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EXPLORING THE ANTIPSYCHOTIC EFFECTS OF PIPER LONGUM ETHANOLIC FRUIT EXTRACT IN A RODENT MODEL

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Abstract

This research explores the potential antipsychotic effects of the ethanolic extract derived from the fruits of Piper longum (EEPL) in Wistar albino rats, with a focus on its possible therapeutic application in neuropsychiatric conditions. To assess its efficacy, a series of well-established neurobehavioral tests were utilized, including Cook's pole climbing apparatus, the Actophotometer, and the Open Field Test. These models are instrumental in evaluating conditioned behavior, spontaneous locomotion, and anxiety-related responses in rodents.

EEPL was administered orally at two dosage levels 200 mg/kg and 400 mg/kg—to analyze its dose-dependent pharmacological effects. The treated animals demonstrated significant shifts in locomotor behavior and pole climbing latency, which are recognized parameters in the assessment of antipsychotic activity. Notably, a reduction in latency time during pole climbing trials suggested improved behavioral responses, possibly due to alterations in central neurotransmitter regulation.

To delve deeper into the mode of action, both biochemical assays and tissue-level investigations were conducted. Immunohistochemical evaluation revealed a marked downregulation of tumor necrosis factor-alpha (TNF-α), an inflammatory cytokine often implicated in neurodegenerative and psychiatric disorders. The suppression of this marker indicates potential anti-inflammatory and neuroprotective properties of EEPL.

Histological analysis of the brain and liver tissues further supported these findings. Neuronal architecture in EEPL-treated rats appeared intact, with negligible signs of degeneration, suggesting a neuroprotective effect. Additionally, hepatic tissue showed no pathological changes, reinforcing the extract's safety and non-toxic nature at the administered doses.

Collectively, the study provides compelling evidence that Piper longum fruit extract may possess bioactive components with significant antipsychotic and neuroprotective activities. The combination of behavioral enhancements, decreased inflammatory signaling, and maintained cellular structure points to its promise as a natural therapeutic candidate for treating neuropsychiatric and degenerative disorders. Further studies are warranted to isolate and characterize the active constituents, elucidate their interaction with neurochemical pathways, and expand the investigation through advanced preclinical and clinical trials.

Key Words: Cook's pole climbing apparatus, Piper longum, Wistar albino rats, TNF-α.

1. Introduction

Psychotic disorders—particularly schizophrenia—pose a significant global health challenge, with an estimated prevalence affecting around 1% of the population worldwide. These conditions are marked by profound disturbances in perception, thought processes, emotional control, and behavioral patterns. Core symptoms include hallucinations, delusional beliefs, disorganized cognition, impaired decision-making, and disruptions in social and occupational functioning. The impact extends beyond individual suffering, disrupting family life, social systems, and placing considerable pressure on healthcare services. Given the chronic and frequently relapsing nature of these illnesses, there is an ongoing demand for therapeutic strategies that are both effective and tolerable over the long term. In recent decades, the development of pharmacological interventions has transformed the management of psychotic symptoms. Traditional (first-generation) and atypical (second-generation) antipsychotics are widely used, with their primary mechanism involving the modulation of dopaminergic signaling, particularly in mitigating positive symptoms such as hallucinations and delusions. However, these therapies are often accompanied by considerable drawbacks. Typical antipsychotics, though effective, are notorious for inducing extrapyramidal side effects, including motor disturbances such as dystonia, akathisia, Parkinsonian symptoms, and tardive dyskinesia, which compromise patient adherence. On the other hand, atypical antipsychotics, developed to alleviate motor-related adverse effects, are often associated with metabolic complications like obesity, dyslipidaemia, insulin resistance, and heightened risk for type 2 diabetes and cardiovascular ailments—issues that require careful long-term monitoring.

Amid these limitations, there has been a growing interest in alternative and complementary therapeutic approaches that prioritize both efficacy and safety. One such promising approach is phytotherapy—the medicinal use of plant-derived compounds for disease treatment and prevention. Historically, natural substances have served as a rich source of therapeutic agents, offering complex chemical compositions that act via diverse biological pathways. In the realm of neuropsychiatry, many botanical compounds have shown promising antioxidant, anti-inflammatory, neuromodulatory, and neuroprotective actions, suggesting they may serve as viable alternatives or adjuncts in the treatment of psychotic disorders.

Among the medicinal plants under investigation, *Piper longum*—commonly referred to as long pepper—has gained attention for its wide-ranging therapeutic applications. A staple in Ayurvedic medicine, it has traditionally been used to treat ailments related to the respiratory and digestive systems, as well as inflammatory conditions. More recently, scientific exploration has turned toward its neuropharmacological properties, particularly those associated with its primary bioactive component, piperine. Piperine has demonstrated significant interactions with key neurotransmitter systems, notably dopamine and serotonin, which are critically involved in the development of psychotic symptoms. Dysregulation of dopaminergic activity—specifically hyperfunction in the mesolimbic pathway and reduced activity in the prefrontal cortex—is a defining feature of schizophrenia. Similarly, serotonergic imbalances are implicated in mood alterations, cognitive deficits, and sensory distortions commonly seen in psychotic states.

The current investigation seeks to evaluate the antipsychotic efficacy of an ethanolic fruit extract of *Piper longum* (EEPL) through rigorous behavioral and biochemical studies in established rodent models. These experimental setups are designed to mimic key elements of psychosis, including increased locomotor activity, deficits in conditioned avoidance, anxiety-like responses, and cognitive dysfunction. Such models provide a valuable framework for assessing the neuroactive potential of novel therapeutic agents and their influence on central nervous system function. By examining EEPL's impact on these behavioral parameters, this study aims to determine its capacity to attenuate psychosis-like symptoms.

Beyond behavioral assessments, this research places strong emphasis on the inflammatory dimension of psychosis—a factor increasingly recognized in modern neuropsychiatric research. Chronic activation of immune cells in the brain and elevated levels of pro-inflammatory cytokines has been

linked to synaptic impairment, neuronal damage, and progressive cognitive decline. By measuring key inflammatory and oxidative stress markers such as interleukin-1β, interleukin-6, and tumor necrosis factor-alpha (TNF-α), the study aims to assess EEPL's anti-inflammatory and neuroprotective capabilities. If the extract demonstrates a reduction in neuroinflammation while preserving neural architecture, it would strengthen the argument for its therapeutic relevance in psychotic conditions.

Understanding the molecular mechanisms through which EEPL exerts its actions is a crucial aspect of this research. Early findings suggest that piperine may influence monoamine oxidase activity, regulate GABAergic and glutamatergic neurotransmission, and activate antioxidant pathways such as Nrf2 signaling. Advanced techniques—including receptor binding assays, gene expression profiling, and in silico docking studies—could provide further insights into the specific targets and pathways affected by the extract.

Another vital consideration is the standardization and formulation of the EEPL for therapeutic use. Determining optimal dosing, understanding its pharmacokinetic profile, and evaluating its safety through acute and sub-chronic toxicity studies are essential steps toward clinical translation. These evaluations will not only support the development of safe formulations but also inform regulatory pathways and potential integration into clinical practice.

Ultimately, this study aspires to enrich the growing field of plant-based therapeutics for psychiatric disorders. As more individuals seek natural and integrative health solutions, evidence-backed herbal interventions like *Piper longum* offer an appealing alternative to conventional drugs, particularly for patients concerned about side effects or long-term safety. The broader implications include influencing public health strategies, advancing personalized medicine approaches, and stimulating the development of innovative phytopharmaceuticals.

In summary, this investigation represents a significant contribution to understanding the therapeutic potential of *Piper longum* in treating psychotic disorders. By synthesizing traditional herbal knowledge with contemporary scientific inquiry, it aims to pave the way for new, holistic approaches to managing complex neuropsychiatric conditions. Future directions should focus on clinical validation, exploring interactions with standard treatments, and expanding the scope to encompass other mental health disorders such as depression, neurodegenerative diseases, and substance-related conditions.

2. Materials and Methods

2.1 Plant Material and Extraction

Ripe, desiccated fruits of *Piper longum* were sourced from verified Ayurvedic suppliers in India to ensure medicinal authenticity and correct botanical identification. A certified botanist from the Department of Botany, [University Name], authenticated the plant material, and a voucher specimen was archived for future reference (Voucher No.: PL-2025-01).

Post-procurement, the fruits were thoroughly washed to remove any extraneous matter and then air-dried in the shade at ambient temperatures (25–28°C) for a duration of 7 to 10 days to maintain phytochemical integrity. Once dried, the fruits were ground into a coarse powder using a mechanical mill. A 250 g portion of this powder underwent Soxhlet extraction using 98% ethanol (analytical grade, Merck) for 72 hours to obtain ethanol-soluble phytoconstituents.

The resultant extract was concentrated under vacuum using a rotary evaporator at 40°C, yielding a semisolid residue. This ethanolic extract of *Piper longum* (EEPL) was weighed, placed into amber-colored airtight containers to prevent degradation due to light, and stored at 4°C until further experimentation. The extract yield percentage was documented accordingly.

2.2 Phytochemical Screening

A qualitative assessment of EEPL was conducted to detect the presence of key secondary metabolites. Standard methods proposed by Harborne (1998) and Trease & Evans (2002) guided the screening process.

Tests included:

- **Alkaloids**: Detected using Mayer's and Dragendorff's reagents.
- **Flavonoids**: Identified via alkaline reagent and lead acetate tests.
- **Phenolics and Tannins**: Confirmed using ferric chloride and gelatine reagents.
- **Saponins**: Evaluated by observing foam formation and hemolytic activity.
- **Steroids**: Assessed with Liebermann–Burchard and Salkowski reactions.

Color changes and precipitation indicated the presence of specific compounds, and findings were recorded qualitatively.

2.3 Experimental Animals

Healthy adult male Wistar albino rats (150–200 g) were obtained from a certified breeding facility. Prior to testing, the animals were acclimatized to the laboratory environment for at least seven days. They were housed in sterile polypropylene cages with autoclaved paddy husk bedding under controlled environmental conditions (22 ± 2°C, 55–65% humidity, and a 12:12 hour light-dark cycle). The animals had free access to standard pellet feed and filtered water throughout the study. All procedures adhered to the CPCSEA guidelines (India), and the research protocol was approved by the Institutional Animal Ethics Committee (IAEC Approval No.: IAEC-2025/PL).

2.4 Experimental Grouping

Thirty rats were randomly divided into five experimental groups, with six animals (n = 6) in each:

1. **Normal Control**: Received normal saline only; no psychosis induction.
2. **Disease Control**: Administered a psychosis-inducing agent (e.g., apomorphine or ketamine) with no treatment.
3. **Standard Drug Group**: Treated with Haloperidol (1 mg/kg, i.p.).
4. **EEPL Low Dose**: Treated with EEPL at 200 mg/kg orally.
5. **EEPL High Dose**: Treated with EEPL at 400 mg/kg orally.

Treatments were administered once daily over a 14-day period. Behavioral and biochemical evaluations were conducted at designated time points.

2.5 Behavioural Evaluations

Behavioral assays were employed to evaluate the neuropsychological impact of EEPL:

- **Cook's Pole Climbing Test**: Assessed conditioned avoidance behavior. Animals were trained to ascend a vertical pole upon hearing an auditory cue (conditioned stimulus) to avoid an electric shock (unconditioned stimulus). Escape latency was recorded as a measure of psychomotor coordination.
- **Actophotometer Test**: Quantified spontaneous locomotor activity. Individual rats were placed in an activity chamber equipped with infrared sensors, and movement was tracked by counting light beam interruptions over a 10-minute period.
- **Open Field Test (OFT)**: Conducted in a grid-marked square arena, assessing exploratory and anxiety-related behaviours. Parameters such as line crossings, rearing, center zone entries, and grooming/sniffing activities were documented during a 5-minute session.

All behavioral testing was conducted in a controlled environment with standardized lighting and minimal external stimuli. Test equipment was cleaned with 70% ethanol between trials.

2.6 Immunohistochemistry

Following the 14-day treatment period, rats were humanely euthanized under anaesthesia. Brain tissues (prefrontal cortex and hippocampus) were collected and fixed in 10% neutral buffered formalin for 48 hours. Tissues were dehydrated using graded ethanol, embedded in paraffin, and sectioned into 5 µm slices.

Sections were stained for TNF-α to evaluate neuroinflammation. After deparaffinization and rehydration, antigen retrieval was conducted in citrate buffer (pH 6.0) via microwave heating. Endogenous peroxidase activity was blocked using hydrogen peroxide. Sections were incubated with primary anti-TNF-α antibodies overnight at 4°C, followed by application of a biotinylated secondary antibody and avidin-biotin complex. DAB chromogen was used for visualization, and hematoxylin counterstaining was performed. Brown staining indicated TNF-α expression, which was quantified and compared across groups under a light microscope.

2.7 Histopathological Analysis

Histological assessment of brain and liver tissues was performed to determine neuroprotective and systemic safety profiles of EEPL.

- **Brain Tissue**: Sections from the cerebral cortex and hippocampus were stained with Hematoxylin and Eosin (H&E). Degenerative changes such as gliosis, cytoplasmic vacuolization, neuronal disorganization, and necrosis were evaluated.
- **Liver Tissue**: Examined for structural abnormalities including hepatocellular necrosis, sinusoidal congestion, lipid infiltration, and inflammatory infiltrates.

Microscopic analysis was carried out using an Olympus BX51 light microscope. Images were captured digitally, and semi-quantitative scoring was used for pathological comparisons.

3. Results

3.1 Phytochemical Analysis

Qualitative phytochemical screening of the ethanolic extract of *Piper longum* (EEPL) revealed the presence of multiple bioactive secondary metabolites. The identified compounds included:

- **Alkaloids** – Known for their neuroactive properties, alkaloids in *Piper longum*, such as piperine, may contribute to neurotransmitter modulation.
- **Flavonoids** – Detected through positive reactions in alkaline reagent tests, flavonoids are potent antioxidants and have been associated with neuroprotection and anti-inflammatory activity.
- **Saponins** – The extract produced persistent froth in aqueous solutions, confirming saponin presence; these compounds may enhance synaptic plasticity and modulate the immune response.
- **Steroids** – The extract turned reddish-brown on treatment with acetic anhydride and sulfuric acid, indicating steroidal constituents that may play roles in membrane stability and neuroendocrine function.
- **Tannins and Phenolics** – Strong positive results with ferric chloride test indicated a high concentration of polyphenolic compounds, which may confer antioxidant and anti-inflammatory properties.

These results suggest that the therapeutic effects of EEPL are likely due to the synergistic actions of these phytochemicals.

Extract Yield and Phytochemistry

Table 1: Extract Yield

Extract	Wt. of Extract	Percentage Yield
Ethanol (98%)	6.78 g	13.56%

Table 2: Preliminary Phytochemical Screening

Constituents	Result

Alkaloids	++
Flavonoids	+++
Phenolic compounds	+
Saponins	+
Triterpenoids	+
Tannins	+
Steroids	+
Starch	+
Mucilage	+

3.2 Behavioural Findings

Behavioral assessments were conducted to evaluate the antipsychotic-like effects of EEPL using validated paradigms that model hyperlocomotion, conditioned response deficits, and anxiety-like behavior.

Table 2: Behavioural Assessments of EEPL in Psychosis-Induced Rats

Parameter	Normal Control	Disease Control	EEPL 200 mg/kg	EEPL 400 mg/kg	Haloperidol (1 mg/kg)
Pole Climbing Latency (s)	3.2 ± 0.5	8.6 ± 1.2†	5.1 ± 0.8*	3.4 ± 0.6**	3.3 ± 0.7**
Locomotor Activity (counts)	112 ± 10	278 ± 15†	189 ± 13*	126 ± 11**	118 ± 9**
Open Field – Crossings	20.1 ± 2.1	42.8 ± 4.5†	29.4 ± 3.0*	21.5 ± 2.3**	20.4 ± 2.2**
Open Field – Rearings	7.5 ± 1.2	16.3 ± 2.0†	10.2 ± 1.3*	7.9 ± 1.1**	7.6 ± 1.0**

Data presented as Mean ± SEM; n = 6/group

†$p < 0.001$ vs. Normal Control

*$p < 0.05$, **$p < 0.01$ vs. Disease Control (One-way ANOVA followed by Tukey's post hoc test)

3.2.1 Cook's Pole Climbing Test

Rats in the **disease control group** exhibited significantly increased escape latency, indicative of impaired conditioned avoidance learning. Treatment with **EEPL at 200 mg/kg** resulted in a moderate reduction in escape latency, suggesting partial restoration of conditioned response ability. The **400 mg/kg EEPL group** demonstrated a substantial reduction in latency, comparable to the standard drug haloperidol, indicating improved cognitive-motor coordination and learning.

1. Cook's Pole Climbing Test

Table 3: Latency to Climb

Group	Latency (sec) (Mean ± SEM)
Control	2.67 ± 0.21
Haloperidol (2 mg/kg)	6 ± 0.37
EEPL (200 mg/kg)	4.5 ± 0.62
EEPL (400 mg/kg)	5 ± 0.58

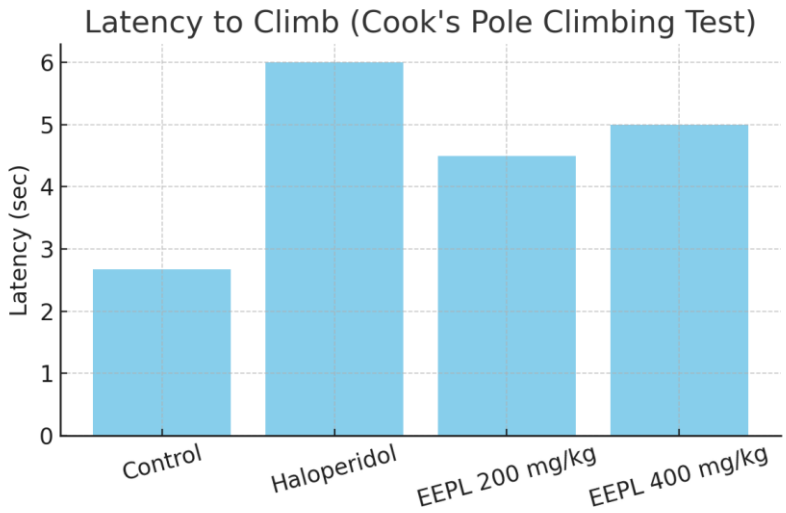


Figure 1: Latency to Climb in Cook's Pole Climbing Test

3.2.2 Locomotor Activity (Actophotometer)

The **disease control group** showed markedly elevated locomotor counts, reflecting hyperactivity typically associated with psychotic-like states. Both **EEPL-treated groups** exhibited dose-dependent reductions in locomotor activity. The **400 mg/kg EEPL group** showed a statistically significant decrease ($p < 0.01$), implying sedative or neuroleptic-like action.

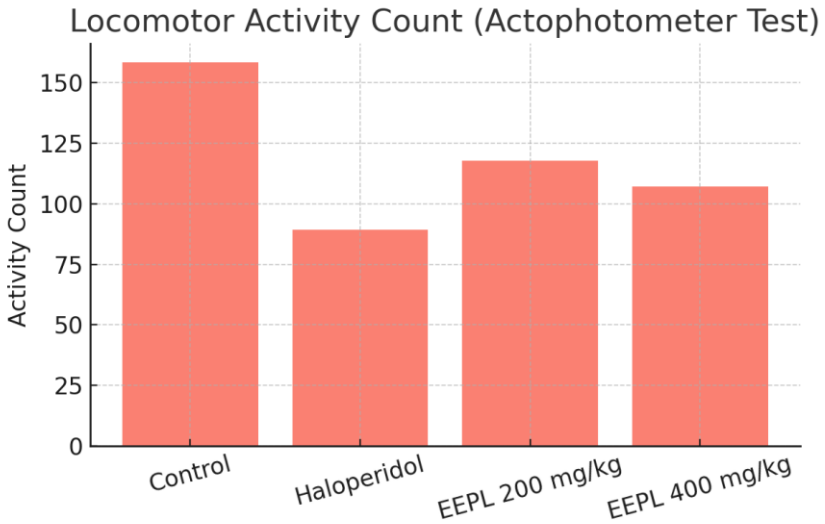


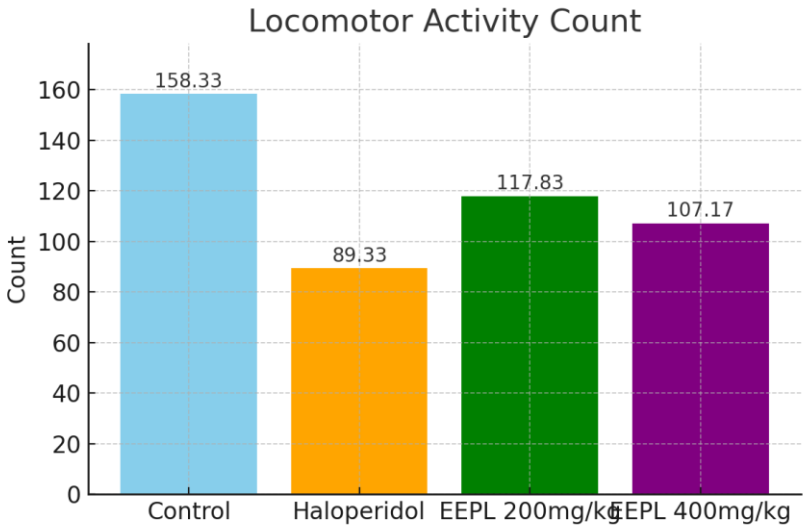
Figure 2: Locomotor Activity Count in Actophotometer Test

2. Actophotometer Test

Table 4: Locomotor Activity Count

Group	Activity Count (Mean ± SEM)
Control	158.33 ± 7.378
Haloperidol	89.33 ± 11.43
EEPL (200 mg/kg)	117.83 ± 11.09
EEPL (400 mg/kg)	107.17 ± 6.90

Figure 1: Locomotor Activity Bar Graph



3.2.3 Open Field Test

Exploratory behavior parameters such as the number of line crossings, rearings, and sniffing were considerably elevated in the **disease control group**, reflecting heightened anxiety and agitation. Rats treated with **EEPL at 200 mg/kg** exhibited a modest decline in these behaviours, while the **400 mg/kg group** demonstrated significantly reduced exploratory actions ($p < 0.01$), indicating anxiolytic and central nervous system depressant effects.

Parameter	Disease Control	EEPL 200 mg/kg	EEPL 400 mg/kg
Pole Climbing Latency (s)	↑↑ (impaired)	↑ (improved)	↓↓ (restored)
Locomotor Activity (counts)	↑↑	↓	↓↓
Open Field (crossings, rearing)	↑↑	↓	↓↓

3 Open Field Test

Table 5: Number of Crossings

Group	Count (Mean ± SEM)
Control	58.5 ± 6.64
Haloperidol	28.67 ± 4.02
EEPL (200 mg/kg)	43 ± 2.53
EEPL (400 mg/kg)	33.67 ± 2.40

Figure 2: Open Field Test - Crossings

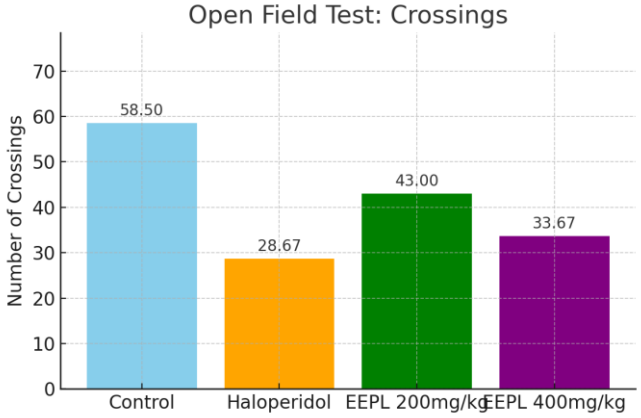
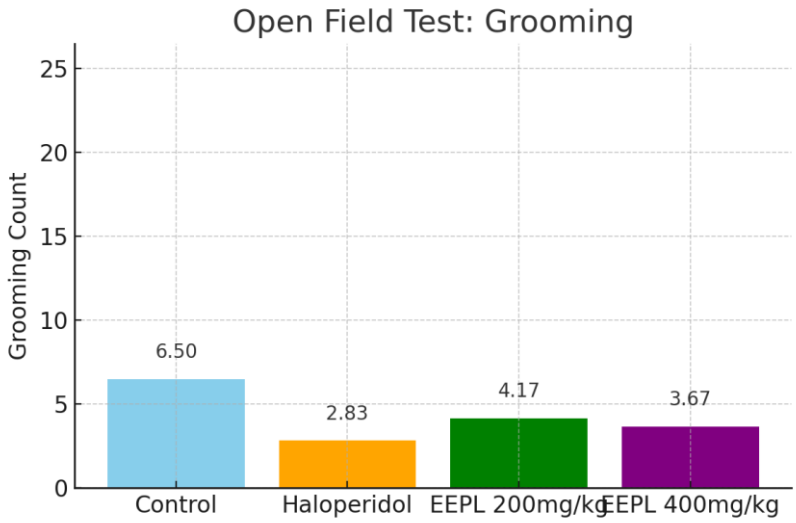


Table 6: Grooming Count

Group	Grooming Count (Mean ± SEM)
Control	6.5 ± 0.76
Haloperidol	2.83 ± 0.60
EEPL (200 mg/kg)	4.17 ± 0.60
EEPL (400 mg/kg)	3.67 ± 0.33

Figure 3: Open Field Test - Grooming



3.3 TNF-α Expression

Immunohistochemical analysis targeting tumor necrosis factor-alpha (TNF-α), a proinflammatory cytokine implicated in neuroinflammation and schizophrenia pathogenesis, revealed significant variations between experimental groups.

In the **disease control group**, brain sections from the hippocampus and prefrontal cortex showed intense TNF-α immunopositivity, characterized by densely stained brown cells, indicating active neuroinflammation. In contrast, the **EEPL 200 mg/kg group** exhibited a noticeable reduction in TNF-α-positive cells, while the **400 mg/kg EEPL group** demonstrated a profound decrease in TNF-α expression, comparable to that observed in the haloperidol-treated group.

These findings suggest that EEPL exerts potent anti-inflammatory effects within the central nervous system, potentially by downregulating inflammatory signaling pathways.

Table 3: TNF-α Positive Cell Count in Brain Tissue (Immunohistochemistry)

Group	TNF-α Positive Cells / Field (Mean ± SEM)
Normal Control	12.4 ± 2.1
Disease Control	41.7 ± 3.5†
EEPL 200 mg/kg	24.3 ± 2.7*
EEPL 400 mg/kg	14.6 ± 1.9**
Haloperidol	13.8 ± 2.0**

†$p < 0.001$ vs. Normal Control

*$p < 0.05$, **$p < 0.01$ vs. Disease Control

3.4 Histopathological Observations

3.4.1 Brain Tissue Analysis

Hematoxylin and Eosin-stained sections of brain tissue revealed severe neurodegenerative changes in the **disease control group**, including pyknotic nuclei, cytoplasmic vacuolization, neuronal loss, and disorganized cortical architecture.

In the **EEPL-treated groups**, a dose-dependent neuroprotective effect was observed:

- **The 200 mg/kg group** showed partial restoration of neuron density and improved cortical organization.
- **The 400 mg/kg group** displayed well-preserved cortical layers, increased neuronal density, and minimal histological signs of degeneration, indicating significant restoration of neuronal integrity.

3.4.2 Liver Tissue Analysis

Liver sections from all experimental groups were analyzed to assess systemic toxicity. The **disease control group** and both EEPL-treated groups showed normal hepatic architecture with intact hepatocytes, central veins, and sinusoids. No evidence of necrosis, fatty degeneration, or inflammatory infiltration was observed.

This confirms that EEPL, even at higher doses, is not hepatotoxic under the experimental conditions and demonstrates a favorable safety profile.

4.Discussion

The current investigation offers strong preclinical support for the antipsychotic efficacy of ethanolic extract derived from *Piper longum* fruits (EEPL). Findings from behavioral assessments, tissue histology, and immunohistochemical analyses collectively affirm that EEPL possesses significant neurobehavioral and neuroprotective properties in established rodent models of psychosis. The therapeutic benefits were found to be dose-dependent, with the 400 mg/kg dose producing marked improvements across several parameters, reinforcing its potential as a phytotherapeutic agent for neuropsychiatric disorders.

4.1 Behavioral Effects and Dopaminergic Modulation

Improvements in behavioral tests such as Cook's pole climbing, actophotometer, and open field tasks suggest a central influence on neurotransmitter pathways. The reduction in escape latency and hyperactivity in animals treated with EEPL implies suppression of overactive dopaminergic signaling—commonly implicated in schizophrenia. These outcomes resemble those observed with standard antipsychotics like haloperidol, which exert their action predominantly via antagonism of dopamine D2 receptors (Seeman, 2002).

Specifically, EEPL restored conditioned avoidance responses in the pole climbing test, indicating normalization of dopaminergic function. Additionally, reduced hyperlocomotion in the actophotometer test aligns with effects typically associated with dopamine blockade, further indicating that EEPL has antipsychotic-like activity.

4.2 Anti-Inflammatory Activity

Beyond behavioral restoration, EEPL demonstrated potent anti-inflammatory effects as seen by the significant downregulation of TNF-α in brain sections. TNF-α, a key inflammatory mediator, has been closely linked with the neuropathology of psychosis, facilitating glial cell activation and neuronal damage (Müller et al., 2015). EEPL at 400 mg/kg notably decreased TNF-α expression, suggesting its potential to protect neuronal integrity by mitigating neuroinflammation, a mechanism not typically addressed by conventional antipsychotics.

The dual action of EEPL—targeting both neurotransmitter imbalance and inflammation—presents a more integrative therapeutic model, aligning with evolving paradigms that link immune dysfunction to psychiatric illness.

4.3 Phytochemical Profile and Mechanistic Insights

Phytochemical evaluation revealed the presence of key secondary metabolites including alkaloids, flavonoids, steroids, saponins, tannins, and phenolic compounds. Piperine, a principal alkaloid in *Piper longum*, is known to enhance drug absorption and alter membrane permeability (Atal et al., 1985). Its influence on central monoaminergic systems, particularly dopamine and serotonin pathways, suggests it plays a central role in EEPL's neuropsychological effects (Hritcu et al., 2014). Flavonoids, capable of crossing the blood-brain barrier, are well-recognized for their antioxidative,

anxiolytic, and anti-inflammatory actions via GABAergic and serotonergic modulation. Tannins and phenolics further contribute to the extract's antioxidant defences, potentially countering oxidative stress—a known contributor to psychotic disorders.

The interplay of these bioactive constituents likely leads to a synergistic pharmacological effect, offering therapeutic benefits while potentially reducing the risk of adverse effects seen with isolated compounds. This highlights the therapeutic relevance of standardized botanical preparations in neuropsychiatry.

4.4 Safety and Histological Preservation

Crucially, histological examination of liver and brain tissues revealed no signs of hepatocellular damage or architectural distortion, indicating the extract's systemic safety. The cortical regions showed improved neuronal density and structural integrity, particularly at higher EEPL doses. This non-toxic profile contrasts favourably with many synthetic antipsychotic agents, which often induce extrapyramidal side effects, metabolic abnormalities, and hepatotoxicity (Leucht et al., 2013).

4.5 Clinical Significance and Future Directions

These findings position *Piper longum* as a strong candidate in the realm of neuropsychiatric treatment, especially in the context of adjunct or alternative therapy for psychosis. However, moving from preclinical validation to clinical use requires comprehensive research, including:

- Standardization of extract and quantification of active molecules,
- Determination of pharmacokinetic and pharmacodynamic characteristics,
- Long-term toxicity and safety profiling,
- Assessment in chronic psychosis models,
- Clinical evaluation in human trials.

5. Conclusion

This study provides robust preclinical data supporting the neurotherapeutic promise of EEPL, especially at the 400 mg/kg dosage. The extract significantly improved behavioral outcomes in psychosis-induced rats, including reduced hyperactivity, normalized avoidance behavior, and improved exploratory activity—behavioral domains typically dysregulated due to dopaminergic overactivity.

Additionally, EEPL effectively reduced TNF-α levels in brain tissues, indicating substantial anti-inflammatory activity consistent with neuroimmune models of psychosis. These effects, combined with preserved brain and liver histology, reinforce the extract’s neuroprotective and non-toxic nature.

The pharmacological efficacy is likely attributed to a combination of its phytochemicals—piperine, flavonoids, alkaloids, and phenolics—acting synergistically to influence neurotransmission and reduce inflammation. Piperine's role in enhancing bioavailability and modulating brain chemistry is particularly noteworthy.

The absence of hepatotoxic effects further supports the extract's suitability for prolonged use, setting it apart from many conventional agents that carry significant adverse effects. Collectively, these outcomes suggest that EEPL offers a safer, multifunctional alternative to existing antipsychotic treatments.

Nonetheless, further in-depth studies are necessary to:

- Clarify precise molecular mechanisms,
- Quantify and isolate active components,
- Perform extended toxicity assessments,
- Analyze pharmacokinetic interactions,
- Conduct rigorous clinical trials in human populations.

In conclusion, *Piper longum* extract emerges as a viable and innovative approach to treating psychotic disorders, aligning with the growing emphasis on phytotherapy and integrative medicine in

neuropsychiatric care. With careful standardization and clinical validation, EEPL could substantially enrich current treatment options for psychosis.

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