented yet[1], [3]. Several drugs, such as rivastigmine and donepezil, are used to treat this condition as inhibitors of Acetyl-cholinesterase (AChE) licensed by a variety of global food and drug companies.[4] Despite the use of these inhibitors to control the role of AChE, there is a growing need to seek new medications. Therefore, several studies for this reason were aimed at new natural compounds with potential antioxidant properties and with very low side effects have been reported. As a consequence, the use of imitative herbal medicines for AD treatment is on the increase. As is already known, acetylcholine is the key neurotransmitter that plays a vital role in AD. For this reason, several trials have been performed to use AChE suppressors. [5], [6], [7]

Fenugreek is one of the most important plants with antioxidant properties. Its seeds and leaves are used for food and also in traditional medicines. Some studies stated that trigonelline, a compound isolated from fenugreek, exhibited nerve regeneration and enhanced memory activity in AD-induced mice. Its seeds and leaves are used in food and herbal medicine as well. Fenugreek seeds have been found to have a variety of compounds, such as steroidal. The seeds of which are rich in choline, alkaloid, flavonoid, polyphenol antioxidants and other hydroxy-aromatic components which help it in exhibiting anti-oxidant, anti-inflammatory and neuro-protective properties. Therefore, the purported efficacy of this herb in in enhancing cognition was explored in the current study.[8–12](Figure 1)

2. Materials and Methods

2.1 Drugs and chemicals

Piracetam (Nootropil injection, a commercial product), standard nooropic agent. Commercially available scopolamine hydrobromide was purchased froma local pharmacy. Acetylthiocholine iodide, 5, 5′-dithiobis-2-nitrobenzoic acid, DPPH (2, 2, diphenyl-1-picryl hydrazil radical) was procured from Fluka Chemie (Buchs, Switzerland). All the other chemical agents used were of analytical grade available in our chemistry lab.

2.2 Animals

Male Swiss albino mice were procured from the commercial supplier and breeder in Riyadh, Saudi Arabia. Animal studies were performed after obtaining necessary permission from Institutional Animal Ethics Committee (IAEC UCP/18-19/01). After procuring the mice, they were acclimatized for 7 days and housed in groups of six under standard laboratory condition with relative humidity of 45-55% and light/ dark cycle of 12 hours. They were fed with synthetic standard pellet diet available locally and were supplied water *ad libitum*. Male mice weighing between 25-35 gm were used in this study and were fasted for 3 hrs prior to any administration of vehicle/standard/extract. All the experimental procedures were carried out as per the protocol in a dimly lit room during the light period (8:00 to 16:00 hour).

2.3 Experimental design

2.3.1 Preparation of fenugreek seed extract

The fresh *Fenugreek seeds* were purchased locally. The seeds were washed with tap water and dried in the shade at room temperature for 2 days. Then the dried seeds were kept for germination for 1 day in a covered muslin cloth. The germinated seeds were dried and powdered and sieved. Different concentrations (5%, 10% and 20% w/v) were prepared in water and stirred for two hours in magnetic stirrer and then centrifuged for 1 hour at 5000rpm. Supernatant was collected for administration.

2.3.2 Determination of antioxidant activity of FSE

Method followed as per the available references and consists of taking 3 ml of 0.05 mM DPPH in methanol with 30 µL of the different concentrations of the extract in phosphate buffer (pH 7.4), mixed well and kept in dark for 20 min followed by reading absorbance. Blank reading was taken for DPPH alone without any extract. IC50 values for the different extracts were calculated and percentage inhibition was calculated as,

% inhibition = [(Ab - As)/ Ab] *100, where Ab is control absorbance, As- sample absorbance. Vitamin C 1mM and 1mM vitamin E were used as positive control[13,14].

2.3.3 Acute toxicity test

Acute toxicity test of fenugreek seed extract was carried as per the method described in OECD Test Guidelines 425 (Up and Down Procedure) [15] wherein a single albino mouse was given 2000 mg/kg p.o. as single dose and observed for first 30 min, then for 4 h. After survival of treated mouse, 4 additional mice were administered with the same dose under same conditions. Observed for 2 days for any signs of toxicity or death.

2.3.4 Assessment of cognitive performance

For all experimental procedures, all groups of treatments were blinded to the investigators to avoid any bias. The apparatus used for testing were cleaned with 5% alcohol before using each mouse to remove any animal cues. All the experiments were conducted in dim light and were video recorded for offline analysis

2.3.4.1 Animal groupings

Mice were divided into following groups each containing six, group I: Control (Distilled water 10ml/kg, p.o.), group II: Standard (Piracetam, 200 mg/kg, IP.), group III: Low dose of FSE (5%), po Group IV: Medium dose of FSE (10%, po), Group V: High dose of FSE (20%, p.o). They were fasted for 3 h prior to the administration but water was supplied *ad libitum*. All the groups of mice were administered respective treatment as shown in the protocol (Figure 2).

2.3.4.2 Elevated plus-maze

The Elevated Plus maze (EPM) used was fabricated locally with wood and dimensions meeting the published literature. It had two open arms and two closed arms, crisscrossing each other forming a plus. The closed arms and open arms were 25 cm × 10 cm × 20 cm and 25 cm × 10 cm respectively with a central platform of 10 X 10 cm area. The entire maze was elevated to a height of 90 cm with a wooden column. All procedures were conducted in a dimly lit dark room[16–18]. All the parameters were recorded using a web cam fixed above EPM to the roof and connected to a computer for recording and offline analysis. On day 15, 1 h after the dose, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) i.e. the time taken by mouse with all its four legs to move into one of the enclosed

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arms was recorded as the initial transfer latency (L1) on the first day. If the animal does not enter into one of the enclosed arms within 90 s, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for next 10 s and then returned to its home cage. Retention of this learned-task was examined again 24 h later (L2). The whole apparatus was thoroughly cleaned with 5% alcohol before placing each animal in the maze to avoid animal cues. The inflexion ratio (IR) was calculated by the following formula, (IR) = (*L2 – L1*)/ *L1*, Where *L1* is the initial TL (s) on 1st day and *L2* is the TL (s) on the 2nd day.

2.3.4.3 Novel object recognition task[19–21]

The apparatus was made up of wood of a rectangular box measuring 50 cm × 50 cm × 50 cm. It was placed in dimly lit dark room. All the parameters were recorded using a video camera. Mice were divided into following groups each containing six. They were fasted for 3 hrs prior to the administration but water was supplied *ad libitum*. On day 17, 1 h after the dose, each mouse was tested in a 30 cm X 30 cm rectangular box. The test consists of 3 phases, 1. habituation session 2. training session 3. test session. All animals were given one habituation session in which they were allowed to explore the apparatus (without objects) for 10 min. For the training session, each mouse was placed into the box with two identical objects (1 and 2) and allowed to explore for 5 min (training). The time spent by the animal exploring each object and also the time spent by the animal exploring both objects and the box were measured. 24 h after the training, one of the objects was replaced with a novel object (object number 3, novel) and the other object is same as used for training (1, familiar object). Each mouse was individually tested and video graphed for 5 minutes. Time spent by mouse exploring objects determined.

2.3.4.4 Scopolamine induced amnesia in mice using above tests

In the second set of experiment same groups and treatment period were maintained but scopolamine (1 mg/kg, IP) was administered to all the groups one hour after last dose on day 19 in the respective tests (EPM and NORT) and then examined to record as above. Retention of this learned-task was examined again 24 h later and parameters were calculated as per the procedure in above respective methods.

2.4 Statistical Analysis

All the results were expressed as mean ± standard error. The data were analyzed using ANOVA followed by tukey's multiple comparison post hoc test. p <0.05 were considered as significant. The statistical analysis was done using the SPSS software package for Windows, version 20, Chicago, USA.

3. Results

3.1Antioxidant activity of FSE

The decrease in DPPH absorption in the presence of varying concentrations of extract was monitored and it was noticed that the extract showed a dose dependent decrease in the absorbance of DPPH radical. IC50 value for the extract was found to be 9.93 µg/ml. These results indicated an antioxidant potential of seed. (Table 1)

3.2 Effect of FSE on transfer latency (TL) in elevated plus maze

Effect of FSE on TL in mice were recorded with elevated plus maze apparatus where piracetam 200 mg/kg and FSE with three different dose levels (w/v) i.e. 5%, 10% and 20%, treated groups have shown a decrease in

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transfer latencies leading to corresponding increase in inflexion ratios as compared to normal control. But statistically significant effect ($P < 0.05$) was observed with high dose 20 % of FSE and piracetam ($P < 0.05$). (Figure 3)

3.3 Effect of FSE on transfer latency in scopolamine induced amnesic mice in EPM

The effect of the vehicle, scopolamine (1 mg/kg), FSE (5%, 10% and 20%) and piracetam (200 mg/kg) are shown in Figure 4. The scopolamine alone treated group showed a significant ($P < 0.01$) increase in TL values on the acquisition as well as on the retention days (decrease in inflexion ratio) as compared to vehicle control mice, indicating an impairment in learning and memory. Whereas in the acquisition as well as retention trial FSE demonstrated dose dependent decrease in the TL (increase in inflexion ratio) when compared to the scopolamine alone treated group ($P < 0.01$). Piracetam (200 mg/kg IP.) exhibited marked decrease ($P < 0.01$) in TL in comparison with the scopolamine. However, FSE at the dose levels 20% and 10% showed a comparable decrease in the TL($P < 0.05$).

3.4 Effect of FSE for object exploration in mice using novel object recognition test

Effect of FSE on inflexion ratios in mice were recorded with elevated plus maze apparatus. Piracetam 200 mg/kg and FSE with three different dose levels i.e. 100, 200 and 400 mg/kg, treated groups have shown decrease in transfer latencies leading to increase in inflexion ratios when compared to control. But statistically significant effect ($P < 0.05$) was observed with high doses i.e. 10 and 20% of FSE groups only indicating a dose dependent nootropic like effect. Piracetam also has increased the inflexion ratio very significantly ($P < 0.01$). (Figure 5)

3.5 Effect of FSE on time spent exploring in scopolamine induced dementia in mice using NORT

The effect of the vehicle, scopolamine (1 mg/kg, po), FSE (5%, 10% and 20%) and piracetam (200 mg/kg) were evaluated at the end of treatment period. The scopolamine (1 mg/kg) control group showed a significant ($P < 0.01$) increase in exploration time for novel object on the acquisition as well as on the retention days (decrease in discrimination index) as compared to vehicle control mice, indicating an impairment in learning and memory. In the acquisition as well as retention trial, FSE demonstrated dose dependent decrease in the exploration time (increase in DI) as compared to the scopolamine control group. Piracetam (200 mg/kg IP.) exhibited marked decrease ($P < 0.01$) in exploration time in comparison with the scopolamine control group. (Figure 6)

4. Discussion

Alzheimer's disease is a neurogenerative condition associated with a decrease in cognitive ability.[4] Given the seriousness and high prevalence of this disease, the allopathic medical system has failed to provide a suitable cure.[22] The present study therefore concentrated on investigating the memory enhancing function of the FSE in a chemical-induced amnesia models. In this study the exteroceptive model was used for evaluating the nootropic activity (memory enhancing) of FSE on learning and memory processes, which was indicated by decreased transfer latency and increased inflexion ratio in EPM. The interoceptive models used were amnesia induced by scopolamine, which was indicated by prevention of fall in transfer latency and inflexion ratio in EPM[16–18]. The present study suggests that FSE possesses memory enhancing activity in view of its decreased transfer latency and increased inflexion ratio in EPM. This suggests that the FSE has pronounced nootropic effect which was comparable to nootropil (standard) in the study. FSE also exhibited a facilitatory effect on the retention of memory in scopolamine induced amnesic mice.

Similarly, in another group of models used by NORT, scopolamine substantially increased the e xploration period suggesting that scopolamine induced cognitive impairment in this model. Pret reatment with different doses of FSE greatly increased the ability of the treated mice to identify

novel artifacts. Administration of different doses of FSE led to enhancement in indices of memory in normal as well as scopolamine induced memory impaired mice in EPM as well as NORT tests in the present. It is well known that cholinergic neuronal systems play an important role in cognitive deficiencies associated with AD, aging and neurodegenerative diseases.[23,24] In our study, amnesia caused by scopolamine is evident from the results obtained and its reversal with the prior treatment of FSE indicating the activation of cholinergic system by FSE. Also, the ability of the FSE to scavenge the oxidative free radical and to prevent induced tissue damage by its potential antioxidant activity in the DPPH free radical scavenging assay contributes to its cognition enhancing effect. In addition, FSE has phenolic and flavonoid compounds which are proven antioxidants. There are also numerous studies on the antioxidant capacity of fenugreek seeds.[25–27] These findings indicate that they have antioxidant ability to prevent chemically mediated memory deficits. As a consequence, it can be concluded from these findings that FSE may provide a potential advantage in the amelioration of Alzheimer's disease type memory loss due to its probable potential for activation of the cholinergic system and/or free radical scavenging capability that can provide neuroprotection in the prevention or management of this disease. The effects observed with FSE are in agreement with the previous published studies wherein fenugreek extract as well as its primary constituent, trigolline exhibited potential cognitive effect in various chemically induced cognitive deficit models such as.[24,26–30]

Although this research was not an exhaustive adventure to draw any conclusions, it is proof of our hypothesis. However, more studies are required to further investigate the potential effects of FSE on AChE in various parts of the brain, amyloid beta plaques, the role of other neurotransmitters such as glutamate, gamma aminobutyric acid (GABA) and catecholamines.

5. Conclusions

In this study, we concentrated on exploring FSE's ability to improve memory in laboratory mice as well as reversing chemically induced memory deficits in experimental mice. The results of the invitro studies have shown that FSE is an antioxidant and the results of the in vivo analysis have concluded that FSE has nootropic function in the absence of cognitive deficits and has also been effective in preventing chemically induced memory deficits in experimental mice. The mechanism by which FSE has shown these properties can be related to its antioxidant, neuroprotective properties, its choline content or activation of acetylcholine system in brain. In the light of above, it may be worthwhile to explore the potential of these seeds in the management of AD patients

Author Contributions:

"Conceptualization, SMH.; methodology, SMH.; software, MS and SN.; validation, NA, FA and MA.; formal analysis, SMH.; investigation, NA, FA and MA.; resources, MS and SN.; data curation, SMH.; writing—SMH.; writing—review and editing, SMH, MS and SN; supervision, SMH.; project administration, NA, FA and MA. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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320 **1. Table**

321 **2. Table 1. Antioxidant activity of FSE using DPPH free radical scavenging activity**

3. S.No	**4. Concentration of extract (µg/ml of FSE)**	**5. Inhibitory activity (%)**	**6. IC50 µg/ml**
7. 1	8. 10	9. 50	10. 9.93
11. 2	12. 20	13. 83	
14. 3	15. 30	16. 85	
17. 4	18. 40	19. 86	
20. 5	21. 50	22. 86	
23. 6	24. 60	25. 87	
26. 7	27. 70	28. 89	
29. 8	30. 80	31. 90	
32. 9	33. 90	34. 90	
35. 10	36. 100	37. 90	

322 **38.**

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40. Figures

Figure 1 Image of leaves and seeds of *Trigonella foenum graecum* and chemical structure of trigonelline

Leaves of *Trigonella foenum graecum* Seeds of *Trigonella foenum graecum* Chemical structure of trigonelline

41.

42.

328 **44. Figure 2: Detailed protocol for the experimental design for normal and scopolamine induced cognitive**
329 **deficit in mice**

330 **45.**

331 **46.**

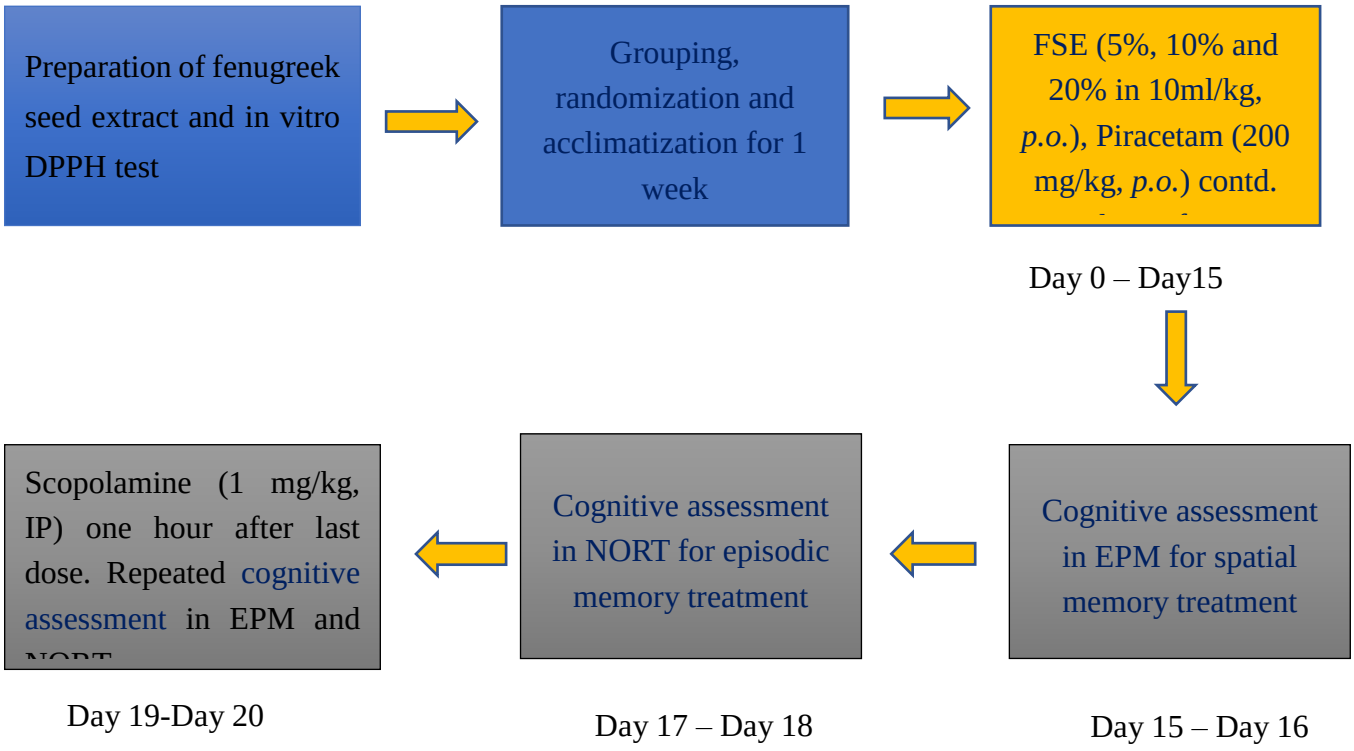
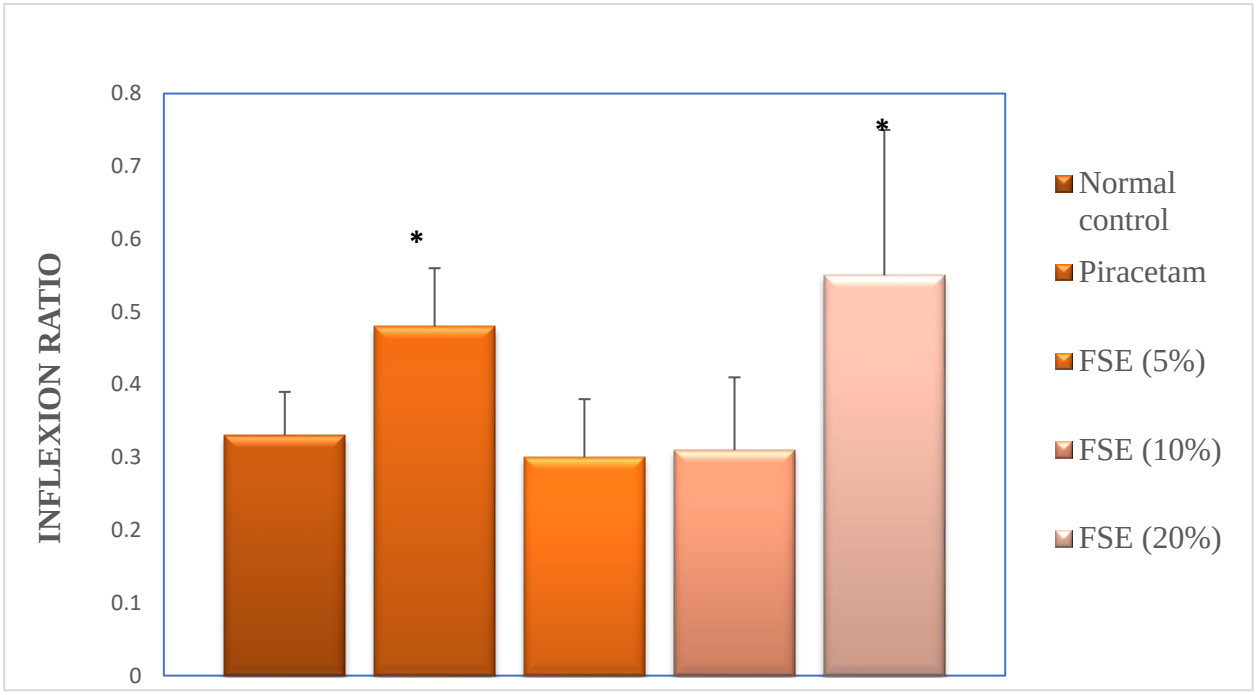
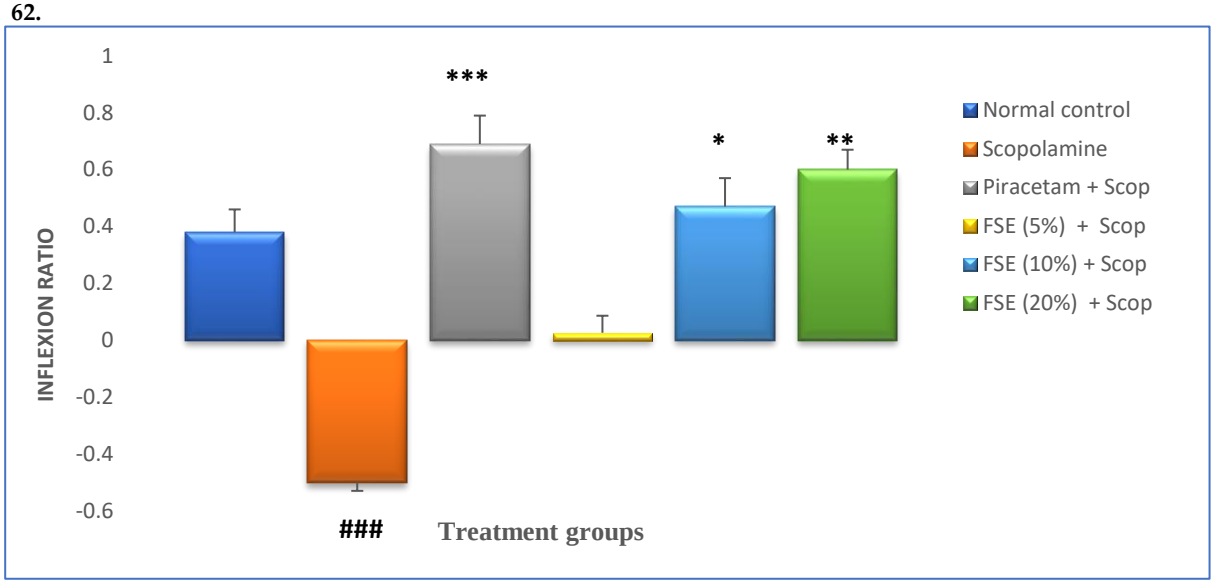


Fig 3: Effect of FSE on inflexion ratio in mice in elevated plus maze



61. Figure 4: Effect of FSE on inflexion ratio in scopolamine induced amnesic mice in elevated plus maze

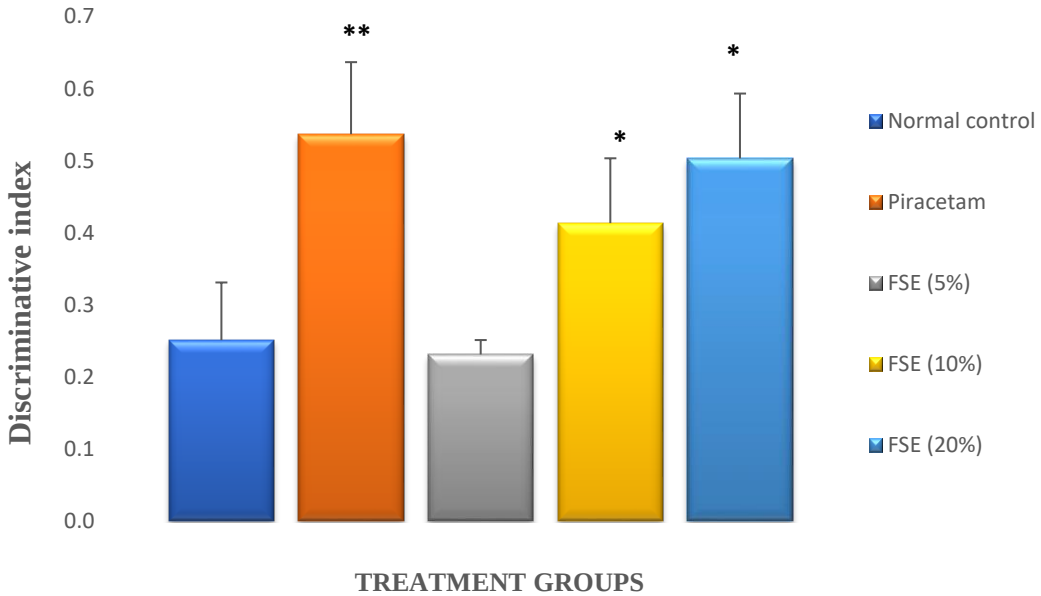
62.



71. Statistical analysis by one-way ANOVA followed by Dunnett's't' test. Values are expressed as mean ± S.E.M (n = 6). *p<0.05,.**p<0.01 compared with normal control group. ##p<0.01when compared with disease control (Scopolamine)

72.

73. Figure 5: Effect of FSE on discrimination index in mice in novel object recognition test



84. Statistical analysis by one-way ANOVA followed by Dunnett's' test. Values are expressed as mean ± S.E.M (n = 6). *p<0.05, **p<0.01 compared with normal control group

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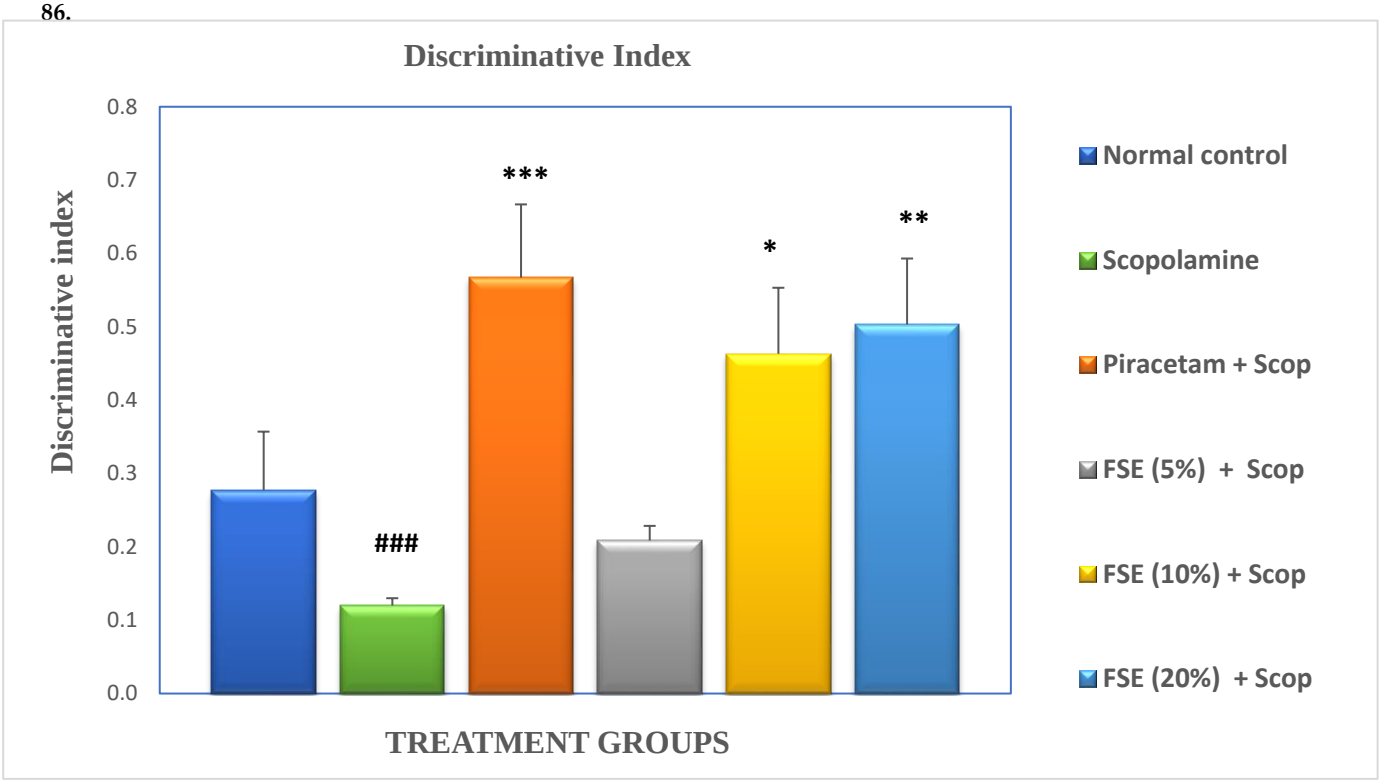
378 **85. Figure 6: Effect of FSE on discrimination index in scopolamine treated mice in novel object recognition**
379 **test**

380 **86.**



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387 **89.** Statistical analysis by one-way ANOVA followed by Dunnett's' test. Values are expressed as mean ± S.E.M
388 (n = 6). *p<0.05, **p<0.01 compared with scopolamine control group. ##p<0.01when compared with normal
389 control