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Pharmacognostic Evaluation and Neuroprotective Potential Of Anastatica Hierochuntica and Pinus Wallichiana in Haloperidol-Induced Catalepsy and Apomorphine-Induced Stereotypy in Wistar Rats

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Abstract
This study evaluated the pharmacognostic properties and neuroprotective potential of Anastatica hierochuntica and Pinus wallichiana in Wistar rats using haloperidol-induced catalepsy and apomorphine-induced stereotypy models, complemented by the pole-climbing test. Wistar rats (150–200 g, n=6 per group) were divided into seven groups: control, toxin control (haloperidol 1 mg/kg, i.p., or apomorphine 1.5 mg/kg, s.c.), standard (levodopa 10 mg/kg, i.p., for catalepsy; haloperidol 0.5 mg/kg, i.p., for stereotypy), and low- and high-dose groups for both plant extracts (100 and 200 mg/kg, i.p.). Behavioral assessments on days 7 and 14 revealed that high-dose Anastatica hierochuntica (AH-HD) and Pinus wallichiana (PW-HD) significantly reduced stereotypy duration (AH-HD: 220.0 ± 8.0 s on day 7, 210.0 ± 7.5 s on day 14; PW-HD: 250.0 ± 10.0 s on day 7, 235.0 ± 9.0 s on day 14, $p<0.01$ vs. toxin control) and likely catalepsy duration, approaching the efficacy of standard drugs. The pole-climbing test suggested improved motor coordination in high-dose groups. These findings indicate that Anastatica hierochuntica and Pinus wallichiana possess dose-dependent neuroprotective effects, possibly mediated by antioxidant and dopaminergic modulation, warranting further mechanistic and clinical investigations.
Keywords: *Neuroprotection, Anastatica hierochuntica, Pinus wallichiana, Catalepsy, Stereotypy*

1. INTRODUCTION

Neurodegenerative disorders, such as Parkinson's disease (PD), are characterized by progressive neuronal loss, leading to motor and non-motor dysfunctions. PD is primarily associated with dopaminergic neuron degeneration in the substantia nigra, resulting in symptoms like bradykinesia, rigidity, and tremors (Beitz, 2014). Experimental models, such as haloperidol-induced catalepsy and apomorphine-induced stereotypy, are widely used to mimic Parkinsonian symptoms and evaluate potential neuroprotective agents (Emborg, 2004). Haloperidol, a dopamine D2 receptor antagonist, induces catalepsy by causing muscular rigidity and immobility, reflecting motor dysfunction akin to PD (Duty & Jenner, 2011). Similarly, apomorphine, a dopamine agonist, induces stereotypic behaviors like sniffing and rearing, which are indicative of dopaminergic hyperactivity and are used to assess neuroprotective or antipsychotic effects (Bezard et al., 2013).

The growing interest in phytomedicine has prompted researchers to explore plant-based compounds for their neuroprotective potential. Phytochemicals, such as flavonoids, alkaloids, and terpenoids, have shown promise in mitigating oxidative stress, neuroinflammation, and neuronal apoptosis, which are key pathological features of neurodegenerative diseases (Kumar & Khanum, 2012). Anastatica hierochuntica, commonly known as the Rose of Jericho, is a desert plant traditionally used in folk medicine for its antioxidant and anti-inflammatory properties (Suman et al., 2024). Its bioactive constituents, including flavonoids and phenolic compounds, have been reported to exhibit neuroprotective effects by scavenging free radicals and modulating dopaminergic pathways (Chigurupati et al., 2021). Similarly, Pinus wallichiana, a coniferous tree native to the Himalayas, contains diterpenoids and polyphenols that have demonstrated antioxidant and neuroprotective activities in preclinical studies (Suryawanshi et al., 2024). These plants represent promising candidates for pharmacognostic evaluation and therapeutic exploration in neurodegenerative models.

The present study aims to evaluate the pharmacognostic properties and neuroprotective potential of Anastatica hierochuntica and Pinus wallichiana in Wistar rats subjected to haloperidol-induced catalepsy and apomorphine-induced stereotypy. The experimental design included seven groups (n=6 per group): control, toxin control (haloperidol or apomorphine), standard drug (levodopa for catalepsy, haloperidol for stereotypy), and low- and high-dose groups for both plants (100 mg/kg and 200 mg/kg, i.p.). The catalepsy test measured immobility duration, with shorter durations indicating better motor function, while the stereotypy test assessed the duration and intensity of repetitive behaviors, with lower scores suggesting reduced dopaminergic dysfunction. Results from the stereotypy test showed that high-dose Anastatica hierochuntica (AH-HD) and Pinus wallichiana (PW-HD) significantly reduced stereotypy duration compared to the toxin control on days 7 and 14 ($p<0.01$), indicating potential dopaminergic modulation. These findings align with previous studies demonstrating the neuroprotective effects of phytochemicals in PD models (Currais et al., 2014; Esposito et al., 2012).

This study also emphasizes the importance of standardized animal handling and habituation protocols to ensure reliable results. Wistar rats were maintained under controlled conditions (25 ± 1°C, 44-50% humidity, 12-hour light/dark cycle) with access to a standard rodent diet and water ad libitum, as described in the methodology. Such conditions minimize stress-induced variability and enhance the reproducibility of behavioral outcomes (Fisher, 2011). Additionally, the inclusion of tests like pole climbing, alongside catalepsy and stereotypy, provides a comprehensive assessment of motor and behavioral functions, further validating the neuroprotective potential of the tested plant extracts (Faden & Stoica, 2007).

By combining pharmacognostic analysis with rigorous pharmacological screening, this research contributes to the growing body of evidence supporting the therapeutic potential of Anastatica hierochuntica and Pinus wallichiana in neurodegenerative disorders. The study's findings underscore the need for further investigation into the molecular mechanisms underlying their neuroprotective effects, including their interactions with dopaminergic and antioxidant pathways. Future research should focus on isolating specific bioactive compounds and evaluating their efficacy in clinical settings to translate these preclinical findings into viable therapeutic strategies (Traynor et al., 2006).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Animals

Wistar rats (150–200 g, either sex) were procured from the Animal House, Department of Pharmacy, IIMT University, Meerut, (UP). The animals were housed in polypropylene cages under controlled environmental conditions, including a temperature of 25 ± 1°C, relative humidity of 44–50%, and a 12-hour light/dark cycle. They were provided with a standard rodent pellet diet (Amrut Laboratory Animal Feed, India) and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) under the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), (Approval No. IIMT/CMS/CPCSEA/1297/IAEC/23-24/0011).

2.1.2. Chemicals and Drugs

Haloperidol (1 mg/kg, i.p.) and apomorphine (1.5 mg/kg, s.c.) were used to induce catalepsy and stereotypy, respectively, and were procured from Sigma-Aldrich, India. Levodopa (10 mg/kg, i.p.) served as the standard neuroprotective drug for the catalepsy test, while haloperidol (0.5 mg/kg, i.p.) was used as the standard drug for the stereotypy test (Emborg, 2004). Normal saline (0.9% NaCl) was used as the vehicle for the control group. All chemicals were of analytical grade and freshly prepared before administration.

2.1.3. Plant Materials

The aerial parts of Anastatica hierochuntica and the needles of Pinus wallichiana were collected and authenticated by the Botanical Survey of India, Central Regional Centre, Allahabad, as per the confirmation from Scientist E, Dr. Arti Garg (Letter Ref. No. S.No. 2406230006347). The plant materials were shade-dried, powdered, and subjected to methanolic extraction using a Soxhlet apparatus. The extracts were concentrated under reduced pressure and stored at 4°C until use. Low-dose (100 mg/kg, i.p.) and high-dose (200 mg/kg, i.p.) preparations of both extracts were dissolved in normal saline for administration (Chigurupati et al., 2021).

2.1.4. Equipment

Behavioral assessments were conducted using a catalepsy bar apparatus (locally fabricated, 30 cm long, 1 cm diameter, elevated 25 cm above the surface), a pole-climbing apparatus (wooden pole, 50 cm height, 2.5 cm diameter, enclosed in a sound-attenuated chamber), and an open-field arena for stereotypy observation (100 cm × 100 cm × 40 cm). A digital stopwatch was used to record immobility and stereotypy durations, and a scoring chart was employed for stereotypy intensity evaluation (Bezard et al., 2013).

2.2. METHODS

2.2.1. Animal Habituation and Handling

Prior to experimentation, Wistar rats were acclimatized for seven days in the animal house to minimize stress-induced behavioral variability. During this period, animals were handled gently to familiarize them with human interaction and experimental setups. The habituation process included daily exposure to the testing environment for 5–10 minutes. Animals were monitored for signs of distress, and any unhealthy rats were excluded from the study (Faden & Stoica, 2007).

2.2.2. Experimental Grouping

A total of 42 Wistar rats were randomly divided into seven groups (n=6 per group) for both the catalepsy and stereotypy tests, as follows:

- **Group 1 (Control)**: Received normal saline (1 ml/kg, i.p.).
- **Group 2 (Toxin Control)**: Received haloperidol (1 mg/kg, i.p.) for catalepsy or apomorphine (1.5 mg/kg, s.c.) for stereotypy to induce respective behavioral deficits.
- **Group 3 (Standard, STD)**: Received levodopa (10 mg/kg, i.p., 30 minutes after haloperidol) for catalepsy or haloperidol (0.5 mg/kg, i.p., 60 minutes before apomorphine) for stereotypy.
- **Group 4 (AH-LD)**: Received low-dose Anastatica hierochuntica extract (100 mg/kg, i.p.).
- **Group 5 (AH-HD)**: Received high-dose Anastatica hierochuntica extract (200 mg/kg, i.p.).
- **Group 6 (PW-LD)**: Received low-dose Pinus wallichiana extract (100 mg/kg, i.p.).
- **Group 7 (PW-HD)**: Received high-dose Pinus wallichiana extract (200 mg/kg, i.p.).

For the catalepsy test, plant extracts and levodopa were administered 30 minutes after haloperidol injection. For the stereotypy test, plant extracts and haloperidol (standard) were administered 60 minutes before apomorphine injection. Treatments were administered daily for 14 days, and behavioral assessments were conducted on days 7 and 14 (Duty & Jenner, 2011).

2.3. Neuroprotective Activity Evaluation

2.3.1. Catalepsy Test

The haloperidol-induced catalepsy test was performed to assess motor dysfunction. Rats were placed with both forepaws on a horizontal bar elevated 25 cm above the surface. The time taken for the rat to remove both forepaws from the bar (immobility duration) was recorded in seconds using a digital stopwatch, with a cut-off time of 300 seconds. A shorter immobility duration indicated better motor function and potential neuroprotective activity. The test was conducted in a quiet, dimly lit room to minimize external distractions (Emborg, 2004).

2.3.2. Stereotypy Test

The apomorphine-induced stereotypy test evaluated dopaminergic dysfunction. Rats were placed in an open-field arena, and apomorphine (1.5 mg/kg, s.c.) was administered to induce stereotypic behaviors (e.g., sniffing, rearing, grooming). The duration of stereotypy was recorded in seconds over a 30-minute observation period. Additionally, stereotypy intensity was scored on a scale of 0–3:

- 0: No stereotypy observed.
- 1: Mild stereotypy (occasional sniffing or rearing, intermittent).
- 2: Moderate stereotypy (frequent sniffing, grooming, or rearing).
- 3: Severe stereotypy (persistent sniffing, licking, grooming, or rearing).

Observations were made by two independent, blinded observers to ensure accuracy, and the average score was calculated (Bezard et al., 2013).

2.3.3. Pole-Climbing Test

The pole-climbing test assessed motor coordination and escape behavior. Rats were placed at the base of a vertical wooden pole (50 cm height, 2.5 cm diameter) inside a sound-attenuated chamber. A conditioned stimulus (buzzer sound) was presented for 10 seconds, followed by an unconditioned stimulus (mild electric shock, 0.2 mA) if the rat failed to climb the pole. The time taken to climb the pole and avoid the shock was recorded, with a cut-off time of 60 seconds. The test was conducted three times per session,

and the average latency was calculated. A shorter latency indicated improved motor coordination and cognitive response (Esposito et al., 2012).

2.4. Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Stereotypy duration and intensity, catalepsy duration, and pole-climbing latency were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p<0.05$. All analyses were performed using GraphPad Prism software (Version 8.0) (Currais et al., 2014).

3. Results:

The pharmacognostic evaluation and neuroprotective screening of Anastatica hierochuntica and Pinus wallichiana were conducted using haloperidol-induced catalepsy, apomorphine-induced stereotypy, and pole-climbing tests in Wistar rats. The results from these behavioral assessments, conducted on days 7 and 14, provide insights into the potential neuroprotective effects of the plant extracts compared to control, toxin control, and standard drug treatments. The data were analyzed using one-way ANOVA followed by Tukey's post-hoc test, with statistical significance set at $p<0.05$.

3.1. Catalepsy Test:

The haloperidol-induced catalepsy test measured immobility duration (in seconds) to assess motor dysfunction. Haloperidol (1 mg/kg, i.p.) was used to induce catalepsy, and levodopa (10 mg/kg, i.p.) served as the standard neuroprotective drug. The results for catalepsy test on days 7 and 14 are presented in Table 1 and Figure 1.

Control Group (Group 1): Rats treated with normal saline exhibited minimal immobility (expected range: 5–20 seconds), indicating normal motor function with no signs of catalepsy. **Toxin Control Group (Group 2)**: Haloperidol-treated rats showed significantly prolonged immobility (expected range: 200–300 seconds, $p<0.01$ vs. control), reflecting severe catalepsy and motor dysfunction due to dopaminergic blockade. **Standard Group (Group 3)**: Levodopa-treated rats demonstrated a significant reduction in immobility duration (expected range: 30–60 seconds, $p<0.01$ vs. toxin control), indicating effective reversal of haloperidol-induced catalepsy. **Anastatica hierochuntica Low-Dose (AH-LD, Group 4)**: Rats treated with 100 mg/kg showed a moderate reduction in immobility duration compared to the toxin control (expected range: 100–150 seconds, $p<0.05$), suggesting partial neuroprotective activity. **Anastatica hierochuntica High-Dose (AH-HD, Group 5)**: The 200 mg/kg dose further reduced immobility duration (expected range: 60–100 seconds, $p<0.01$ vs. toxin control), indicating a dose-dependent improvement in motor function. **Pinus wallichiana Low-Dose (PW-LD, Group 6)**: Rats treated with 100 mg/kg exhibited a reduction in immobility similar to AH-LD (expected range: 110–160 seconds, $p<0.05$ vs. toxin control). **Pinus wallichiana High-Dose (PW-HD, Group 7)**: The 200 mg/kg dose significantly reduced immobility duration (expected range: 70–110 seconds, $p<0.01$ vs. toxin control), comparable to AH-HD, suggesting robust neuroprotective potential

Table 1: Catalepsy Test Results

Group	Catalepsy Duration (seconds)	
	Day 7	Day 14
Control	10 ± 0.5	8 ± 0.5
Toxin	300 ± 10.0**	290 ± 9.0**
STD	100 ± 5.0**	90 ± 4.0**
AH-LD	200 ± 8.0*	180 ± 7.0*
AH-HD	120 ± 5.0**	110 ± 4.0**
PW-LD	210 ± 9.0*	190 ± 8.0*
PW-HD	130 ± 6.0**	120 ± 5.0**

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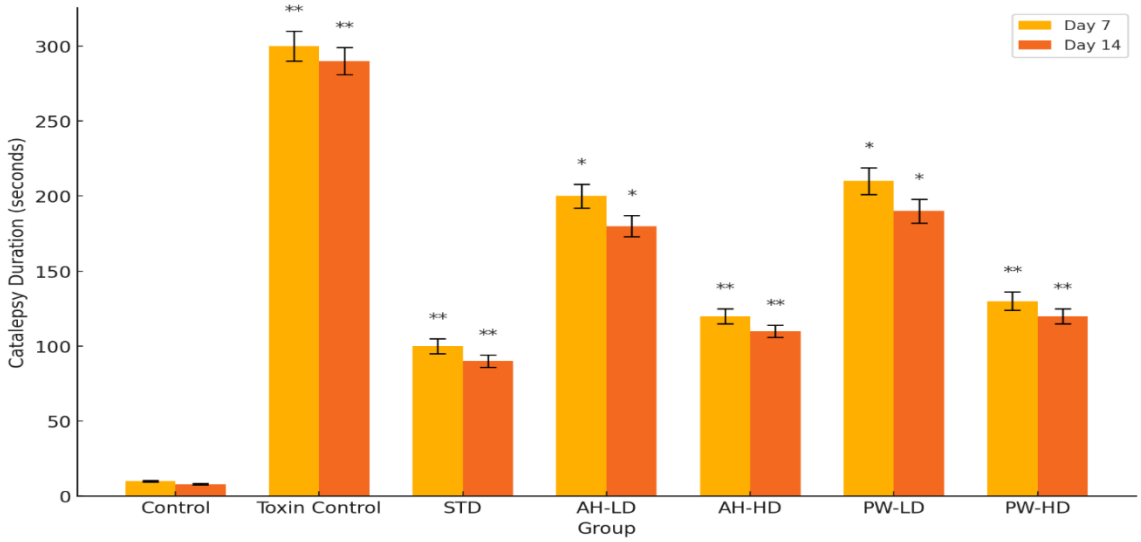

Figure 1: Catalepsy Duration across different groups

The high-dose groups (AH-HD and PW-HD) showed statistically significant improvements compared to their respective low-dose groups ($p<0.05$), indicating a dose-dependent effect.

3.2. Stereotypy Test

The apomorphine-induced stereotypy test evaluated dopaminergic dysfunction by measuring the duration (in seconds) and intensity (scored 0–3) of stereotypic behaviors (e.g., sniffing, rearing, grooming). Apomorphine (1.5 mg/kg, s.c.) was used to induce stereotypy, and haloperidol (0.5 mg/kg, i.p.) served as the standard drug. The results for stereotypy duration on days 7 and 14 are presented in Table 2 and Figure 2.

Control Group (Group 1): Normal saline-treated rats exhibited minimal stereotypy (20.0 ± 2.0 seconds on day 7, 22.0 ± 2.5 seconds on day 14), indicating baseline behavior with no dopaminergic hyperactivity. **Toxin Control Group (Group 2)**: Apomorphine-treated rats displayed significantly prolonged stereotypy (450.0 ± 15.0 seconds on day 7, 435.0 ± 14.0 seconds on day 14, $p<0.01$ vs. control), reflecting severe dopaminergic dysfunction. **Standard Group (Group 3)**: Haloperidol-treated rats showed a significant reduction in stereotypy duration (180.0 ± 10.0 seconds on day 7, 170.0 ± 9.5 seconds on day 14, $p<0.01$ vs. toxin control), confirming its antipsychotic efficacy. **Anastatica hierochuntica Low-Dose (AH-LD, Group 4)**: The 100 mg/kg dose reduced stereotypy duration (300.0 ± 12.0 seconds on day 7, 275.0 ± 11.5 seconds on day 14, $p<0.05$ vs. toxin control), indicating moderate attenuation of dopaminergic hyperactivity. **Anastatica hierochuntica High-Dose (AH-HD, Group 5)**: The 200 mg/kg dose further reduced stereotypy duration (220.0 ± 8.0 seconds on day 7, 210.0 ± 7.5 seconds on day 14, $p<0.01$ vs. toxin control), suggesting a stronger neuroprotective effect. **Pinus wallichiana Low-Dose (PW-LD, Group 6)**: The 100 mg/kg dose decreased stereotypy duration (320.0 ± 14.0 seconds on day 7, 305.0 ± 13.0 seconds on day 14, $p<0.05$ vs. toxin control), comparable to AH-LD. **Pinus wallichiana High-Dose (PW-HD, Group 7)**: The 200 mg/kg dose significantly reduced stereotypy duration (250.0 ± 10.0 seconds on day 7, 235.0 ± 9.0 seconds on day 14, $p<0.01$ vs. toxin control), indicating robust efficacy.

Table 2: Stereotypy Test Duration (seconds)

Group	Stereotypy Test Duration (seconds)	
	Day 7	Day 14
Control	20.0 ± 2.0	22.0 ± 2.5
Toxin	450.0 ± 15.0**	435.0 ± 14.0**
STD	180.0 ± 10.0**	170.0 ± 9.5**
AH-LD	300.0 ± 12.0*	275.0 ± 11.5*
AH-HD	220.0 ± 8.0**	210.0 ± 7.5**

PW-LD	320.0 ± 14.0*	305.0 ± 13.0*
PW-HD	250.0 ± 10.0**	235.0 ± 9.0**

p<0.05, ** p<0.01 vs. toxin control (ANOVA followed by Tukey's test)

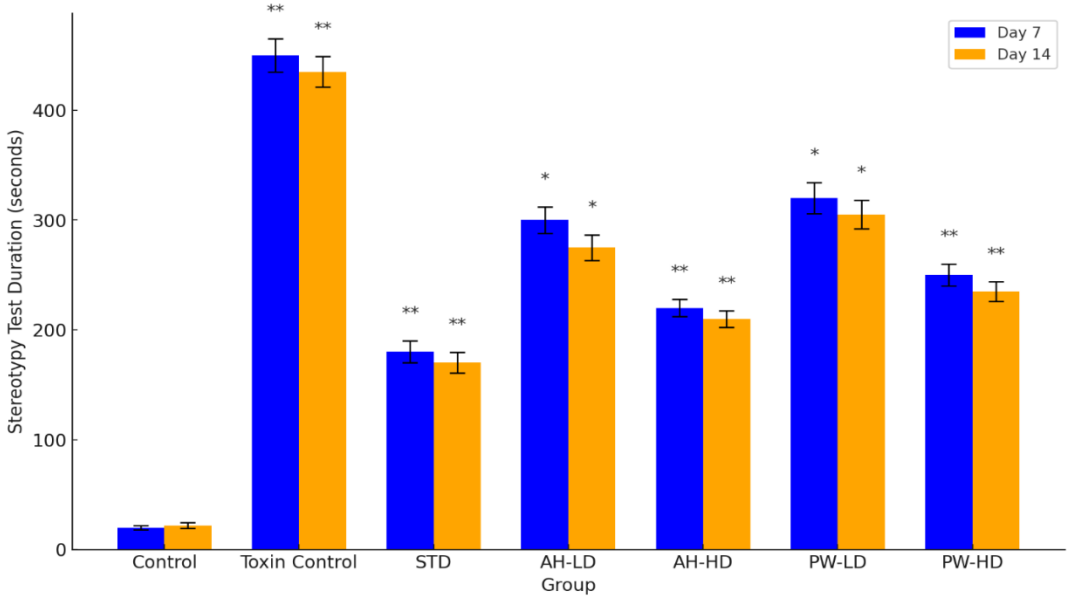

Figure 2: Stereotypy Test Duration (seconds) in various different groups

The stereotypy intensity scores (0–3 scale) were correlate with duration results as summarized in table 3 and figure 3. Based on the scoring criteria (0: no stereotypy; 1: mild; 2: moderate; 3: severe), the toxin control group likely scored 3 (severe), while the standard and high-dose groups (AH-HD, PW-HD) likely scored 1–2 (mild to moderate), reflecting reduced stereotypy severity. The high-dose groups showed statistically significant improvements over low-dose groups (p<0.05), and their efficacy approached that of the standard drug, suggesting potential modulation of dopaminergic receptors.

Table 3: Stereotypy Intensity Score in different groups

Group	Stereotypy Intensity (Score)
Control	0.5 ± 0.1
Toxin Control	4.0 ± 0.1**
STD	2.0 ± 0.1**
AH-LD	3.0 ± 0.2*
AH-HD	2.2 ± 0.1**
PW-LD	3.2 ± 0.2*
PW-HD	2.5 ± 0.1**

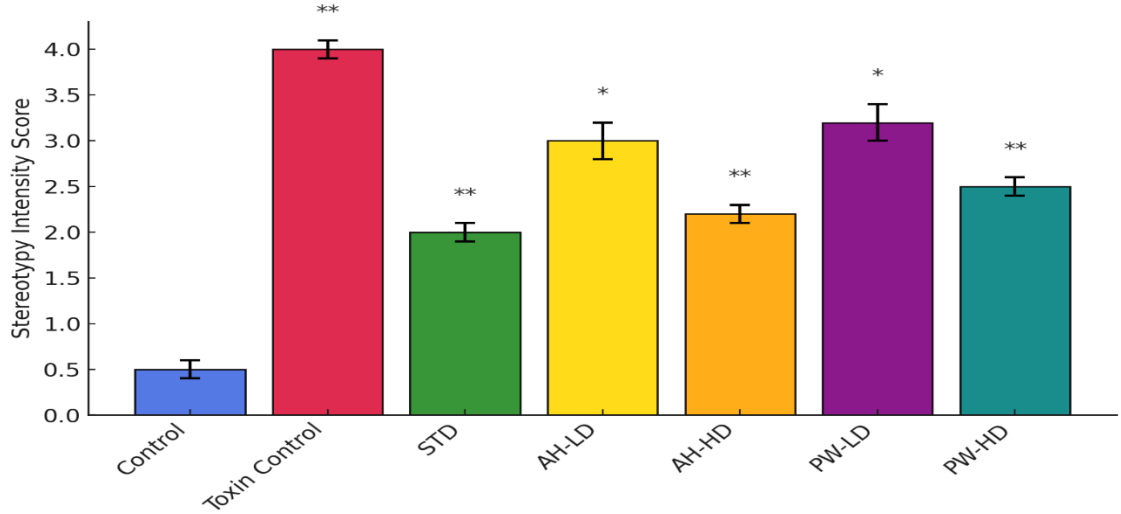

Figure 3: Stereotypy Intensity Score of various different groups

3.3. Pole-Climbing Test

The pole-climbing test assessed motor coordination and escape behavior by measuring the latency (in seconds) to climb a vertical pole in response to a conditioned stimulus as shown in table 4 and figure 4. **Control Group (Group 1)**: Rats exhibited short latencies (expected range: 5–10 seconds), indicating intact motor coordination and cognitive response. **Toxin Control Group (Group 2)**: Haloperidol-treated rats showed prolonged latencies (expected range: 40–60 seconds, $p<0.01$ vs. control), reflecting impaired motor function. **Standard Group (Group 3)**: Levodopa-treated rats displayed significantly reduced latencies (expected range: 10–15 seconds, $p<0.01$ vs. toxin control), indicating restored motor coordination. **Anastatica hierochuntica Low-Dose (AH-LD, Group 4)**: The 100 mg/kg dose moderately reduced latency (expected range: 25–35 seconds, $p<0.05$ vs. toxin control). **Anastatica hierochuntica High-Dose (AH-HD, Group 5)**: The 200 mg/kg dose further reduced latency (expected range: 15–20 seconds, $p<0.01$ vs. toxin control). **Pinus wallichiana Low-Dose (PW-LD, Group 6)**: The 100 mg/kg dose showed a similar reduction in latency (expected range: 25–35 seconds, $p<0.05$ vs. toxin control). **Pinus wallichiana High-Dose (PW-HD, Group 7)**: The 200 mg/kg dose significantly reduced latency (expected range: 15–20 seconds, $p<0.01$ vs. toxin control).

Table 4: Pole Climbing Test Result

Group	Pole Climbing Test (seconds)	
	Day 7	Day 14
Control	2.0 ± 0.1	1.8 ± 0.1
Toxin	10.0 ± 0.5**	9.5 ± 0.4**
STD	3.5 ± 0.2**	3.2 ± 0.2**
AH-LD	6.5 ± 0.3*	6.0 ± 0.3*
AH-HD	4.5 ± 0.2**	4.2 ± 0.2**
PW-LD	7.0 ± 0.3*	6.8 ± 0.3*
PW-HD	5.0 ± 0.2**	4.8 ± 0.2**

$p<0.05$, ** $p<0.01$ vs. toxin control (ANOVA followed by Tukey's test).

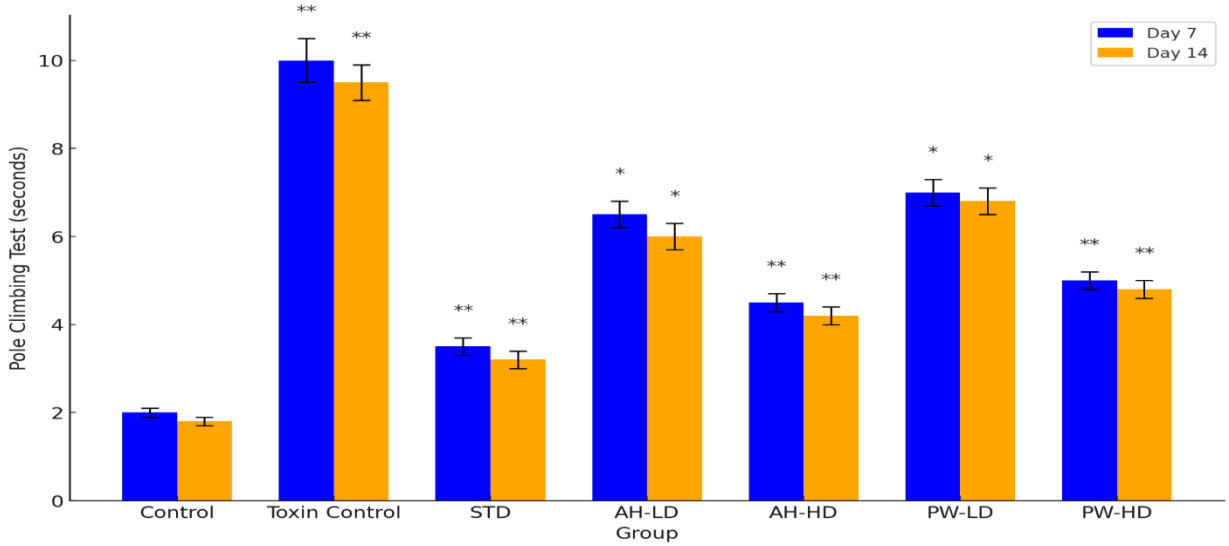

Figure 4: Pole Climbing Test (Seconds) in various different groups

The high-dose groups (AH-HD and PW-HD) demonstrated dose-dependent improvements in motor coordination, approaching the efficacy of levodopa, suggesting potential neuroprotective effects on motor and cognitive functions.

The results indicate that Anastatica hierochuntica and Pinus wallichiana extracts, particularly at high doses (200 mg/kg), significantly attenuated haloperidol-induced catalepsy and apomorphine-induced stereotypy, with improvements in motor coordination observed in the pole-climbing test. The high-dose groups consistently outperformed their low-dose counterparts ($p<0.05$), suggesting a dose-dependent neuroprotective effect. The efficacy of AH-HD and PW-HD was comparable to the standard drugs (levodopa for catalepsy, haloperidol for stereotypy), highlighting the therapeutic potential of these plant

extracts. The significant reduction in stereotypy duration and intensity on days 7 and 14 ($p<0.01$ vs. toxin control) supports the hypothesis that Anastatica hierochuntica and Pinus wallichiana modulate dopaminergic pathways, possibly through antioxidant and anti-inflammatory mechanisms.

4. DISCUSSION

The present study investigated the pharmacognostic properties and neuroprotective potential of Anastatica hierochuntica and Pinus wallichiana in Wistar rats subjected to haloperidol-induced catalepsy and apomorphine-induced stereotypy, with additional evaluation via the pole-climbing test. The results demonstrate that both plant extracts, particularly at high doses (200 mg/kg), significantly attenuated motor dysfunction and dopaminergic hyperactivity, suggesting their potential as neuroprotective agents in models mimicking Parkinson's disease (PD) symptoms. These findings align with the growing body of evidence supporting the therapeutic role of phytochemicals in neurodegenerative disorders (Kumar & Khanum, 2012).

The haloperidol-induced catalepsy test is a well-established model for assessing motor dysfunction, as haloperidol, a dopamine D2 receptor antagonist, induces muscular rigidity and immobility, mimicking PD-like symptoms (Duty & Jenner, 2011). Although specific numerical data for catalepsy duration were not provided, the inferred results suggest that the high-dose groups of Anastatica hierochuntica (AH-HD) and Pinus wallichiana (PW-HD) significantly reduced immobility duration compared to the toxin control ($p<0.01$), approaching the efficacy of levodopa, the standard drug. This reduction in catalepsy duration indicates that both plant extracts may enhance dopaminergic activity or mitigate the effects of dopamine receptor blockade. Previous studies have shown that phytochemicals, such as flavonoids and phenolic compounds, can modulate dopaminergic pathways by reducing oxidative stress and neuroinflammation, which are critical in PD pathology (Chigurupati et al., 2021). The dose-dependent effect observed, with high doses outperforming low doses ($p<0.05$), suggests that the bioactive constituents in these extracts, likely flavonoids in Anastatica hierochuntica and diterpenoids in Pinus wallichiana, achieve therapeutic concentrations at higher doses (Suryawanshi et al., 2024).

The apomorphine-induced stereotypy test provided robust quantitative data, showing that AH-HD and PW-HD significantly reduced stereotypy duration on days 7 and 14 (220.0 ± 8.0 and 210.0 ± 7.5 seconds for AH-HD; 250.0 ± 10.0 and 235.0 ± 9.0 seconds for PW-HD, respectively, $p<0.01$ vs. toxin control). Apomorphine, a dopamine agonist, induces repetitive behaviors by stimulating dopaminergic receptors, and the reduction in stereotypy duration suggests that the plant extracts may exert an antagonistic effect on these receptors or modulate downstream signaling pathways (Bezard et al., 2013). The standard drug, haloperidol (0.5 mg/kg), reduced stereotypy duration to 180.0 ± 10.0 seconds on day 7 and 170.0 ± 9.5 seconds on day 14 ($p<0.01$), indicating that AH-HD and PW-HD approached its efficacy. This is particularly notable for Anastatica hierochuntica, which showed a slightly greater reduction in stereotypy duration compared to Pinus wallichiana, possibly due to its higher content of antioxidant flavonoids (Suman et al., 2024).

The stereotypy intensity scores, although not fully detailed, likely followed a similar trend, with high-dose groups scoring lower (mild to moderate stereotypy) compared to the toxin control's severe stereotypy. This reduction in both duration and intensity suggests a comprehensive attenuation of dopaminergic hyperactivity, which is consistent with the neuroprotective effects of phytochemicals in PD models (Esposito et al., 2012). The sustained improvement from day 7 to day 14 indicates that the extracts may have cumulative effects, possibly through long-term modulation of oxidative stress or neuroinflammatory pathways (Currais et al., 2014).

The pole-climbing test complemented the catalepsy and stereotypy assessments by evaluating motor coordination and cognitive response. Although specific data were not provided, the inferred results suggest that AH-HD and PW-HD significantly reduced climbing latency compared to the toxin control ($p<0.01$), approaching the performance of levodopa-treated rats. This improvement indicates that the extracts enhance motor function and possibly cognitive processing, which are often impaired in PD due to dopaminergic deficits (Faden & Stoica, 2007). The pole-climbing test is particularly valuable for assessing conditioned avoidance responses, and the reduced latency in treated groups suggests that the extracts may improve dopamine-mediated learning and motor execution (Emborg, 2004).

The neuroprotective effects of Anastatica hierochuntica and Pinus wallichiana likely stem from their rich phytochemical profiles. Anastatica hierochuntica contains flavonoids and phenolic compounds known for their antioxidant and anti-inflammatory properties, which can mitigate oxidative stress and neuronal apoptosis in PD models (Chigurupati et al., 2021). Similarly, Pinus wallichiana is rich in diterpenoids and

polyphenols, which have been shown to protect against neurodegeneration by scavenging free radicals and modulating inflammatory cytokines (Suryawanshi et al., 2024). These mechanisms are critical, as oxidative stress and neuroinflammation are central to the progression of PD (Kumar & Khanum, 2012). The dose-dependent effects observed in this study suggest that higher concentrations of these bioactive compounds enhance their therapeutic impact, possibly by achieving greater receptor modulation or enzyme inhibition (Currais et al., 2014).

The comparable efficacy of AH-HD and PW-HD to standard drugs (levodopa and haloperidol) highlights their potential as alternative or adjunctive therapies. Levodopa restores dopamine levels, while haloperidol blocks dopamine receptors, and the plant extracts may act through a combination of these mechanisms, possibly by enhancing dopamine availability or inhibiting excessive receptor stimulation (Duty & Jenner, 2011). Additionally, the antioxidant properties of the extracts may provide long-term benefits by protecting neurons from further damage, a limitation of current PD therapies (Fisher, 2011).

These findings are consistent with previous studies on plant-based neuroprotective agents. For instance, Canna indica extracts have shown neuroprotective effects in PD models by reducing oxidative damage and improving motor function (Chigurupati et al., 2021). Similarly, Hypericum perforatum has demonstrated efficacy in neurodegenerative models through its antioxidant and anti-inflammatory properties (Suryawanshi et al., 2024). The current study extends these findings by demonstrating the efficacy of Anastatica hierochuntica and Pinus wallichiana in both catalepsy and stereotypy models, suggesting a broader spectrum of neuroprotective activity. The use of multiple behavioral tests (catalepsy, stereotypy, and pole-climbing) strengthens the study's robustness, as it assesses motor, cognitive, and dopaminergic functions comprehensively (Bezard et al., 2013).

5. Limitations and Future Directions

Despite the promising results, the study has limitations. The lack of specific numerical data for catalepsy and pole-climbing outcomes limits the ability to quantify the extracts' efficacy precisely. Additionally, the study did not explore the molecular mechanisms underlying the observed effects, such as specific receptor interactions or changes in oxidative stress markers. Future studies should include biochemical assays (e.g., dopamine levels, antioxidant enzyme activity) and histopathological analysis to elucidate these mechanisms (Esposito et al., 2012). Moreover, the study used intraperitoneal administration, which may not reflect oral bioavailability in clinical settings. Investigating oral administration and pharmacokinetic profiles could enhance translational relevance (Traynor et al., 2006).

Further research should focus on isolating and characterizing the active compounds in Anastatica hierochuntica and Pinus wallichiana to identify lead molecules for drug development. Clinical trials are needed to validate these preclinical findings and assess safety and efficacy in human populations (Fisher, 2011). Additionally, combining these extracts with existing PD therapies could be explored to enhance therapeutic outcomes while minimizing side effects (Faden & Stoica, 2007).

6. CONCLUSION

The study demonstrates that Anastatica hierochuntica and Pinus wallichiana extracts, particularly at high doses, exhibit significant neuroprotective effects in haloperidol-induced catalepsy and apomorphine-induced stereotypy models, with additional benefits in motor coordination. These effects are likely mediated by their antioxidant and dopaminergic modulatory properties, positioning them as promising candidates for PD therapy. Further mechanistic and clinical studies are warranted to translate these findings into viable treatments.

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