Readers Insight
Publisher of open access books and journals
PJMLS OPEN ACCESS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/wj3yca76	Copyright © All rights are reserved by Corresponding Author
Vol. 9 No. 1, 2026: pp. 201-208	
www.readersinsight.net/pjmls	**Revised:** March 09, 2026 **Accepted:** March 17, 2026
Submission: December 29, 2025	**Published Online:** March 31, 2026

EVALUATION OF NEUROBEHAVIORAL RESPONSES AND ANTIOXIDANT ACTIVITY FOLLOWING FENUGREEK ADMINISTRATION IN RATS

Irfan Sajid[1]*, Hafsah Khan[1], Shoaib Ahmed[1], Lubna Anis[1], Munawar Abbas[1], Palwasha Abrar[1], Syed Ikhlaq Hussain[2]

[1]**Department of Biochemistry**, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan
[2]**Department of Zoology,** Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan

***Corresponding Author:** Irfan Sajid. E. mail: irfan.sajid@fuuast.edu.pk

Abstract

Fenugreek seeds are small, yellowish brown and bitter in taste. It is used as an old medicinal plant as Ayurvedic and Chinese medicine. Present study is designed to evaluate neurobehavioral effects following fenugreek seeds administration in male albino rats. Twelve albino rats were categorized into control and test groups. Control was given normal saline while test was given 200 mg/kg fenugreek seeds aqueous solution in oral form for three weeks. Force swimming test used for depression-like symptoms and anxiety by using elevated plus maze test. While memory and learning functions were evaluated by using Morris water maze test. Antioxidant activities evaluated by measuring levels of malondialdehyde (MDA) and Catalase activity in brain. The effect 200 mg/kg for three weeks of fenugreek seeds significantly increase the memory functions and muscles strength accompanied by notable antioxidant effects on malondialdehyde and catalase enzymes levels. While a non-significant effect observed on depression-like symptoms and anxiety functions. It is concluded that fenugreek administration significantly effects on memory functions ($p<0.01$) and on antioxidant enzyme activity ($p<0.05$) show a beneficial outcome.

Keywords: *Antioxidant, Depression, Fenugreek, Memory, Medicinal*

INTRODUCTION

Fenugreek seeds (*Trigonellafoenum-graecum*), alternatively referred as fenugreek, bird's foot, hayseed (1). Previous literature shows that it is the oldest medicinal plant and rich in vitamins and minerals, high in protein and serves as a significant source of diosgenin (2). It is renowned for its detoxifying and antioxidant properties (3).

Some previous studies reported that fenugreek as a traditional Chinese herb used as a remedy for many CNS diseases (4). Riba *et al.,* (2019) studies showed that cognitive function and neurotoxicity can be improved with the administration of fenugreek seed, suggesting a potential to influence central nervous system outcomes (5). Prior studies suggested when mice were exposed to arsenic, there is an impairment of acetyl and butyrylcholinesterase, superoxide dismutase and glutathione reductase activities and brain-derived neurotrophic factor levels were found in the brain, compared to control mice (3). The antidepressant-like effects found after fenugreek seeds administration, have been reported sing in animal models of severe depression (6). Investigation was done to see the effect of fenugreek seeds administration on pre-menopausal discomforts, along with its impact on endocrine balance and homeostasis (7). Further reported that Fenugreek seeds that fenugreek seeds may have anti-depressant properties due to their content of certain compounds like flavonoids and saponins, which could help alleviate symptoms of depression.

Supplementing Alzheimer's disease (AD) patients with fenugreek seed extract demonstrates reported positive effects on memory functions, life processes, blood pressure and specific oxidative index parameters (8). The administration of fenugreek seed extract alleviates cognitive dysfunction associated with diabetes in rats (9). The fenugreek extract does not exhibit any gastrointestinal stimulant or relaxant

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

activity; despite it inhibits the acetylcholine esterase (AchE) enzyme, indicating their potential to improve memory (10). The consequences of methanol extract from fenugreek seeds were assessed in mice (11). It has been observed that administering fenugreek seed powder (with different percentage composition mixed with animal feed) protected against memory and learning impairments induced by AlCl3 (12). Scientists want to examine how adding fenugreek seed extract to the diet, either by itself or together with choline-DHA, affect the levels of BDNF and the branching of dendrites in pyramidal neurons in different hippocampal regions in rats that have had their ovaries removed (13).

Previous studies may report about individual effects on different behavioral parameters, but purpose of current study is to explore the effect of fenugreek seeds on neurobehavioral parameters in rats correlating with their antioxidant activities comprehensively evaluate the potential neuroprotective and therapeutic properties, offering new insights into their use for treating both neurological conditions.

METHODOLOGY

Twelve Albino Wister rats (150-180g) were purchased and received from Dow University OJHA Campus. All animals were kept individually in a standard environment given by guidelines. Animals were randomly assigned as controls and test groups. Test group received 200 mg/kg for 3 weeks while control was given normal saline. Behavioral activities were monitored after 3 weeks of drug administration. After monitoring behavioral activities, animals were decapitated and blood was collected and plasma samples were used for antioxidant activities. Experiment was conducted after the approval of Institutional Animal Care Ethical Committee (# EIRB-09003/2024).

BEHAVIOURAL TESTS

MORRIS WATER MAZE TEST (MWM)

The effects on learning and memory functions were monitored by using MWM test that was designed, and working is as given in (14). In training phase each rat is placed in opaque water making sure that animal is facing wall of tank. So, rats will be given a total 120 second to allocate hidden platform. If rat successfully find out platform, then the rat will be stay leave for 10 seconds and if it fails then gently guided to locate the platform. After training session test session perform after 24 hrs of training session. Use of this apparatus is to find out latency time (Time taken to reach at platform).

FORCE SWIMMING TEST

This behavioral despair test is used to assess the effects of drugs possess antidepressant like effects and force swimming apparatus used for this type of analysis. Detail of protocol for this apparatus is given as in (14). Purpose of this apparatus is to evaluate depression like symptoms by noting immobility time.

ELEVATED PLUS MAZE TEST

It is one of an important behavioural model for evaluation of anxiety functions (14) in rats. Test is performed in one testing session only in which rat placed individually at one end of open arm carefully noted that central platform is away and then latency (time taken to enter close arm by rat with all four paws in seconds) was observed and noted.

ANTIOXIDANT ACTIVITIES

DETERMINATION OF CATALASE (CAT)

Catalase activity is measured using brain sample and reported procedure was followed as in (22). For the filtrate brain tissue used and prepare homogenate in phosphate buffer. Filtrate was taken and mixed H_2O_2 and phosphate buffer. The % inhibition of catalase was determined, and solutions are read at 620 nm and get absorbance.

DETERMINATION OF MDA CONTENTS

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

MDA estimation in brain sample was done which is reported as according to (22). In a test tube brain homogenate were taken and then proceed to reaction by introducing in a water bath. After cooling the mixture centrifugation done, absorbance was read at 532 nm using supernatant.

RESULTS

EFFECT OF FENUGREEK SEEDS ON ANXIETY FUNCTIONS

Effect of oral fenugreek seeds administration on anxiety functions in rats is shown in Fig. 1(a). Data analysis done by using student's t test showed a not significant effect of fenugreek seeds administration on behaviour (t value = 0.33, df = 10, p>0.05). These results represent that the effect of fenugreek seeds administration showed a nonsignificant decreased (p>0.05) in anxiety in fenugreek seeds treated rats as compared to control rats.

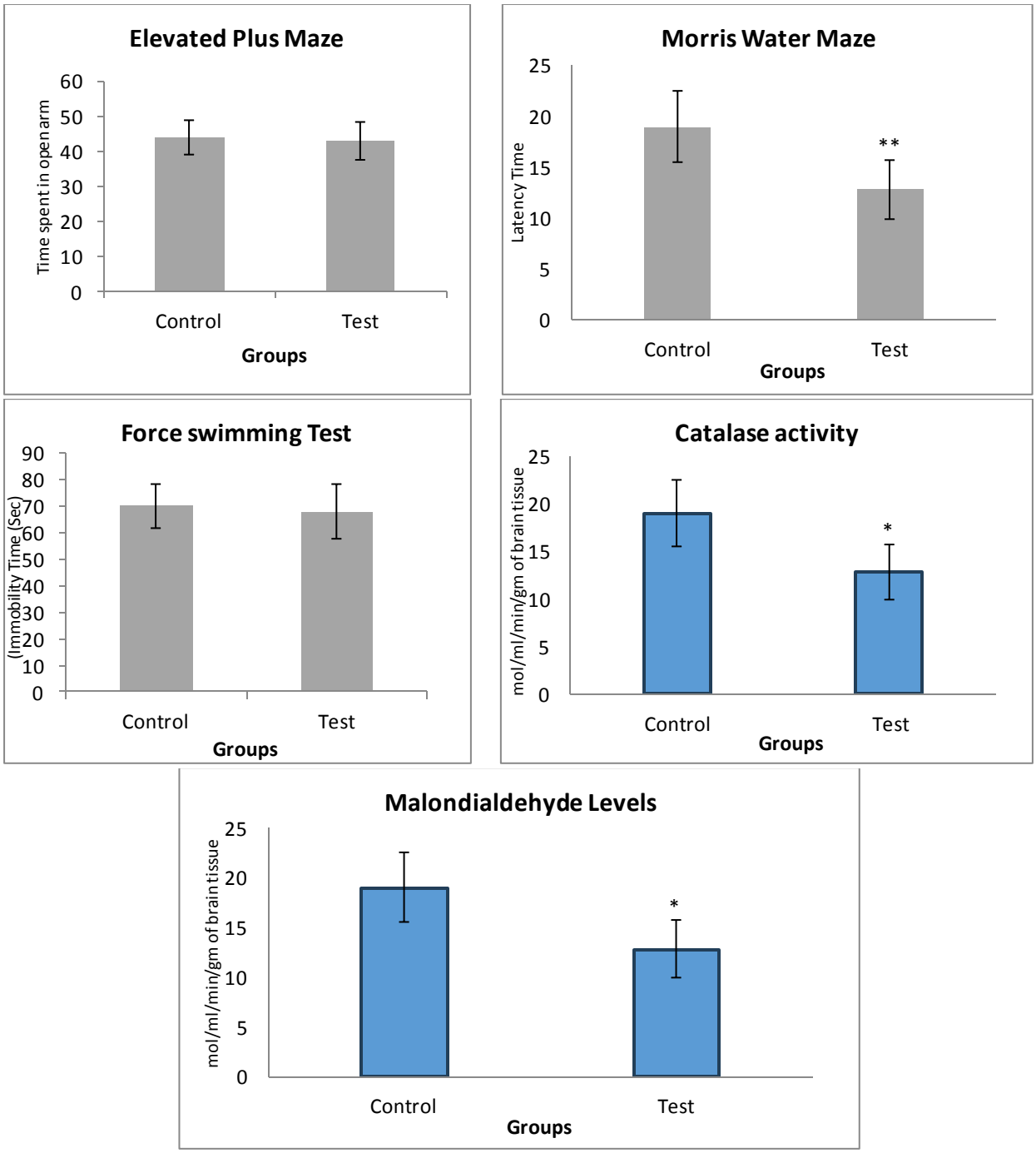

Fig. 1 (a). Values are represented as mean ±SD. Data analyzed by student's test revealed a not significant effect (P>0.05);
(b). Values are represented as mean ±SD. Data analyzed by student's test revealed a significant effect **(P<0.01);
(c). Values are represented as mean ±SD. Data analyzed by student's test revealed a non significant effect (P>0.05);
(d). Values are represented as mean ±SD. Data analyzed by student's test revealed a significant effect *(P<0.05);
(e). Values are represented as mean ±SD. Data analysed by student's test revealed a significant effect *(P<0.05)

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

EFFECT OF FENUGREEK SEEDS ON MEMORY FUNCTIONS

Effect of fenugreek seeds administration on behaviours in rats is shown in Fig. 1(b). Data analysis performed by using student's t test showed a significant effect of fenugreek seeds administration on behavior (t value = 3.29, df = 10, $p<0.01$). These results represent that the effect of fenugreek seeds administration showed a significant increased ($p<0.01$) in memory in fenugreek seeds treated rats as compared to control rats.

EFFECT OF FENUGREEK SEEDS ON DEPRESSION LIKE SYMPTOMS

Effect of fenugreek seeds administration on behaviours in rats is shown in Fig. 1(c). Data analysed by student's t test showed a non-significant effect of fenugreek seeds administration on behaviour (t value = 0.33, df = 10, $p>0.05$). These results represent that the effect of fenugreek seeds administration represent non-significant decreased ($p>0.05$) in depression in fenugreek seeds treated rats as compared to control rats.

EFFECTS OF FENNEL SEEDS ON CATALASE ACTIVITY

Fig. 1(d) shows the effect of fenugreek extract on catalase antioxidant enzyme. Data analyzed by the t-Test reveals the significant effect on administration of fenugreek seeds extract significantly increased ($t=5.61$, $df=10$, $p<0.05$) catalase enzyme as compared to control.

EFFECT ON MDA CONTENT

Fig. 1(e) shows MDA levels that has mentioned as a lipid peroxidation marker in experiment showing oxidative stress and redox signalling. Data analysed by the t-test reveals the significantly decreased ($t=2.23$, $df=10$, $p<0.05$) effect of fenugreek seeds administration of MDA content LPO as compared to test.

DISCUSSION

Present study showed that three weeks fenugreek seeds oral administration of 200 mg/kg significantly improving memory functions and antioxidant enzyme activity levels, but no significant effects observed on anxiety functions and depression-like symptoms. Previous research showed that animals were exposed to arsenic showed more anxiety-like behavior compared to the control group when tested using elevated plus maze but when they were administered with fenugreek powder effects were attenuated and anxiety effects decreased as compared to the mice that were only exposed to arsenic (3).Scientists reported that fenugreek seeds, specifically a compound called 4-HI, had antidepressant and antianxiety effects in rats that were subjected to social isolation and stress. These effects were likely due to the involvement of various mechanisms that help to relieve stress (6). But some other studies showed that nootropic and anxiolytic activities of the acetone-soluble fraction from the methanol extract of Fenugreek seeds were assessed. Results revealed a significant effect of ethanolic on decreasing anxiety like functions tested in plus maze apparatus (15). Current study revealed no anxiety-like symptoms using the plus maze test. The results showed that rats treated with fenugreek seeds nonsignificantly decrease the time spent in the open arm as compared to the control group. These findings are not as such consistent with a previous study and it could be due to the dose of fenugreek or duration of administartion.

Researchers evaluated the effects of fenugreek seeds on learning and memory functions in mice at various doses. The findings suggest that these seeds administration enhances the learning and memory processes and could serve as a promising anti-amnesic agent (11). Prema *et al.,* (2016) investigated the neuroprotective impact of fenugreek seed powder against an experimental Alzheimer's disease model induced by aluminium chloride. It protected the AlCl3-induced memory and learning impairment (12). The randomized control trial was to examine how fenugreek seed extract impacts memory, depression, and quality of life. Preliminary findings indicate that supplementation with fenugreek seed extract in Alzheimer's patients' yields positive effects on memory (12). Present study observed that rats treated with fenugreek seeds showed significantly reduced latency time and learning and memory improved in the rats,

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

as they spent less time in other areas and quickly reached to the platform as compared to the control group, indicating beneficial effects on memory functions.

Some scientists reported fenugreek seeds containing naturally branched-chain amino acid, 4-HI which is derived from socially isolated rats. Their results showed decreased immobility and climbing time and increased swimming time (6). In another study, the impact of fenugreek seeds methanol extract on depression in mice. The researchers assessed depression lie symptoms and the findings indicated a decrease in immobility time, suggesting an anti-depressant effect (6). The finding indicated that extract of fenugreek seeds and seeds notably decreased immobility time during the test in a dose-dependent manner (16). Present study aimed to investigate the effects of fenugreek seeds on the force swimming test in rats as a measure of immobility time and fatigue resistance. In conversely to our initial hypothesis, the fenugreek seeds administration non significantly improves the swimming performance compared to the control group. Current research reveals that fenugreek seeds significantly affect malondialdehyde levels and catalase activity in blood. Current results show that fenugreek seed administration decrease oxidative stress by decreasing the lipid peroxidation activity. Previous research also shows that fenugreek seeds found to be lower the serum lipid peroxide levels (17) and NDF extracting from coconut and black gram reduce the lipid peroxidation (18). Chances are that high levels of saponins and NDF are found in fenugreek seeds could be attributed to lower the MDA level so decrease the lipid peroxidation in tissues leading to reduce in plasma levels too. Addition of fenugreek seeds to the diet reduced lipid peroxidation. It increased the levels of glutathione and beta-carotene while decreasing the alpha-tocopherol content. The level of ascorbic acid remained unchanged (19).

Present study represents that catalase enzyme activity significantly increased so that oxidative stress got decrease. Scientists explained that catalase is an important antioxidant that helps reduce oxidative stress significantly by breaking down cellular hydrogen peroxide into water and oxygen (20). Some studies reported that Fenugreek may elevate the catalase enzyme which protects cell membranes by neutralizing free radicals, transferring of hydrogen atoms or electrons, and precipitating metal ions, thereby safeguarding tissues from interaction with reactive oxygen species (21).

In another research, researcher treated diabetic rats with Fenugreek seeds, Termis seeds, and Mixture of fenugreek and termis seeds which led to significant increases in catalase (CAT) activities and reduced GSH content (22). These results indicate that Fenugreek and Termis seeds may help to restore the antioxidant defense system by regulating antioxidant enzyme activities. Decreased GSH levels could shield cells from reactive oxygen species (ROS) toxicity (23) while CAT is crucial in neutralizing free radicals (20). Based on the above past mentioned research findings and recent study it can speculate that fenugreek seeds have components that are flavonoids, saponins and trigonelline these all can cross the blood brain barrier so we can hypothesize that flavonoids could potentially help in replenishing the antioxidant enzymes function in the brain and prevent the neuronal degeneration. Such studies could be design in future in order to completely understand their mechanism. Antioxidant enzymes are innate proteins with distinct abilities to scavenge free radicals so that oxidative stress can be reduced, saponins can enhance memory and trigonelline found in cerebral cortex after crossing blood brain barrier and can provide protection to nerves against conditions such as Alzheimer's disease, Parkinson's disease, and depression.

CONCLUSION

In conclusion, present study reveals the significant effects of fenugreek seed administration on memory functions but on mood or other behaviors and similarly enhancing antioxidant system shows healthy effects on brain and in body. Further studies needed to evaluate the mechanisms of fenugreek seeds by performing neurochemical estimations like dopamine, serotonin and adrenalin as well as flavonoids.

Conflict of interest:

Authors declare no conflict of interest.

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Funding:

No external funding or financial support was received for this study.

Author s' contributions:

IS Conducted the experimental work and data collection; SM Conceptualized and Supervised the study; FT done the analysis of results; NHS and SW worked for write up; AS provided the experimental support and supervision.

Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

References:

1. Visuvanathan T, Than LTL, Staslas J, Chew SY, Vellasamy S. Revisiting Trigonella foenum-graecum L.: Pharmacology and Therapeutic Potentialities. Plants. 2022; 11:1450.
2. Wenfeng L, Jie D, Jiecheng H, Liang Y, Rong Y, Lingkun Z, Jian S, Zeping H, Fangmei X, Jinhua H, Yanqing G. Integration of molecular docking, molecular dynamics and network pharmacology to explore the multi-target pharmacology of fenugreek against diabetes. J Cell Mol Med. 2023;27(14):1959-1974.
3. Islam J, Islam Z, Haque N, Khatun M, Islam F, Hossain S, Hoque MA, Nikkon F, Hossain K, Saud ZA. Fenugreek seed powder protects mice against arsenic-induced neurobehavioral changes. Curr Res Toxicol. 2023;14(5):100114.
4. Zhou J, Chan L, Zhou S. Trigonelline: A plant alkaloid with therapeutic potential for diabetes and central nervous system disease. Curr Med Chem. 2012;19(21):3523-31.
5. Riba A, Feki A, Ben Salem M. Neuroprotective effect of fenugreek seed extract on aluminum chloride-induced neurotoxicity in rats. Physiol Behav. 2019; 199:63-70.
6. Kalshetti PB, Alluri R, Mohan V, Thakurdesai PA. Effects of 4-hydroxyisoleucine from Fenugreek Seeds on Depression-like Behaviour in Socially Isolated Olfactory Bulbectomized Rats. Pharmacognosy Magazine. 2015;11: S388-S396.
7. Khanna A, John F, Das S, Thomas J, Rao J, Maliakel B, Krishnakumar IM. Efficacy of a novel extract of fenugreek seeds in alleviating vasomotor symptoms and depression in perimenopausal women: A randomized, double-blinded, placebo-controlled study. J Food Biochem. 2020;44: e13507.
8. Foroumandi E, Javan R, Moayed L, Fahimi H, Kheirabadi F, Neamatshahi M, Shogofteh F, Zarghi A. The effects of fenugreek seed extract supplementation in patients with Alzheimer's disease: A randomized, double-blind, placebo-controlled trial. Phytother Res. 2023; 37:285-294.
9. Kodumuri PK, Thomas C, Jetti R, Pandey AK. Fenugreek seed extract ameliorates cognitive deficits in streptozotocin-induced diabetic rats. J Basic Clin Physiol Pharmacol. 2019; 30:2018-0140.
10. Ghayur MN, Abdalla M, Khalid A, Ahmad S, Gilani AH. Trigonella foenum-graecum Methanolic Extract on Isolated Smooth Muscles and Acetylcholinesterase Enzyme: An In Vitro and Mechanistic In Silico Investigation. Biomed Res Int. 2022;4849464.
11. Assad T, Khan RA, Rajput MA. Effect of Trigonella foenum-graecum Linn. seeds methanol extract on learning and memory. Metab Brain Dis. 2018; 33:1275-1280.
12. Prema A, Thenmozhi AJ, Manivasagam T, Essa MM, Akbar MD, Akbar M. Fenugreek Seed Powder Nullified Aluminium Chloride Induced Memory Loss, Biochemical Changes, Aβ Burden and Apoptosis via Regulating Akt/GSK3β Signaling Pathway. PLoS One. 2016;11(11): e0165955.
13. Konuri A, Kumar MRB, Kiranmai SR, Gourishetti K, Phaneendra MYS. Supplementation of fenugreek with choline–docosahexaenoic acid attenuates menopause induced memory loss, BDNF and dendritic arborization in ovariectomized rats. Anat Sci Int. 2021; 96:197-211.
14. Anis L, Haider S, Sajid I, Ahmed S, Saeed A, Bano M. Repeated oral administration of flaxseeds induced antidepressant and anxiolytic effects in rats. FUAAST J Biol. 2016; 6:41-46.
15. Assad T, Khan RA. Effect of methanol extract of Trigonella foenum-graecum L. seeds on anxiety, sedation and motor coordination. Metab Brain Dis. 2017; 32:343-349.
16. Wang J, Cheng C, Xin C, Wang Z. The Antidepressant-like Effect of Flavonoids from Trigonella Foenum-Graecum Seeds in Chronic Restraint Stress Mice via Modulation of Monoamine Regulatory Pathways. Molecules. 2019; 24:1105.
17. Bafadam S, Beheshti F, Khodabakhshi T, Asghari A, Ebrahimi B, Sadeghnia HR, Mahmoudabad M, Niazmand S, Hosseini M. Trigonella foenum-graceum seed (fenugreek) hydroalcoholic extract

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

improved the oxidative stress status in a rat model of diabetes-induced memory impairment. Horm Mol Biol Clin Investig. 2019;39.
18. Brunna CBB, Patrícia F, Aliny S, Graziela A, Elayne C, Fernanda A, Elisabeth W, Edson L. Association of mate tea (Ilex paraguariensis) intake and dietary intervention and effects on oxidative stress biomarkers of dyslipidemic subjects. Nutrition. 2012;28(6):657-64.
19. Saber AS, Samah MY. Effect of fenugreek seed extract on adriamycin-induced hepatotoxicity and oxidative stress in albino rats. Toxicol Ind Health. 2012;28(10):876-85.
20. Rahimi RA, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidant in the management of diabetes and its complications. Biomed Pharmacother. 2005; 59:365-373.
21. Descalzo AM, Sancho AM. A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. Meat Sci. 2008; 79:423-436.
22. Marzouk M, Soliman AM, Omar TY. Hypoglycemic and antioxidative effects of fenugreek and termis seeds powder in streptozotocin-diabetic rats. Eur Rev Med Pharmacol Sci. 2013; 17:559-565.
23. Zhijuan Z, Hui Z, Peng Z, Rong L, Jinyu Z, Jiyuan L, Danmei H, Rui H, Fenglin T, Jie L, Demei X, Chenlu Z, Xin T, Yuanlin M, Patrick K. D2HGDH Deficiency Regulates Seizures through GSH/Prdx6/ROS-Mediated Excitatory Synaptic Activity. Adv Sci (Weinh). 2025;12(13): e2404488.

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2026 Authors. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.