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RESEARCH ARTICLE

Ferulic Acid- and Quercetin-based Extracts Enhanced Neuropharmacological Potential in Swiss Albino Mice: Molecular Dynamics Simulation, Molecular Docking, ADMET, and Biological Investigation

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ARTICLE HISTORY

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Abstract: ***Introduction*****:** Neurological disorders impair cognitive, emotional, and behavioural functions, leading to a reduced quality of life. Due to the limitations of conventional therapies, the present study aimed to develop and evaluate a polyherbal formulation containing *Foeniculum vulgare*, *Emblica officinalis*, and *Ocimum sanctum* for its neuroprotective and anxiolytic potential.

Methods**:** A hydroalcoholic extract of the selected plants was combined in a 3:1:3 ratio to prepare a polyherbal formulation. Phytochemical analysis using Thin Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) confirmed the presence of ferulic acid and quercetin as major constituents. Computational approaches, including molecular docking and molecular dynamics simulations, were employed to evaluate the interaction of these compounds with GABA receptors. ADME profiling was conducted to assess pharmacokinetic suitability, and in vivo studies on Swiss albino mice were performed to determine anxiolytic and antistress activities, along with acute toxicity evaluation.

Results**:** The formulation showed strong binding affinity of ferulic acid and quercetin to GABA receptor sites, comparable to diazepam. ADME analysis revealed favourable pharmacokinetic and drug-likeness properties. In vivo behavioural studies demonstrated significant anxiolytic and antistress effects ($p < 0.05$) without any observable signs of acute toxicity.

Discussion**:** The results indicate that the synergistic interaction of bioactive compounds enhances GABAergic modulation, contributing to the observed neuroprotective and anxiolytic effects. These findings align with previous reports on the neuroactive potential of flavonoid-rich herbal extracts, supporting the therapeutic relevance of polyherbal formulations in neuropharmacology.

Conclusion**:** The developed polyherbal formulation demonstrated promising neuroprotective, anxiolytic, and antistress effects through GABAergic modulation, supported by both computational and experimental evidence. It may serve as a safe, natural, and effective alternative for managing neurological disorders.

Keywords: Neuropharmacological potentials, synergistic effect, computational studies, fennel, amla, tulsi.

1. INTRODUCTION

Many plant-derived natural products are reported to have beneficial effects against CNS disorders, one of their advantages being their low side-effect profile compared to chemically synthesised medications [1–5]. Alkaloids, terpenoids, polyphenols, and flavonoids are the largest groups of plant-derived natural products and have therapeutic potential in CNS disorders [6–17]. These compounds usually act as agonists or antagonists of various neurotransmitters through direct binding to neuroreceptors and/or by interfering with neurotransmitter metabolism [18–22]. These bioactive compounds have shown significant roles in various CNS disorders through direct interaction with neurotransmitter systems, including sedative, anxiolytic, antipsychotic, cognitive-enhancing, cholinergic-upregulating, and antidepressant effects [23-44].

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This research uniquely combines the synergistic effects of fennel, amla, and tulsi for neuropharmacological benefits, focuses on the bioactive compounds ferulic acid and quercetin, and integrates traditional phytochemical screening with advanced computational methods [45]. Previous studies have shown potent anxiolytic behaviour associated with quercetin and ferulic acid present in fennel, amla, and tulsi.

Lijing Deng et al. studied ferulic acid and feruloylated oligosaccharides and reported significant potential in alleviating symptoms of anxiety and depression by targeting the gut microbiome. These natural compounds help restore microbial balance, promoting the growth of beneficial bacteria that produce mood-regulating metabolites such as short-chain fatty acids and neurotransmitter precursors. By enhancing microbial metabolism, they strengthen the gut–brain axis, which plays a crucial role in emotional and cognitive health. This biochemical interaction leads to improved neural signalling and reduced inflammation, both of which are associated with better mental well-being. Their plant-derived origin and multifaceted mechanism make them promising candidates for integrative approaches to mood disorders [46].

Susi Mara Soecki Sborg et al. studied the effects of ferulic acid and demonstrated positive outcomes in treating depressive symptoms by evaluating its anxiolytic activity and possible mechanism of action using the light–dark test in zebrafish. To assess anxiolytic activity, the light–dark preference test was performed after exposure of the animals to ferulic acid or the positive controls (clonazepam or fluoxetine) [47].

B. Lee reported that quercetin administration significantly increased the time spent and the number of entries into the open arms of the elevated plus maze (EPM) test, along with increased centre-zone crossings in the open-field test, effectively ameliorating anxiety-like behaviours. These beneficial effects were observed in a mouse model established by lateral ventricle lipopolysaccharide (LPS) injection, which mimics anxiety- and depression-like phenotypes. Mechanistically, the anti-anxiety action of quercetin is closely associated with the inhibition of interleukin-6 (IL-6) and interleukin-1β (IL-1β) expression in the hippocampus [48].

2. MATERIALS AND METHODS

2.1. Plant Materials

The fruits of *Foeniculum vulgare* and *Emblica officinalis*, and the aerial parts of *Ocimum sanctum*, were selected and collected from Haridwar city of Uttarakhand in September 2019 and stored in a cool, dry place (0–4 °C) for 2–3 weeks in breathable packaging.

Amla: Harvested in late winter to early spring when fully ripe. It was stored at low temperatures (6 °C) for several months using breathable packaging. The plant materials were identified and authenticated by the Department of Botany, IFTM University, Moradabad, U.P. (Ref. 2019/SOS/BOT/75), and by Deendayal Research Institute, Arogyadham, Chitrakoot, Satna (M.P.) (Ref. AD/AS/Consult/-2019-20/09), as shown in Fig. (**1a-c**).

2.2. Physicochemical Analysis

Physicochemical parameters of the plant materials were assessed following standard pharmacopeial procedures [49–51]. These included the determination of ash values (total ash, acid-insoluble ash, water-soluble ash, and sulphated ash), extractive values (hydroalcoholic extracts), and loss on drying.

2.3. Preliminary Phytochemical Screening

The hydroalcoholic extracts of fennel, amla, and tulsi were subjected to preliminary phytochemical screening using standard qualitative tests to identify the presence of alkaloids, flavonoids, phenols, tannins, saponins, and glycosides [52–55]. In addition, High-Performance Thin-Layer Chromatography (HPTLC) analysis was performed for marker-based identification. Ferulic acid (phenolic acid) and quercetin (flavonoid) were selected as standard reference compounds, and their presence in the extracts was confirmed by matching Rf values and densitometric profiles [56, 57].

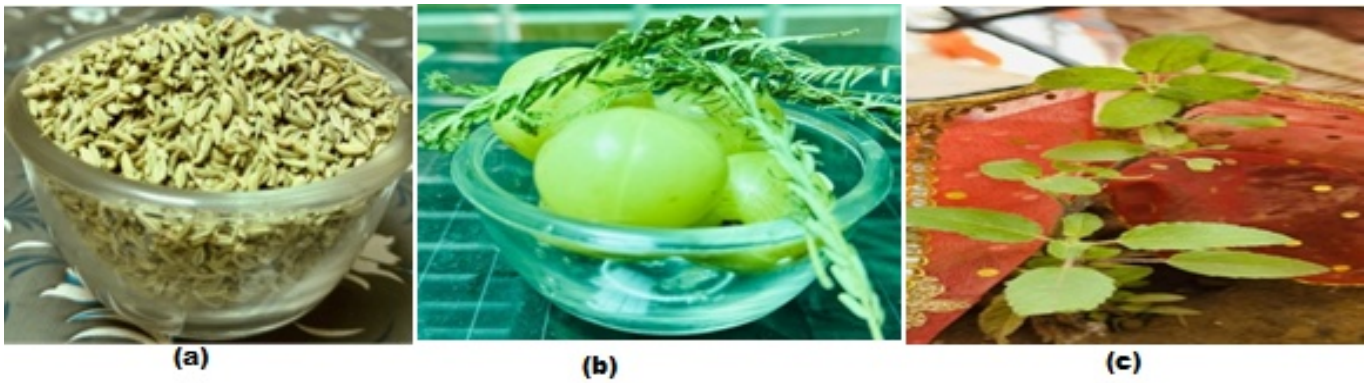


Fig. (1). (a) Fruit of *Foeniculum vulgare,* **(b)** Fruit of *Emblica officinalis,* (**c**) Aerial part of *Ocimum sanctum.* (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Physicochemical parameters of hydroalcoholic extracts of plant material.

Physicochemical Parameter	Fruit of *Foeniculum vulgare*	Fruit of *Emblica officinalis*	Aerial part of *Ocimum sanctum*
		Value (% w/w)	
Total ash	4.56	2.32	9.46
Acid-insoluble ash	1.22	1.66	2.69
Water-soluble ash	0.46	0.98	4.62
Sulphated ash	3.66	2.23	7.23
Extractive value	84.34	66.55	79.39
Loss on drying	7.88	7.66	10.09

Table 2. Preliminary phytochemical screening of hydroalcoholic extracts of plant material.

Test	Hydroalcoholic Extract of *Foeniculum vulgare*	Hydroalcoholic Extract of *Emblica officinalis*	Hydroalcoholic Extract of *Ocimum sanctum*
Carbohydrates	+	+	+
Amino acids	+	+	+
Steroids	+	+	+
Alkaloids	+	+	+
Tannins and phenolic compounds	+	+	+
Saponin glycosides	+	+	+
Flavonoids	+	+	+

Note: + = Present; - = Absent

2.4. Computational Studies

Computational analyses were carried out to predict the neuropharmacological potential of the identified phytoconstituents. Molecular docking studies were performed using the AutoDock 4.2 algorithm to evaluate binding affinities with selected protein targets. The docking grid was defined around the active site, and binding poses were validated based on binding energy scores and hydrogen-bonding interactions. The most stable ligand–protein complexes were further subjected to molecular dynamics (MD) simulations using GROMACS (100 ns runs with explicit water model and periodic boundary conditions) to assess structural stability. ADME properties (absorption, distribution, metabolism, and excretion) and drug-likeness were predicted using SwissADME. The best docked poses were visualised using UCSF Chimera, and ligand–protein interactions were analysed with BIOVIA Discovery Studio Visualizer [58–62].

2.5. Biological Evaluation

The animals used in this study were procured from a CPCSEA-registered breeder in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Healthy Swiss albino mice of either sex, aged 6–8 weeks and weighing 25–30 g, were used, with six animals included in each experimental group.

Biological evaluation of the polyherbal formulation was performed in Swiss albino mice. Acute oral toxicity studies were carried out as per OECD guidelines (423/425), with careful monitoring of behavioural, autonomic, and neurological responses up to 14 days. Neuropharmacological activities were assessed using standard behavioural paradigms, including the Elevated Plus Maze (EPM), Open Field Test (OFT), and Rotarod test [63–70]. Mice were randomly assigned to three groups (n = 6 per group), and all experimenters were blinded to treatment allocation to reduce bias.

2.6. Statistical Analysis

All experimental results are expressed as mean ± standard error of the mean (SEM). Data were analysed using GraphPad Prism 5 software. One-way analysis of variance (ANOVA) was performed, followed by appropriate post hoc tests to identify group differences. A significance threshold of $p \leq 0.001$ was chosen to minimise Type I error, given the multiple behavioural and computational endpoints evaluated. This stringent cutoff enhances the reliability of our findings [71–75].

3. RESULTS AND DISCUSSIONS

3.1. Physicochemical Parameters

Physicochemical parameters of the hydroalcoholic extracts were determined, and the results are reported in Table **1**.

3.2. Preliminary Phytochemical Screening

The results of the preliminary phytochemical screening and HPTLC analysis of hydroalcoholic extracts of plant materials are given in Table **2** and Fig. (**2a-c**) for the presence or absence of various phytoconstituents.

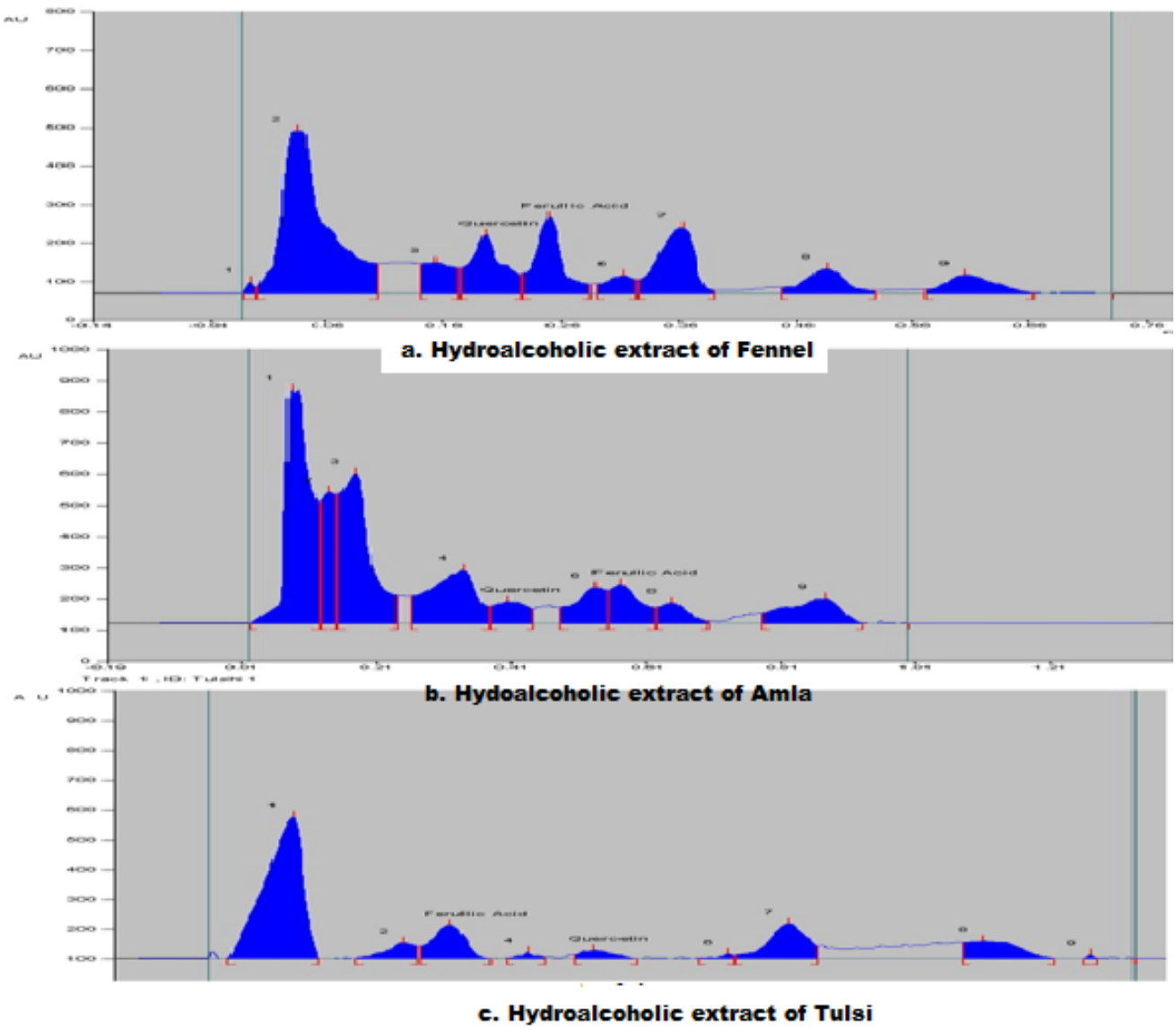


Fig. (2). HPTLC chromatogram of **(a)** hydroalcoholic extract of fennel, **(b)** hydroalcoholic extract of amla, **(c)** hydroalcoholic extract of tulsi. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 3. Docking analysis of ligands and standard drugs.

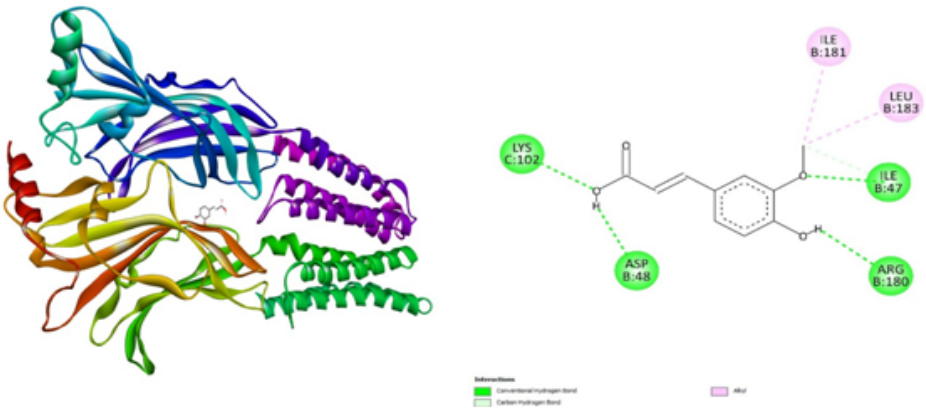
Compounds	Estimated Free Energy of Binding	Estimated Inhibition Constant, Ki
Ferulic acid	-6.16 kcal/mol	30.44 µM
Quercetin	-8.31 kcal/mol	0. 8133 µM
Diazepam	-7.38 kcal/mol	3.90 µM

3.3. Computational Studies

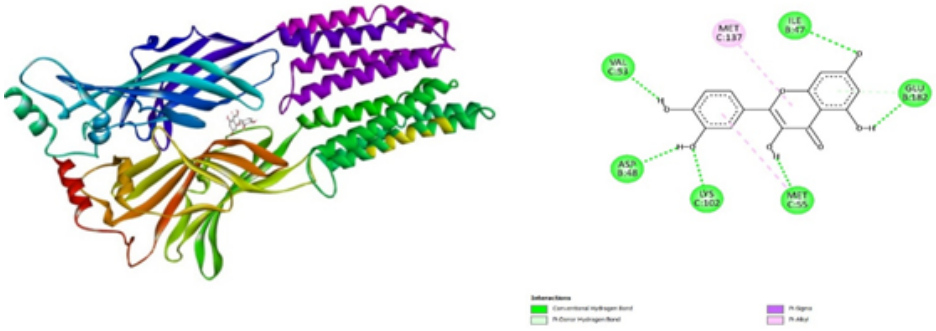
3.3.1. Docking Analysis

The free binding energy, a measure of the binding affinity of the compounds and the standard drug, expressed in kcal/mol, was estimated using the AutoDock 4 algorithm, and the results are shown in Table **3**.

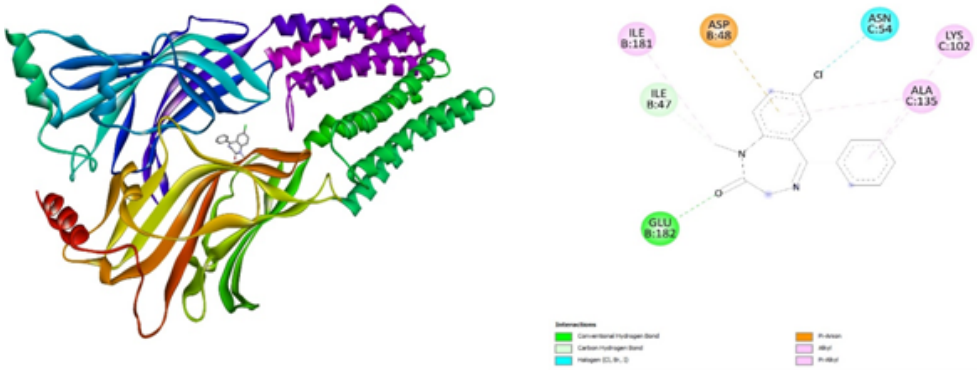
The inhibition constant (Ki), a measure of the dissociation of the ligand–protein complex, was also estimated through molecular docking. Lower values of the inhibition constant indicate stronger inhibition of the ligand–protein complex. The estimated inhibition constant for quercetin was lower than that of the standard drug diazepam, indicating a higher binding affinity.



Molecular docking of ferulic acid with receptor 3D & 2D



Molecular docking of Quercetin with receptor 3D & 2D



Molecular docking of Diazepam with receptor 3D & 2D

Fig. (3). The protein ligand interactions of ferulic acid, quercetin, and diazepam. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.3.1.1. Protein Ligand Interactions

The interactions between the best docked pose of the ligands with the human $GABA_A$ receptor were further analysed with respect to the formation of hydrogen bonds and other non-bonded interactions. The analysis of the protein and ligand interactions indicates that the tested compounds bind around the same region of the $GABA_A$ receptor to which the standard diazepam binds. The compound quercetin exhibited the highest free binding energy values and also exhibited the maximum number of hydrogen bond interactions with the receptor. The protein ligand interactions of ferulic acid, quercetin, and diazepam are shown in Fig. (**3**).

3.3.2. Molecular Dynamics (MD) Simulations

The MD study of the compounds was performed using GROMACS 2024.1 on the Ubuntu 22.04 LTS operating system, along with CUDA toolkit 12.4. The parameters of the ligands required for MD studies were obtained from the webserver SwissParam. The structural behaviour of the target protein and ligands over the duration of the MD simulation was analysed using GROMACS 2024.1 and UCSF Chimera 1.17.1 software. The best-docked conformations of the phytoconstituents were subjected to MD simulations, and the Root Mean Square Deviation (RMSD) was calculated to determine the stability of the complexes.

The RMSD of Cα after least-squares fitting to the protein (Fig. **4**) in the ferulic acid–protein complex varied from 0.2 nm to 0.6 nm. The RMSD of the quercetin–protein complex (Fig. **5**) varied from 0.1 nm to 0.5 nm, while the RMSD of the diazepam–protein complex (Fig. **6**) varied from 0.5 nm to 3.0 nm. All ligands tested showed stable conformations of the protein–ligand complexes when compared to the standard drug.

The residue-wise Root Mean Square Fluctuation (RMSF) of Cα was also evaluated for the ligands (Figs. **7–9**). Most residues exhibited RMSF values in the range of 0.3–0.5 nm. The RMSF values exhibited by all tested compounds were similar to those of the diazepam–protein complex.

The radius of gyration (R_g) is a measure of the compactness of the structure. The radius of gyration was calculated for the ligands (Figs. **10–12**). The R_g of ferulic acid, quercetin, and diazepam complexes varied within the range of 3.15–3.2 nm. Short-range interaction energies, namely Coulombic and Lennard–Jones energies, were evaluated for the protein–ligand complexes (Figs. **13-15**). All complexes showed favourable interaction energies when compared to the diazepam–protein complex.

The number of hydrogen bonds during the MD simulation was also evaluated using GROMACS (Figs. **16** and **17**). The ferulic acid–protein complex exhibited multiple hydrogen bonds; quercetin formed 1–3 hydrogen bonds throughout the simulation, while the standard diazepam did not form any hydrogen bonds with the protein.

3.3.2.1. Interaction Energies of Protein-ligand

Total interaction energies of protein-ligand complexes were also evaluated using GROMACS. Ferulic acid and quercetin exhibited higher values of total interaction energies when compared to the standard antianxiety drug diazepam, as shown in Table **4**.

3.3.3. ADME Properties

Ferulic acid (194.18 g/mol) and quercetin (302.24 g/mol) showed lipophilicity within the range of standard drugs, indicating their ability to penetrate lipophilic cell membranes. Most commercially available drugs have molecular weights ranging from 200 to 600 Daltons, and the molecular weight of ferulic acid and quercetin falls within this range. Molar refractivity (MR) is a size descriptor related to molecular weight and polarizability. For example, ferulic acid has a molar refractivity of 51.63, and quercetin has 78.03.MR is calculated as the ratio of liquid density and is influenced by the refractive index of the liquid. In this study, we used molar refractivity as a physicochemical property to evaluate the target compounds and standard drugs, providing valuable information for drug design and optimization. Topological Polar Surface Area (TPSA) helps predict the ability of a molecule to permeate cell membranes, with lower TPSA generally indicating better membrane permeability. For instance, ferulic acid has a TPSA of 66.76, and quercetin has 131.36. Lipinski's Rule of Five is a set of guidelines to evaluate the drug-likeness of a compound, suggesting that a molecule with no more than one violation of these rules (such as molecular weight ≤ 500 Daltons, log P ≤ 5, *etc*.) is likely to be an orally active drug in humans. In this study, all the target compounds adhered to Lipinski's rules with zero violations. These parameters are essential for assessing the pharmacokinetics of drug candidates. The ADME properties were determined, and the results are shown in Table **5**.

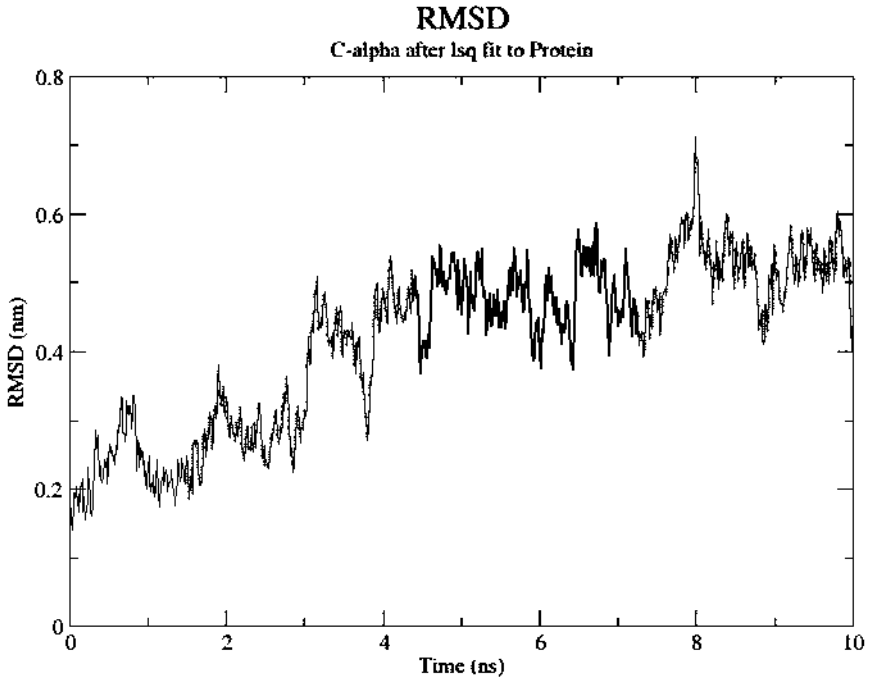


Fig. (4). Root Mean Square Deviation (RMSD) of ferulic acid-protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

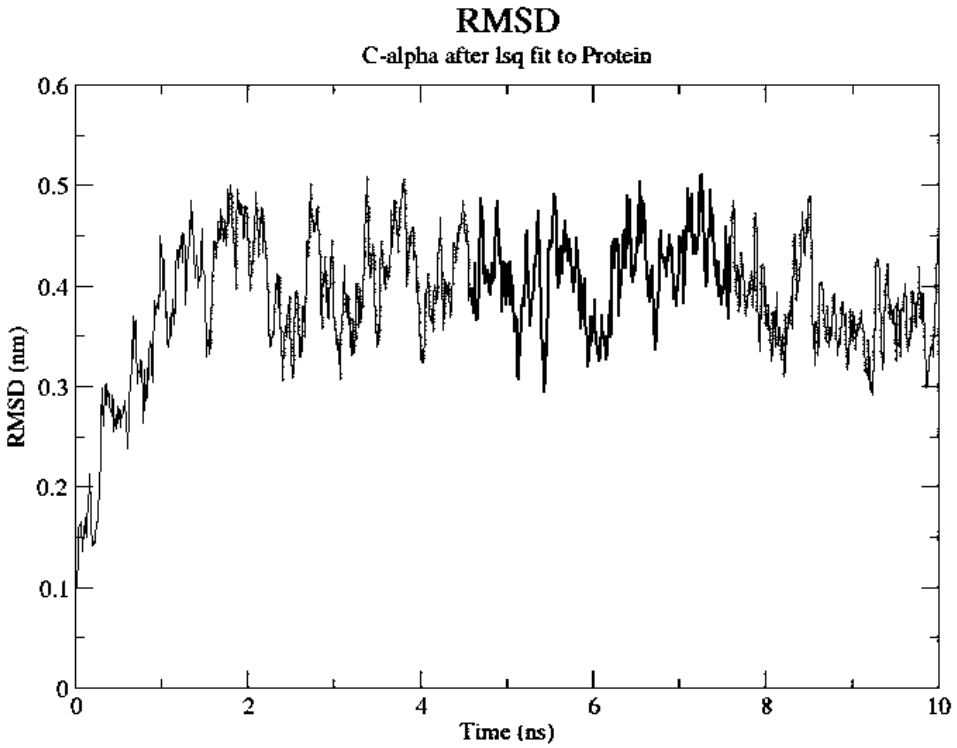


Fig. (5). Root Mean Square Deviation (RMSD) of quercetin-protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

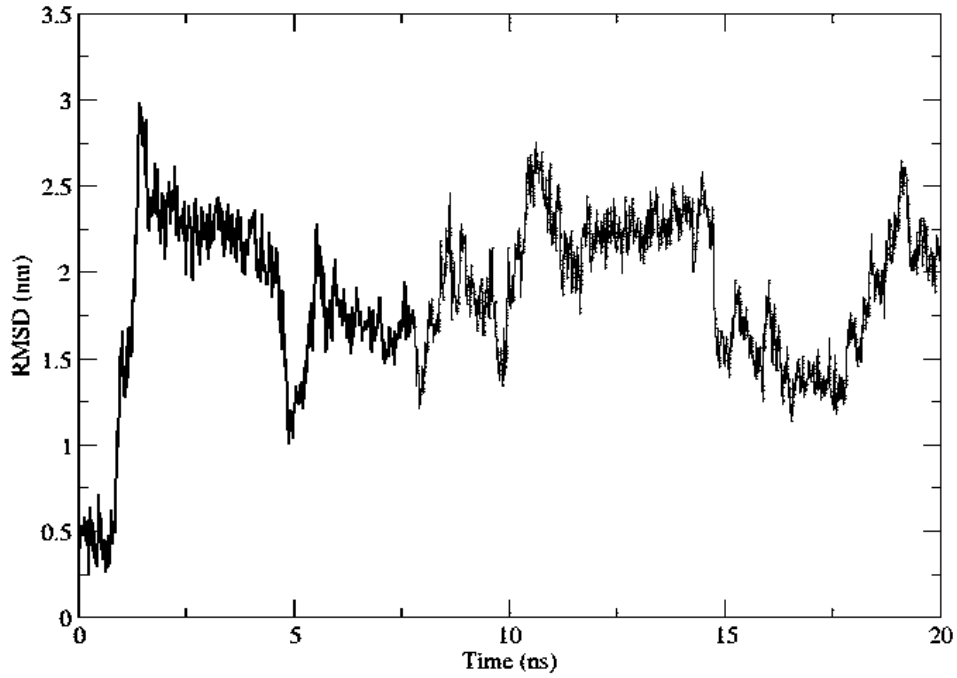


Fig. (6). Root Mean Square Déviation (RMSD) of diazepam-protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

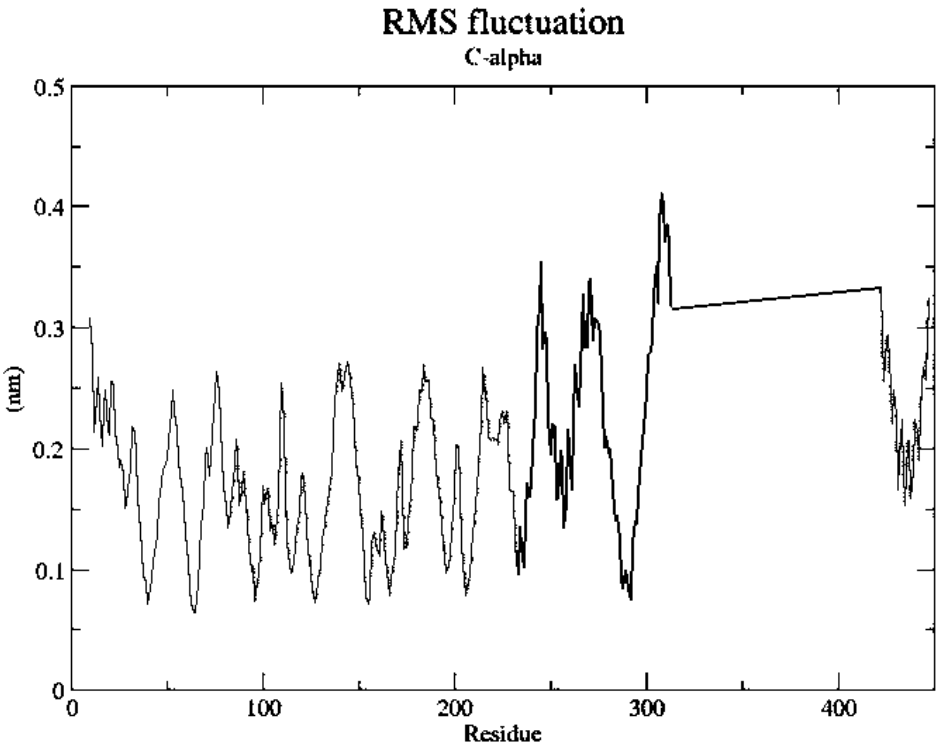


Fig. (7). Root Mean Square Fluctuation (RMSF) of ferulic acid-protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

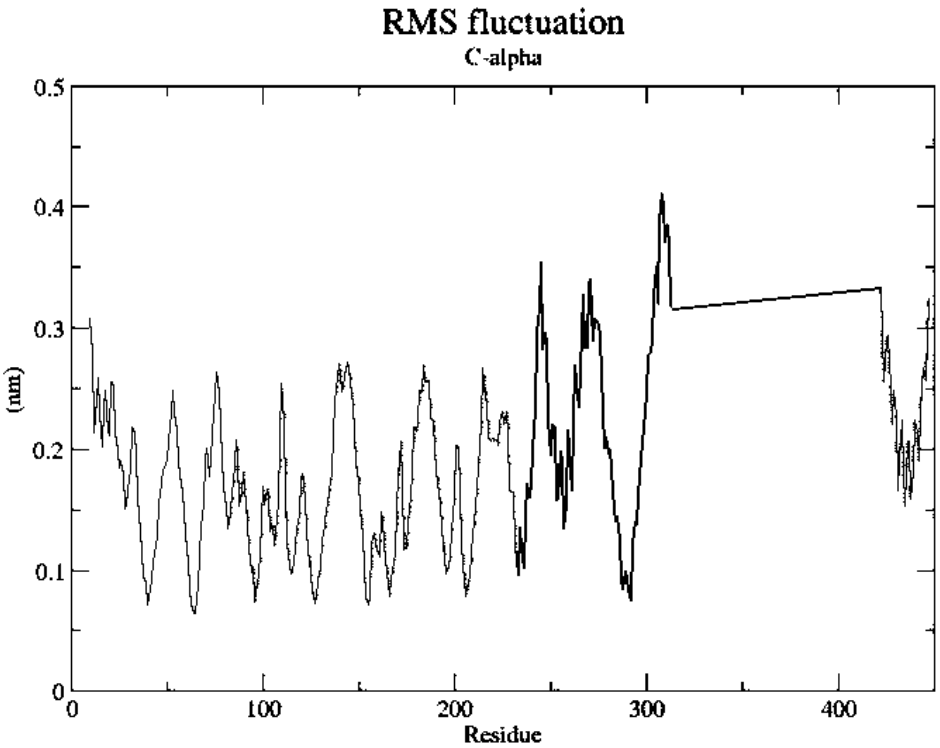


Fig. (8). Root Mean Square Fluctuation (RMSF) of quercetin-protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

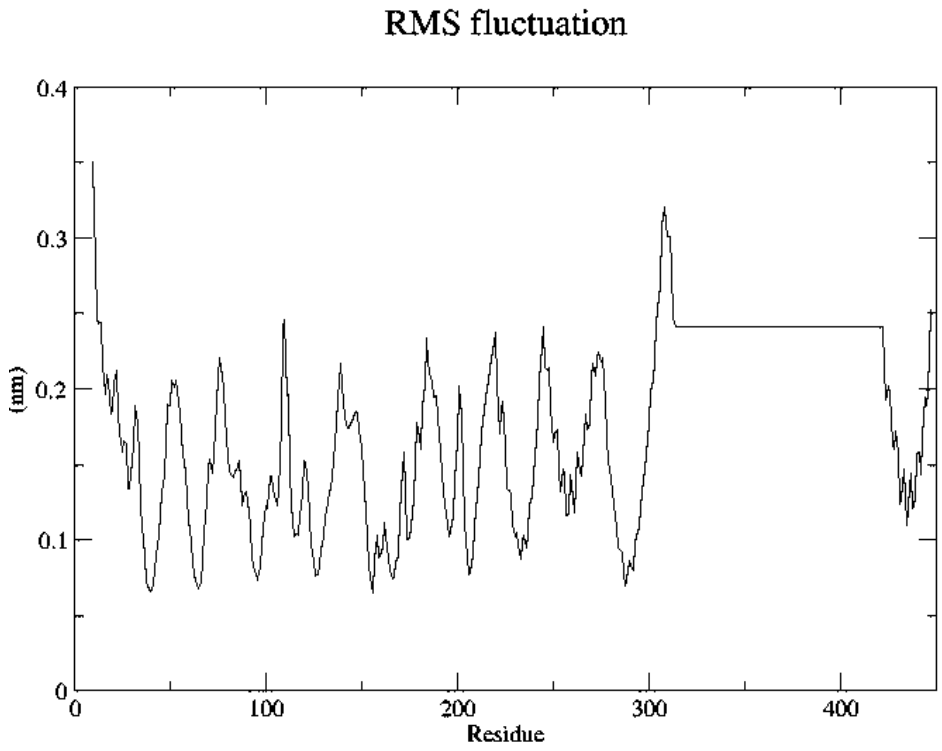


Fig. (9). Root mean square fluctuation (RMSF) of diazepam-protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

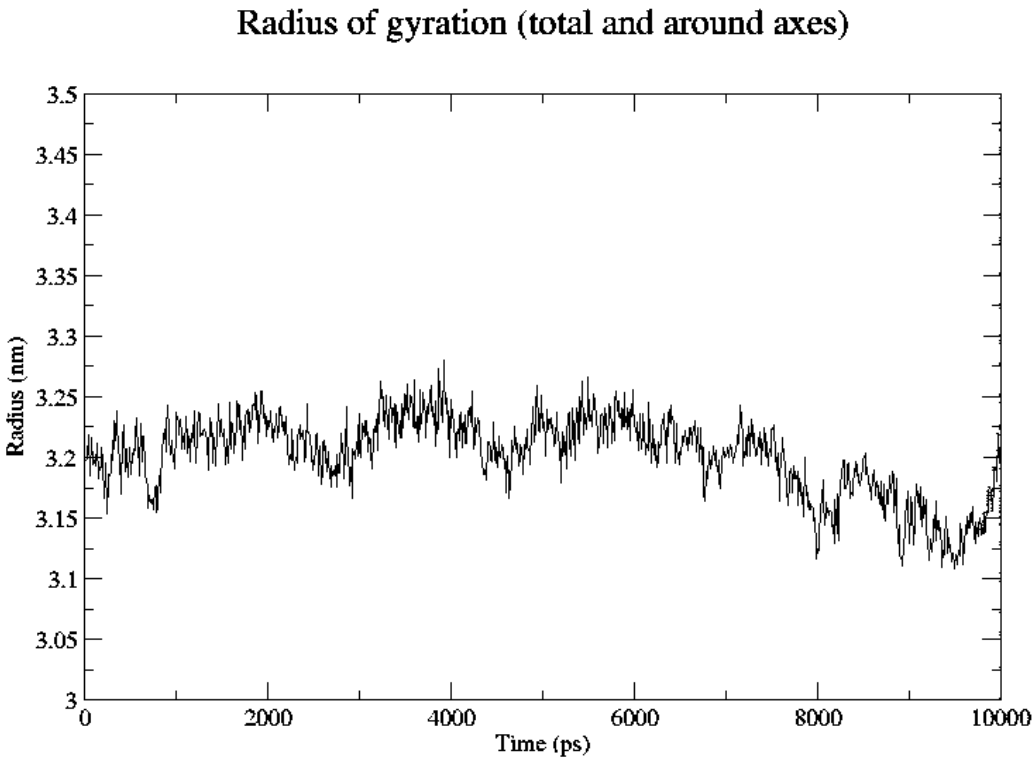


Fig. (10). Radius of gyration of ferulic acid- protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

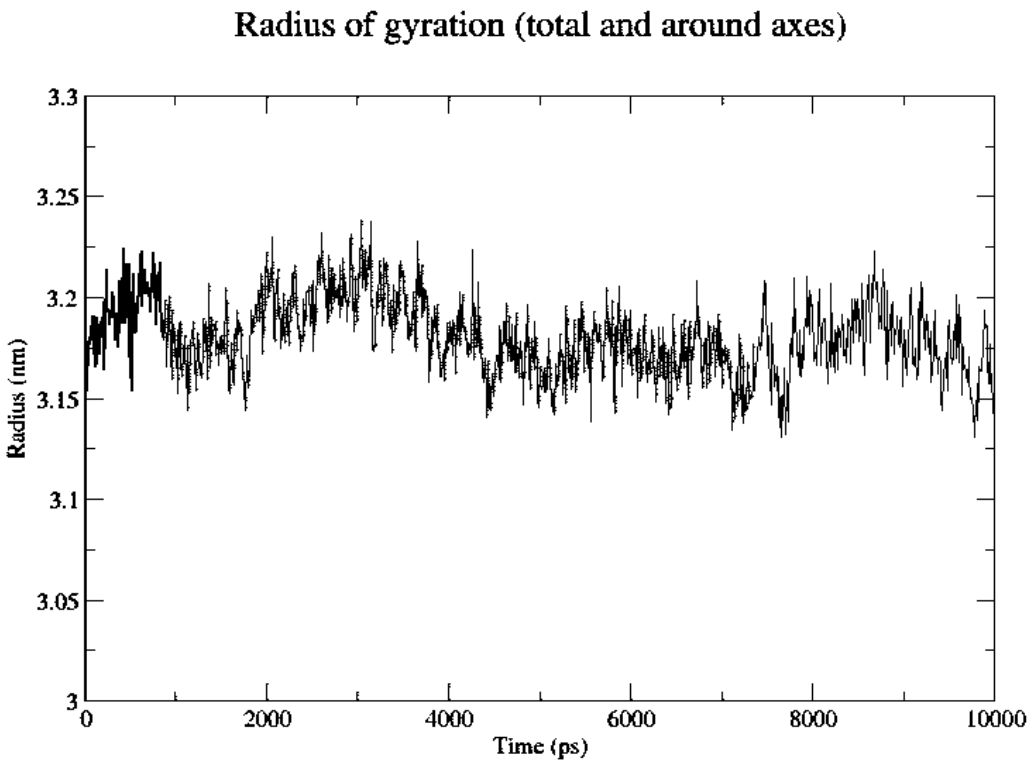


Fig. (11). Radius of gyration of quercetin-protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

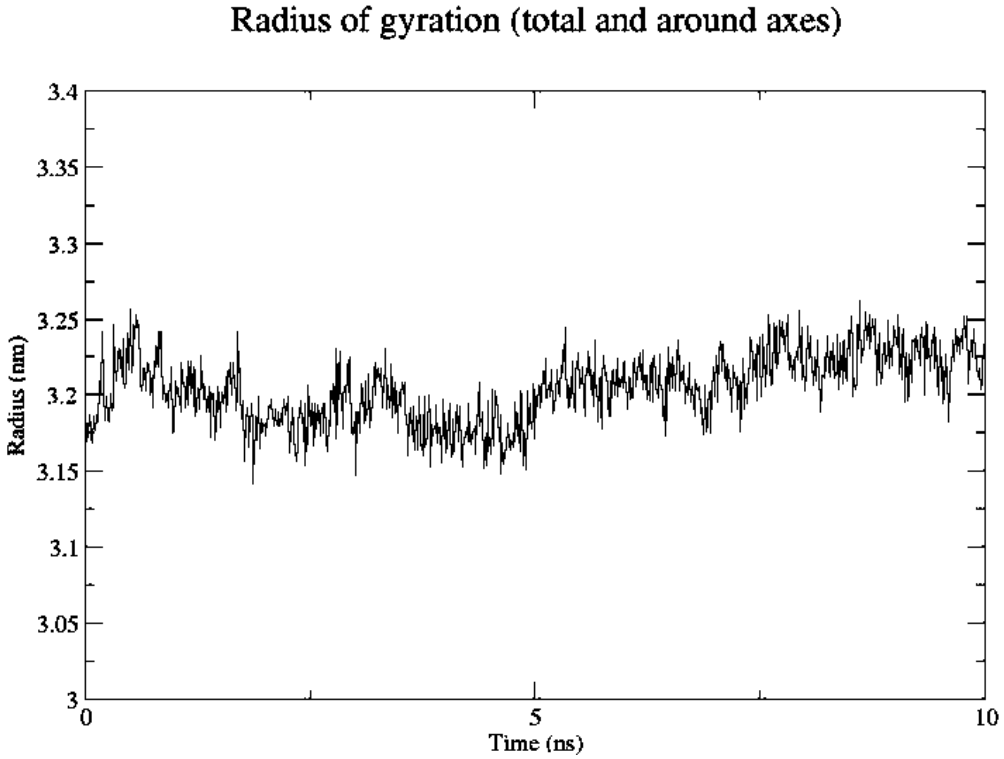


Fig. (12). Radius of gyration of diazepam-protein. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

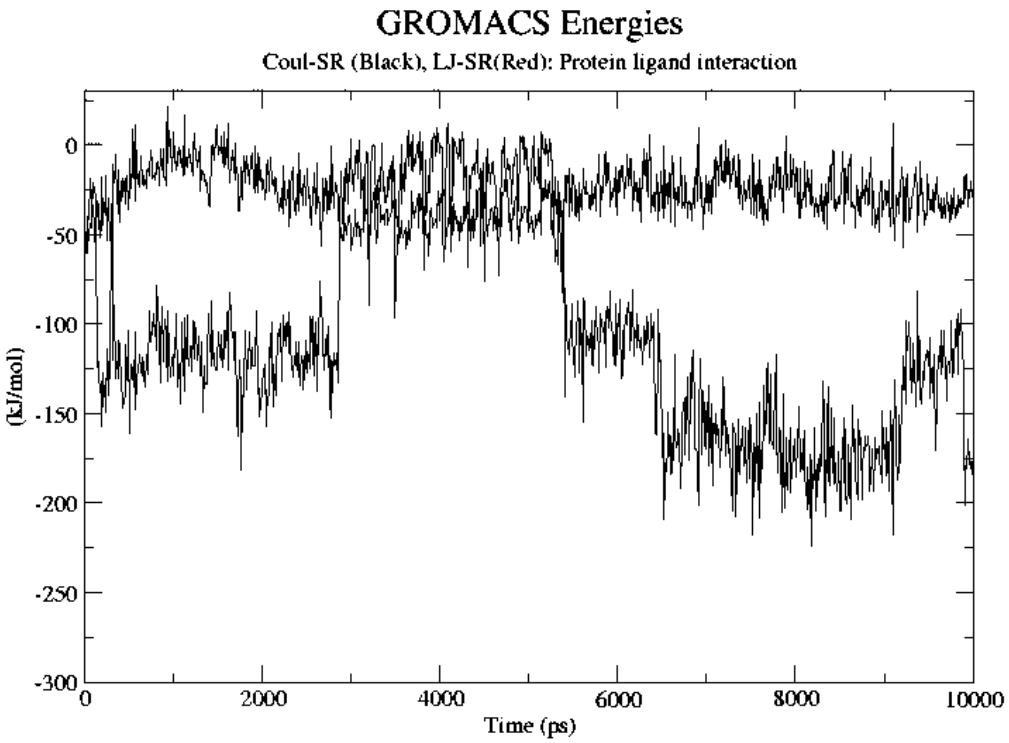


Fig. (13). GROMACS energies of Ferulic acid-Protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

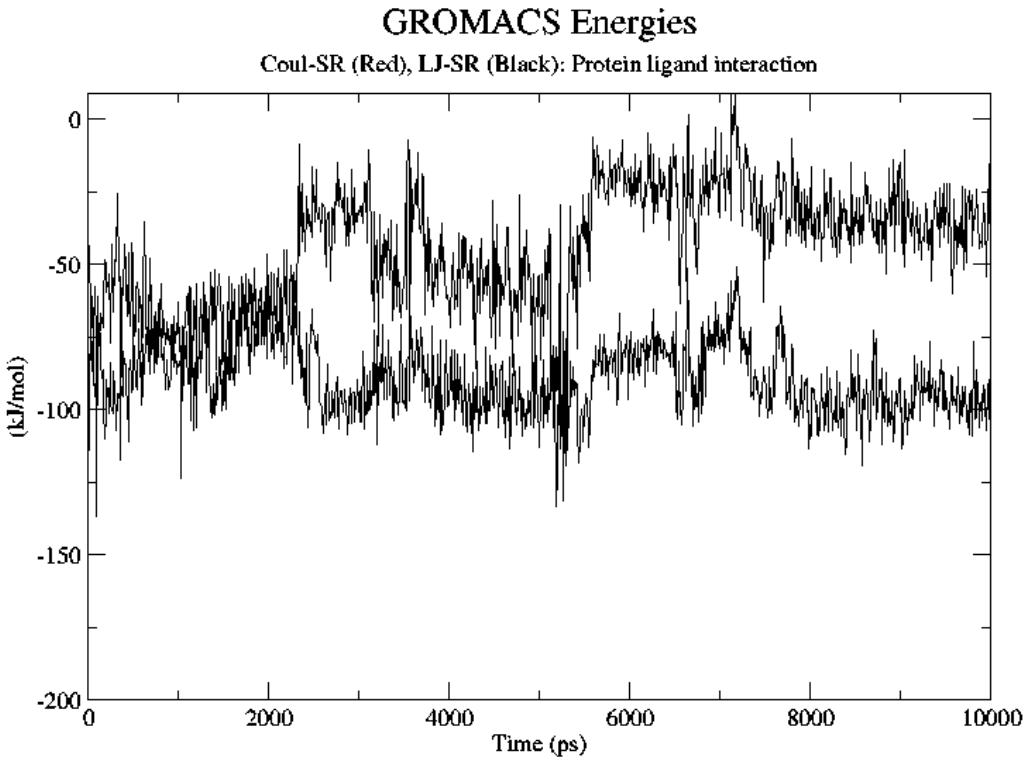


Fig. (14). GROMACS energies of quercetin-protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

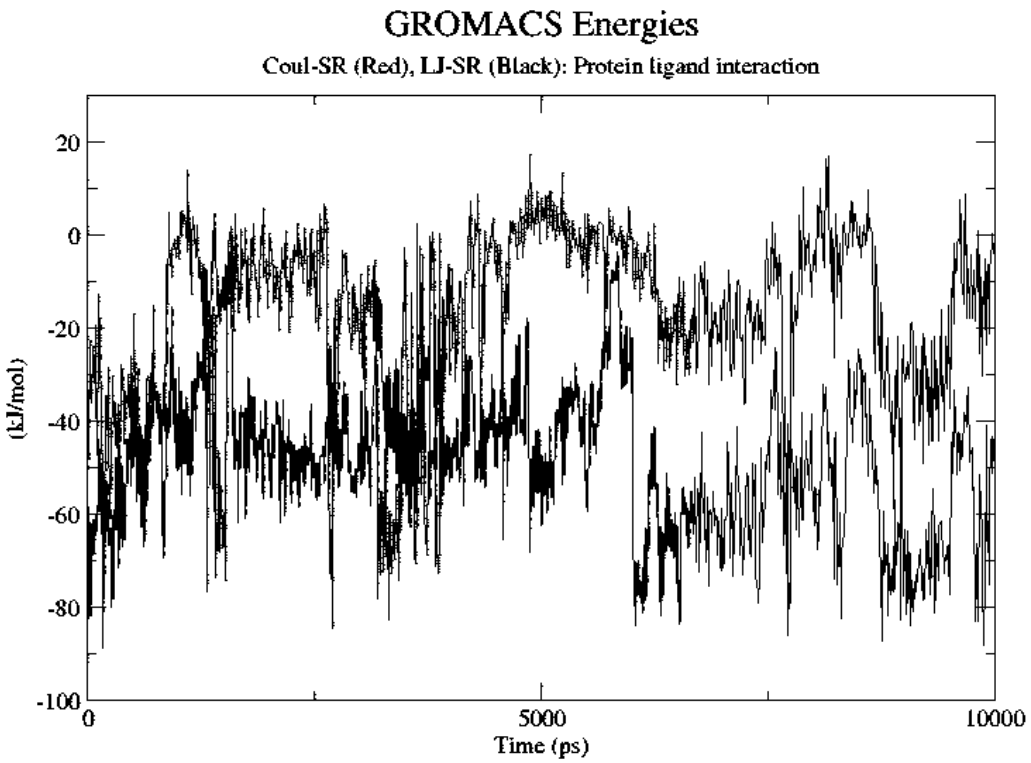


Fig. (15). GROMACS energies of diazepam-protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

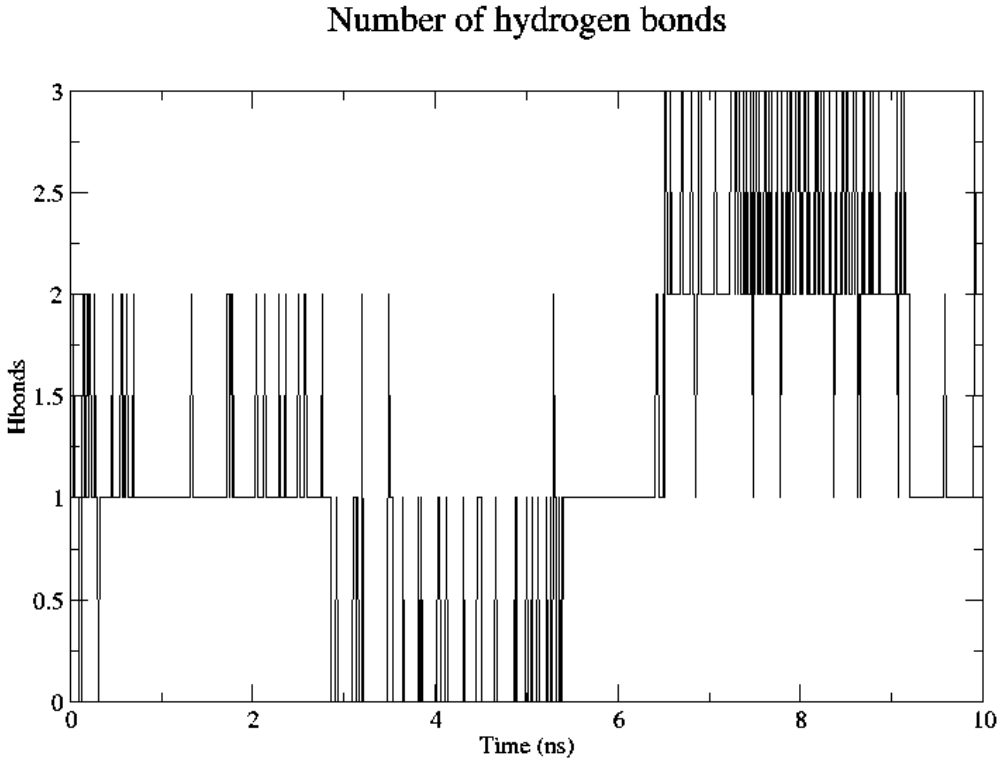


Fig. (16). Number of hydrogen bonds of ferulic acid-protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

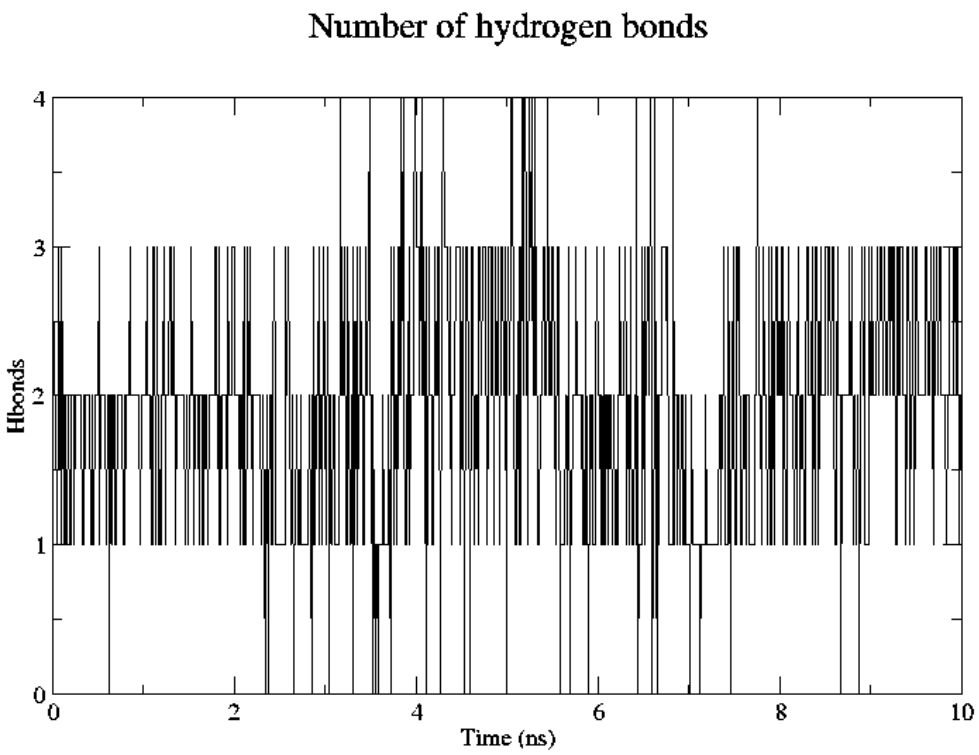


Fig. (17). Number of hydrogen bonds of the quercetin-protein complex. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 4. Interaction energies of protein-ligand (kJ/mol).

Energy	Ferulic Acid	Quercetin	Diazepam
Coul-SR: Protein-LIG	-105.478	-44.8992	-17.112
LJ-SR: Protein-LIG	-28.1217	-88.9032	-49.1632
Total Interaction energy	-133.5999	-133.8024	-66.2752

Table 5. ADME properties of ferulic acid, quercetin, and diazepam.

S. No.	Physicochemical Properties	Ferulic Acid	Quercetin	Diazepam
1	Molecular Weight	194.18	302.24	284.74
2	No. of heavy atoms	14	22	20
3	No. of rotatable bonds	3	1	1
4	Solubility	Soluble	Soluble	Poorly Soluble
5	No. of hydrogen bond acceptors	4	7	2
6	No. of hydrogen bond donors	2	5	0
7	GI absorption	High	High	High
8	BBB permeant	Yes	No	Yes
9	Synthetic accessibility	1.93	3.23	3.00
10	Bioavailability score	0.85	0.55	0.55
11	Lipinski filter	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
12	Molar refractivity	51.63	78.03	87.95
13	TPSA	66.76	131.36	32.67
14	Log P	1.36	1.23	2.97

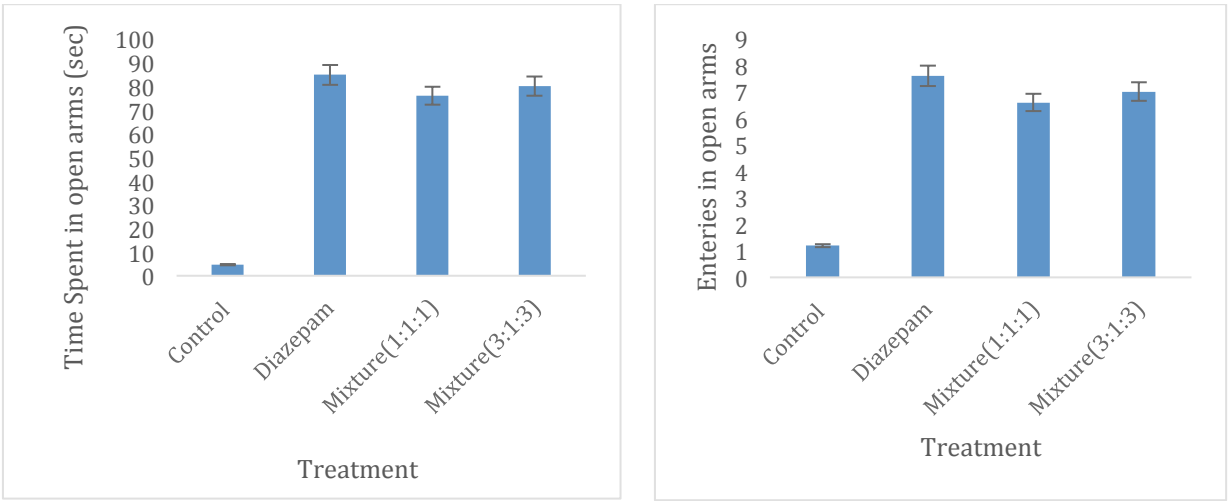


Fig. (18). Observation of time spent in open arms and entries in open arms by the Elevated Plus Maze test. All values are mean ±SEM (n=6); *$p< 0.1$ when compared to control. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.3.4. Acute Oral Toxicity Studies

Acute oral toxicity of a polyherbal formulation was performed, and none of the toxicity symptoms (Loss of reflex, tremors, convulsion, salivation, sleep, diarrhea, and death) were observed.

3.4. Neuropharmacological Potentials for Polyherbal Formulation

The neuropharmacological potential of a polyherbal formulation containing different composition ratios, i.e., fennel:amla:tulsi (1:1:1) and (3:1:3), was evaluated at a dose of 200 mg/kg. It was observed that the hydroalcoholic extract of fennel:amla:tulsi in the 3:1:3 ratio produced the most synergistic effect. The sample size of n = 6 per group was chosen in accordance with commonly accepted practices in preclinical neuropharmacological studies, where group sizes of 5–8 animals are frequently employed to obtain statistically valid results while adhering to ethical principles. This number was also guided by our institutional ethics committee approval, which emphasises minimizing animal use in line with the 3R principles.

3.4.1. Elevated Plus Maze Test

The Elevated Plus Maze (EPM) test is a widely used behavioural experiment designed to assess anxiety-like behaviour in rodents, such as mice and rats. The maze is shaped like a plus sign, with two opposite arms enclosed by walls (closed arms) and the other two arms exposed without walls (open arms). The entire structure is elevated above the ground. Rodents typically exhibit an aversion to open spaces, resulting in them spending more time in the closed arms of the maze. This natural tendency to avoid open arms is used as an indicator of anxiety-like behaviour.

Mice treated with the polyherbal mixture showed a substantial increase in the number of open arm entries and a decrease in the time spent in the closed arms, indicating reduced anxiety-like behaviour. The formulation in the 3:1:3 ratio was found to be the most effective, as shown in Fig. (**18**).

3.4.2. Open Field Test

The Open Field Test (OFT) is a common behavioural experiment used to assess anxiety, locomotor activity, and exploration in rodents. It involves placing the animal in a large, enclosed arena and observing its movement patterns, frequency of rearing, and time spent in the centre versus the periphery. Increased time in the centre and higher activity levels generally indicate lower anxiety. This test helps evaluate the effects of various treatments or genetic modifications on anxiety-related behaviours.

When compared to the control, diazepam showed a considerable reduction in anxiety, while the polyherbal mixture (fennel:amla:tulsi in a 3:1:3 ratio) significantly increased the number of rearings, the number of squares crossed, and the number of aided rearings. The results are shown in Fig. (**19**).

3.4.3. Rota Rod Test

The Rota Rod test is a widely used experimental method to assess motor coordination and balance in rodents. This test involves placing the animal on a rotating rod and measuring the time it can remain on the rod without falling. A decrease in this time indicates impaired motor function. The Rota Rod test is commonly used to evaluate the effects of drugs or genetic modifications on locomotor abilities.

In this study, compared to the control animals, diazepam caused a considerable decrease in the locomotor score, indicating impaired motor coordination and balance. Additionally, both doses of the polyherbal mixture (fennel:amla:tulsi in a 3:1:3 ratio) significantly reduced the locomotor score, as shown in Fig. (**20**).

3.4.4. Hole Board Test

The Hole Board test is used to evaluate exploratory behaviour and anxiety in rodents. The test involves an arena with evenly spaced holes in the floor, where the animal's head-dipping behaviour is observed. Increased head dipping suggests reduced anxiety and higher curiosity levels.

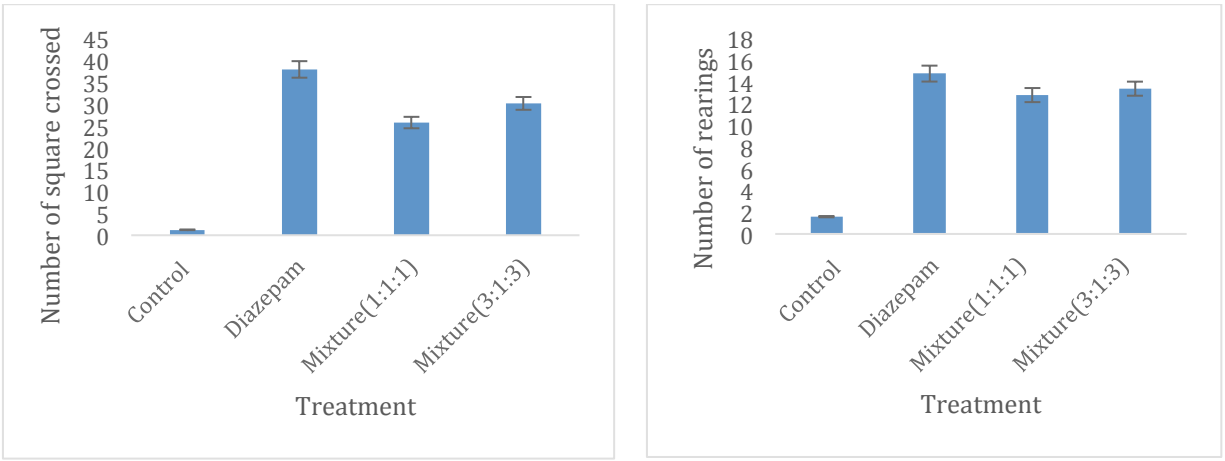


Fig. (19). Observation of the number of squares crossed and the Number of rearings by the open field test. All values are mean ±SEM (n=6);*p< 0.1 when compared to control. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

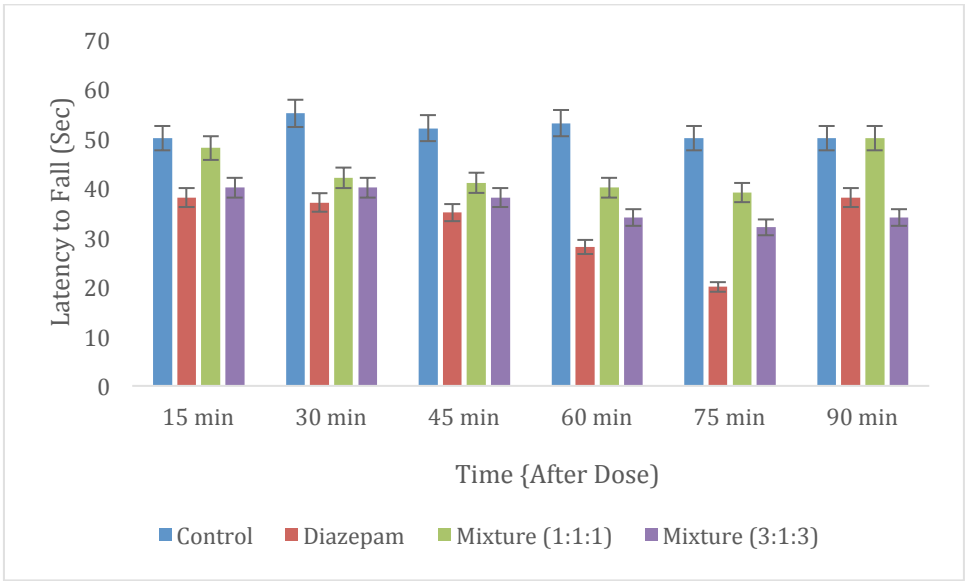


Fig. (20). Observation of the Rota Rod test. All values are mean ±SEM (n=6); *p< 0.1 when compared to control. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

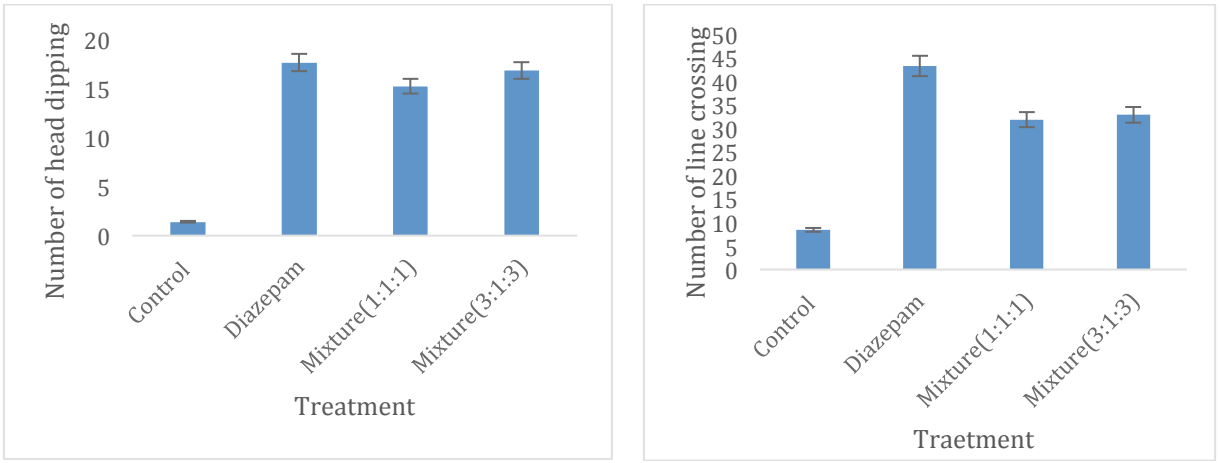


Fig. (21). Observation of the number of head dips and the number of lines crossing by the hole board test. All values are mean ±SEM (n=6);*p< 0.1 when compared to control. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

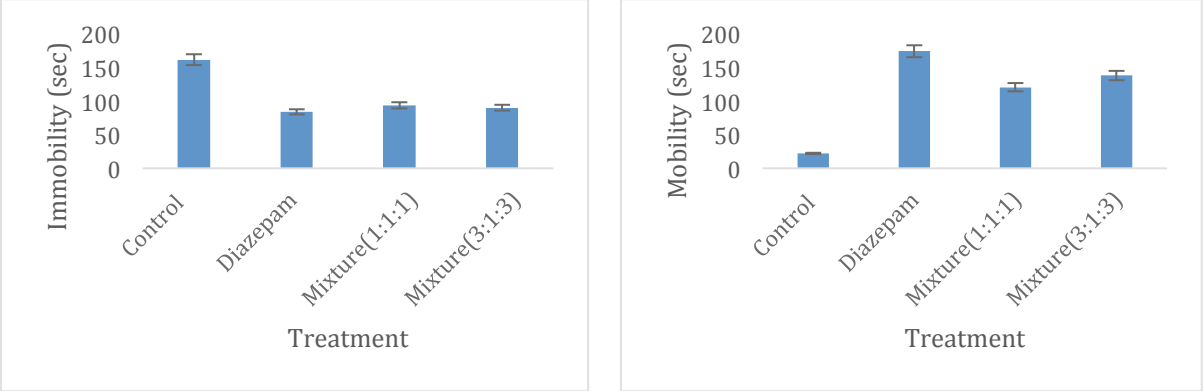


Fig. (22). Observation of Immobility and mobility by the tail suspension test. All values are mean ±SEM (n=6);*p< 0.1 when compared to control. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Compared to control animals, diazepam-treated animals showed a considerable increase in the number of line crossings and head dips. The polyherbal mixture (fennel:amla: tulsi in a 3:1:3 ratio) also increased the number of line crossings and head dips, indicating reduced anxiety and heightened curiosity. The results are shown in Fig. (**21**).

3.4.5. Tail Suspension Test

The Tail Suspension Test (TST) is used to evaluate depressive-like behaviour in rodents. In this test, the animal is suspended by its tail, and its behaviour is observed, particularly focusing on the duration of immobility. Increased immobility time is indicative of depressive-like behaviour, while decreased immobility suggests an antidepressant-like effect.

Compared to control animals and those treated with diazepam, both test groups receiving the polyherbal mixture (fennel:amla:tulsi in a 3:1:3 ratio) demonstrated a dose-dependent decrease in immobility time. This indicates that the mixture has potential antidepressant-like effects. The results are shown in Fig. (**22**).

4. DISCUSSION

The polyherbal formulation (fennel:amla: tulsi, 3:1:3) demonstrated notable neuropharmacological activity, most likely due to the synergistic actions of ferulic acid and quercetin. Molecular docking and dynamics studies confirmed strong interactions with GABA $_A$ receptors, while behavioural findings in Swiss albino mice suggested anxiolytic and antistress effects.

Despite these promising outcomes, several limitations restrict the overall interpretation. Similar to many previous studies in herbal pharmacology, the current study suffers from a small sample size and a short duration of in vivo testing, which limits statistical power and the ability to assess long-term efficacy and safety. Previous studies on single or combined herbal extracts have also faced challenges of potential bias due to inadequate blinding, unclear randomisation, and selective reporting, raising concerns about reproducibility.

Furthermore, generalisability remains limited, as findings obtained from one mouse strain under controlled laboratory conditions may not extend to other animal models or human populations. Mechanistic insights were inferred primarily from *in silico* analyses rather than validated through biochemical assays (*e.g.,* neurotransmitter levels, oxidative stress markers) or gene expression studies, leaving the therapeutic pathways insufficiently clarified. Although ADME and toxicity predictions were favourable, as in previous studies, these computational results require experimental pharmacokinetic and toxicological validation.

5. FUTURE PROSPECTS AND APPLICATIONS OF RESEARCH

5.1. Therapeutic Development

The synergistic effects of ferulic acid and quercetin in plant extracts could lead to the development of new therapeutic agents for stress and anxiety disorders. These natural compounds have shown promising results in neuropharmacological tests, indicating their potential as alternative treatments with fewer side effects compared to synthetic drugs.

5.2. Personalized Medicine

With the integration of computational methods such as ADME profiling and molecular docking, it is possible to tailor treatments to individual patients. By understanding how these compounds interact with specific targets in the brain, personalised treatment plans can be developed to maximise efficacy and minimise adverse effects.

5.3. Mechanistic Insights

Our research can provide valuable insights into the mechanisms through the GABA receptor pathway by which ferulic acid and quercetin exert their effects. This knowledge can be used to identify new therapeutic targets and pathways for drug development.

5.4. Combination Therapies

The study's findings on the synergistic effects of these compounds suggest that combination therapies could be

more effective than single-agent treatments. This approach could be explored further in clinical trials to enhance treatment outcomes for patients with stress and anxiety disorders.

CONCLUSION

The study aimed to design and evaluate a polyherbal formulation for its neuropharmacological and synergistic effects. Preliminary phytochemical screening of the hydroalcoholic extracts confirmed the presence of ferulic acid and quercetin. Molecular docking indicated strong binding of these compounds to the GABA $_A$ receptor, and molecular dynamics simulations demonstrated favourable RMSD, RMSF, radius of gyration, and interaction potentials, comparable to diazepam. ADME analysis revealed that the pharmacokinetic properties of ferulic acid and quercetin were within the range observed for diazepam. Among the tested combinations, the 3:1:3 ratio of fennel, amla, and tulsi at 200 mg/kg produced the most significant effects in animal models. These results support the efficacy and safety of the formulation in managing neurological disorders.

RESEARCH INVOLVING PLANTS

The plant materials were identified and authenticated by the Department of Botany, IFTM University, Moradabad, U.P. (Ref. 2019/SOS/BOT/75), and by the Deendayal Research Institute, Arogya Dham, Chitrakoot, Satna, M.P. (Ref. AD/AS/Consult/-2019-20/09), ensuring compliance with scientific standards. The study was conducted with official approval from IFTM University, Moradabad, for the researcher as a registered scholar. All research activities, including plant collection, authentication, and experimentation, complied with institutional guidelines and ethical standards established for academic research.

STUDY LIMITATIONS

The study was limited by a small sample size and short experimental duration, which may restrict generalizability. Biochemical validations, such as neurotransmitter or oxidative stress markers, were not performed to support behavioral findings. Computational results remain predictive and require experimental confirmation. Furthermore, the use of a single mouse strain may not capture interspecies variability.

AUTHORS' CONTRIBUTIONS

BS was responsible for data curation, JKS contributed to writing, reviewing, and editing, AK handled validation, and SK oversaw the methodology.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of MET's Institute of Pharmacy (Regd. No. 1867/Po/Re/S/16/CPCSEA; Ref. No. MET/GOI/FOP/028; IAEC Approval No. MET-FOP/IAEC/04/2021).

HUMAN AND ANIMAL RIGHTS

All procedures were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, U.S.A.) and CPCSEA guidelines to ensure humane handling and ethical treatment of animals.

This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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