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Original Research Article

Evaluation of Anti-Anxiety and Antioxidant Activity of Ethanolic Extract of Fenugreek (Trigonella Foenum Greacum) Seed in Mice

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Abstract

Introduction: Trigonella foenum-graecum, commonly known as fenugreek, is used to enhance the flavor, color, and texture of food and for medicinal purposes in many traditional systems. Oxidative stress occurs due to an imbalance between the production and removal of free radicals. Anxiety is a mental condition characterized by excessive apprehensiveness about real or perceived threats, typically leading to avoidance behaviours and often to physical symptoms such as increased heart rate and muscle tension.

Aim & Objectives: To evaluate the anti-anxiety and antioxidant activity of the ethanolic extract of fenugreek seed in Wistar rats.

Materials & Methods: A total of 42 mice were taken & divided them in to 7 groups, six in each. Groups I, II, III, IV, and V were treated with normal saline (25ml/kg), Diazepam 1mg/kg, fenugreek 200mg/kg, fenugreek 400mg/kg, and diazepam 1 mg/kg + fenugreek 400mg/kg, respectively, for 7 days and studied for antianxiety effect. To study the antioxidant effect, along with groups I, III, and IV, 2 more groups were taken and designated as groups VI and VII. The formal one was given vitamin C alone, and the latter one was given fenugreek 400mg/kg + vitamin C.

Results: The results revealed that EETFG at 200 mg/kg and 400mg/kg exhibited dose-dependent antianxiety and also antioxidant effect comparable to the standard drug diazepam (1 mg/kg) and vitamin C, respectively. A synergistic antianxiety and antioxidant effect was observed when EETFG was combined with diazepam and vitamin C, respectively.

Conclusion: The study concludes that EETFG showed a significant antianxiety activity and antioxidant effect as it increased the duration of time spent in the open arm (Elevated plus maze method)and light chamber (Light-dark model) by mice and showed low serum MDA and high TAC level.

Keywords: Antianxiety, Antioxidant, Trigonella Foenum Greacum, Oxidative Stress, Superoxide Dismutase, Malondialdehyde.

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Introduction

Oxidative stress is defined as an imbalance between the production and scavenging of free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), resulting in their accumulation in the body. [1] In aerobic life, oxygen is the main component. However, in certain conditions, this oxygen may be harmful to cells when it creates reactive species. [2]

Oxidative damage to proteins, DNA, and lipid is associated with chronic degenerative diseases. Free radicals are implicated in more than 80 diseases, including diabetes mellitus, atherosclerosis, cataract, rheumatism, and other autoimmune diseases like aging, etc. To safeguard the cells and the body's oxygen system from reactive oxygen species (oxidative stress), humans have developed a highly advanced and intricate antioxidant defense system. This system acts by suppression of the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements.

Anxiety disorders comprise a range of symptoms and consist of generalized anxiety disorder, obsessive–compulsive disorder, panic disorder, post-traumatic stress disorder, separation anxiety disorder, social phobia, specific phobias, and acute

stress. [3] Anxiety impacts one in eight individuals globally and has emerged as a significant area of study within psychotherapy. [4] The occurrence of anxiety disorders stands at 30.5% for women and 19.2% for men, respectively. Anxiety disorders have focused on GABA, serotonergic, noradrenergic mechanisms, and neuropeptides. Recent evidence indicates a role for adenosine and cholecystokinin in the onset of anxiety. [3] Numerous medications are available to address anxiety disorders; however, they often result in various systemic side effects. This highlights the necessity to create a treatment for anxiety disorders that has no or minimal side effects.

Trigonella foenum-graecum (TFG) has been utilized in traditional Indian medicine for centuries, owing to its wide array of medicinal benefits. [5] It has been employed for its hypoglycemic, [6] hypocholesterolemic, [7] antioxidant, [8] antirheumatic, [3] appetite-stimulating, [4] gastroprotective [9] properties, as well as for treating burns, assisting in labor and delivery, addressing gynecological issues, [10] enhancing milk production, [11] alleviating dyspepsia, rickets, hemorrhoids, chronic cough, [12] managing both acute and chronic inflammation, [13] providing analgesic,14 effects, reducing fever, relieving abdominal colic, treating boils, and carbuncles. [15] Additionally, it exhibits antitumor, antiviral, antimicrobial, and hypotensive properties [16, 17] acts as a laxative (18 serves as an anthelmintic, aids in the treatment of leprosy and bronchitis, functions as a diuretic, and is considered an aphrodisiac, beneficial for dropsy, hair loss, and enlargement of the liver and spleen. [19, 20] The bioactive compounds extracted from fenugreek seeds encompass saponins (such as fenugreekine and diosgenin), alkaloids (including trigonelline, gentianine, and carpaine), amino acids, flavonoids, some of which function as insulin secretagogues (like 4-hydroxyl isoleucine and arginine), nicotinic acid, coumarins, mucilaginous fibers (Galactomannan), along with various vitamins and minerals. [21, 22] The current research aims to explore the anti-anxiety and antioxidant properties of this fenugreek seed extract.

Material and Methods

It was an animal-based experimental study conducted from August 2013 to October 2013 in the Department of Pharmacology, Mamata Medical College, and Khammam. The study was initiated after prior permission from the Institutional Ethics Committee (IAEC); the power of the test is 80%.

Preparation of Plant Extract: 100 g of dry powder of Trigonella foenum-graecum was continuously extracted for 48 hr with 90% ethanol in a Soxhlet apparatus. The collected extract was stored at 0-4 °C until it is used.

Experimental Animals: Laboratory breeds of albino mice of either sex weighing between 15 and 30g were procured from the animal house of Mamata Medical College, Khammam. The animals were housed in standard cages, and temperature was maintained at 23 ± 5.00C with a 12-h light/dark cycle. The experimental protocol has been approved by the Institutional Animal Ethics Committee.

Toxicity study of fenugreek extract: Based on the literature, the Trigonella foenum-graecum seed extract acute oral LD50 was found to be >5 g/kg. [23] So, the doses to be used in this study were fixed as 200mg/kg and 400 mg/kg. The fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for toxicity studies. [24]

Experimental design: Animals were divided into seven groups. Each group contains six animals

- Group I – Control (25ml/kg)
- Group II – Diazepam 1mg/kg (Standard)
- Group III – Test-1 (Fenugreek – 200 mg/kg)
- Group IV – Test-2 (Fenugreek – 400 mg/kg)
- Group V –Fenugreek – 400 mg/kg + Diazepam 1mg/kg
- Group VI- vitamin C (Standard)
- Group VII-Vitamin C+ test 2 (Fenugreek – 400 mg/kg)

The animals of all groups were treated for 7 days.

Anti-Anxiety Activity was evaluated by two methods: 1. Light-Dark Model, 2. Elevated plus maze model

Elevated plus maze model: The EPM apparatus consisted of two open arms (16×5cm) and two closed arms (16×5×12cm) emanating from a common central platform (5×5cm). The two pairs of identical arms are opposite to each other. The entire apparatus was elevated to 25cm above the floor level. The animals received the treatment as per the schedule, 1 hour before the start of the session. At the beginning of the session, the mouse was placed at the centre of the maze, its head facing the open arm. The number of entries and the time spent in closed and open arms were recorded during a period of 5 minutes. Observation period. An entry is defined as the presence of all four paws in the arm. [25]

Light-Dark Model: The apparatus used was an open-top wooden box. The box was divided by a barrier possessing a doorway (7.5 x 5 cm), which mice could cross in two chambers of measures (20 x 30 x 35 cm), painted black and illuminated with dimmed red light, and a bright chamber (30 x 30 x 35 cm) painted white and illuminated with a 100-W white light source. Mice were placed individually in the centre of the light zone and observed for the next 5 minutes for the number of crossings between

the two compartments and the time spent in the light and dark areas. The suppression of exploratory activity in the light compartment caused by bright illumination is antagonized by anxiolytic agents, and anxiogenic agents exacerbate the behavioural suppression. The simplicity of the model provides a rapid means for the assessment of the action of agents capable of modifying anxiety. [25,26] Blood samples were collected by retro-orbital plexus method from all treatment groups and sent to the central laboratory for assessment of oxidative stress. The parameters taken for assessment of oxidative stress were TAC (Total Antioxidant Capacity) and MDA (Malondialdehyde). The formal one was estimated by thiobarbituric acid reactive substance assay (TBARS), and later by ferric reducing ability of plasma (FRAP) method.

Statistical Analysis: The obtained data were analyzed by using One-Way Analysis of Variance (ANOVA) followed by Dunnets test. Results were expressed as Mean ± SEM. A p-value < 0.05 was considered statistically significant.

Results

The duration of time mice spent in the open arm was recorded in the elevated plus maze method, and time spent in the light chamber was recorded in the light-dark model for evaluation of Anti-Anxiety activity.

Elevated plus maze model: When compared with the group I (normal saline), mice from group II (diazepam 1 mg/kg) spent a longer time in the open arm (214.2±8.01sec), which was statistically significant ($p<0.05$). Group-III (145.8±5.84 sec) and group-IV (173.3±9.31 sec) spent significantly longer duration of time in the open arm when compared to the control group ($p<0.05$), but less than the duration spent by mice from group II. Group-V treated with Fenugreek-400mg/kg and diazepam 1mg/kg, spent a longer duration of time (222.8±3.76 sec) in the open arm as compared with group-I and group II, which was statistically significant ($p<0.05$).Table-1 and Figure -1.

Table 1: Effect of fenugreek extract on Time spent in open arm (sec).

	Treatment groups	Mean±SD
Group – I	Control	93.33±4.08
Group – II	Diazepam 1mg/kg	214.2±8.01
Group – III	Test 1 (Fenugreek – 200mg/kg)	145.8±5.84
Group – IV	Test 2 (Fenugreek – 400mg/kg)	173.3±9.31
Group – V	Test 2 + Diazepam 1mg/kg	222.8±3.76

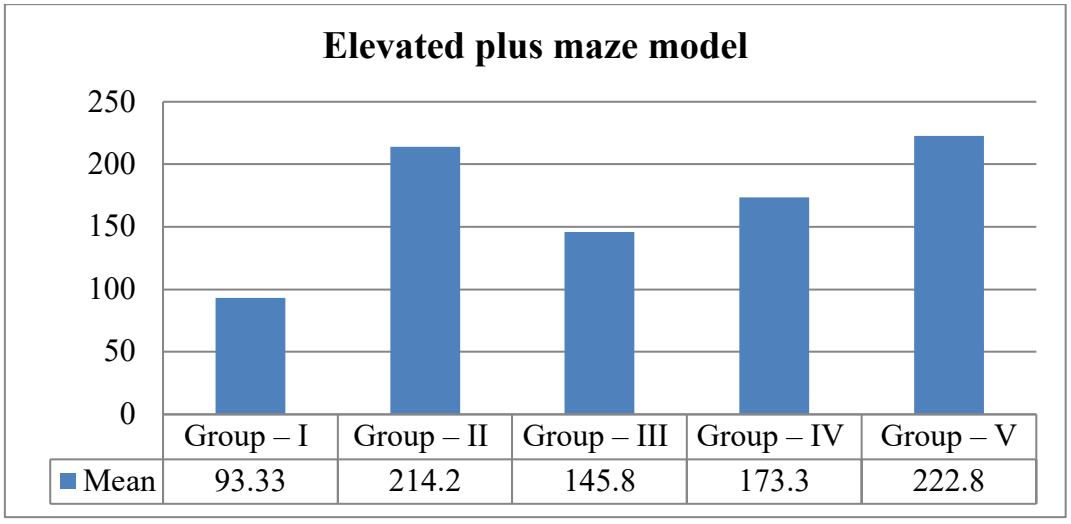


Figure 1: Effect of fenugreek extract on Time spent in open arm sec.

Light – Dark model: Mice from the group II treated with diazepam 1mg/kg spent significantly longer time (226.7±13.29) in the light chamber than the control group ($p<0.05$). Group-III (152.5 ± 5.24 sec) and group-IV (167.5 ± 7.58 sec) spent significantly longer duration of time in the light chamber when compared to the control group ($p<0.05$), but less than the duration spent by mice from group II.

Group-V treated with Fenugreek-400mg/kg and diazepam 1mg/kg, spent a longer duration of time (230.4±7.36 sec) in the light chamber as compared with group-I and group II, which was statistically significant ($p<0.05$). Table-2 and Figure 2.

Table 2: Effect of fenugreek seed extract on Time spent in light chamber

	Treatment groups	Mean±SD
Group – I	Control	108.3±6.83
Group – II	Diazepam	226.7±13.29
Group – III	Test 1 (Fenugreek – 200mg/kg)	152.5±5.24
Group – IV	Test 2 (Fenugreek – 400mg/kg)	167.5±7.58
Group – V	Test 2 + Diazepam	230.4±7.36

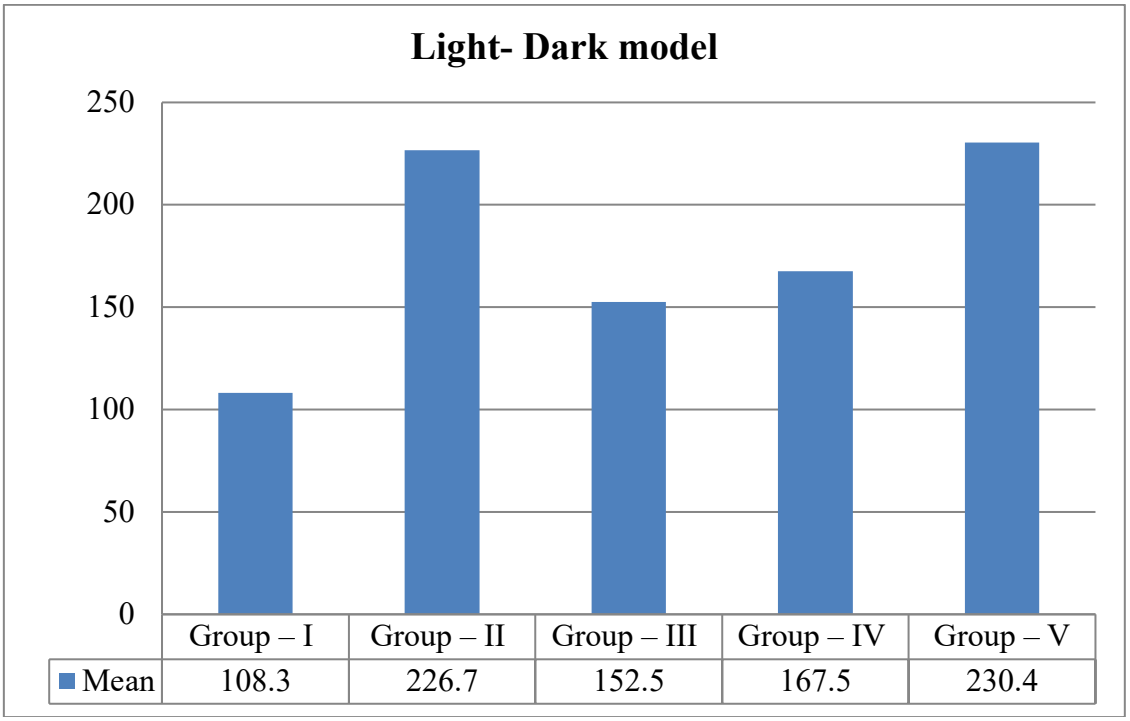
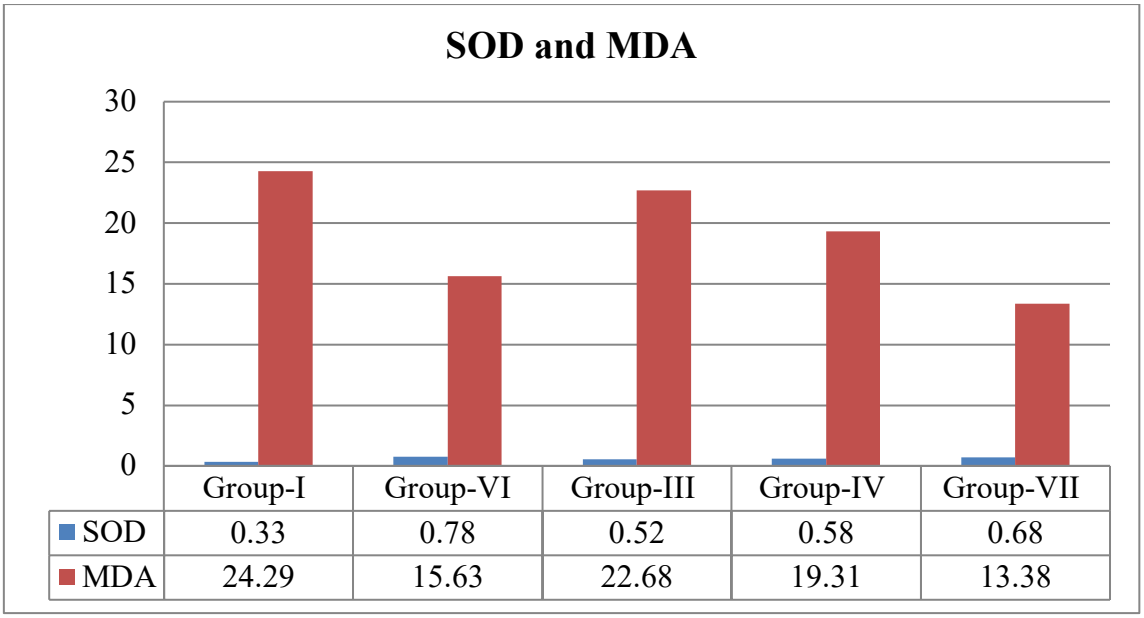


Figure 2: Effect of fenugreek seed extract on Time spent in light chamber

Estimation of SOD and MDA levels: The mean of SOD levels are more in group III and IV when compared with group I (control), which was statistically significant($p<0.05$). But, when it was compared with the group VI that received vitamin C, both group III and IV showed low mean SOD values. When compared with groups I and VI, high mean SOD values were observed in group VII, which was statistically significant ($P<0.05$). When compared with group-I, the mean of MDA levels was less in group III and IV, which was statistically significant ($p<0.05$), but when compared with group VI, both the groups showed more MDA levels. MDA values were very low in group VII when compared with group I and group VI, which was statistically significant ($p<0.05$).

Table-3: Effect of fenugreek seed extract on SOD and MDA

	Treatment groups	SOD (Mean± SD)	MDA (Mean± SD)
Group-I	Control	0.33±0.03	24.29±0.33
Group-VI	Vitamin C	0.78±0.04	15.63±0.46
Group-III	Test 1 (Fenugreek – 200mg/kg)	0.52±0.01	22.68±0.33
Group-IV	Test 2 (Fenugreek – 400mg/kg)	0.58±0.01	19.31±0.39
Group-VII	Test 2 +Vit C	0.68±0.01	13.38±0.16



	Group-I	Group-VI	Group-III	Group-IV	Group-VII
SOD	0.33	0.78	0.52	0.58	0.68
MDA	24.29	15.63	22.68	19.31	13.38

Figure 3: Effect of fenugreek seed extract on SOD and MDA

Discussion

Present research study was aimed to estimate antianxiety and antioxidant effect of ethanolic of fenugreek (Trigonella foenum greacum) seed extract in wistar rats. Plants are used as a source of medicine in the past centuries and today scientists and the general public recognize their value as a source of new or complimentary medicinal products. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people. [27]

Trigonella foenum-graecum, commonly known as fenugreek belongs to the family of Leguminosae which is an annual, herbaceous and aromatic plant and is commonly used as a condiment and seasoning in food preparations; is assumed to possess nutritive and restorative properties and has been used in folk medicine for centuries for a wide range of diseases. Fenugreek contains saponins that are transformed in the gastrointestinal tract into sapogenins. Saponins in this plant include; Sarsapogenin, Yuccagenin, Smilagenin and, the most important saponin in this plant is Diosgenin. [27] Fenugreek seeds contain 50-percent fiber (30-per-cent soluble fiber and 20-percent insoluble fiber) that can slow the rate of postprandial glucose absorption28 and improve the digestion. Fenugreek seeds, contain oils, alkaloids, amino acids (lysine, argenine, tryptophan, threonin, valyn and methionin) and mucilages such as the most famous galactomannan, this also contain vitamins A, C, D, B1 and, minerals calcium, iron and zinc. [29] The World Health Organization (WHO) has recommended that the use of herbs should be encouraged in India.30 Development of phytomedicines is relatively inexpensive and less time consuming. It is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years. [31] Anxiety is an emotional state, unpleasant in nature and is associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat. It is normal reaction to stress and is characterized by palpitations, fatigue, nausea and shortness of breath. Despite a phenomenal development of modern drug industry, medicinal plants still constitute an important part of pharmacopoeias in both the developed and developing countries. These plants are important elements of traditional medicine and can be developed as potential drug, after scientific validation. [32]

Out of many possibilities to modify maze tests (e.g. water maze, the Y-maze, the radial maze, and the elevated plus maze), Elevated plus maze have found acceptance in many laboratories. The test has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect. [33] The fear due to height induces anxiety in the animals when placed on the elevated plus maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by preference to remain at safer places. Anxiolytic agents are expected to increase time spent by the animal in the open arms. [25]

The alteration in the time spent in open arm is considered more sensitive to the drug effect than the number of entries. Fenugreek extracts)200mg/ kg and400mg/kg) showed significant ($P<0.05$) reduction in the anxiety by EPM

(Elevated plus maze method)with reduction of time spent in closed arms. Although, the duration of time spent by mice treated with Fenugreek extracts 400 mg/kg longer than the duration of time spent by mice treated with Fenugreek extracts 200mg/kg, which indicates that Fenugreek extracts showed dose dependent anxiolytic effect. Whereas the mice received Fenugreekextract 400mg/kg along with diazepam showed even more anxiolytic (spent more time in the open arm) effect than mice received diazepam alone that indicates the additive synergistic anxiolytic effect of fenugreek and diazepam. Table-1& Figure -1

When another method was used i.e. Light-Dark model for estimation of anxiolytic effect of fenugreek extract, Fenugreek extracts)200mg/kg and400mg/kg) showed significant($P<0.05$) reduction in the anxietyby mice spending more time in the light chamber. Although, the duration of time spent in the light chamber by mice treated with Fenugreek extracts 400 mg/kg longer than the duration of time spent by mice treated with Fenugreek extracts 200mg/kg, which indicates that Fenugreek extracts showed dose dependent anxiolytic effect. Whereas the mice received Fenugreekextract 400mg/kg along with diazepam showed even more anxiolytic (spent more time in the light chamber) effect than mice received diazepam alone. Table-2& Figure -2 Hence, it can be hypothesized that the extracts used in the combination showed a synergistic action through GABAergic mechanism to produce anxiolytic effect.

Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases. Free radicals are implicated for more than 80 diseases including diabetes mellitus, atherosclerosis, cataract, rheumatism, and other auto immune disease like aging etc. Antioxidants exert their mode of action by suppression the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements.34 Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. The mean of SOD levels are more in group III and IV when compared with group-I (control), which was statistically significant($p<0.05$). But, when it was compared with group VI received vitamin C, both group III and IV showed low mean SOD values. When compared with group I and VI, high mean SOD values were observed in group VII, which was statistically significant ($P<0.05$). When compared with group-I, the mean of MDA levels were less in group III and IV, which was statistically significant ($p<0.05$), but when compared with group-VI, both the groups showed more MDA levels. MDA values were very low in group VII when compared with group I and also group VI, which was statistically significant ($p<0.05$).Table& Figure -3 SOD is an enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen, [35] hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes.36 Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical–mediated damage and oxidative stress. [37]

Conclusion

The study concludes that ethanolic extract of fenugreek (Trigonella foenum greacum) seeds showed a significant antianxiety effect as it has increased the duration of time spent in open arm (Elevated plus maze method)and light chamber (Light-dark model) by mice. The extract also showed a significant antioxidant effect. The extract showed a marked synergistic anxiolytic and antioxidant effect when combined with diazepam and vitamic C respectively. However, further studies are required to identify the phytochemical compound in the extract and their mechanism which might be responsible for their anxiolytic and antioxidant action.

The present research study aimed to estimate antianxiety and antioxidant effects of the ethanolic extract of fenugreek (Trigonella foenum-graecum) seed extract in Wistar rats. Plants have been used as a source of medicine for centuries, and today scientists and the general public recognize their value as a source of new or complementary medicinal products. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people. [27]

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seeds contain oils, alkaloids, amino acids (lysine, arginine, tryptophan, threonine, valine, and methionine), and mucilages such as the most famous galactomannan, this also contain vitamins A, C, D, B1, and minerals calcium, iron, and zinc. [29] The World Health Organization (WHO) has recommended that the use of herbs should be encouraged in India. [30] Development of phytomedicines is relatively inexpensive and less time-consuming. It is more suited to our economic conditions than allopathic drug development, which is more expensive and spread over several years. [31]

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