Anti-Cataleptic Activity Of Pine Oil In Haloperidol Induced Mice Model

Rudhali Lilhare¹, Amol Bondre¹, Dr. Rajesh Mujariya¹, Dr. Manjeet Singh¹, Poonam Bihone¹

¹Institute of Pharmaceutical Science and Research (IPSR), Balaghat(M.P), India

Abstract: The current study examines the effects of pine oil by inducing catalepsy with the antipsychotic medication haloperidol. It results in progressive neurodegeneration at a certain dose and for a predetermined amount of time. It was given intraperitoneally half an hour after the test and treatment medications. There were five groups in all, with six animals in each. The first group was managed. Second place went to the sickness control group, which included haloperidol-induced cataplexy. Low dosages of test and therapy medications made up the third group. Haloperidol and pine oil were utilized by the fourth group at 300 µl, and nutmeg oil and haloperidol at 600 µl by the last group. Behavioral measures were measured on the seventh, fourteenth, and twenty-first days of the study. The actophotometer, rotarod apparatus, climbing pole, and catalepsy bar were utilized to measure the behavioral parameter. The action of the pine oil was analyzed using the behavioral characteristics.

Keywords: Catalepsy, Haloperidol, mice, pine oil, nutraceutical, parkinson’s disease, natural remedies

I. INTRODUCTION

1.1 Catalepsy
A neurological condition known as catalepsy is characterized by ongoing muscle rigidity and a lack of motion in the area where one’s limbs are sustained and in a suitable, rigid state. The illness encouraged a deadening of responses to environmental cues and a decreased sensitivity to discomfort. A somnolent state with little voluntary movement and response, as well as unusually flexible muscles, are the common symptoms of catalepsy. Muscle flexibility allows for the molding of the complete body and its component components into unique postures that can be maintained for an extended period of time (Ingram et al., n.d.). Catalepsy is a condition characterized by sluggish movement and tonic immobility in several body parts (Hagenaars et al., 2006). Haloperidol is a type of butyrophenone neuroleptic drug that was first administered to treat schizophrenia. It is classified as a strong-affinity dopamine antagonist. Since it is now known that dopamine plays a crucial role in many of these unpleasant symptoms, haloperidol and
other dopamine antagonists are used in palliative care to treat delirium, nausea, and vomiting symptoms (Prommer, 2012).

1.2 Haloperidol induced catalepsy
For several decades, psychotic disorders were successfully treated via the powerful neuroleptic medication Haloperidol (Froemming et al., 1989). Whenever nigrostriatal dopamine is disrupted using dopaminergic antagonists including haloperidol, rats as well as other experimental creatures may experience catalepsy, which is indicative of Parkinson’s disease alongside other illnesses. Additionally, it recently demonstrated that stiffness in Parkinson’s disorder shows up to be well simulated by haloperidol-induced catalepsy, with similar EMG measurements in both (Field et al., 2000).

1.3 Parkinson’s Disease (PD)
Among the nation's most prevalent neurodegenerative conditions, Parkinson's dopamine in the region known as the substantia nigra (SN). Despite the precise causes of PD are unidentified, evidence establishes inflammation as well as oxidative disease (PD), has been described by a degeneration of neurons that manufacture stress as contributing factors to its etiology. Nigrostriatal dopaminergic (DA) neurons ultimately decline in the course of Parkinson's disease (PD), a prevalent neurodegenerative illness. The decline of striatal levels of dopamine, particularly can trigger aberrant motor behaviors such as stationary tremors, stiffness, and bradykinesia, is one of the most obvious biochemical alterations in Parkinson's disease (PD) (Viveros-Paredes et al., 2017). In addition to the classification of Parkinson's disease as a movement-based illness, it has grown increasingly apparent that an extensive list of symptoms that are not related to movement, which includes mental retardation, autonomic disorders, difficulty falling asleep, depression as well, and hyposmia (impaired smell), encompass all aspects of the disease and significantly add to the as a whole (Fahn, 2008).

2. plant profile

Pine Plant

![Fig.1 Pine Plant](image)

2.1 Scientific Classification
Kingdom: Plantae
Order: Pinales
Family: Pinaceae
Genus: Pinus
Species: Pinus sylvestris
Binomial name: Pinus strobus
d
Vernacular name: Pine Oil, Forest Pine, Pine Needle, Oleum FoliiPini Sylvestris

**TERRPE DERIVATIVES, USUALLY COMPRISING ABOUT 70% ALPHA TERPINOL. IN SUCH A SCENARIO, THE TEST RESULTS SHOW THAT THE ANTI-FUNGAL PROPERTIES OF THE DISINFECTING AGENTS HAVE AN ADVANTAGE OVER FUNGICIDAL ONES (ACTION OF PINE OIL ON SOME FUNGI OF THE SKIN, IN VITRO, N.D.).** NUMEROUS SPECIES OF PINE TREES IN THE GENUS PINUS COMMONLY CONTAIN ALPHA-PINENE, A BICYCLIC MONOTERPENE MOLECULE. PREVIOUS STUDIES HAVE DEMONSTRATED THAT A-PINENE POSSESSES a wide range of pharmacological properties, such as antioxidant and antinociceptive behavior. Additionally, it has been shown that a-pinene has a strong anti-inflammatory effect when conditions are diseased (Khoshnazar et al., 2020).

It has been reported that -pinene has hypnotic and anxiety-relieving effects when taken orally. Essential pine oils have several different biochemical effects; these actions include being anti-inflammatory, antimicrobial, analgesic, and stress-relieving. The great majority of them are monoterpenes, including limonene, 3-carene, terpinene, and -pinene. -pinene increases the decay time of GABAergic transmission between neurons, which promotes NREMS via influencing the GABA receptor's BZD binding area. Up till now, it had been demonstrated that breathing (-)-pinene had hypnotic and anxiety-reducing properties (H. Yang et al., 2016).

### 2.2 Chemical Composition of Pine Essential Oil

The procedure known as gas chromatography by mass spectrometry was implemented to establish the identification of 23 ingredients, collectively constituted 95.79% of the extracted oil. The elementary constituents include limonene (17.00%), linalool (24.47%), plus α-terpineol (30.2%), eugenol (2.14%), caryophyllene (3.14%), anethole (14.57%)(Zeng et al., 2012).

### 2.3 Therapeutic Uses

#### 2.3.1 Anti-inflammatory activity

The main components of oils that are essential are monoterpenes, that happen to be organic compounds that correspond to the terpenes biochemical category. Analyzing the medicinal qualities of monoterpenes exhibiting anti-inflammatory capabilities is crucial(De Cássia Da Silveira E Sá et al., 2013).

#### 2.3.2 Antioxidant activity

Employing the highly stable radical, DPPH, the antioxidant capability of the volatile ingredient’s species P. armandii has been investigated on the basis of how well they were able to contribute hydrogen or scavenge reactive oxygen species(X. Yang et al., 2010).

#### 2.3.3 Anxiolytic activity

In accordance with reports that whenever inhaled, the compound pinene exhibits sedative and anxiety-reducing qualities. Inhalation of -pinene was recently demonstrated to exert anxiolytic impacts on mice during an elevated-plus-maze assessment, reported according to recent research reported by Satou et al(H. Yang et al., 2016).

#### 2.3.4 Wound healing activity

The resinous substance of the Pinus sylvestris species Pinus nigra can be applied locally to heal wounds in Turkish traditional medicines. Five diverse Pinus species had the essential fatty acids extracted from their cones and needles investigated for their capability to encourage wound recovery in vivo(Süntar et al., 2012).
2.3.5 Antimicrobial activity
the oil that is essential from Picea abies L.'s young shoots' antibacterial qualities. Gram-positive and Gram-negative bacteria were subjected to testing to discover how the oil influenced them in their final days as well as certain fungi. (Kartner et al., 1991).

2.3.6 Anti-aging activity
It is commonly referred to as inus turpentines has applications in cosmetics that are anti-aging, external anti-irritants in the management of rheumatic conditions also muscles difficulties, as well as healing. Pine is employed in Chinese medicine to serve as a herbal painkiller, anti-rheumatic, as well as for anti-aging therapies (Saber et al., 2021).

Materials and Methods
4.1 Animals
Healthy Male Swiss albino mice weighing between 25-40 gm body weight were used for the study. Animals were obtained from the Central Animal (Pre-clinical) Research Facility (Registration Number – 393/PO/Re/S/02/CPCSEA Dt.19.07.2002; protocol no.2022-23-08). Animals were housed in well-ventilated polypropylene cages and maintained under standard conditions (at 25 ± 2 0C, 1212 hr LD (Light and Dark) cycle) in the departmental animal house. The animals were fed with standard pelletized feed and water was provided.

3.EXPERIMENTAL WORK
3.1 Materials
3.1.1 Drugs
Pine oil was obtained from (Green Leaf HERBAL). Levodopa tablet was obtained from (local medical store). Haloperidol & Sterile saline were purchased from a local pharmacy.

3.1.2 Chemicals
Distilled water and Tween 80 solution were taken from laboratory of college. Tween 80 solution was used to prepare emulsion of drug. For 300µl oil 0.2µl Tween 80 was added in 50ml of water. For 600 µl oil 0.2µl Tween 80 was added in 25 ml of water. Fresh saline solution was brought from local pharmacy.

3.2.3 Dosage and concentrations
For In-vivo studies, Pine oil was used at doses 300µl/kg and 600µl/kg orally as low dose, and high dose respectively. Standard drug levodopa 20mg/kg given orally as 20mg is suspended in 10 ml of distilled water. According to literature survey, pine oil is insoluble in water so it is convenient to prepare emulsion of pine oil by means of using Tween 80 solution as emulsifying agent as per dose. And haloperidol is soluble in saline solution so 0.4µl is suspended in 10 ml saline water. Doses were calculated as per literature and survey.

4. Preparation of doses
4.1. Pine oil
Pine oil is categorized into essential oil. Pine oil is highly volatile in nature. Pine oil is administered orally at doses of 300µl and 600µl per kg weight of the mice in the form of emulsion. Emulsion was prepared by using bottle method in Falcon tube as it was appropriate method for emulsion preparation of volatile oil.

For 300µl/kg Dose: 300µl Pine oil was mixed with 1.8ml of prepared Tween along with 1.8ml of water in Falcon tube. Further volume made up to 10ml with water.

For 600µl/kg Dose: 600µl Pine oil was mixed with 900µl prepared Tween 80 solution along with 2.7ml of water in Falcon tube. Further volume made up to 10ml with water.

Levodopa was administered orally at a dose of 20mg/kg. Initially levodopa tablet was triturate in mortar and pestle and then required amount was suspended in 10ml of distilled water. In present study haloperidol injection is use as inducing agent for catalepsy. Haloperidol 0.4µl was dissolve in 10 ml of saline solution.
Procedure
The neuroprotective effect of Pine oil studied in haloperidol induce catalepsy in mice. The catalepsy was induced by consecutive administration of haloperidol 20mg/kg for 21 days. Following one-week acclimatization period, mice were randomly divided into six groups, each group containing six animals (n = 6), and subjected to various drug treatments for 21 days. The first normal group i.e control group of animals were kept as it is without any drug treatment. The second positive control group were intraperitoneally administered with Haloperidol 1mg/kg once daily for 21 days. The group 3 and 4 received Pine oil orally at dose 300µl/kg and 600µl/kg dose, respectively for 21 days with haloperidol 1mg/kg. Fifth group i.e standard group receive Levodopa20mg/kg with haloperidol 1mg/kg.

Table 1. Experimental animal groups

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Group</th>
<th>Drug treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control group</td>
<td>No any Treatment</td>
</tr>
<tr>
<td>2.</td>
<td>Positive control</td>
<td>1 mg/kg (i.p.)</td>
</tr>
<tr>
<td>3.</td>
<td>Pine oil + Haloperidol</td>
<td>300µl/kg (p.o.)</td>
</tr>
<tr>
<td>4.</td>
<td>Pine oil + Haloperidol</td>
<td>600µl/kg (p.o.)</td>
</tr>
<tr>
<td>5.</td>
<td>Standard group (Haloperidol + Levodopa)</td>
<td>20mg/kg (p.o.)</td>
</tr>
</tbody>
</table>

5. Behavioral parameters
- Bar catalepsy (Evaluation of Posture instability)
- Actophotometer test (Evaluation of Locomotor activity)
- Rotarod test (Evaluation of muscle coordination and muscle strength)
- Pole test (Evaluation of Bradykinesia)

5.1 Catalepsy bar test

Principle
Musculoskeletal stiffness is a frequent complaint in the development of Parkinson's disease, and for screening for it, animals’ catalepsy is popular; via catalepsy, arms and legs persist in any position that is positioned on the bar. The extrapyramidal network is frequently examined utilizing the bar catalepsy procedure. The duration of time during which the animal maintained in its original posture while keeping its front feet lifted and lying on a 3-cm-high (0.9-cm-diameter) bar can be determined through the typical bar test. Whenever an animal raises its head around in an attempt to explore either once its hind feet move off the bar, catalepsy is believed to have occurred stopped and visualize animals if an animal remains longer than 10 seconds or fails to change its posture within 10 seconds, the condition is cataleptic (Sharma et al., 2018).

Procedure
1. Bring out animal from the cage.
2. Put the paws over the 3 cm bar.
3. Count the amount of time until the animal withdrawn its paws or changed its position.
4. Removing animals while paws are taken away or when it turns its head in an exploring attitude.
5. 5 minutes being the maximum period allowed.
5.2 Locomotor activity

**Principle**
The basic concept in evaluating locomotor activity in Parkinson disease lies in the fact that every sign and symptom, especially muscular rigidity, dyskinesia, and gait difficulties, diminish it. Parkinson’s condition generates a reduction in levels of dopamine, which inhibits typical motion or generates uncontrolled movement. Actophotometer is utilized for assessing the activity of locomotion. The substrate has IR rays that recognize movements as it crossed them, and breaking the line and presenting the score instantaneously (R26, n.d.)

**Procedure**
1. Turn on the Actophotometer.
2. Animal is kept in the Actophotometer.
3. Note Number of light rays cross by the animal in 5 mins.
4. After 5 mins turn off the Actophotometer and remove out animal from it.

**Rotarod Test**

**Principle**
Rotarod examination is employed for determining an animal's impairment in movement. The rotarod testing was carried out for assessing the extent to which the animal was able to preserve its posture after being rotated across a 1-inch rod at different rotational motions per min. The rotarod assessment's fundamental concept is that it is challenging individuals with Parkinson's disease to maintain their balance or grasp devices in their hands (Guzmán-Gutiérrez et al., 2012).

**Procedure**
1. Switch on the Rotarod Apparatus.
2. Kept an animal on the Rod.
3. Hold for two minutes, then subsequently raise the speed of rotarod by 6 RPM.
4. When an animal drops, remove it from rod and speeds up another time for 2 minutes at a speed of 12 RPM.
5. Repeat this procedure up to 30 RPM, after which separate every animal and record Reading of each.

5.3 Pole Climbing Test

**Principle**
Mice’s balancing, orientation in space, and motor integration are investigated using a pole. A mice is positioned on high of the pole having its forehead facing upside down while performing the usual pole test, and the duration that it requires for it to turn around and decline towards the base of that the pole is monitored. It is essential to give pre-training to animals before this test (Miller et al., 2015).

**Procedure**
1. Take an animal from the cage.
2. Animals have been placed on the top of the pole with their heads pointing upward down.
3. Mice will be facing downward and descent towards the pole’s base.
4. Five trials are made for the duration it requires to make a T turn and decline to the pole's base.
6. Results and Discussion

6.1. Effect of Pine oil on catalepsy time in Haloperidol-Induced catalepsy in mice

Dunnett’s post hoc multiple comparison testing was employed to investigate the data following two-way ANOVA. These values are mean SEM, and there were six participants in each group. When comparison to the normal control team, log \( \#\#\#p<0.001, ***p<0.001 \) when compared to disease control.

6.2. Effect of Pine oil on locomotor activity in Haloperidol-Induced catalepsy in mice

Dunnett’s post hoc multiple comparison testing was employed to investigate the data following two-way ANOVA. These values are mean SEM, and there were six participants in each group. When comparison to the normal control team, log \( \#\#\#p<0.001, ***p<0.001 \) when compared to disease control.
6.3 Effect of Pine oil on motor impairment/motor coordination in Haloperidol-Induced catalepsy in mice

Dunnett’s post hoc multiple comparison testing was employed to investigate the data following two-way ANOVA. These values are mean SEM, and there were six participants in each group. When comparison to the normal control team, log \( \#\#p<0.001 \), \( **p<0.001 \) when compared to disease control.

6.4 Effect of Pine oil on gait impairment in Haloperidol-Induced catalepsy in mice

![Graph showing the effect of Pine oil on motor impairment/motor coordination in mice](image1)

![Graph showing the effect of Pine oil on gait impairment in mice](image2)
Dunnett’s post hoc multiple comparison testing was employed to investigate the data following two-way ANOVA. These values are mean SEM, and there were six participants in each group. When comparison to the normal control team, log ##p 0.001 and ***p 0.001, respectively when compared to disease control.

7. Conclusion
In conclusion, our finding revealed that oral administration of Pine oil 300µl/kg and 600µl/kg for 21 days along with induction of Parkinson’s disease by Haloperidol (1mg/kg), shows dose-dependent protective effect. Most effective dose of Pine oil is 600µl/kg compare to 300µl/kg. It may actually reduce neuronal damage and it can also lower oxidative stress in brain tissue. Pine oil protects neurons from damage and improve dopaminergic transmission by acting as an antioxidant.

8. References
1. ACTION OF PINE OIL ON SOME FUNGI OF THE SKIN, IN VITRO. (n.d.).


