"Evaluation of antiparkinson activity of methanolic extract of *Hyoscyamus niger L.* seeds & *Curcumin* combination in Swiss albino mice."

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ABSTRACT:

Aim: "Evaluation of antiparkinson activity of methanolic extract of *Hyoscyamus niger L.* seeds & *Curcumin* combination in Swiss albino mice."

Objective: To evaluate the antiparkinson activity of a combination of methanolic extract of *Hyoscyamus niger L.* seeds (MEHN) and *Curcumin* in a haloperidol-induced parkinsonism in Swiss albino mice by behavioral models, biochemical estimation, histopathology of brain tissue, statistical analysis.

Methods: Parkinsonism induced in male Swiss albino mice. Animals were divided into 8 groups (n=6). Group-1 Normal control group received (1% Tween 80, 0.25ml/kg p.o.), Group-2 served as disease control (Haloperidol 1mg/kg i.p.), Group-3 as a standard control (syndopa 10mg/kg p.o.), Group 4 & 5 treated as individual dose (MEHN-500 mg/kg, p.o. & *Curcumin*-200mg/kg, p.o.), Group 6, 7 & 8 treated in combinations (MEHN (30%)-150 mg/kg, p.o. + *Curcumin*(70%)-140 mg/kg, p.o. = 290 mg/kg, p.o.), (MEHN (50%)-250 mg/kg, p.o. + *Curcumin* (50%)-100 mg/kg, p.o.= 350mg/kg p.o.), (MEHN (70%)-350 mg/kg, p.o. + Curcumin(30%)-60 mg/kg, p.o.= 410mg/kg p.o.). Dopamine levels in brain tissue were measured, and histopathological analysis was performed on brain tissue. Phytochemical screening of extracts was conducted. The dose-dependent effects were statistically analyzed using ANOVA followed by Tukey-Kramar multiple comparison test.

Results: The combination of MEHN and *curcumin* demonstrated a significant protective effect against haloperidol-induced motor impairments. The treated group 30:70 combination of MEHN and *curcumin* showed significantly reduced catalepsy scores and improved locomotor activity as compare to disease control group and other individual treatment and combination treatment group. Histopathological studies revealed neuroprotection in the substantia nigra. Neuroprotective effect due to presence of alkaloids (hyoscyamine, hyoscine and scopolamine), flavonoids (rutin, quercetin, and kaempherol), (0.03% of L-DOPA). MEHN reported as to possess dopaminergic, acetylcholine, antioxidant, anti-inflammatory activity. *Curcumin*, the major polyphenolic component from *Curcuma longa* reported as crosses the blood brain barrier and has acetylcholine, anti-oxidant, anti-inflammatory property. *Curcumin* possessed activities for management of PD symptoms, which reduced cataleptic time and increased locomotor activity in treatment group as compared to disease control group. *Curcumin* has decreased intracellular ROS production, and prevented from alpha-synuclein aggregation. *Curcumin* has reported to prevent protein activity from inflammatory cytokines transcription factor NF-alphaB, pro-inflammatory cytokines (TNF-alpha, IL-1B, and IL-1alpha), and increased BDNF-alpha activity. It activates PI3K/AKT pathway in the brain. Several curcumin-associated targets identified in PD, including (HSP90AA1, TP53, MAPK1, AKT1, GSK3B, MAPT, STAT3, and SIRT1). Results strongly support neuroprotective effects through PI3K/AKT activation.

Conclusion: The combination of *Hyoscyamus niger L.* seed extract and *Curcumin* exhibits potent antiparkinsonian activity in experimental mice, with dopaminergic, anticholinergic, antioxidant, anti-inflammatory, and neuroprotective mechanisms of *Hyoscyamus niger L.* and *Curcumin*. The combination shows synergistic activity on PD. Their complementary mechanisms modulation of cholinergic/dopaminergic systems and oxidative stress support their potential as safer alternatives to conventional PD therapies.

Keywords: Anti-parkinsonian activity, MAO-B inhibitor, Antioxidant, Hydroxyl radical, Akinesia, Catalepsy, Haloperidol, L-DOPA, Dopamine.

1. Introduction:

Parkinson's disease (PD) is a chronic degenerative disorder primarily affecting the neurons of the basal ganglia, first described by James Parkinson in 1817 (Sayyaed, et al., 2023). PD is the second-most prevalent neurodegenerative disorder, affecting approximately 11.77 million people worldwide, resulting from significant loss of dopamine-producing neurons in the substantia nigra pars compacta (SNpc) (Luo Y et al., 2025, Putha et al., 2025). The substantia nigra, located in the midbrain, plays a crucial role in movement control and reward processing. It consists of two distinct parts: the pars compacta (SNpc) and the pars reticulata (SNpr). The SNpc sends dopaminergic projections to the striatum, while the SNpr sends GABAergic projections to the thalamus and superior colliculus (Nicola et al., 2000, Al-kuraishy et al., 2024). The neuropathological hallmarks include progressive loss of neuromelanin-containing dopaminergic neurons in the SNpc and the presence of eosinophilic, intracytoplasmic, proteinaceous inclusions termed Lewy bodies (LBs) in surviving neurons (O'Hara et al., 2020, Al-kuraishy et al., 2024). Symptoms include such as tremor, rigidity, bradykinesia, and postural instability (Perren et al. 2020, Tolosa et al. 2021).

Parkinson's disease characterized by a progressive sequence of pathophysiological changes. Lewy body (LB), a pathologic characteristic of dopaminergic neurons, improved in PD, which described as pathophysiological as degradation or dopaminergic neuronal loss located in the SN. Aggregation of LB contains a wide range of proteins including ubiquitin alpha-synuclein and ubiquitin, which impair optimal neuron function. Aging and environmental stress, contribute to neuropathology. Environmental contamination (e.g., pesticides), Cellular aging in neurons in the brain over time is caused by this inflammatory process (Crowley et al. 2019). In PD, the substantia nigra degenerates, destroying the nigrostriatal pathway. The neurochemical basis of PD is the ensuing reduction in striatal dopamine. The impairment in striatal dopaminergic transmission seems to depend on and be sufficient for the emergence of PD motor symptoms. Dopamine is the precursor of levodopa. Individual dopamine does not cross the BBB. Levodopa actively transported into the brain, where levodopa converted into dopamine in the brain. In the periphery of the brain, medication decarboxylated dopamine. Because of that, it requires a large dose of levodopa (Ishiguro et al. 2021). Acetylcholine (ACh) released by cholinergic interneurons in the striatum. In PD, leading to excessive acetylcholine release and interrupting information transfer from motor command centers in the cerebral cortex. α-Synuclein is an unfolded protein that folds into a toxic form, damaging dopaminergic neurons in PD (Aosaki et al., 2010). Reactive oxygen species in PD such as hydroxyl radical (OH•), superoxide anion (O2), and hydrogen peroxide (H2O2) are synthesized because within the mitochondria there is physiological metabolism of molecular oxygen. In ETS (electron transport chain), the mitochondrial complexes I and III produce Superoxide anion which are very reactive and can easily cross the mitochondrial membrane where it is reduced to H2O2. Additionally, various nitric oxide synthases (NOS) create nitric oxide (NO), a transient reactive nitrogen species (RNS), which combines with thiols and reduced glutathione (GSH) to form disulfides, sulfenic, sulfonic, and s-nitrosothiols. Additionally, peroxynitrite (ONOO) can created when oxygen (O2) and nitric oxide (NO) are combined (Hollville et al. 2020). Aggregation of α-synuclein leads to neurodegeneration and influenced by oxidative stress, gene mutations, and overexpression. Mitochondrial dysfunction plays a key role in PD, with reduced mitochondrial complex I activity causing mitochondrial damage and cell death. Mutations in PD-related genes (e.g., Parkin, PINK1, DJ1) impair mitochondrial function, linking mitochondrial damage to dopamine accumulation and lysosomal dysfunction. The brain in PD often appears normal, with mild atrophy of the frontal cortex and occasional ventricular dilation. The main noticeable change is in the brainstem, where nearly all cases show a loss of dark pigmentation in the substantia nigra pars compacta (SNpc) and the locus coeruleus((Aryal and Lee 2019 , Moon et al., 2015) .

Hyoscyamus niger L.: Known as *Khurasani ajwain* and *Black henbane*, belonging to the *Solanaceae* family. The scientist associated with the binomial name of *Hyoscyamus niger L.* is Carl Linnaeus (Naseem et al., 2023). The seeds are rich in tropane alkaloids like hyoscyamine, scopolamine, flavonoids like rutin, quercetin, kaempferol and L-DOPA (Sengupta et al., 2011), L-DOPA crosses the blood-brain barrier and it converted into dopamine, helping relieve Parkinson's symptoms (Sengupta et al., 2011, Khatri et al., 2015). Phytochemical studies have shown that *Hyoscyamus* species contain alkaloids, flavonoids, tannins, terpenes, saponins, carbohydrates, cardiac

glycosides, and anthraquinones. They have several health benefits, including anti-Parkinson, anti-diabetic, antioxidant, anticancer, insecticidal, antiasthmatic, antiallergic, antidiarrheal, antisecretory, and blood pressure-lowering effects. They also protect the heart and liver, help manage high uric acid levels, treat Parkinson's disease, prevent seizures, improve mood, and have anticholinergic effects due to their tropane alkaloids (Al-Snafi et al., 2018).

Curcumin is the principal curcuminoid of the popular Indian spice turmeric, family (*Zingiberaceae*). The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric. (Maheshwari et al ., 2006, Akram et al., 2010). *Curcumin* can help reduce inflammation, prevent atherosclerosis, and assist in post-surgery recovery (Chanda et al., 2019). It has anti-Parkinson's, anti-inflammatory, anti-HIV, antibacterial, antioxidant, and other therapeutic effects. *Curcumin* can neutralize harmful molecules called reactive oxygen species (ROS), including superoxide, hydroxyl radicals, and nitric oxide. It also inhibits enzymes that cause inflammation, such as cyclooxygenase (COX-I and COX-II). *Curcumin* helps protect cells from oxidative damage and prevents harmful substances from binding to DNA (El-Shamarka et al., 2023, Shen et al., 2011, Khatri et al 2016, Sharma et al., 2018, King et al., 2011, Rajeswari et al ., 2008, Mehla et al., 2010, Yang et al., 2014).

2. Material and Methods:

2.1 Collection and validation of the plant materials:

The seeds of *Hyoscyamus niger L.* was purchased from Kirti enterprises, Hadapsar, Pune. Authenticated by Dr. Harshad M. Pandit, Ph.D. (Botany), Azad Nagar Gem CHS Ltd, Mumbai, MS, India. The authenticated specimen numbers assigned as *Hyoscyamus niger L.* – Specimen #: vck 24120732. *Curcumin* purchased from Nacton Biolife Sciences Pvt. Ltd. Noida.

2.2 Preparation of Hyoscyamus niger L. seeds extract:

The *Hyoscyamus niger L.* seeds weighed 400 g and grinded to powdered form. This powdered material macerated with 600 ml of methanol. This mixture kept at room temperature for 4 days. The resulting methanol solution was filtered using Whatman No. 1 filter paper to remove solid residues from the liquid extract. To concentrate the extract, the methanol evaporated using a rotary evaporator at 40°C. Percentage yield obtained to be 4.32 % w/w (Sengupta et al., 2011, Khatri et al., 2015).

2.3 Phytochemical analysis:

Phytochemical analysis of the methanolic extract of seeds of *Hyoscyamus niger (L.)* (MEHN) revealed the presence of secondary metabolites like flavonoid, alkaloids, phenols, Terpenoids glycosides and tannins (Tyagi et al., 2017, Shaikh et al., 2020) .

2.4 Chemicals:

Haloperidol obtained from Divine Laboratories Gujrat, and Syndopa tablet procured from med world pharmacy Kharadi, Pune.

2.5 Animals:

Healthy 96 male Swiss Albino Mice (25-30 grams) purchased from the Crystal Biological solutions, Uralidevachi. Mice was 8 to 10 weeks adults. Animals were kept in polycarbonate cages with the bedding of corncobs that was changed every three days under standard laboratory conditions (27 ± 2 °C) and relative humidity of 45-55 % under 12h light: 12h dark cycle. The mice given free access to water and standard diet pellets ad libitum. The experimental protocol met the National Guideline (CCSEA) and the Animal Ethics Committee of the institute approved animals. Proper care taken to minimize animal suffering.
Animals were randomly divided into eight experimental groups (n=6) as follows;

- Group 1: Mice treated with normal 1% Tween 80 (0.25ml/kg p.o.); this served as normal control group.

- Group 2: Mice were administered intraperitoneal injection of haloperidol (1mg/kg i.p.); this served as disease control group.
- Group 3: Mice were orally treated with standard drug syndopa (10mg/kg p.o.); served as standard control group.
- Group 4: MEHN-500 mg/kg, p.o. served as treatment group.
- Group 5: *Curcumin*-200mg/kg, p.o. served as treatment group .
- Group 6: MEHN (30%)-150 mg/kg, p.o. + *Curcumin* (70%)-140 mg/kg, p.o. = 290 mg/kg, p.o.
- Group 7: MEHN (50%)-250 mg/kg, p.o. + *Curcumin* (50%)-100 mg/kg, p.o. = 350mg/kg p.o.
- Group 8: MEHN (70%)-350 mg/kg, p.o. + *Curcumin* (30%)-60 mg/kg, p.o. = 410mg/kg p.o.

2.6 Induction of Parkinson's disease

Parkinson's disease induced intravenous injection of haloperidol 1mg/kg, for 1 week in alternate days prior the therapeutic treatment (Dovonou et al., 2023, Varty et al., 2008).

2.7 Catalepsy bar test:

Catalepsy is the inability of an animal to correct an externally imposed posture. Catalepsy was measured by placing animal on a flat horizontal surface with both hind limbs placed on a bar which is 4 cm above from the ground surface and measured catalepsy score in sec. cut off time was 5 min (Sengupta et al., 2011).

2.8 Actophotometer test:

Measurement of locomotor activity of mice by using actophotometer. Mice were placed in the actophotometer. A continuous beam of six lights which fall on corresponding photoelectric cells. Photoelectric cell get activated when animal cross the beam light. Cut off time was 5 min. Before locomotor task, animal acclimatize for 2 min. Result recorded and express in terms of total beam counts per 5 min (Gosavi et al., 2020).

2.9 Biochemical parameter

Dopamine level estimation

Whole brain removed, weighed, and cut into fine pieces and homogenized in 5 mL of 5% HCl-butanol and centrifuged at 2000 rpm for 10 minutes. The supernatant collected, mixed with 2.5 mL of heptane and 0.3 mL of 0.1 M HCl, shaken thoroughly, and centrifuged again. The lower aqueous phase collected for dopamine analysis. For estimation, 0.02 mL of this phase was reacted with HCl, sodium acetate buffer (pH 6.9), and iodine solution. The oxidation reaction halted with sodium thiosulphate in 5 M NaOH, followed by the addition of acetic acid and heating to 100°C for 6 minutes. After cooling, fluorescence measured at 330–375 nm using a spectrofluorimeter, and dopamine levels determined by comparing fluorescence with a blank and standard (Chaitra et al., 2016).

2.10 Histopathology of brain

The brain tissue was carefully isolated and immediately fixed in 10% neutral buffered formalin to preserve its structural integrity. Following fixation, the samples were processed and embedded in paraffin wax. Thin sections measuring 3–5 µm in thickness cute using a microtome, with serial sections of 3 µm obtained for detailed histological analysis. The sections stained using the haematoxylin-eosin (H&E) staining method to highlight cellular and tissue morphology. The stained slides examined under a microscope (40x) to assess histopathological changes and structural alterations in the brain tissue (Chaitra et al., 2016, Chandrashekhar et al., 2010).

2.11 Statistical analysis

Statistical analysis of data performed using one way ANOVA of variance followed by Tukey-Kramar multiple

comparison test using Graph pad prism software 10.4.2. Data were expressed as Mean ± SEM (n=6), and difference were considered as statistically significant *p<0.05, **p<0.01, ***p<0.001 when compared with disease treated group ###p<0.001 (Fowles et al., 2013)

3. Result:

3.1 Physical properties:

The methanolic extract of *Hyoscyamus niger L.* (MEHN) appeared dark brown to black in color and exhibited a bitter taste with a characteristic pungent odor. It was found to be soluble in methanol, ethanol, and other organic solvents, but poorly soluble in water. The extract had a pH of 5.5 and yielded 4.32% w/w.
Curcumin was a yellow-orange solid with a slight bitter taste and a distinct aroma. It was poorly soluble in water but freely soluble in 1% Tween 80, ethanol, methanol, acetone, and DMSO. Curcumin had a pH of 4.8 and maintained its yellowish color in solution.

3.2 Phytochemical analysis of Methanolic extract of *Hyoscyamus niger L.* (MEHN):

The MEHN showed the presence of following phytochemicals like alkaloids, phenols, terpenoids, and flavonoids (Table 1)

Table 1: Phytochemical analysis of MEHN

Sr. no	Test name	Results of MEHN
1.	Wagner's test	Alkaloids present
2.	Ferric Chloride test	Phenols present
3.	Salkowski Test	Terpenoids present
4.	Shinoda's Test	Flavonoids present
5.	Borntrager’s Test	Glycosides absent
6.	Braymer’s test	Tannis absent

3.3 Behavioral models: Catalepsy bar and Actophotometer test

3.3.1 Catalepsy bar test:

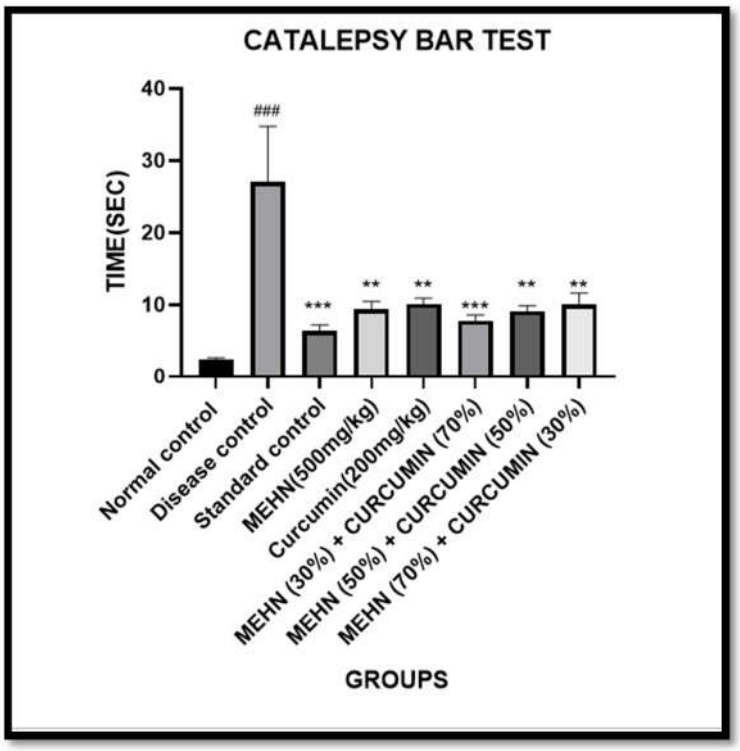


Fig 1: Effect of methanolic extract of *Hyoscyamus niger (L.)* with *curcumin* combination in haloperidol induced catalepsy in mice Values are expressed as Mean ± SEM (n=6). Statistical analysis carried out by One-way ANOVA followed by Tukey-Kramar multiple comparison test *p<0.05, **p<0.01, ***p<0.001 when compared with disease control group, ###P<0.001 when disease control compared with normal control group, MEHN: methanolic extract of *Hyoscyamus niger (L.)*, i.p.: intraperitoneal route, p.o.: oral route.

In Catalepsy bar test the normal control group showed no behavioral changes, while the disease control group exhibited a marked increase in catalepsy from day 7 to 21 (###P<0.001). Standard control group shows significantly reduced catalepsy (, ***p<0.001). MEHN (500 mg/kg) and curcumin (200 mg/kg) also reduced catalepsy individually, but curcumin being slightly more effective (**p<0.01). Among the combinations, MEHN 30% + Curcumin 70% (290 mg/kg) group displaying the most pronounced improvement by day 21, test 3 group showed statistically significant results (***p<0.001) than (###P<0.001 , *p<0.05, **p<0.01) respectively (Fig 1)

3.3.2 Actophotometer test:

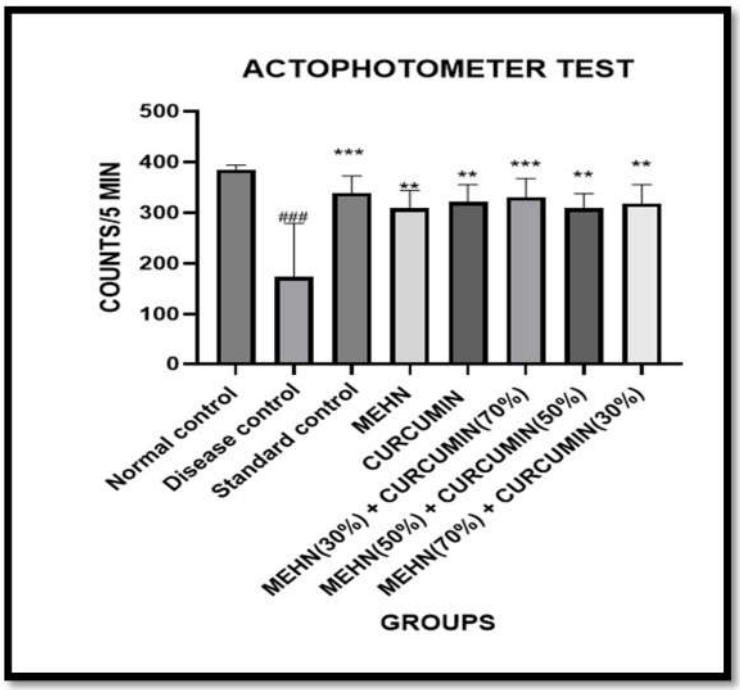


Fig 2: Effect of methanolic extract of *Hyoscyamus niger (L.)* with *curcumin* in haloperidol induced locomotor activity in mice Values are expressed as Mean ± SEM (n=6). Statistical analysis was carried out by One-way ANOVA followed by Tukey-Kramar multiple comparison test *p<0.05, **p<0.01, ***p<0.001 when compared with disease control group, ###P<0.001 when disease control compared with normal control group. MEHN: Methanolic extract of *Hyoscyamus niger (L.)*, i.p.: intraperitoneal route, p.o.: oral route.

In actophotometer test, the normal control group showed no behavioral changes, while the disease control group exhibited reduced locomotor activity over 21 days (###P<0.001). Standard control group significantly improved locomotor counts (***p<0.001). Individually, MEHN (500 mg/kg) and curcumin (200 mg/kg) increased locomotor activity, with curcumin showing a slightly better effect (**p<0.01). MEHN 30% + Curcumin 70% group displaying the most pronounced improvement by day 21, test 3 group showed statistically significant results (***p<0.001) than (###P<0.001 , *p<0.05, **p<0.01) respectively (Fig 2).

3.4 Estimation of dopamine level in brain tissue:

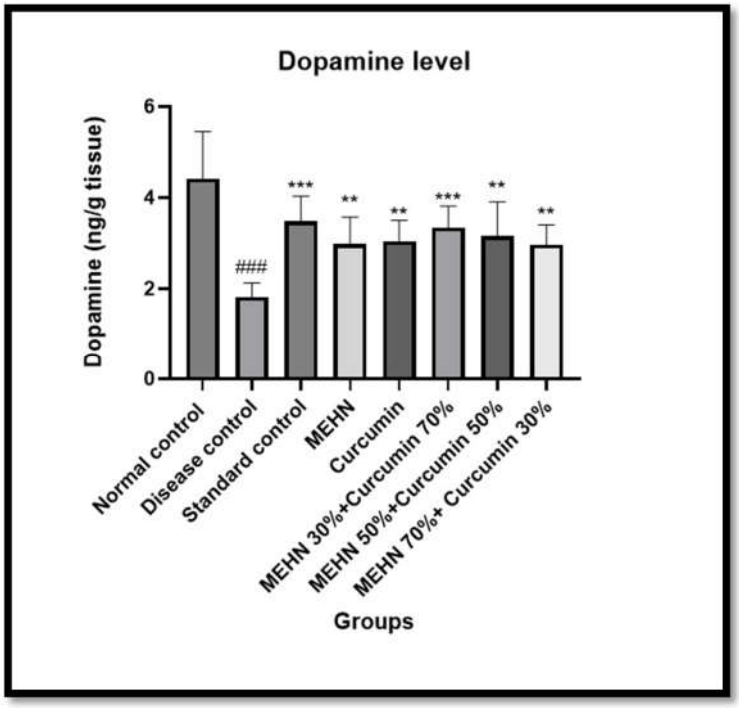


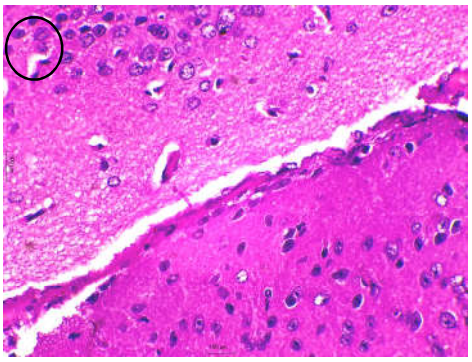
Fig. 3. Estimation of dopamine level in brain tissue by spectrofluorimetry in Swiss albino mice Values are expressed as Mean ± SEM (n=6). Statistical analysis was carried out by One-way ANOVA followed by Tukey-Kramar multiple comparison test *p<0.05, **p<0.01, ***p<0.001 when compared with disease control group, ###P<0.001 when disease control compared with normal control group. MEHN: Methanolic extract of *Hyoscyamus niger (L.)*, i.p.: intraperitoneal route, p.o.: oral route.

The Normal control group showed baseline dopamine level. The Disease control showed a significant reduction to 1.82±0.429 ng/g (###p<0.001 vs Normal control), confirming Parkinsonian induction. The Standard group restored dopamine to 4.65±0.647 ng/g (***p<0.001 vs Disease control). MEHN (500 mg/kg) and *Curcumin* (200 mg/kg) alone yielded 2.86±0.857 and 2.94±0.665 ng/g, respectively, showing moderate neuroprotection. Combination tests showed improved effects, with Test 3 (150 mg/kg MEHN + 140 mg/kg *Curcumin*) achieving the highest dopamine level of 3.68±0.235 ng/g(***p<0.001 vs Disease control), suggesting synergistic and dose-dependent benefits with a higher *Curcumin* ratio. (Fig 3)

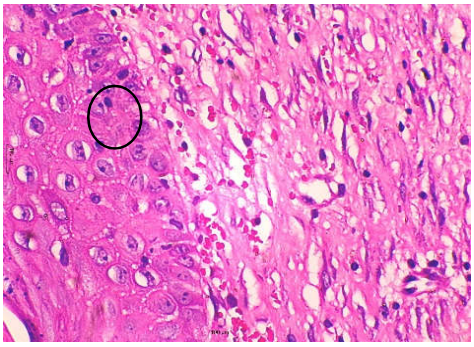
3.5 Histopathology of brain tissue

Histopathological evaluation of brain tissues revealed distinct differences across the experimental groups. The normal control group exhibited normal neuronal morphology without any signs of degeneration. In contrast, the disease control group showed significant neuronal shrinkage, vacuolation, and gliosis, indicating moderate pathology (+++). The standard treatment group displayed mostly preserved neurons with slight vacuolation, reflecting minimal changes (+++). MEHN (Test 1) demonstrated mild neuronal damage (+) with improved architecture compared to the disease control. *Curcumin* alone (Test 2) showed minimal degeneration (++) and better neuronal preservation. Among the combination treatments, Test 3 (MEHN 30% + *Curcumin* 70%) exhibited the best neuroprotection with well-preserved neurons and very few pathological changes (+++). Test 4 (MEHN 50% + *Curcumin* 50%) showed mild histological alterations (++), whereas Test 5 (MEHN 70% + *Curcumin* 30%) presented moderate pathology (++), likely due to a reduced antioxidant contribution from curcumin. Overall, combination therapies especially with a higher proportion of *curcumin* provided enhanced histopathological protection against haloperidol-induced neurotoxicity. (Fig 4)

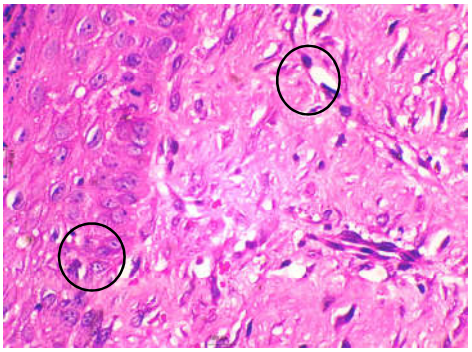
Histopathology of brain tissue (40x):



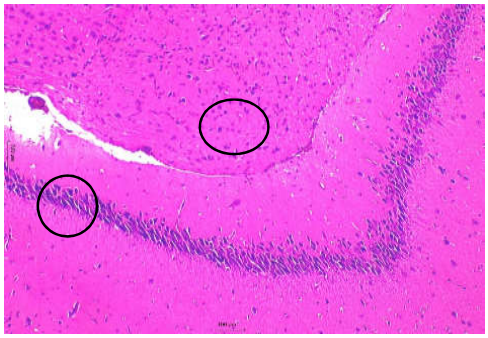
Normal control



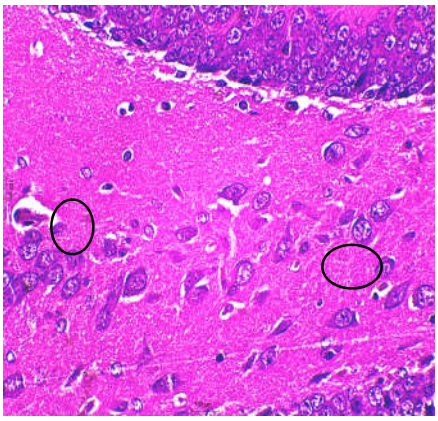
***Curcumin* (Test 2)**



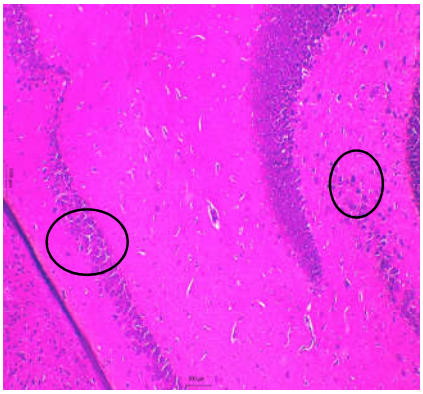
Disease control



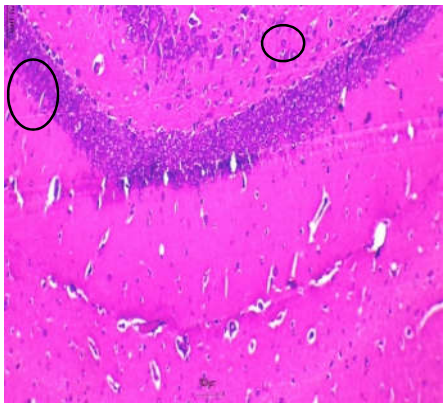
MEHN (30%) + *Curcumin* (70%) (Test 3)



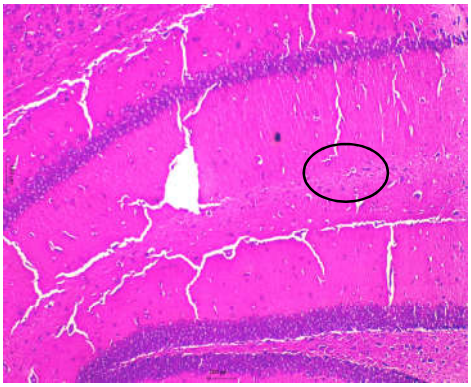
Standard control



MEHN (50%) + *Curcumin* (50%) (Test 4)



***MEHN* (Test 1)**



MEHN (70%) + *Curcumin* (30%) (Test 5)

Fig. 4: Histopathology of brain tissue

Table 2: Observations of histopathology of brain tissue:

Sr. No	Groups	Histopathological Observations	Overall Pathological Grade
1	Normal control	Normal neuronal morphology, no signs of degeneration	0
2	Disease control	Neuronal shrinkage, vacuolation, gliosis	Moderate (+++)
3	Standard control	Mostly preserved neurons, slight vacuolation	Minimal (+)
4	MEHN (Test 1)	Mild neuronal damage, improved architecture vs haloperidol	Mild (++)
5	*Curcumin* (Test 2)	Minimal degeneration, improved neuronal preservation	Minimal (+)
6	MEHN (30%) + *Curcumin* (70%) (Test 3)	Well-preserved neurons, very few pathological changes	Minimal (+)
7	MEHN (50%) + *Curcumin* (50%) (Test 4)	Mild changes, including occasional vacuolated neurons and slight gliosis, better than AEMP alone	Mild (++)
8	MEHN (70%) + *Curcumin* (30%) (Test 5)	Mild-to-moderate vacuolation, early gliosis, and some neuronal loss, especially due to reduced antioxidant defense	Mild (++) to Moderate (+++)
Note: Overall Grade score as- 0 = No Abnormality Detected, Minimal changes (+), Mild changes (++), Moderate changes (+++), Severe changes (++++).			

4. Discussion:

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in a dopamine deficit in the striatum and the manifestation of cardinal motor symptoms such as tremors, rigidity, and bradykinesia (Sayyaed, et al., 2023). The human brain is a very complex organ made up of different regions, each responsible for important body and behavior functions. One of these regions is the basal ganglia, a group of structures deep inside the brain that help control movement, learning habits, and emotions. Inside the basal ganglia is a part called the substantia nigra, especially a section known as the par's compacta, which produces dopamine (Crowley et al. 2019). Dopamine is a crucial messenger in the brain that helps control smooth and coordinated movements by sending signals through a pathway called the nigrostriatal pathway, which helps maintain the balance between different brain circuits that either increase or decrease movement(Maheshwari et al ., 2006, Akram et al., 2010). In PD neurodegeneration cause in SNpc in basal ganglia. Despite the availability of pharmacological interventions like levodopa and dopamine agonists, these treatments are primarily symptomatic and are often associated with significant long-term complications, including dyskinesia and motor fluctuations (Perren et al. 2020, Tolosa et al. 2021). Consequently, there is a pressing need for alternative therapeutic strategies that can address both the symptoms and underlying neurodegenerative processes of PD.

The neuroprotective and antiparkinsonian effects of a methanolic extract of *Hyoscyamus niger L*. (Khurasani Ajwain, Black henbane) seeds in combination with *curcumin*, bioactive compound of *Curcuma longa* (turmeric), were evaluated in an experimental model of haloperidol-induced Parkinsonism. The rationale for this combination lies in their complementary mechanisms: *Hyoscyamus niger* possesses anticholinergic, antioxidant, and dopaminergic modulatory properties, while curcumin exhibits potent antioxidant, anti-inflammatory, and mitochondrial protective effects (Sengupta et al., 2011, Khatri et al., 2015). The *Hyoscyamus niger L* modulating dopaminergic neurotransmission and reducing oxidative stress. Its seeds contain tropane alkaloids such as hyoscyamine and scopolamine, and little amount of L-Dopa. Which restore the balance between acetylcholine and dopamine in the striatum, thereby ameliorating Parkinsonian symptoms. *Hyoscyamus niger L.* demonstrated significant inhibition of mitochondrial monoamine oxidase-B (MAO-B), along with a reduction in hydroxyl radical formation and lipid peroxidation, suggesting a robust neuroprotective profile (Sengupta et al., 2011, Khatri et al., 2015). *Curcumin* extensively documented to exert neuroprotective effects in several PD models, including those induced by 6-hydroxydopamine, MPTP, rotenone, and lipopolysaccharides. *Curcumin* exerts its effect through multiple mechanisms, including suppression of pro-inflammatory cytokines, inhibition of α-synuclein aggregation, and protection of mitochondrial function. It also scavenges reactive oxygen species (ROS), modulates apoptotic signaling pathways, and upregulates neurotrophic factors such as brain-derived neurotrophic factor (BDNF), contributing to neuronal survival. Additionally, it influences molecular pathways such as Nrf2/ARE and NF-κB, contributing to cellular protection in neurodegenerative conditions(El-Shamarka et al., 2023, Shen et al., 2011, Khatri et al 2016, Sharma et al., 2018, King et al., 2011, Rajeswari et al ., 2008, Mehla et al., 2010, Yang et al., 2014).

Haloperidol, a typical antipsychotic, disrupting dopamine transmission in the striatum and causing muscle rigidity and catalepsy [39], used to treat schizophrenia and psychiatric disorders. Haloperidol works by binding to postsynaptic dopaminergic D2 receptors in the brain. These receptors are involved in the transmission of dopamine, a neurotransmitter that plays a key role in mood, behavior, and cognitive function. By blocking these receptors, haloperidol reduce the effects of dopamine, noradrenaline, and serotonin in the striatum (Dovonou et al., 2023, Varty et al., 2008).

The methanolic extract of *Hyoscyamus niger L.* (MEHN) appeared dark brown to black in color and exhibited a bitter taste with a characteristic pungent odor. It found to be soluble in methanol, ethanol, and other organic solvents, but poorly soluble in water. The extract had a pH of 5.5 and yielded 4.32% w/w. In comparison, curcumin was a yellow-orange solid with a slight bitter taste and a distinct aroma. It was poorly soluble in water but freely soluble in 1% Tween 80, ethanol, methanol, acetone, and DMSO. Curcumin had a pH of 4.8. Phytochemical analysis of the methanolic extract of seeds of *Hyoscyamus niger (L.)* (MEHN) revealed the

presence of several key secondary metabolites. Flavonoids detected using the Shinoda's test, indicating the presence of flavonoids known for their antioxidant and anti-inflammatory properties. *Hyoscyamus niger (L.)* seed contain rutin, quercetin, kaempherol flavonoids. The Wagner's test confirmed the presence of alkaloids such as hyoscyamine, scopolamine that are pharmacologically active constituents often associated with neuroactive effects. Phenols identified using the ferric chloride test, suggesting potential antioxidant activity in the extract. *H. niger* seed contain gallic acid phenol. Terpenoids also found to be present, as shown by the Salkowski test (Tyagi et al., 2017, Shaikh et al., 2020).

The study assessed the effect of methanolic extract of *Hyoscyamus niger* (MEHN) with curcumin on haloperidol-induced catalepsy in mice using the bar test over 21 days. Disease control group significantly increased catalepsy time (44.5±0.37 sec), indicating Parkinsonian symptoms. Standard control group effectively reduced catalepsy to 4.8±0.67 sec by day 21 (***$p < 0.001$). MEHN (500 mg/kg) and curcumin (200 mg/kg) alone also showed reductions, to 7.6±0.74 and 8.6±0.41 sec respectively. Combination therapies had stronger effects: the best result was from Test 3 (150 mg/kg MEHN + 140 mg/kg curcumin), reducing catalepsy to 5.7±0.63 sec (***$p < 0.001$), close to Standard control group effect. Higher combination doses (Test 4 and 5) also showed significant improvement, confirming synergistic neuroprotective effects.

Effect of methanolic extract of *Hyoscyamus niger (L.)* with *curcumin* combination in haloperidol-induced locomotor activity in mice, indicated how various treatments influence motor function over time in a model of Parkinson's disease. The test uses locomotor activity counts (per 5 minutes) as an index for general motor function, where lower counts reflect reduced activity typically associated with Parkinsonian symptoms.

Locomotor activity assessed over 21 days. The normal control group maintained high locomotor activity (~394 to 386 counts), serving as a baseline. The disease control group showed a marked decline (301.8 to 56.42 counts), confirming Parkinson-like symptoms. Standard control group significantly restored activity (298.2 to 378.4 counts). MEHN alone (200 mg/kg) improved activity (276.8 to 358.5 counts), suggesting neuroprotection. Curcumin alone (200 mg/kg) also maintained high activity, indicating protective effects. The best result came from the combination in Test 3 (150 mg/kg MEHN + 140 mg/kg curcumin), with activity rising from 289.4 to 375.4 counts by day 21, showing strong synergistic effects.

Methanolic extract of seeds of *Hyoscyamus niger L.* showed reduced cataleptic time in experimental mice because MEHN reported to possess dopaminergic, anticholinergic, antioxidant properties. *Hyoscyamus niger L.* seeds has 0.03% of L-DOPA content which was confirmed by HPLC and the presence of L-DOPA responsible for dopaminergic activity (Sengupta et al., 2011, Khatri et al., 2015). Hyoscyamine and hyoscine are two major tropane alkaloids showed anticholinergic property which reduced acetylcholine level by blocking muscarinic receptor in the striatum, capacity to increased level of dopamine and have neuroprotective activity, also possesses Ca+ channel blocking property, MEHN has a strong MAO-B inhibitory property. MEHN restored the activity of antioxidant enzymes (SOD, GPX, and CAT) GSH level, decreased lipid peroxidation level (MDA level), and decreased the production of intracellular H_2O_2. *Curcumin*, the major polyphenolic compound from *Curcuma longa* is gaining wide interest as a potential remedy for neurodegenerative disorders because of its ani-oxidant and anti-inflammatory properties. In this study found that, *Curcumin* was most effective for reduction in cataleptic time and managing the symptoms of PD. *Curcumin* reported to possess activities for management of PD symptoms. It is inhibit active astrogliosis, stabilized the altered level of glutathione homeostasis. *Curcumin* decreased intracellular ROS production, reduced oxidative damage, *Curcumin* mitigated enhanced acetylcholine esterase level, alter alpha-synuclein aggregation. *Curcumin* prevent protein activity from inflammatory cytokines transcription factor NF-alphaB, pro-inflammatory cytokines (TNF-alpha, IL-1B, and IL-1alpha), and increased BDNF-alpha activity. Curcumin prevented from alpha-synuclein aggregation (El-Shamarka et al., 2023, Shen et al., 2011, Khatri et al 2016, Sharma et al., 2018, King et al., 2011, Rajeswari et al ., 2008, Mehla et al., 2010, Yang et al., 2014). *Curcumin* reported as it activates PI3K/AKT pathway in the brain. Associated targets identified in PD, including HSP90AA1, TP53, MAPK1, AKT1, GSK3B, MAPT, STAT3, and SIRT1. Gene oncology (GO)

enrichment analysis revealed key biological processes such as regulation of apoptosis, lipopolysaccharide response, nitric oxide biosynthesis, aging, and PI3K signaling. Oxidative stress, inflammation, and neuronal apoptosis are central to PD pathogenesis, the modulation of these processes by curcumin via the PI3K/AKT. The down-regulation of genes like Cyp3a44 and Fmo3 and up-regulation of protective genes such as Reg3b reinforce curcumin's role in maintaining intestinal barrier function and reducing systemic inflammation. *Curcumin* reduced inflammation by activates of PI3K/AKT signaling pathway. Results strongly support curcumin's neuroprotective effects through PI3K/AKT activation (Cai B et al., 2024).

In this study evaluated brain dopamine levels and histological changes in haloperidol-induced Parkinsonian mice to assess the neuroprotective effects of *Hyoscyamus niger L.* (MEHN) and *curcumin*, individually and in combination. Biochemical analysis revealed that normal control mice exhibited the highest dopamine levels (4.41 ± 0.679 ng/g), serving as the baseline for healthy brain function. In contrast, the disease control showed a significant decline in dopamine levels (1.81 ± 0.429 ng/g), confirming the drug's neurotoxic effect. A standard antiparkinsonian drug, significantly restored dopamine levels to 3.47 ± 0.647 ng/g. MEHN (500 mg/kg) and curcumin (200 mg/kg), when administered individually, produced a moderate increase in dopamine levels. However, the most notable improvement observed with the combination of MEHN (150 mg/kg) and curcumin (140 mg/kg), which elevated dopamine to 3.34 ± 0.235 ng/g, indicating a strong synergistic effect.

Histological examination supported these findings. Brain tissues from the normal control group showed healthy neuronal morphology (grade 0), while the disease control group displayed substantial neurodegenerative changes, such as neuronal shrinkage and gliosis, graded as Moderate (+++). Standard control group resulted in well-preserved neurons (+++). MEHN and curcumin alone provided mild to minimal protection. Notably, the 30:70 MEHN-curcumin combination (Test 3) demonstrated the significant histological outcome, with well-preserved neurons and minimal pathological changes.

5. Conclusion: The combination of *Hyoscyamus niger L.* seed extract and *curcumin* exhibits potent antiparkinsonian activity in experimental mice, likely through antioxidant, anti-inflammatory, and neuroprotective mechanisms. The combination shows synergistic activity on PD. This combination may serve as a promising adjuvant or alternative in the management of Parkinson's disease. The combined use of MEHN and curcumin provides a promising, multi-targeted phototherapeutic strategy for PD. Their complementary mechanisms modulation of cholinergic/dopaminergic systems and oxidative stress support their potential as safer alternatives or adjuncts to conventional PD therapies.

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