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Evaluation of Anti-Anxiety Effect of *Perilla Frutescens* Seed Extracts on Experimental Animals

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Abstract Background: *Perilla Frutescens* (PF) has traditionally been used in depression-related diseases, as a sedative and anti-oxidant agent. Objective: This study intended to appraise the anxiolytic effects of PF on experimental animals using n-hexane extract (NHE) and ethanolic extract (EE) of PF. Methods: The animals were divided into six separate groups, with each group comprising 6 Swiss albino mice of either sex. PF seeds powder is extracted with NHE, and EE and given orally at a dosage ranging from 1-2 ml/kg of body weight. To evaluate anxiolytic behavior, we employed several tests, including the Elevated Plus Maze (EPM), light-dark exploration test (LDE), open-field test (OFT), Photoactometer (PA), and hole-board test (HBT). Additionally, we assessed various biochemical parameters such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). Result: In the EPM model, both extracts exhibited a significant rise in the percentage of time spent and the number of entries into the open arm. The LDE test demonstrated a prolonged duration of entry into the light box and an increased period spent in the light compartments. Moreover, in the OFT, there was a notable rise in the number of squares crossed. Locomotor activity is measured with a PA, and lower cut-off numbers distinguish extract-treated groups from diazepam-treated normal control groups. The HBT demonstrated a dose-dependent increase in the number of head dips. Conclusion: Both extracts exhibited notable anxiolytic activity, but the most favorable outcome was achieved by administering a 2 ml/kg dose of NHE of PF in all the models. The present research suggests that chemical constituents of PF are an acceptable candidate for the prophylaxis of AD.

Keywords *Perilla frutescens*, Anxiolytics, Anxiety, Elevated Plus Maze, Light-Dark Exploration Test, Open-Field Test, Photoactometer, Hole-Board Test

1. Introduction

Anxiety is a typical and uncomfortable condition characterized by tension, apprehension, and psychological defense in response to an unpleasant or threatening situation caused by known or unknown sources. Anxiety Disorder (AD) is a commonly encountered psychological illness in clinical practice, affecting more than 10-20% of the population. GAD is characterized by alterations in mood, behavioral patterns, health and well-being, and cognitive ability. Obsessive-compulsive disorder, disordered eating patterns, post-traumatic stress disorder, panic attacks, and various specific phobias are often associated with manifestations of anxiety [1], despite having an extensive list of undesirable effects, including sedation, neuromuscular relaxation, ataxia, dementia, and tolerance with continued usage. Additional treatment alternatives encompass drug-based approaches and behavioral interventions. Medication-based treatment

typically involves antianxiety medications such as those in the benzodiazepine (BDZ) category, antidepressants, and beta blockers. It has been reported that many herbal products are less harmful than synthetic medications. Many herbal products have been suggested as being less harmful than synthetic medications and have strong psychological benefits [2].

An aromatic yearly herb from the Lamiaceae family, *Perilla frutescens* (L.) Britt. is widely cultivated throughout many Asian zones. The stalks, leaflets, and seeds of the plant include more than hundreds of constituents, including monoterpenes, sesquiterpenes, triterpenoids, flavonoids, polyphenols, fatty acids, phytosterols, etc. Most of these are linked to the plant's therapeutic properties, including those with anti-inflammatory, free radical scavenging, antitumor, antimicrobial, and antidepressant properties. The seeds are a significant provider of omega-3 (specifically alpha-linolenic acid, ALA), omega-6 (linoleic acid), and omega-9 fatty acids (oleic acid) [3]. Furthermore, PF seeds are rich in compounds such as rosmarinic acid, luteolin, chrysoleriol, quercetin, catechin, caffeic acid, and ferulic acid. Major chemical compounds of PF are fatty acids, which diminish anxiety by modifying GABAergic neurotransmission. Changes in GABAA receptor neurotransmission cause hyperpolarization, which is dependent on chloride ion channel opening frequency, and Chloride ion channels play a role in anxiolytic effects [4]. Gang Zhao et al. prove that luteolin and apigenin, are two compounds that possess actions that enhance monoamine uptake [5]. Oleic acid exhibited a dose-dependent increase in norepinephrine, dopamine, and serotonin levels [6]. Rosmarinic acid lowered plasma corticosterone levels and enhanced the expression of hippocampal GR, MR, and BDNF [7]. Oxidative stress within the brain has the potential to disrupt the nervous system, and it has likewise been linked to the presence of depressive symptoms, anxiety-related conditions, and increased levels of anxiety [8].

A major chemical compound of PF is fatty acid, which diminishes anxiety by modifying GABAergic neurotransmission. Changes in GABAA receptor neurotransmission cause hyperpolarization, which is dependent on chloride ion channel opening frequency, and chloride ion channels take part in anxiolytic-like actions [4]. Certain literature proves that luteolin and apigenin, are two compounds that possess actions that enhance monoamine uptake [5]. In a dose-dependent manner, oleic acid significantly increased norepinephrine, dopamine, and serotonin levels [6]. The rosmarinic acid reduced plasma corticosterone levels and increased the expression of hippocampal GR, MR, and BDNF [7]. Oxidative damage in the brain hurts the nervous system and has also been related to depression, anxiety disorders, and high anxiety levels [8].

2. Materials and Methods

2.1. Experimental Animals

The experiments were conducted by the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA). The Institutional Animal Ethical Committee approved the experimental protocol (Approval number:CPCSEA/SNLPCP/IAEC/22/01/127).

In this research, a combined total of 36 Swiss albino mice, each weighing within the weight range of 20g to 25g, were sourced from the Jay Research Foundation located in Vapi, Gujarat. The animals were kept in a controlled environment with a temperature of 25 ± 1 ℃ and a relative humidity of 50 ± 55%. Lighting was regulated with a 12:12-hour light/dark cycle, and the mice had unrestricted access to food and water within polypropylene cages.

2.2. Drug

The Diazepam (DZP) ampoules, containing 10 mg per 2 ml, were procured from Sun Pharma Laboratories Ltd. in India [9,10].

2.3. Plant Material

PF seeds were procured from Earth Expo, Bhavnagar, Gujarat. Their authenticity was verified by Dr. B. R. Patel, who holds the position of Associate Professor of Botany at the Patidar Gin Science College in Bardoli, District Surat, Gujarat. (Authen. 03/2022 Botany) on January 27th, 2022.

2.4. Preparation of Extract and Administration

2.4.1. Preparation of n-hexane Extract

About 50gm of *Perilla* seeds were winnowed and cleaned off all the foreign particles and sun-dried for 4-5 days. The seeds were then crushed and made into fine powder. Then that powder was extracted in a Soxhlet extractor for 36hr with n-hexane (1:10). The solvent was evaporated entirely and the extract thus achieved was used for subsequent investigations [10,11].

2.4.2. Preparation of Ethanolic Extract

About 50gm of Perilla seeds were winnowed and cleaned off all the foreign particles and sun-dried for 4-5 days. The Perilla seeds were then crushed and made into a fine powder. The powder was then extracted with a Soxhlet extractor in 1 liter (1000 gm) of 70% ethanol. The ethanolic solution was stirred for four hours and then left to stand overnight. Subsequently, the solution underwent filtration using Whatman filter paper no.1, and the resultant solution was utilized for further analysis [12].

2.5. Preliminary Phytochemical Studies

Both extracts were screened for the presence of phytoconstituents. The extracts were tested for flavonoids, alkaloids, phenol, carbohydrates, saponins, glycosides, tannins, steroids, resin, amino acids, and fixed oils with all respective testing procedures as described in the textbook of Biren Shah [13].

2.6. Experimental Design

Swiss albino mice (15-20 gm) were used. The animals were housed in conventional conditions under a 12-hour light/dark cycle, and they had continuous availability of food and water throughout the experiment. One hour before initiation of the experiments, the rats were given time to acclimate to the laboratory setting. All trials took place during the light period, and the animals were subdivided into six groups, each consisting of six individuals.

- Group I Normal group
- Group II Standard group: Diazepam 1 mg/kg p.o.
- Group III – n-hexane extract of *PF* 1 ml/kg p.o. [14].
- Group IV – n-hexane extract of *PF* 2 ml/kg p.o. [15].
- Group V– Ethanolic extract of *PF* 1 ml/kg p.o. [14].
- Group VI – Ethanolic extract of *PF* 2 ml/kg p.o. [15].

2.7. Pharmacological Evaluation for Anxiolytic Activity

2.7.1. Elevated plus-maze Model: [16]

The evaluation of anxiolytic and anxiogenic effects of drugs commonly involves the use of the EPM, which is a widely utilized experimental model for studying anxiety. This maze consists of two open arms and two closed arms, each measuring $50 \times 10 \times 40$ cm, elevated to a height of 50 cm. The drug was administered to the animals 30 minutes before the test. After this waiting period, each animal was placed individually in the center of the maze, and the study recorded several parameters, including the number of entries into both the open and closed arms, as well as the percentage of time spent in these open and closed arms. Before beginning the next test to get rid of any filth or odor, clean it with 70% ethanol. Subsequently, the next group of mice can be introduced into the apparatus.

2.7.2. Light-Dark Model: [17]

This LDE apparatus comprises wooden boxes measuring 60 cm in length, 40 cm in width, and 35 cm in depth. A wooden board with a 10×10 cm^2 aperture at ground level was employed, distributing the boxes into two equal partitions, and connecting them. One of these partitions is coated in black and includes a wooden lid. Transport the mice from their housing cages to the testing room. It is advisable to allow them to acclimate to the new environment for 30 minutes before commencing the experiment. Place the experimental mice within the brightly illuminated section of the model and insert the cage into the system, making certain that the dark section is positioned towards the rear. Allow the mice to move freely between the two compartments for 5 minutes. Make the apparatus uncontaminated with 70% ethanol before initiating the assessment or eradicating any dust debris or odor amassed on the apparatus. Subsequently, the next mouse can then be inserted into the apparatus.

2.7.3. Open-field Test: [18]

The open field comprised of wood and measured fifty cm in (l), fifty cm in (w), and twenty-five cm in (h). The floorboards of the open field were separated into 8cm $\times$ 8cm squares, with 16 squares on the model. Sixteen squares were separated on the floorboard and the others nearby to the walls as the edge. Every other mouse was gently placed in the center of the field, and the number of squares they crossed was documented during a five-minute interval. After this initial five minutes, the mice were taken outside from the apparatus, and the board was cleaned with ethanol. It was considered that a mouse had crossed from one square to another when all four paws had passed, and this was recorded during the five-minute observation period.

2.7.4. Photoactometer: [19]

The identical photoelectric cells were arranged to receive a steady beam of radiation via six lights. The photoelectric cells will become active when a mouse crosses the light beam and thus cuts off (crossing) the light beams hitting upon that. This crossing is counted automatically for 10 minutes by the PA. The cut-off no. was taken as a resulting factor of the mice as locomotor activity.

2.7.5. Hole-board Test: [20]

The Hole Board Test (HBT) incorporated a paradigm characterized by novelty and uncertainty. Head dipping was utilized as a common indicator of exploration or curiosity, distinguished from mere motor activity. The apparatus is constructed from a wooden board measuring $42 \times 42 \times 30$ cm, featuring 16 evenly spaced holes, each with a diameter of 2.5 cm. The center of each hole was positioned at a distance of 10 cm from the closest wall of the box. The board was raised so that the mice did not see the bottom at the time of nose poking into the hole. The box was elevated to a height of 15 cm above the ground. The animals were introduced into the center of the hole board, and the hole board was left uncovered for five minutes, allowing the mice to freely explore. Following each trial, the hole board was meticulously cleaned using a 10% ethanol solution and then dried. The observations involved recording both the number and duration of head dips on the board. A head dip was documented when both eyes of the mouse were obscured within the hole.

2.8. Evaluation of the Biochemical Parameters in Brain Tissue [21,22]

For the assessment of biochemical parameters, the mice were subjected to anesthesia with chloroform, followed by decapitation to isolate their brains. The procedure began with rinsing the isolated brain with an ice-cold normal saline solution. After this initial step, the brains were then carefully weighed and subsequently homogenized in ice-cold phosphate buffer with a pH level of 7.4.

2.8.1. Assessment of Superoxide Dismutase (SOD) Level in Brain

To prepare tissue homogenate, the brain sample was homogenized in a 67 mM buffer with phosphate at pH 7.4, with a ratio of 1:3. This process was replicated three times, each involving 1 mL of tris-HCl buffer and 50 µL of homogenate. Following this, 1 mL of a 0.2 mM pyrogallol solution was introduced to the sample as well as control tubes, whereas a blank tube filled with only tris-ethylenediamine tetra-acetic acid buffer at pH 8.2 was used. A spectrophotometer was employed to assess the absorbance at 532 nm after introducing the pyrogallol solution at both 0 and 1-minute time intervals. The assessment of Superoxide Dismutase activity was determined using the formula provided as follows:

% Inhibition of pyrogallol auto-oxidation =
ΔA (test)/ΔA (control) × 100 SOD activity (U/mL) =
% inhibition of pyrogallol auto-oxidation / 50%

2.8.2. Assessment of Catalase (CAT) Activity in the Brain

The tissue homogenate was mixed with a solution comprising 0.01 M phosphate buffer at pH 7.0 and 0.2 M H2O2. To stop the reaction, a mixture of C2H4Cr2O9-2, containing 5% K 2Cr 2O 7 and glacial acetic acid in a 1:3 ratio, was introduced into the resulting mixture. Subsequently, the test tubes were positioned in a water bath and heated for ten minutes. After the mixture had cooled to ambient temperature, the absorbance was then assessed at 570 nm. Catalase activity in the homogenate was subsequently determined using a calibration curve.

2.8.3. Assessment of Glutathione (GSH) Activity in the Brain

The connective tissue of the brain was homogenized in a buffer with a pH of 7.4, which contained 67 mM phosphates. Immediately after homogenization, we introduced 25% trichloroacetic acid (TCA) to the homogenate to precipitate it. The resulting mixture was subsequently subjected to centrifugation at 4 ℃, running for 40 minutes at 4200 rpm. The supernatant received sequential additions of 10 mM DTNB, 7.9 mL of methanol, and 200 mM Tris-HCl buffer with 0.2 M EDTA at a pH of 7.5. In place of the homogenate, a blank consisting of 67 mM phosphate buffer at a pH of 7.4 was employed. Subsequently, the test tubes were vortexed and incubated in an oven at 37 ℃ for 30 minutes. Absorbance at 412 nm was measured using a spectrophotometer, and a glutathione (GSH) standard curve was established through dilution.

2.9. Statistical Analysis

GraphPad Prism 8.0.2 was used to perform statistical analysis, and the results were reported as mean ± SEM (Standard Error of Mean) for parameters related to the anxiolytic models. Subsequently, a one-way analysis of variance (ANOVA) was performed, followed by the utilization of Dunnett's multiple comparison test. Statistical significance was obtained at significance levels of $p<0.05$ and $p<0.01$, respectively.

3. Result

3.1. Preliminary Phytochemical Studies

Ethanol extract showed a high extractive yield of 22.8 % w/v when compared to n-hexane extracts of 19.6 % w/v of PF. Preliminary phytochemical screening discovered that n-hexane extract contains compounds including flavonoids, fats & oil, phenol, and carbohydrate. Ethanol extract contains flavonoids, phenols, carbohydrates, steroids, alkaloids, glycosides, and amino acids.

3.2. Results of Experimental Models

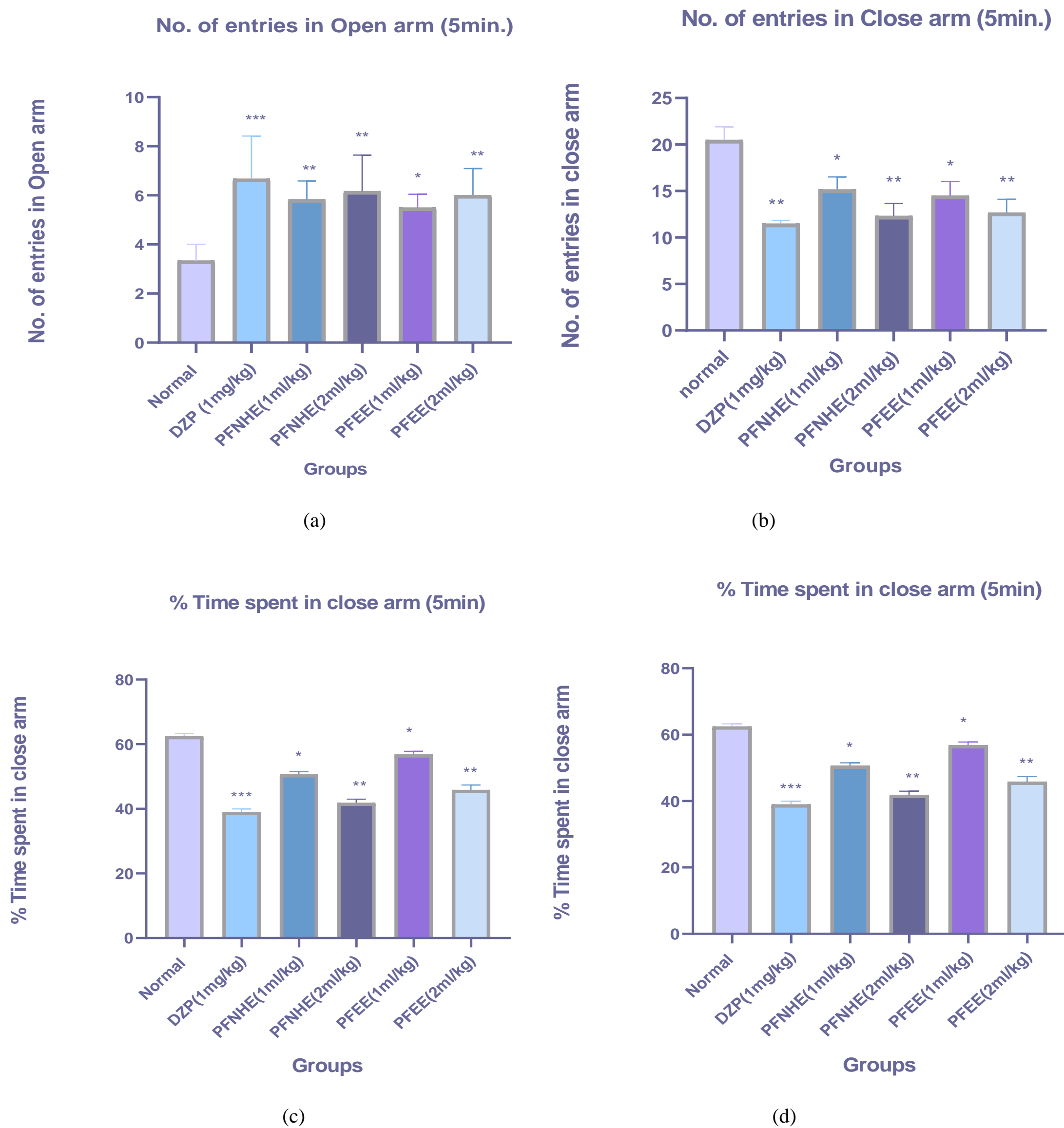
3.2.1. Elevated plus Maze Model

The DZP (1 mg/kg), the NHE, and EE at a dosage of 2 ml/kg substantially enhanced the no. of entries into the open arms of this model. This result (Table 1 and Fig. 1) ensures that the seeds also have some anxiolytic effects.

Table 1. Effect of NHE and EE of *PF* in EPM model in 5 min.

Groups	No. of entries in open-arm	No. of entries in close-arm	% Time spent in Open arm	% Time spent in Close arm
Normal	3.33±1.63	20.5±3.45	9.00±0.894	62.5±1.87
DZP (1 mg/kg)	6.67±1.75***	11.5±0.837**	18.2±1.94***	39.0±2.37***
PFNHE (1 ml/kg)	5.83±0.753**	15.2±3.31*	13.0±2.10*	50.7±2.16*
PFNHE (2 ml/kg)	6.17±1.47**	12.3±3.27**	15.8±2.14**	41.8±2.86**
PFEE (1 ml/kg)	5.50±0.548*	14.5±3.73*	11.3±1.63*	56.8±2.32*
PFEE (2 ml/kg)	6.00±1.10**	12.7±3.56**	14.8±2.40*	45.8±3.82**

Values are mean ± SEM (n=6); Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison test. The signs (***), (**), and (*) indicate values significantly different from the normal group at $P < 0.001$, $P < 0.01$, and $P < 0.05$ respectively.



The signs (***), (**), and (*) indicate values significantly different from the normal group at $^{***}P<0.001$, $^{**}P<0.01$, and $^{*}P<0.05$ respectively.

Figure 1. (a) Effect of NHE and EE on no. of entries in open arm using EPM (b) Effect of NHE and EE on no. of entries in close using EPM. (c) Effect of NHE and EE on % Time spent in the open arm using EPM. (d) Effect of NHE and EE on % Time spent in close arm using EPM

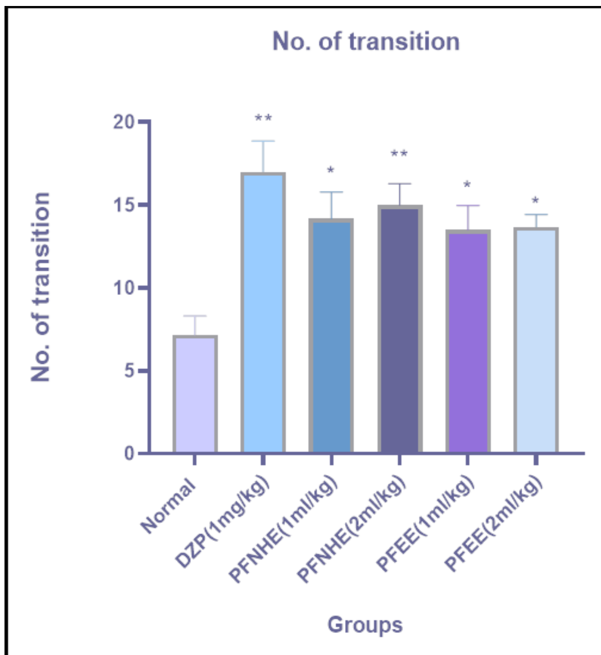
3.2.2. Light-Dark Model

Administering anxiolytics like benzodiazepines and PF seed extract results in an extended duration period inside the light compartment and a greater repetition rate of transitions between the two areas, in contrast to the control or normal group. Table 2 and Fig. 2 prove this result of rodents' intrinsic aversion to bright light.

Table 2. Effect of NHE and EE of *PF* in LDE in 5 min.

Groups	No. of Transition	% Time in Light	% Time in Dark
Normal	7.17±2.79	38.3±2.16	59.5±4.23
DZP (1 mg/kg)	17.0±4.56**	48.3±2.80***	41.7±2.16**
PFNHE (1 ml/kg)	14.2±3.97*	44.0±3.22*	50.0±2.97*
PFNHE (2 ml/kg)	15.0±3.16**	46.7±3.27**	49.8±3.43*
PFEE (1 ml/kg)	13.5±3.62*	43.2±2.32*	53.7±3.72 ns
PFEE (2 ml/kg)	13.7±1.86*	45.2±3.06*	51.3±3.08*

Values are mean ±SEM (n=6); Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison test. The signs (***), (**), and (*) indicate values significantly different from the normal group at P < 0.001, P < 0.01, and P < 0.05 respectively. NS – non-significant



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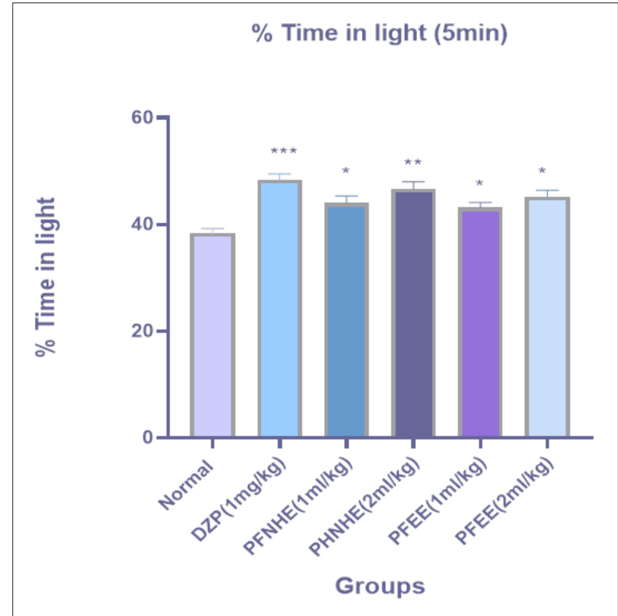

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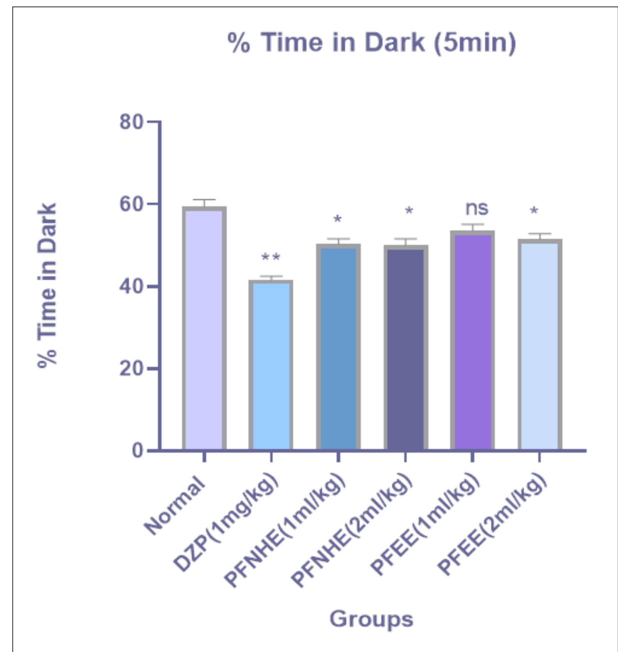

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The signs (***), (**) and (*) indicate values significantly different from normal group at ***P<0.001, **P<0.01 and *P<0.05 respectively

Figure 2. (a) Effect of NHE and EE on no. of transition using LDE. (b) Effect of NHE and EE on % Time in Light using LDE. (c) Effect of NHE and EE on % Time in Dark using LDE

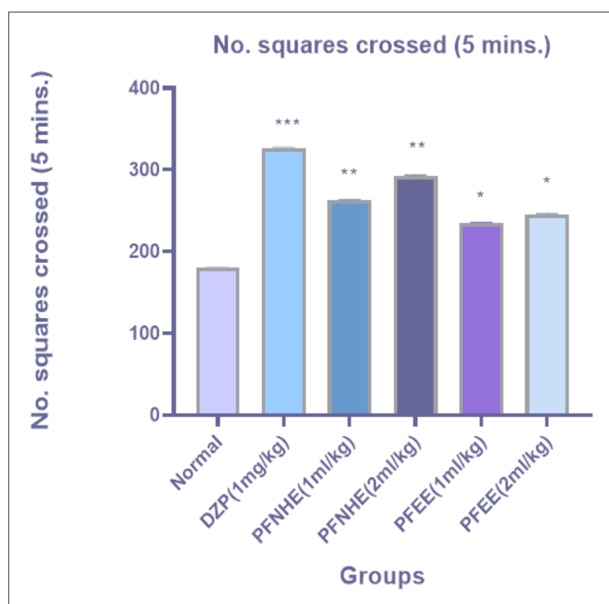
3.2.3. Open Field Test

As seen in Table 3 and Fig 3, the effect of diazepam and PF NHE and EE (1-2 ml/kg) discloses an elevation in the proportion of crossed squares in comparison to the control group indicating an anxiolytic-like effect.

Table 3. Effect of NHE and EE of *PF* in OFT in 5 min.

Groups	No. of Square crossed (5 min.)
Normal	180.3±1.633
DZP (1 mg/kg)	326.3±3.077***
PFNHE (1 ml/kg)	262.8±2.137**
PFNHE (2 ml/kg)	292.8±1.941**
PFEE (1 ml/kg)	234.8±2.639*
PFEE (2 ml/kg)	245.2±3.251*

Values are mean ±SEM (n=6); Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison test. The signs (***), (**), and (*) indicate values significantly different from the normal group at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively. NS – non significant.



The signs (***), (**) and (*) indicate values significantly different from normal group at ***P<0.001, **P<0.01 and *P<0.05 respectively

Figure 3. Effect of NHE and EE on no. of Squares crossed in 5 min. using OFT

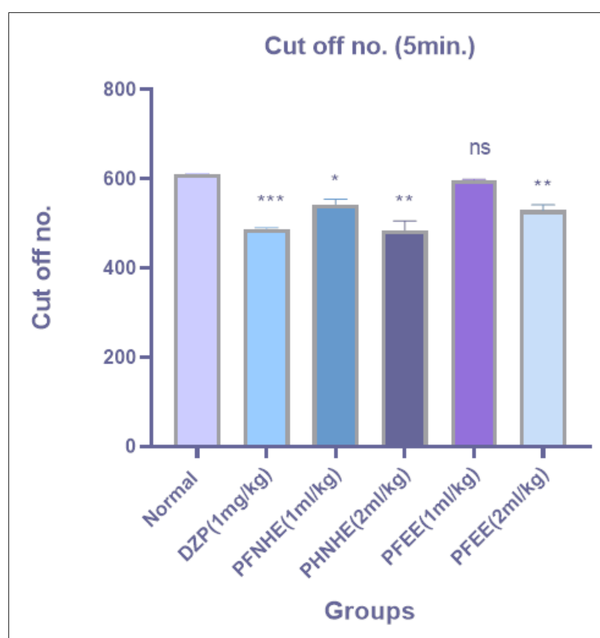
3.2.4. Photoactometer

Mice treated with NHE and EE of PF (1-2 ml/kg), in addition to diazepam (1 mg/kg), demonstrated a substantial reduction in the cut-off number. The decreased locomotor activity observed with both diazepam and PF extracts, as presented in Table 4 and Figure 4, suggests an anxiolytic effect.

Table 4. Effect of NHE and EE of *PF* in PA IN 5 min.

Groups	Cut off No. (5 min.)
Normal	613.500±2.58844
DZP (1 mg/kg)	296.500±3.72827***
PFNHE (1 ml/kg)	532.333±1.63299*
PFNHE (2 ml/kg)	463.333±22.6421**
PFEE (1 ml/kg)	576.667±2.50333 ns
PFEE (2 ml/kg)	517.833±5.34478*

Values are mean ±SEM (n=6); Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison test. The signs (***), (**), and (*) indicate values significantly different from the normal group at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively. NS – non significant.



The signs (***), (**) and (*) indicate values significantly different from normal group at ***P<0.001, **P<0.01 and *P<0.05 respectively

Figure 4. Effect of NHE and EE on cut-off no. using PA

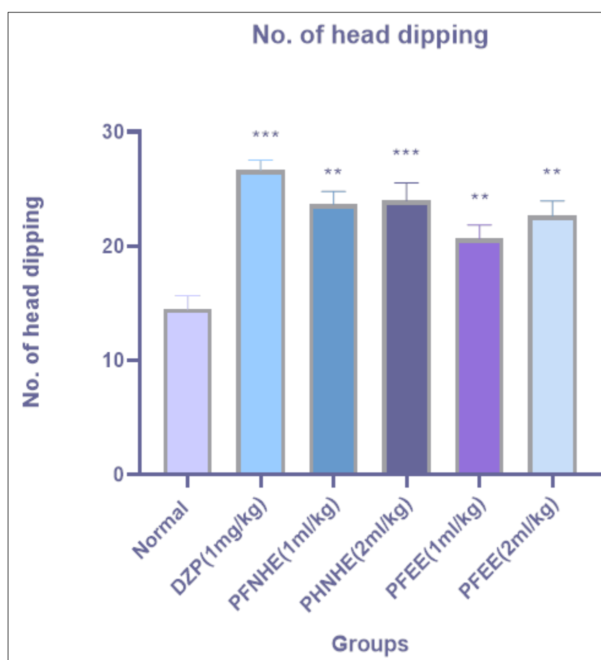
3.2.5. Hole-Board Test

PF significantly enhanced the number and intensity of head poking in this research at dosages of 1 and 2 ml/kg, indicating an anxiolytic impact. Additionally, animals increased visits numbers to the board compared with the normal group (Table 5 and Fig. 5).

Table 5. Effect of NHE and EE of *PF* in HBT

Groups	No. Head dips (5min)
Normal	14.5±2.88
DZP (1 mg/kg)	26.7±2.16***
PFNHE (1 ml/kg)	23.7±2.73**
PFNHE (2 ml/kg)	24.0±3.74***
PFEE (1 ml/kg)	20.7±2.94**
PFEE (2 ml/kg)	22.7±3.20**

Values are mean ± SEM (n=6); Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison test. The signs (***), (**), and (*) indicate values significantly different from the normal group at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively. NS – non significant.



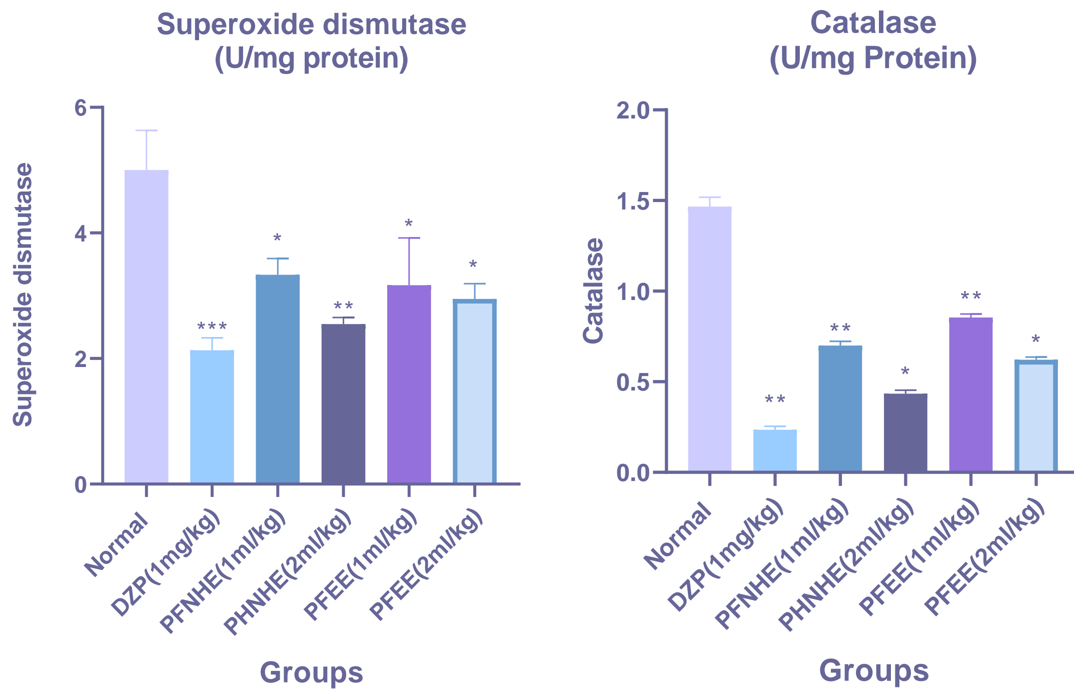
The signs (***), (**) and (*) indicate values significantly different from normal group at ***P<0.001, **P<0.01 and *P<0.05 respectively

Figure 5. Effect of NHE and EE on no. of Head dipping using HBT.

Table 6. Effect of NHE and EE of *PF* on SOD, CAT, GSH Level

Groups	SOD (U/mg protein)	CAT (U/mg Protein)	GSH (ug GSH/mg protein)
Normal	5.67±0.322	1.50±0.125	79.36±2.967
DZP (1mg/kg)	2.12±0.078**	0.269±0.023**	43.70±0.889**
PFNHE (1ml/kg)	3.79±0.260**	0.730±0.033**	62.00±2.086**
PFNHE (2ml/kg)	2.63±0.222**	0.460±0.023**	53.33±1.667**
PFEE (1ml/kg)	4±0.128*	0.888±0.030**	68.66±0.030**
PFEE (2ml/kg)	3.20±0.188**	0.632±0.047**	60.39±1.860**

Values are mean ± SEM (n=6); Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison test. The signs (***), (**), and (*) indicate values significantly different from the normal group at $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively. NS – non significant.



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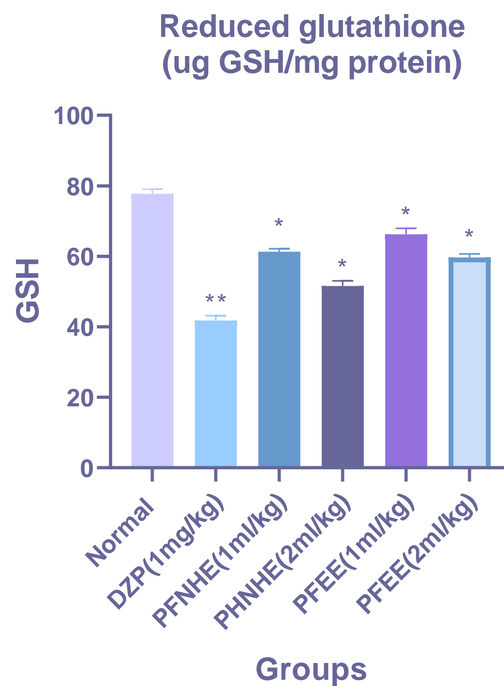

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The signs (***), (**) and (*) indicate values significantly different from normal group at ***P<0.001, **P<0.01 and *P<0.05 respectively

Figure 6. (a) Effect of NHE and EE in Superoxide dismutase (units/mg protein) (Antioxidant) (b) Effect of NHE and EE in Catalase (U/mg protein) (Antioxidant) (c) Effect of NHE and EE in Reduced glutathione (ug GSH/mg protein) (Antioxidant)

3.3. Biochemical Parameter

The generation of oxygen-free radicals associated with anxiety development results in a decrease in GSH, CAT, and SOD levels due to their utilization during oxidative stress and cell breakdown. The diminished levels of Glutathione, Catalase, and SOD in the anxiety control group provide support for this observation. Administering ethanolic and aqueous extracts orally to the mice effectively restored the diminished levels of Glutathione, Catalase, and Superoxide Dismutase, potentially by engaging in the competition to eliminate free radicals.

4. Discussion

AD is represented as the most commonly encountered psychiatric condition in general medical practice. A certain degree of anxiety, whether it's related to known or unknown factors, is a natural physiological response that aids individuals in addressing various challenges in life. The reactions and emotions exhibited by individuals with anxiety disorders typically represent an amplification of those experienced by individuals in their daily lives. Symptoms often include complaints of headaches, tension, a sensation of tightness around the head, palpitations, trembling, dry mouth, excessive sweating, cold extremities, muscle spasms leading to diffuse body discomfort, disabling conditions, and insomnia. These patients may also contend with bowel disturbances and specific phobias, such as the fear of death, insanity, or heart disease. Notably, appetite and libido are generally less affected, and thoughts of suicide are typically absent unless there is an underlying severe depression or major psychosis [23,24]. In an AD, an individual encounters a specific measure of apprehension when confronted with a threatening situation. Anxiety can potentially lead to undesirable and probable immobilizing psychic (such as restlessness or perception of impending dangers) and physiological arousal (often including tachycardia or Dyspnea) if it becomes interminable [25]. Benzodiazepines (BZDs), selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, beta-blockers, and azapirones are major medication classes used to treat anxiety disorders [26]. All the drug classes currently prescribed are known for their side effects, which are both frequent and severe. Similar to Benzodiazepines (BZDs), they lead to unwanted effects such as somnolence, lack of coordination, sedation, muscle weakness, restlessness, and liver toxicity. Additionally, they can have adverse interactions with other central nervous system depressants, particularly alcohol [27,28].

The plant examined in this study, "Perilla Frutescens" (Lamiaceae), is regarded as a potential and promising candidate for the treatment of anxiety disorders. PF is a yearly herb that is mostly found throughout the Himalayas region in India or even Nepal, China, Korea, and Japan. The plant has traditionally been used to treat psychiatric conditions associated with depression, asthma, tumors, allergies, intoxication, congestion, fevers, chills, headaches, and stuffy noses. This plant has the potential to be utilized in the treatment of various diseases and disorders [29-31].

EPM is presently the method of choice for anxiolytic medications, and it has been validated in mice. It is acknowledged as an ethologically sound animal model for anxiety, rooted in rodents' innate fear of heights and open spaces. Because rodents maintain a strategic distance from elevated regions, avoidance of open arms in the Elevated Plus Maze model test is construed as a manifestation of anxious behavior. Fear of open spaces makes mice less likely to enter the maze's open arms, while anxious animals refuse to enter into the open arms of EPM even more. Anxiogenic medications decrease the duration spent in open arms, whereas anxiolytic drugs extend this duration. When compared to DZP (1 mg/kg), the NHE and EE at a dosage of 2 ml/kg substantially enhanced the no. of entries into the open arms of EPM. This ensures that the seeds also have some anxiolytic effects. In this maze, the anti-anxiety effect was likewise demonstrated to be dosage-dependent. The anxiety measures in this test, including the percent of entries into open arms and the time spent in open arms, are responsive to compounds that are known to act through the GABAA receptor complex. This reinforces the use of DZP as a standard in this research.

The anti-anxiety-like effect was also noticed in the LDE. This model has also been extensively utilized to investigate the anti-anxiety activity of pharmacological drugs. This animal model, like LDE, is based on rodents' intrinsic aversion to bright light. During 5 sessions, mice are allowed to move freely in a new environment made up of two separate and distinct compartments, protected (dark) and unprotected (light). Administration of anxiolytic agents like benzodiazepines and PF seed extracts results in an elevation of the time spent in the well-lit section and a rise in the number of transitions between the two areas when compared to the control group.

The Open Field Test (OFT) is a conventional animal model employed to assess the pharmacological characteristics of anxiety, overall motor activity, and exploratory behavior. It utilizes rodents' natural aversion to open, highly lighted areas, as the interaction with the situation produces anxious behavior in rodents. Anxiolytic therapy minimizes the anxiety-related limitation of exploratory behavior. The OFT is utilized to assess the emotional condition of the animal, in which more anxious animals spend much less time in the device's central area and have a lower number of squares crossed in the highly lit area. Anxiety-related behavior is indicated by the animal's natural aversion to an open, highly lit location. Anxiolytic medications diminish mice's scared behavior in open fields. When compared to the control group, both diazepam and PF NHE, along with EE (1-2 ml/kg), exhibit a notable increase in the number of squares crossed, signifying an anxiolytic-like effect.

A PA is a model that determines locomotor- activity. A decrease in locomotor activity suggests a CNS anxiolytic effect. The counts are derived from the animals' movements and their random interruptions of the light beam. After undergoing their respective 30-minute treatments with medication and PF seed, the animals are transferred to a fresh cage within the infrared apparatus. Over the subsequent fifteen minutes, locomotor activity is recorded at 5-minute intervals. The mice were treated with NHE and EE of PF (1-2 ml/kg). Furthermore, the DZP at a dosage of 1 mg/kg notably reduced the cut-off value. Both PF extracts and diazepam decreased locomotor activity, which suggests an anxiolytic effect.

According to the HBT (Hole-Board Test), the presence of an anti-anxiety state in animals can be identified by an elevation in head-dipping behavior, which is sensitive to variations in the animal's emotional state of mind. PF significantly enhanced the number and intensity of head poking in this research at dosages of 1 and 2 ml/kg, indicating an anxiolytic impact. Additionally, animals increased visit numbers to the board compared with the normal group.

When the generation of metabolites derived by oxygen exceeds the brain's protective mechanisms, it can result in oxidative injury affecting DNA (nucleic acids), proteins, and the lipids found in the neural systems, which are high in polyunsaturated fatty acids and vulnerable to oxidative trauma. Such oxidative stress has the potential to interfere with neuronal communication, the functioning of neurons, and the overall neurological activity of the brain. Consequently, oxidative injury in the brain can lead to dysfunction within the nervous system and is additionally linked to the development of AD and increased anxiety levels. The depletion of GSH levels in the brain during AD can be attributed to its extreme usage in defense against oxidative injury. The generation of oxygen free radicals associated with anxiety development results in diminished GSH, CAT, and SOD levels due to their utilization in response to oxidative injury and cellular breakdown, as evident from the reduced levels in the anxiety control group. Oral administration of both ethanolic and aqueous extracts to the mice effectively elevated their GSH, CAT, and SOD levels, likely through a mechanism of competing with free radicals.

5. Conclusions

Studies have shown that PFE exhibited anxiolytic properties across various parameters assessed, including behavior, emotional state, muscle coordination, and locomotor activities. The results of the current study provided evidence that the n-hexane and ethanolic extract of P. frutescens has anxiolytic activity using EPM, LDE, OFT, PA, and HBT, thus supporting the traditional statement that P. frutescens is effective in the treatment of CNS disorders like anxiety. Even when compared to the conventional medicine DZP, the NHE extract in a dosage of 2 ml/kg had almost the same or equivalent results. Furthermore, additional investigation is required to elucidate the promising approach at effect and its implementation in individuals.

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