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Neuroprotective potential of Trimyristin and Dihydroberberine in Parkinson's disease rat models

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ABSTRACT:
Objective: The present investigation aimed to assess the neuroprotective potential of trimyristin and dihydroberberine in Parkinson's disease (PD) rat models. **Methods:** Parkinson's disease (PD) was induced by administration of reserpine (1 mg/kg, s.c., every other day for 3 days) and haloperidol (0.5 mg/kg, i.p. for 7 days) in experimental animals. The treatment groups administered orally trimyristin (10, 30, and 100 mg/kg, p.o.) and dihydroberberine (25, 50, and 100 mg/kg, p.o.), with levodopa–carbidopa as the standard reference drug. Behavioral performance such as orofacial dyskinesia test, catalepsy**,** motor coordination, exploratory and curiosity behaviour, locomotor activity were assessed. Biochemical investigations included the estimation of antioxidant parameters, dopamine and nitrite levels. Furthermore, histopathological examination of brain tissues was conducted to assess neuronal integrity and neuroprotective effects. To support for the dopaminergic effects observed in the in vivo study, molecular docking analysis was performed using the dopamine receptor (PDB ID: 6CM4) as the target protein by utilizing PyRx software. **Results:** Treatment with trimyristin and dihydroberberine significantly improvement in behavioral parameters. Biochemical estimations revealed significantly restored antioxidant enzyme activities, dopamine and nitrite levels in rat brain. Histopathological observations demonstrated improved neuronal integrity in treated groups. Molecular docking studies further supported potential high binding affinity with dopamine receptor. **Conclusion:** The results demonstrate that trimyristin and dihydroberberine possess notable neuroprotective potential, likely by enhancing endogenous antioxidant, restoring dopaminergic neurotransmission and suppressing neuroinflammation, These findings suggest, trimyristin and dihydroberberine could serve as a complementary therapeutic approach that targets oxidative and neurodegenerative mechanisms in PD.

INTRODUCTION:
Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, characterized by progressive loss of dopaminergic neurons and the manifestation of motor impairments[1]. Parkinson's disease (PD) is an extrapyramidal neurological disorder characterized by selective loss of dopaminergic neurons in the substantia

nigra pars compacta (SNpc) and the accumulation of α-synuclein-containing cytoplasmic inclusions, known as Lewy bodies. Parkinson's disease affects approximately 1–3% of the global population aged over 60 years. Several factors have been implicated in its pathogenesis, including neuroinflammation, oxidative stress, free radical accumulation, head trauma, and exposure to environmental toxins. The disease is characterized by both motor and non-motor manifestations, with common symptoms including bradykinesia (slowness of movement), muscular rigidity, resting tremors, postural instability, impaired gait, reduced ability to walk independently, and behavioral disturbances. These symptoms stem from the degeneration of the nigrostriatal dopaminergic pathway, leading to decreased DA levels in the striatum. Levodopa is the most widely used pharmacological agent for the management of Parkinson's disease because it can penetrate the blood–brain barrier and be metabolized into dopamine, thereby restoring dopaminergic neurotransmission and improving motor function[2.] Despite significant advances in elucidating the pathophysiological mechanisms underlying Parkinson's disease (PD), current therapeutic approaches remain predominantly symptomatic. However, their efficacy is limited to symptomatic management, with no significant impact on disease progression. Most available treatments are designed to restore dopaminergic neurotransmission; however, their long-term clinical efficacy is often compromised by declining therapeutic responsiveness and the development of motor complications. Despite their symptomatic efficacy, these interventions do not sufficiently address the multifaceted pathological pathways involved in disease progression, including oxidative stress, mitochondrial dysfunction, neuroinflammation, and progressive neurodegeneration. Although several medications are available, their long-term use is limited by adverse effects that hinder treatment adherence. Currently there is no potent therapeutic approach available for the treatment of neurodegenerative diseases[3.] As a result, natural compounds are being explored for their neuroprotective potential[4.]

Dihydroberberine (D), a natural occurring isoquinoline alkaloid with various bioactivities has demonstrated improved bioavailability and pharmacological activity, including anti-inflammatory and protective effects in metabolic disorders[5.] Trimyristin, a triglyceride isolated from Myristica fragrans (nutmeg), has been reported to be the most effective intervention, exhibiting potent therapeutic and neuroregenerative effects in SA-induced neurotoxic rats [6,7.]

This investigation aimed to elucidate the potential neuroprotective properties of trimyristin (T) and dihydroberberine (D) in mitigating the neuropathological and mnemonic impairments in Parkinson's disease.

MATERIALS AND METHODS:

Animals for Experimentation

Healthy Wistar rats of both sexes (body weight: 200–220 g) were obtained from LACSMI Bio-Farms (Pune, India). Wistar rats were acclimatized in standard polypropylene cages under strictly regulated environmental parameters, including an ambient temperature of 27± 3ºC relative humidity maintained between 50% and 70%, and an automated 12-hour light/dark cycle. The animals maintained unrestricted (*ad libitum*) access to a standardized pellet rodent diet and purified water. The study design was sanctioned by the Institutional Animal Ethics Committee of the Institution (Protocol Approval No. IAEC/2025/02).

Material

Trimyristin and Dihydroberberine were obtained from Yucca Enterprises. Analytical reagents were sourced from standard suppliers. L-DOPA/carbidopa was used as the standard drug for comparison.

Molecular Interaction Analysis (In Silico Study)

To provide mechanistic support for the dopaminergic effects observed in the in vivo study, molecular docking analysis was performed using the dopamine receptor (PDB ID: 6CM4) as the target protein[8.] Biovia Discovery Studio Visualizer was used to create the receptor's three-dimensional structure. Water molecules, heteroatoms, and co-crystallized ligands were eliminated during preparation. H-atoms were then assigned to the protein, followed by structural optimization to refine the binding pocket architecture for optimal docking compatibility.

The PubChem database provided the ligand structures of dopamine and Selegiline, which were then energy minimized to provide stable conformations. Using AutoDock Vina as the docking engine, PyRx software was used to do molecular docking simulations. The generated protein and ligand structures were transformed into pdbqt format, and the known binding area was used to determine the receptor's active binding site.

Docking simulations were executed using the Vina wizard to generate multiple ligand conformations within the

receptor binding pocket. Based on stability of their interaction patterns and binding affinity scores (measured in kcal/mol), the optimal docking postures were chosen. Characterization of the ligand–receptor complexes using Discovery Studio Visualizer identified key spatial interactions, including hydrogen bonds, hydrophobic forces, and π-π configurations, with localized amino acid residues within the binding domain.

Toxicity study
Trimyristin and dihydroberberine acute oral toxicity was assessed using OECD guideline 425 (Up-and-Down approach), a commonly used technique for figuring out the median lethal dosage (LD_{50}) and evaluating test compounds' safety profiles.

Experimental Design
The animals under experimentation were differentiated into nine groups at random, with six rats in each group. (n = 6), to evaluate the neuroprotective effects of Trimyristin and Dihydroberberine for the treatment of Parkinson's disease. Experimental Parkinsonism was established through subcutaneous injections of Reserpine (1 mg/kg) given every other day over a 3-day period and daily intraperitoneal injections of Haloperidol (0.5 mg/kg) for 7 days in rats. Groups studied were:

Group I- vehicle,
Group II- Disease Control Reserpine (1 mg/kg, s.c., every other day for 3 days), or Haloperidol (0.5 mg/kg, i.p. for 7 days).
Group III- Trimyristin (10 mg/kg, p.o.) and Reserpine (1 mg/kg, s.c., every other day for 3 days), or Haloperidol (0.5 mg/kg, i.p. for 7 days).
Group IV- Trimyristin (30 mg/kg, p.o.) and Reserpine (1 mg/kg, s.c., every other day for 3 days), or Haloperidol (0.5 mg/kg, i.p. for 7 days).
Group V- Trimyristin (100 mg/kg, p.o.) and reserpine (1 mg/ kg, s.c., every other day for 3 days), or haloperidol (0.5 mg/kg, i.p. for 7 days).
Group VI- Dihydroberberine (25 mg/kg,p.o.) and Reserpine (1 mg/kg, s.c., every other day for 3 days), or Haloperidol (0.5 mg/kg, i.p. for 7 days).
Group VII- Dihydroberberine (50 mg/kg, p.o.) and Reserpine (1 mg/kg, s.c., every other day for 3 days), or Haloperidol (0.5 mg/kg, i.p. for 7 days).
Group VIII- Dihydroberberine (100 mg/kg, p.o) and Reserpine (1 mg/kg, s.c., every other day for 3 days), or Haloperidol (0.5 mg/kg, i.p. for 7 days).
Group IX- Standard L-dopa + Carbidopa (30 mg/kg, p.o.) and Reserpine (1 mg/kg, s.c., every other day for 3 days), or Haloperidol (0.5 mg/kg, i.p. for 7 days).

Behavioral parameters were evaluated following completion of the treatment period. After completion of the final evaluation, brain tissues were isolated and processed to prepare homogenates for biochemical analysis of catalase, malondialdehyde, superoxide dismutase, glutathione, dopamine and nitrite as well as for histopathology.

Reserpine-induced Parkinson Disease (orofacial dyskinesia)
As an antihypertensive and antipsychotic agent, reserpine causes depletion of central catecholamine reserves. In rats, Reserpine administration produces motor deficits such as dyskinesia, muscular rigidity, tremors, and akinesia-like immobility[9]. Behavioral assessment was examined 1h after the last reserpine injection.

Haloperidol-induced Catalepsy
This model is widely used to screen compounds with potential anti-Parkinsonian activity and the ability to reduce antipsychotic-induced extrapyramidal side effects. Catalepsy was assessed 30 min after haloperidol administration (0.5 mg/kg, i.p.) using the bar test. Animals were placed with their forelimbs on a horizontal bar positioned 6 cm above the surface and their hind limbs on the platform, and the duration of the maintained posture was recorded over a 5-min period[10].

Evaluation Parameters:
Behavioral Assessment
Effect of Trimyristin (T) and Dihydroberberine (D) on orofacial dyskinesia test (vacuous chewing movement and on Tongue protrusions), catalepsy, motor coordination, exploratory and curiosity behaviour, locomotor activity (number of square crossings and number of rearing) was measured[11].

Dissection and homogenization:
The animals were killed using CO_2 euthanasia chamber on the final day, one hour after all behavioral evaluations, and the brains were removed. The divided brains were weighed after being cleaned with isotonic saline solution. It was done using a 10% (w/v) brain tissue homogenate was prepared in ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 1000 × g for 20 min at 4°C to obtain the post-nuclear fraction for catalase assay. Subsequently, the homogenate was further centrifuged at the required speed for 60 min at 4°C, and the supernatant was used for biochemical estimations to obtain the post nuclear fraction for use in additional enzyme experiments. For following tests, an Elico™BL 200 bio spectrophotometer was employed.

II) Biochemical evaluation:
Biochemical analyses were performed 24 h after the last reserpine treatment. After completion of the behavioral studies, the animals were sacrificed and tissues were collected for further evaluation.

Antioxidant evaluation:
Estimation of catalase:
The Luck (1971) method was used to assess catalase activity. H_2O_2 breakdown is assessed using this technique at 240 nm. The necessary assay mixture contained 3 ml of 0.01 M H_2O_2- phosphate buffer (pH 7) and 0.05 ml of tissue homogenate supernatant (10% w/v). At 240 nm, the change in absorbance was seen after 1 minute. In order to determine enzyme activity, the millimolar extinction coefficient of H_2O_2 (0.071) was utilized. The results were represented as milligrams of protein per minute of H_2O_2 decomposition.[12]

Estimation of malondialdehyde (Lipid peroxidation assay):
The method developed by Wills (1966) was employed to quantify lipid peroxidation levels in brain tissue quantitatively. By reacting with thiobarbituric acid at 532 nm, this method evaluated the amount of malondialdehyde (MDA) produced. 1.5 ml of 20% acetic acid, 0.1 ml of tissue homogenate, 0.2 ml of 8% sodium lauryl sulphate (SLS), and 1.5 ml of a 0.8% thiobarbituric acid (TBA) solution make up the reaction mixture. Then, this combination was cooked for one hour on a water bath at 95^0C. It was then given a 15:1 n-butanol and pyridine.

mixture in the amount of 5 ml. After a vigorous shaking, the mixture was centrifuged for 5 min. The organic layer's upper layer's absorbance was measured at 532 nm. The molar extension coefficient of the chromophore was used to convert the values into nM of MDA per milligrams of protein ($1.56x10^5M^{-1}cm^{-1}$)[13]

Estimation of superoxide dismutase (SOD):
The activity of superoxide dismutase was tested using Kono method, which involved inhibiting the reduction of nitrobluetetrazolium chloride (NBT) and measuring it at 560 nm spectrophotometrically. The assay was performed by adding 0.1 mL of 1 mM hydroxylamine hydrochloride to a reaction mixture containing EDTA (0.1 mM), NBT (24 µM), Triton X-100 (0.03% v/v), and the post-nuclear fraction of brain homogenate. Following incubation at 37°C for 20 min, absorbance was measured at 560 nm, and the results were expressed as the percentage inhibition of NBT reduction[14].

Estimation of reduced glutathione:
Reduced glutathione (GSH) levels were estimated using Ellman's method[15]. Briefly, 0.75 mL of tissue homogenate was mixed with an equal volume (0.75 mL) of 4% sulfosalicylic acid to precipitate proteins. The mixture was centrifuged at 4°C for 15 min, and the resulting supernatant was collected for analysis. The assay mixture consisted of 0.5 mL of supernatant, 0.4 mL of 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and 0.1 M phosphate buffer (pH 8.0). The yellow-colored chromogen formed was measured immediately at 412 nm. GSH levels were expressed as µM GSH per mg protein.

Neurochemical analysis:
Estimation of dopamine:
Brain dopamine levels were determined spectrophotometrically following treatment with the test compounds, administered either alone or in combination with amphetamine. Briefly, 1 mL of the tissue homogenate supernatant was mixed with 1 mL of ferric chloride solution (1.5×10^{-2} M) and 1 mL of potassium ferricyanide solution (1.5×10^{-2} M), followed by dilution with distilled water to a final volume of 25 mL. The reaction mixture was allowed to stand for 30 min to facilitate color development. Absorbance was then measured at 735 nm using a UV–visible double-beam spectrophotometer. Dopamine concentration was determined based on the intensity of

the developed color[16]

Estimation of nitrite:
Nitric oxide (NO) production was assessed indirectly by measuring nitrite accumulation in the striatal supernatant using the Griess colorimetric method. Briefly, equal volumes of the supernatant and Griess reagent, comprising 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid, were mixed and incubated at room temperature in the dark for 10 min. Following incubation, absorbance was recorded at 540 nm using a UV–visible spectrophotometer. Nitrite concentrations were calculated from a standard calibration curve and expressed relative to the vehicle-treated control group[17.]

Histopathological analysis:
The rat brains were examined histopathological. Following euthanasia, the brains were immediately excised and fixed in 10% buffered formalin. The brain, which had been alcohol- dehydrated and embedded in paraffin, was sectioned to reveal the hippocampus. Serial sections of 5 µm thickness were prepared from paraffin-embedded tissues using a microtome and subjected to hematoxylin and eosin staining for histological examination. Digital microscope (Olympus, a Japanese company), was used to analyses the sections under a light microscope while taking photomicrographs.

Statistical analysis
Data were expressed as mean ± SEM (n = 6). Statistical significance was determined using one-way ANOVA followed by Dunnett's test. Statistical significance was denoted as ****p<0.0001*** p <0.001, ** p <0.01, * p <0.05 versus the Diseased control group and ## p <0.01, ### p <0.001, #### p <0.0001 versus the vehicle group.

RESULTS:

Molecular Interaction Analysis
To complement the dopaminergic mechanism found in this research, molecular interaction images of standard with the dopamine receptor (PDB ID: 6CM4) were analyzed. Dopamine and Selegiline in Parkinsons disease exhibited robust binding interactions with the receptor's active site, supporting their role in enhancing dopaminergic neurotransmission.

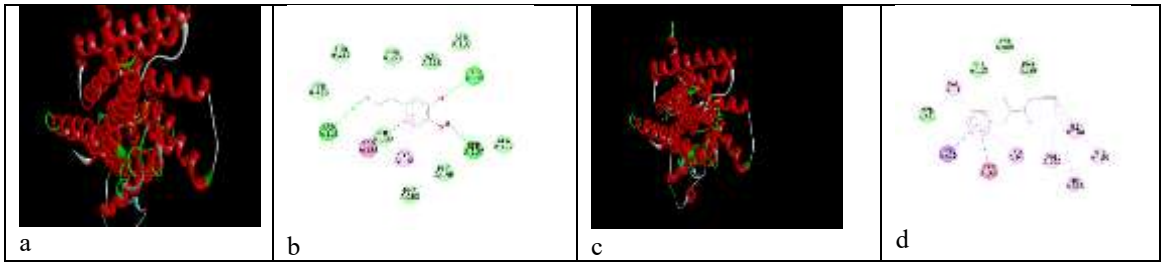

Figure 1. Molecular interactions visualization of; dopamine (a) 3D and (b) 2D; Selegiline with Dopamine receptor
(c) 3D and (d) 2D

Figure 1. Illustrated the molecular interactions between dopamine and Selegiline with the amino acid residues of Dopamine receptor.
The molecular docking results revealed that standard dopamine (Figure 1), (--6.6kcal/mol) and Selegiline (--5.8kcal/mol)

The favorable docking scores and stable ligand–receptor conformations suggest strong and effective interactions within the enzyme's active site. Thus, the *in-silico* analysis complements the experimental outcomes and strengthens the evidence supporting the neuroprotective effects observed in the study. Additionally, the comparative binding efficiency of standard drugs offers a valuable reference for assessing the potential activity of test compounds such as Trimyristin and Dihydroberberine. The molecular docking study demonstrated that both trimyristin and dihydroberberine exhibited binding affinity toward the dopamine receptor (PDB ID: 6CM4). Trimyristin showed a binding energy score of −6.1 kcal/mol, whereas dihydroberberine exhibited a stronger binding affinity with a binding energy score of −9.0 kcal/mol, indicating favorable interactions with the receptor's active site. These favorable docking scores, along with stable ligand–receptor conformations, suggest a high affinity for the enzyme's active site. Molecular interaction findings for Dihydroberberine (2D & 3D) (Fig.)

and Molecular interaction findings for Trimyristin (2D & 3D) (Fig.)

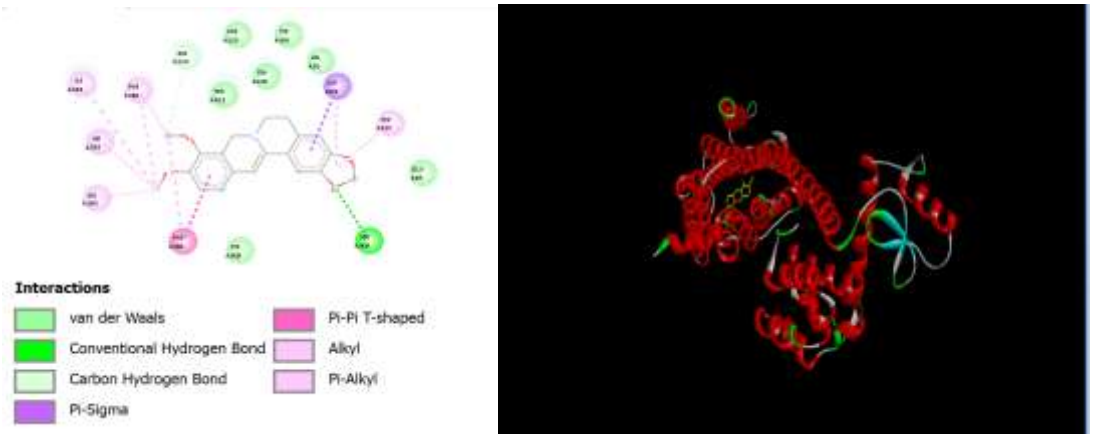

Figure 2. Molecular interaction visualization of Dihydroberberine (2D & 3D)

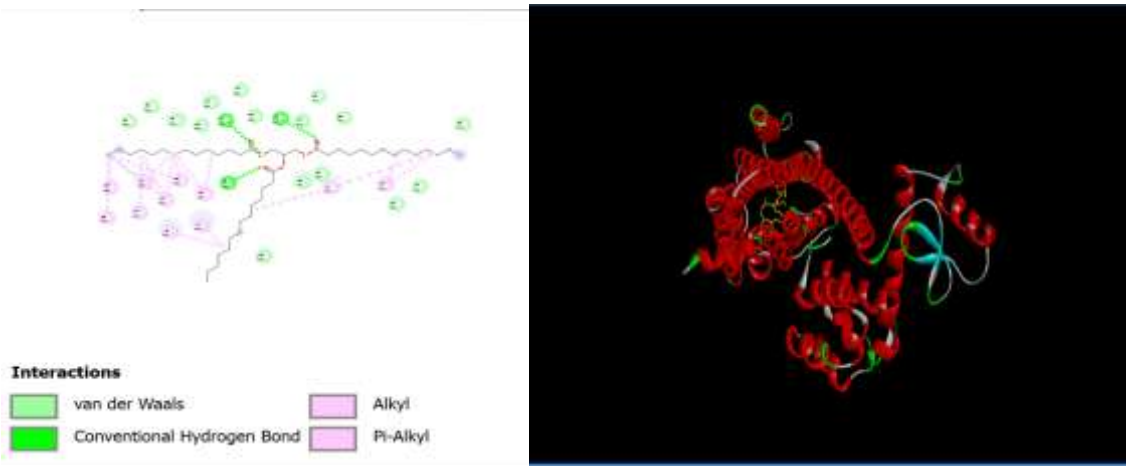

Figure 3. Molecular interaction visualization of Trimyristin (2D & 3D)

Acute Toxicity

The acute oral toxicity study was performed in compliance with OECD Guideline 425 (Up-and-Down Procedure) revealed that both Trimyristin and Dihydroberberine were harmless at 2000 mg/kg. During the 14-day observation period, there were no deaths or obvious indicators of toxicity, suggesting that the test chemicals had a satisfactory safety profile.

Reserpine-induced Parkinson Disease
Behavioral Assessment
Orofacial dyskinesia test
I) Vacuous chewing movements:

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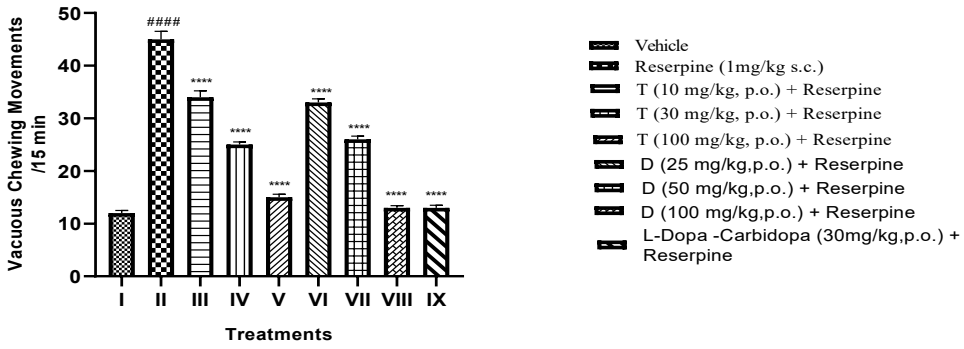

Figure 4. Effect of T & D on vacuous chewing movements by reserpine induced PD

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$

Vacuous chewing movement was increased significantly ($p< 0.0001$) in reserpine treated group as compared to vehicle treated group. Administration of Trimyristin and Dihydroberberine decreased number of vacuous chewing movement significantly ($p< 0.0001$) as compared with Reserpine treated group. L-dopa-carbidopa (30 mg/kg) also decreased number of vacuous chewing movement significantly ($p< 0.0001$) in animals.

II) Tongue protrusion frequency:

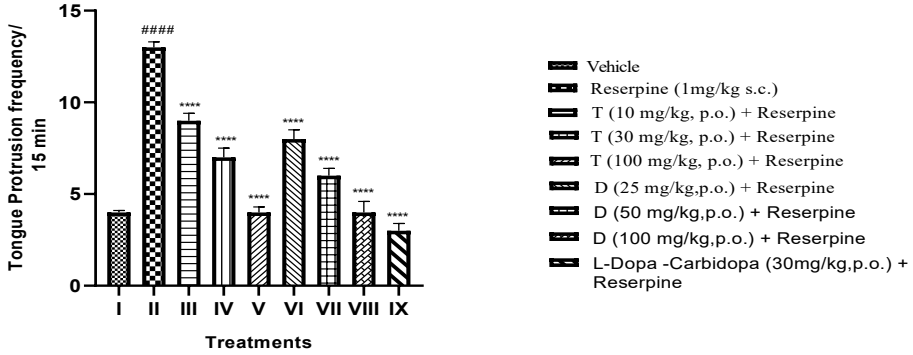

Figure 5a. Elevated Plus maze test open arm
Figure 5. Effect of T & D on tongue protrusion frequency by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

Significant increase in number of tongue protrusion was observed ($p< 0.0001$) in Reserpine treated group as compared to vehicle group. Administration of Trimyristin and Dihydroberberine decreased number of tongue protrusion significantly ($p< 0.0001$) as compared to Reserpine treated group. L-dopa-carbidopa (30 mg/kg) also decreased number of tongue protrusion significantly ($p< 0.0001$) in animals.

B) Catalepsy:

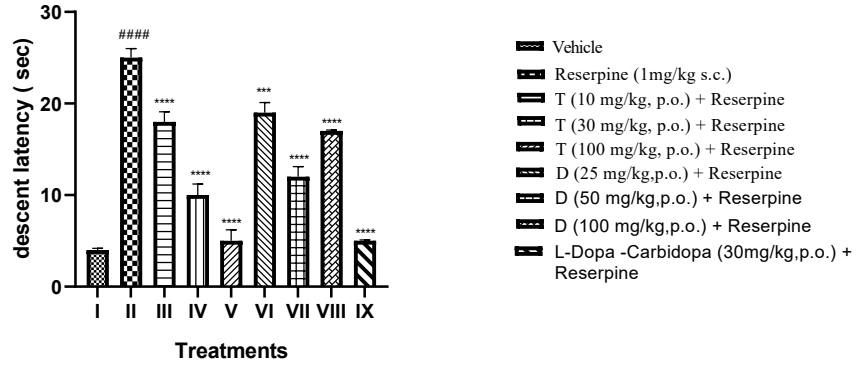

Figure 6. Effect of T & D on descent latency by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$
Reserpine (1 mg per kg) treated animal show increased in catalepsy significantly ($p<0.0001$) compared to the vehicle control group ($p<0.0001$) indicating PD induction. Treatment with Trimyristin and Dihydroberberine in combination with Reserpine significantly ($p<0.0001$) decreased in cataleptic behavior signified by less time spend by rats in a dose-dependent manner. Similarly, L-dopa-carbidopa (30 mg/kg) co-administration with Reserpine reduced catalepsy significantly compared to the Reserpine-treated group ($p<0.0001$).

C) Motor coordination (Rota rod test)

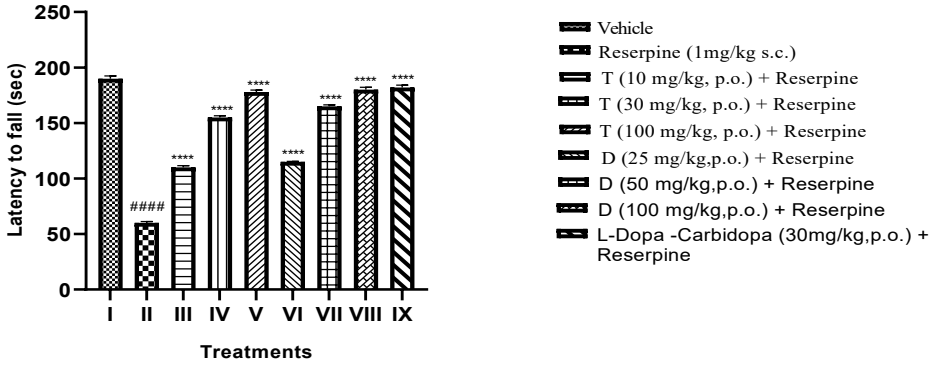

Figure 7. Effect of T & D on latency to fall by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

Reserpine treated group result in a decreased in falling latency significantly as compared to the vehicle group ($p < 0.0001$) due to reduced muscle coordination. Administration of Trimyristin and Dihydroberberine significantly ($p < 0.0001$) improved number of falling latency in a dose dependent manner as compared to Reserpine treated group improving fall of latency and muscle grip function. L-dopa-carbidopa (30 mg/kg) showed significant ($p < 0.0001$) increased as compared with Reserpine treated animals.

D) Exploratory and curiosity behaviour (Hole and board test):

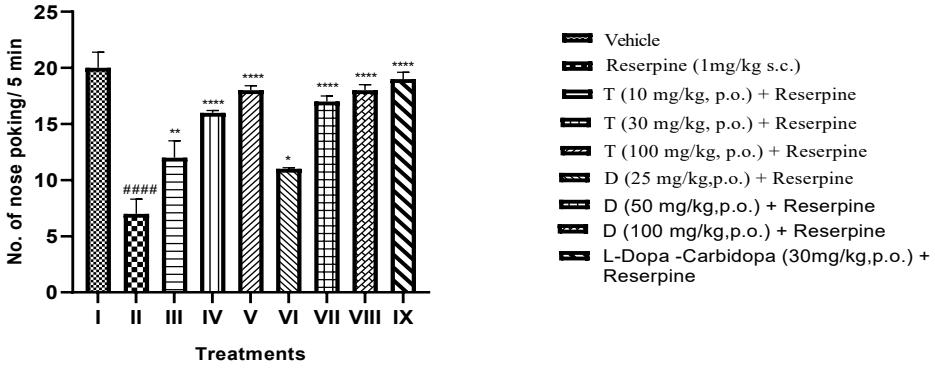

Figure 8. Effect of T & D on no. of nose pocking by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$
Reserpine treated group result in a decreased in the number of nose pokings significantly as compared to the vehicle control (p< 0.0001)). Administration of Trimyristin and Dihydroberberine increased number of nose pokings significantly (p< 0.0001) as compared with Reserpine treated group. L-dopa-carbidopa (30 mg/kg) showed significant (p< 0.0001) increased number of nose pokings as compared with Reserpine treated animals.

E) Locomotor activity (Open field test):
i) no. of square crossings:

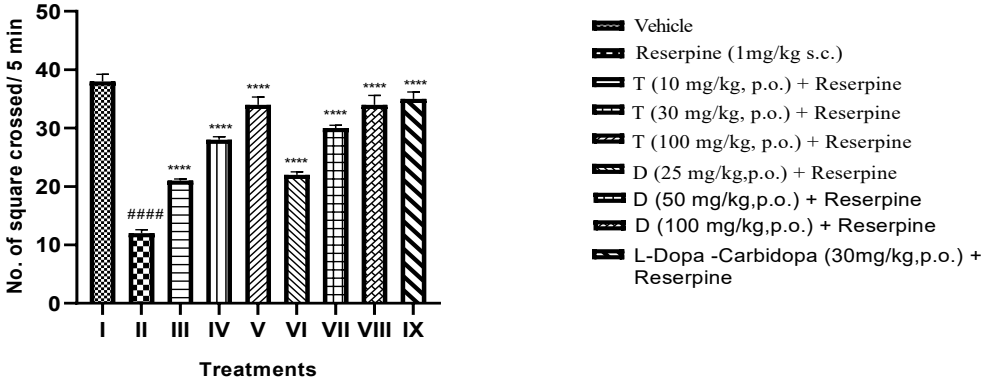

Figure 9. Effect of T & D on no. of square crossed by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

Reserpine treated group resulted in a decreased in the number of square crossings significantly as compared to the vehicle control (p< 0.0001) Administration of Trimyristin and Dihydroberberine significantly (p < 0.0001) increased number of square crossings as compared with Reserpine treated group. L-dopa-carbidopa (30 mg/kg) showed increased number square crossings significantly (p < 0.0001) as compared to Reserpine treated animals.

ii) no. of rearing:

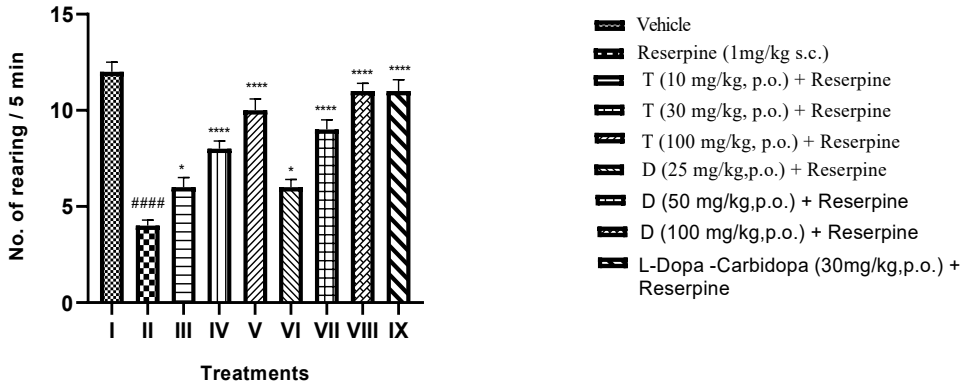

Figure 10. Effect of T & D on no. of rearing by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$
Significant ($p < 0.0001$) reduction in number of rearing was observed in Reserpine treated group as compared to vehicle group. Administration of Trimyristin and Dihydroberberine significantly ($p < 0.0001$) increased number of rearing as compared with Reserpine treated group. L-dopa-carbidopa (30 mg/kg) also significantly ($p < 0.0001$) increased number of rearing in animals.

II) Biochemical evaluation:

- **Antioxidant evaluation:**

A) CAT:

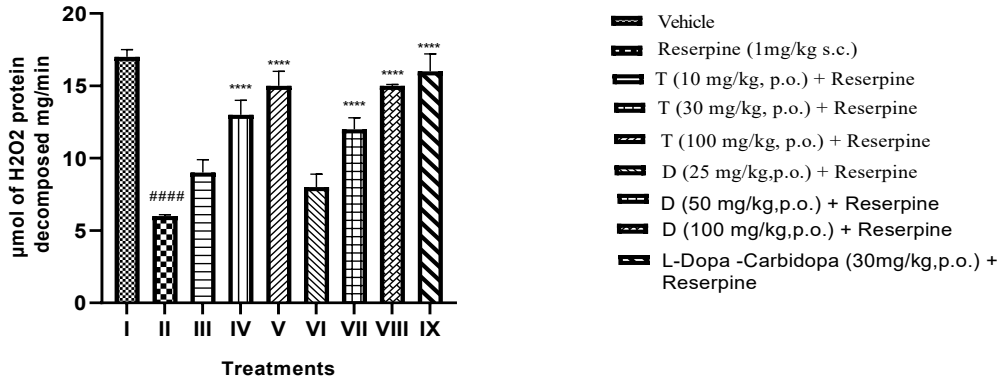

Figure 11. Effect of T & D on catalase level by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$. ns – Non significant
Reserpine induced group showed decreased in CAT levels significantly ($p < 0.0001$) as compared to the control group. Trimyristin and Dihydroberberine ($p < 0.0001$) showed significant increase in CAT levels as compared with the induced group in rat brain. L-dopa-carbidopa (30 mg/kg) group showed significant ($p < 0.0001$) increased in CAT level.

B) LPO:

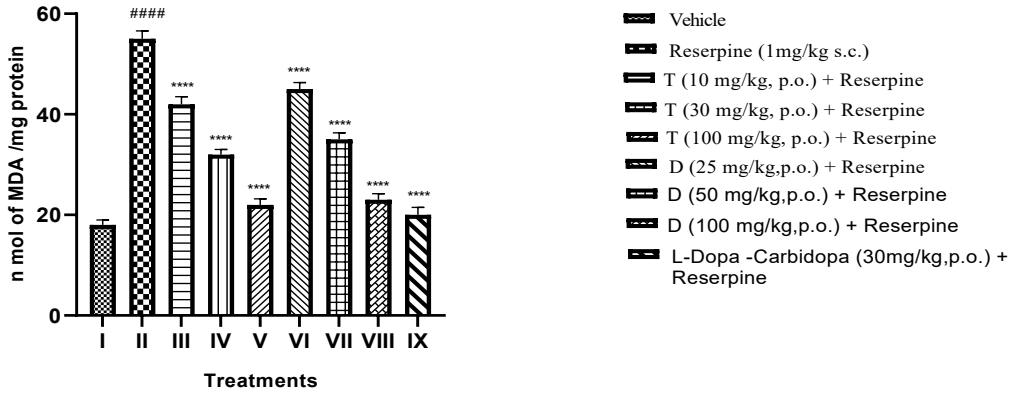

Figure 12. Effect of T & D on lipid peroxidation level by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$.
MDA levels were significantly ($p < 0.0001$) increased in the Reserpine-treated group compared with the vehicle group; while administration of Trimyristin and Dihydroberberine lower levels of LPO significantly ($p < 0.0001$) as compared to Reserpine treated group. L-dopa-carbidopa (30 mg/kg) also reduced the levels of LPO significantly ($p < 0.0001$)

C) GSH:

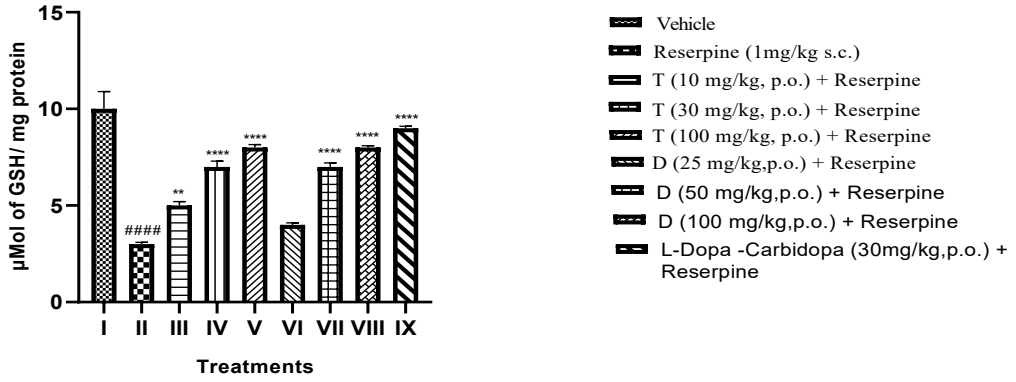

Figure 13. Effect of T & D on Glutathione level by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$. ns – Non significant
GSH levels were significantly reduced ($p < 0.0001$) in the Reserpine-treated group compared with the vehicle group. Administration of Trimyristin and Dihydroberberine showed significant ($p < 0.0001$) rise in levels of GSH as compared with Reserpine treated rats. L-dopa-carbidopa (30 mg/kg) also showed significant ($p < 0.0001$) increase in GSH levels as compared with Reserpine treated group ($p < 0.0001$)

D) SOD:

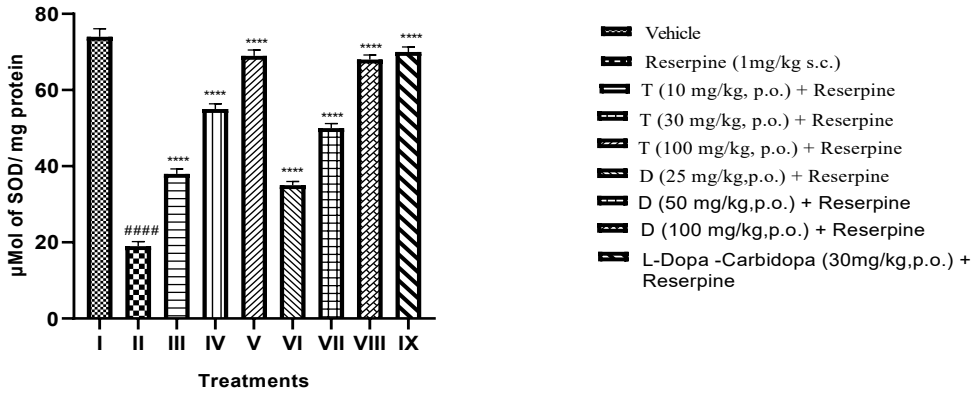

Figure 14. Effect of T & D on superoxide dismutase level by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$
A highly significant reduction ($p < 0.0001$) in SOD levels was observed in the Reserpine-treated group compared with the vehicle-treated group representing neurodegeneration induction in rats. Administration of Trimyristin and Dihydroberberine ($p < 0.0001$) showed significant rise in levels of SOD as compared with Reserpine treated rats. L-dopa-carbidopa (30 mg/kg) also showed increase in SOD levels significantly ($p < 0.0001$) as compared Reserpine treated group ($p < 0.0001$).

Neurochemical analysis:
Dopamine:

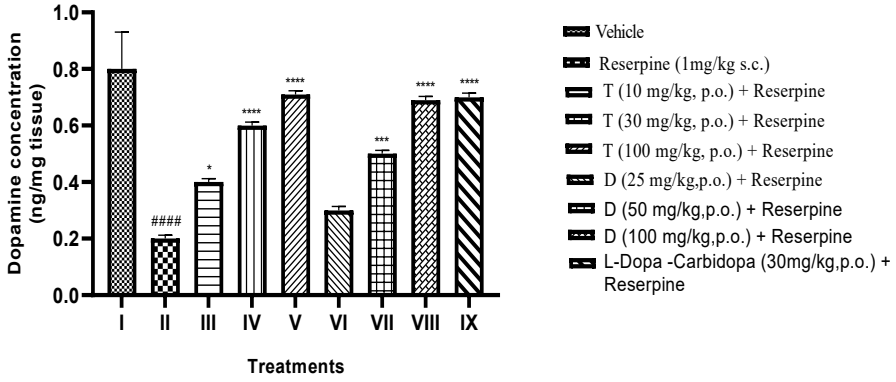

Figure 15. Effect of T & D on dopamine level by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$. ns – Non significant
A highly significant reduction ($p < 0.0001$) in dopamine levels was observed in the Reserpine-treated group compared with the vehicle-treated group. Administration of Trimyristin and Dihydroberberine showed significant dose dependent rise in levels of dopamine as compared with Reserpine treated rats. L-dopa-carbidopa (30 mg/kg) also showed increase in dopamine levels significantly ($p < 0.0001$) as compared with Reserpine treated group ($p < 0.0001$).

NO:

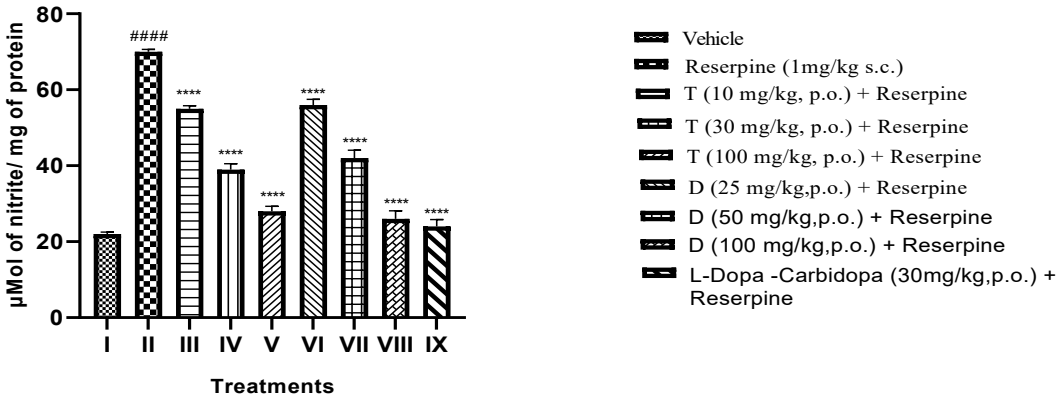

Figure 16. Effect of T & D on nitrite level by reserpine induced PD.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

A significant increase in NO levels was observed in the Reserpine-treated group compared with the vehicle-treated group. While administration of Trimyristin and Dihydroberberine of significantly ($p < 0.0001$) lower levels of NO as compared with Reserpine treated rats. L-dopa-carbidopa (30 mg/kg) also reduced significantly ($p < 0.0001$) the levels of NO.

Histopathological analysis:

I) Vehicle control: normal neuronal cells with prominent nucleus.
II) Reserpine (1 mg/kg, s.c.) induced: more evidence of pyknotic nuclei, vacuolated cytoplasm (Vc) and severe loss of dopaminergic cells.
III) Trimyristin (10 mg/kg, p.o.) + Reserpine (1 mg/kg, s.c.) group indicates less evidence of pyknotic nuclei and neuronal degeneration.
IV) Trimyristin (30 mg/kg, p.o.) + Reserpine (1 mg/kg, s.c.) & vacuolated cytoplasm and pyknotic nuclei was less noticed.
V) Trimyristin (100 mg/kg, p.o.) + Reserpine (1 mg/kg, s.c.) very less pyknotic nuclei and greater number of prominent nuclei neurons indicating less brain tissue damage.
VI) L-dopa-carbidopa (30 mg /kg, p.o.) + Reserpine (1 mg/kg, s.c.) showed optimal sized cells with defined nucleus.
VII)Dihydroberberine (25 mg/kg p.o.) + Reserpine (1 mg/kg, s.c.) group indicates less evidence of pyknotic nuclei and neuronal degeneration.
VIII) Dihydroberberine (50mg/kg, p.o.) + Reserpine (1 mg/kg, s.c.) & vacuolated cytoplasm and pyknotic nuclei was less noticed.
IX)Dihydroberberine (100mg/kg, p.o.) + Reserpine (1 mg/kg, s.c.) very less pyknotic nuclei and greater number of prominent nuclei neurons indicating less brain tissue damage.

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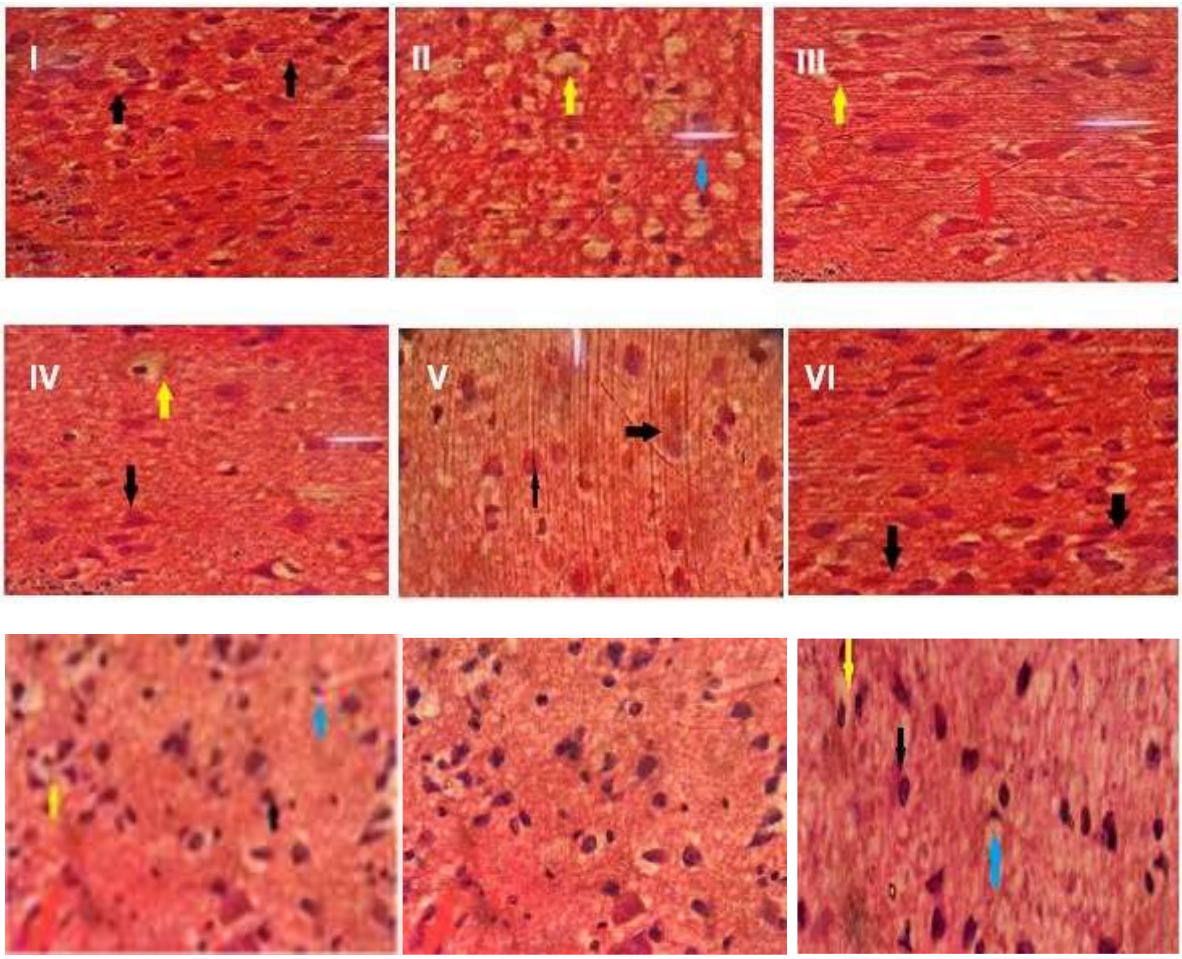

Figure 17: Histopathological changes of substantia nigra pars compacta (SNPc) region of rat's brain Haloperidol-induced Catalepsy

Behavioral Assessment

I) Effect of Trimyristin and Dihydroberberine on vacuous chewing movements:

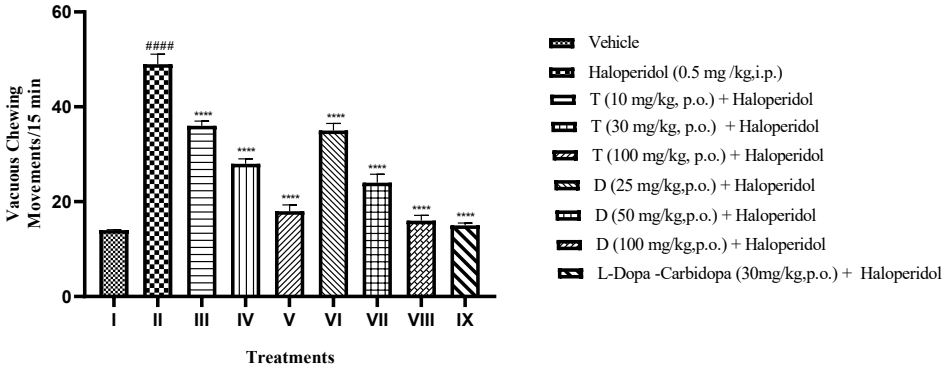

Figure 18. Effect of T & D on vacuous chewing movements by Haloperidol-induced Catalepsy

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

A significant increase ($p < 0.0001$) in vacuous chewing movements was observed in the haloperidol-treated group compared with the vehicle-treated group. Administration of Trimyristin and Dihydroberberine significantly ($p <$

0.0001) reduced number of vacuous chewing movement as compared with haloperidol treated group. L-dopa-carbidopa (30 mg/kg) also significantly ($p < 0.0001$) reduced number of vacuous chewing movement in animals

II) Effect of Trimyristin and Dihydroberberine on Tongue protrusions

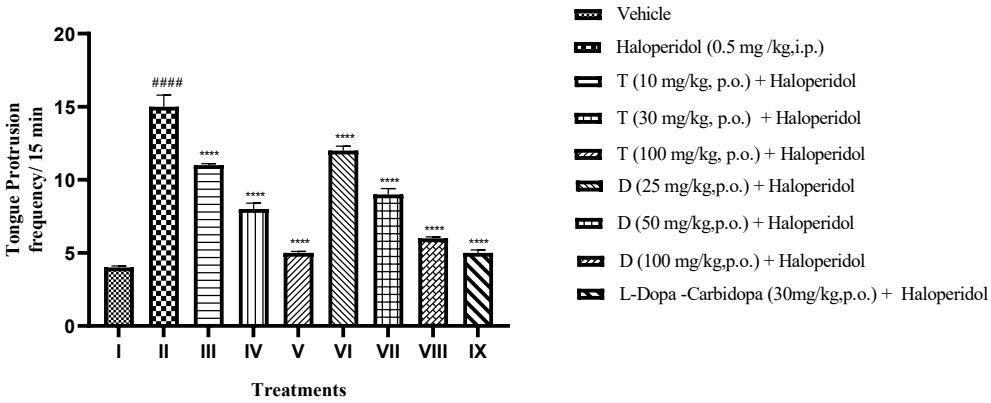

Figure 19. Effect of T & D on Tongue protrusions by Haloperidol-induced Catalepsy

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

A Significant increase ($p < 0.0001$) in number of tongue protrusions were observed in Haloperidol treated group as compared to vehicle group. Administration of Trimyristin and Dihydroberberine significantly ($p < 0.0001$) reduced number of tongue protrusion as compared with haloperidol treated group. L-dopa- carbidopa (30 mg/kg) also reduced number of tongue protrusions significantly ($p < 0.0001$) in animals.

B) Effect of Trimyristin and Dihydroberberine on catalepsy

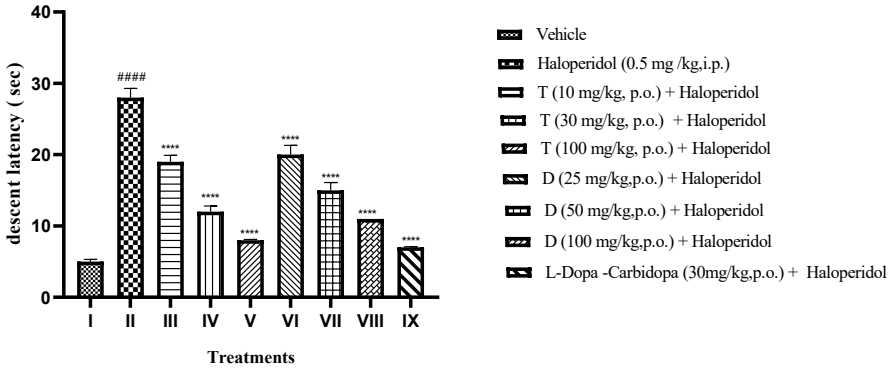

Figure 20. Effect of T & D on catalepsy by Haloperidol-induced Catalepsy

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

Haloperidol treated animal show in a significant increase in catalepsy compared to the vehicle control group ($p < 0.0001$). Treatment with Trimyristin and Dihydroberberine in combination with Haloperidol decreased catalepsy significantly in a dose-dependent manner ($p< 0.0001$). Similarly, L-dopa-carbidopa (30 mg/kg,) co-administration with haloperidol significantly reduced catalepsy compared with the haloperidol-treated group ($p < 0.0001$).

C) Effect of Trimyristin and Dihydroberberine on motor coordination

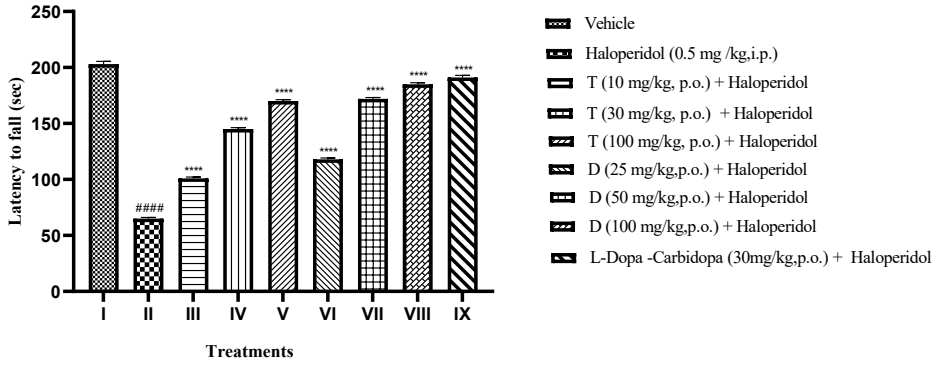

Figure 21. Effect of T & D on motor co-ordination by Haloperidol-induced Catalepsy

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

A highly significant decrease in falling latency ($p < 0.0001$) was observed in the haloperidol-treated group compared with the vehicle-treated group. Administration of Trimyristin and Dihydroberberine significantly ($p < 0.0001$) increased number of falling latency as compared to Haloperidol treated group. L-dopa-carbidopa (30 mg/kg) showed significant ($p<0.0001$) rise as compared with haloperidol treated animals.

D) Effect of Trimyristin and Dihydroberberine on exploratory and curiosity behaviour

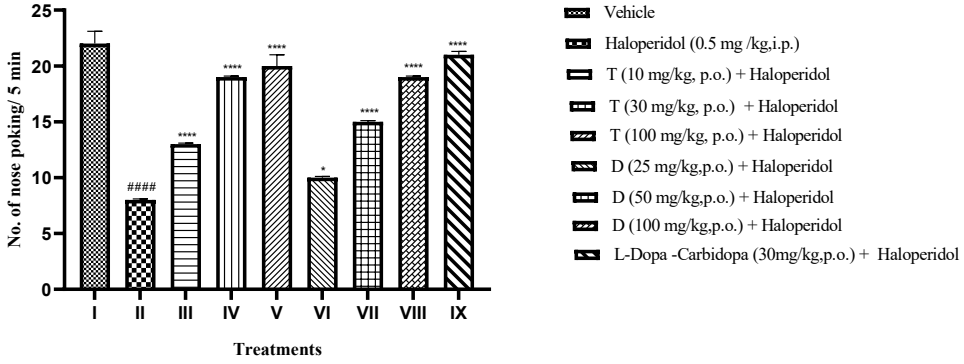

Figure 22. Effect of T & D on exploratory and curiosity behaviour by Haloperidol-induced Catalepsy

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

A significant decrease ($p < 0.001$) in the number of nose pokings was observed in the haloperidol-treated group compared with the vehicle control group. Administration of Trimyristin and Dihydroberberine significantly ($p < 0.001$) increased number of nose pokings as compared to haloperidol treated group. L-dopa-carbidopa (30 mg/kg) showed significant ($p<0.001$) increased number of noses pokings as compared to haloperidol treated animals.

E) Effect of Trimyristin and Dihydroberberine on locomotor activity
i) Effect of Trimyristin and Dihydroberberine on number of square crossings

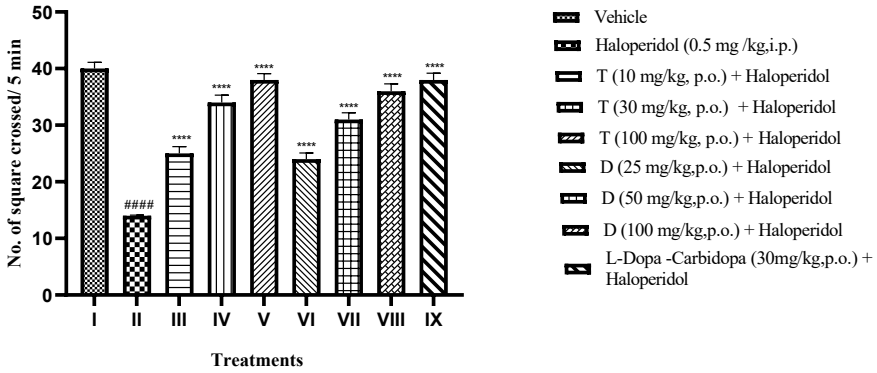

Figure 23. Effect of T & D on number of square crossings by Haloperidol-induced Catalepsy

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

A highly significant decrease ($p < 0.0001$) in the number of square crossings was observed in the haloperidol-treated group compared with the vehicle control group. Administration of Trimyristin and Dihydroberberine significantly ($p < 0.0001$) increased number of square crossings as compared to haloperidol treated group. L-dopa-carbidopa (30 mg/kg) showed increased number square crossings significantly ($p<0.0001$) as compared to haloperidol treated animals.

ii) Effect of Trimyristin and Dihydroberberine on number of rearing

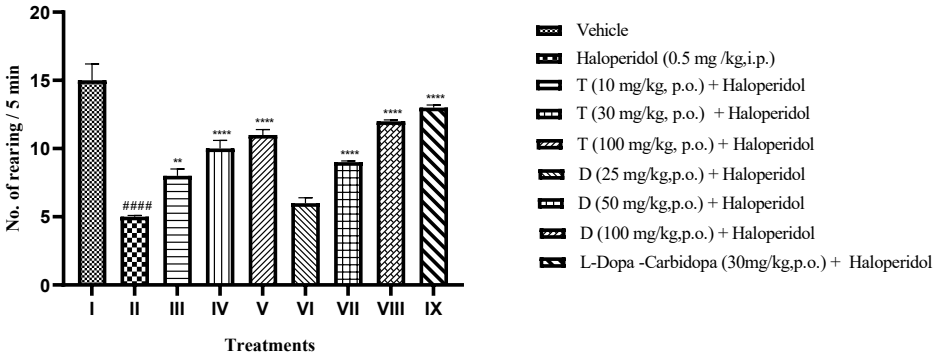

Figure 24. Effect of T & D on number of rearing by Haloperidol-induced Catalepsy

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

A highly significant reduction ($p < 0.0001$) in the number of rearing events was observed in the reserpine-treated group compared with the vehicle-treated group. Administration of Trimyristin and Dihydroberberine increased number of rearing significantly ($p < 0.0001$) as compared to haloperidol treated group. L-dopa-carbidopa (30 mg/kg,) also increased number of rearing significantly ($p < 0.0001$) in animals.

II) Biochemical evaluation:

- **Antioxidant evaluation:**

A) Effect of Trimyristin and Dihydroberberine on CAT level:

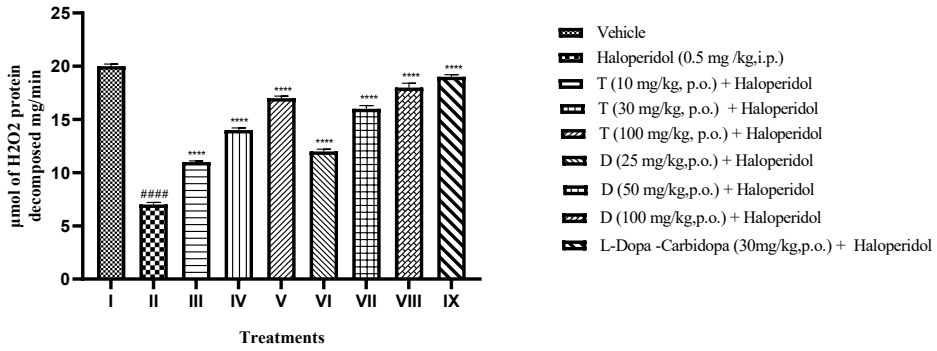

Figure 25. Effect of T & D on catalase level by Haloperidol-induced Catalepsy.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

B) Effect of Trimyristin and Dihydroberberine on LPO level:

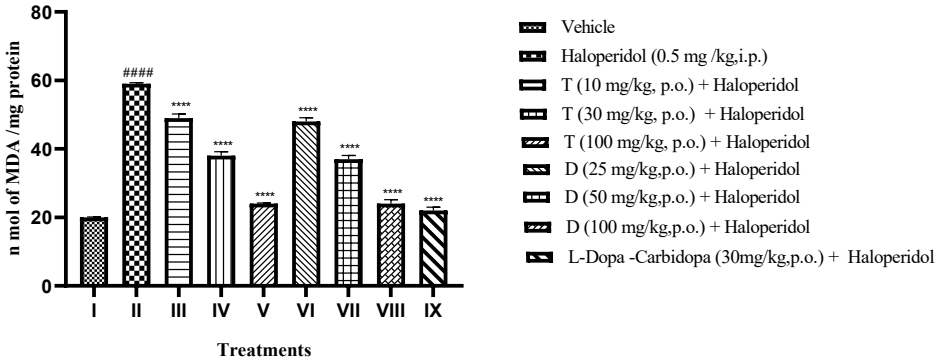

Figure 26. Effect of T & D on LPO level by Haloperidol-induced Catalepsy.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

C) Effect of Trimyristin and Dihydroberberine on GSH level:

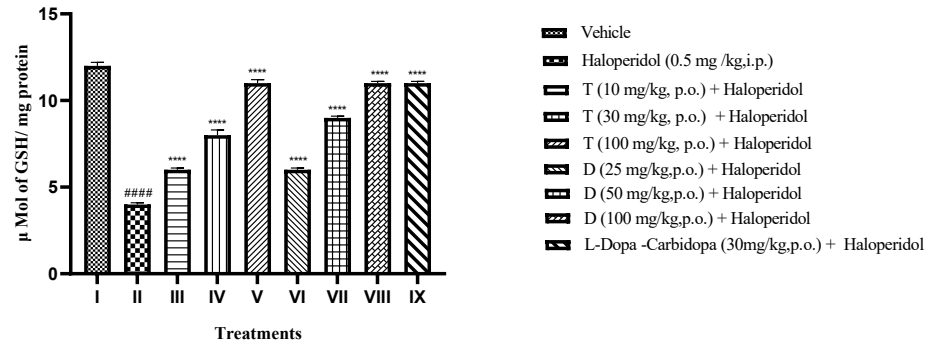

Figure 27. Effect of T & D on GSH level by Haloperidol-induced Catalepsy.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

D) Effect of Trimyristin and Dihydroberberine on SOD level:

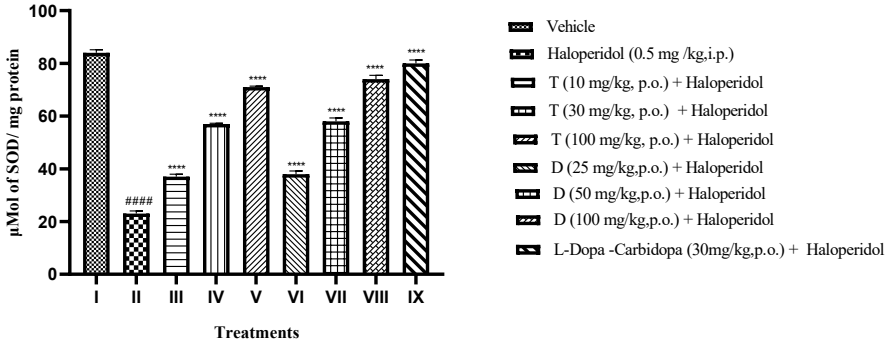

Figure 28. Effect of T & D on SOD level by Haloperidol-induced Catalepsy.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$

The haloperidol-treated group exhibited a highly significant decrease ($p < 0.0001$) in CAT, GSH, and SOD levels, accompanied by a significant increase in MDA levels, compared with the control group. Trimyristin and Dihydroberberine ($p<0.0001$) showed significant rise in CAT, GSH, SOD and decreased MDA level levels as compared to the induced group in rat brain. L-dopa-carbidopa (30 mg/kg,) group showed significant ($p<0.0001$) antioxidant activity.

Neurochemical analysis:
i) Effect of Trimyristin and Dihydroberberine on dopamine

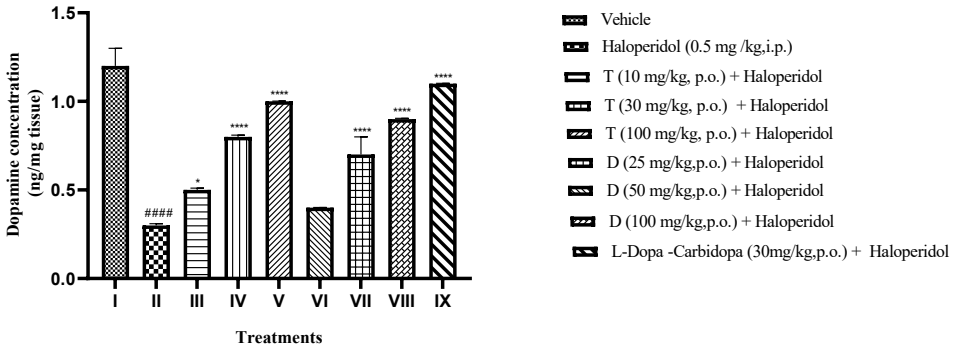

Figure 29. Effect of T & D on dopamine level by Haloperidol-induced Catalepsy.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$. ns – Non significant

Significant ($p < 0.0001$) decreased in levels of dopamine were observed after Haloperidol administration as compared to vehicle group. Administration of Trimyristin and Dihydroberberine showed dose dependent significant rise in levels of dopamine as compared to rats treated with haloperidol. L-dopa-carbidopa (30 mg/kg) also showed significant ($p<0.0001$) increase in dopamine levels as compared to diseases control group.

i) Effect of Trimyristin and Dihydroberberine on NO level

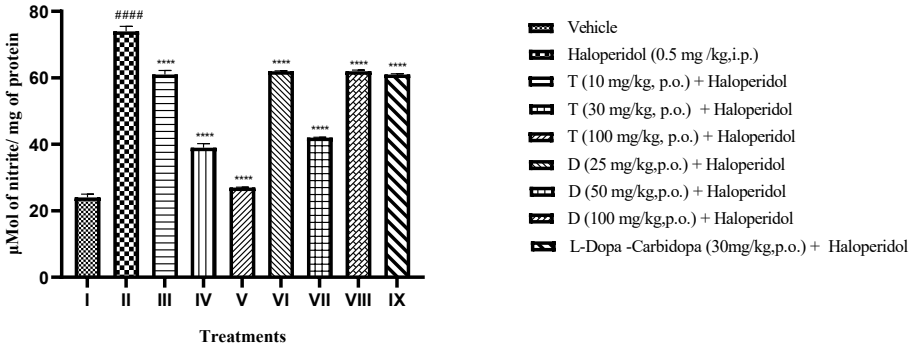

Figure 30. Effect of T & D on NO level by Haloperidol-induced Catalepsy.

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed by one-way ANOVA followed by Dunnett's test. Group II was compared with Group I, and Groups III–VI were compared with Group II. Significance levels were denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p<0.0001$.

A highly significant increase ($p < 0.0001$) in NO levels was observed in the haloperidol-treated group compared with the vehicle-treated group. Administration of Trimyristin and Dihydroberberine showed significant fall in levels of NO as compared haloperidol treated rats. L-dopa-carbidopa (30 mg/kg) also showed significant ($p<0.0001$) decrease in NO levels as compared to diseases control group.

DISCUSSION:

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons, resulting in motor impairments such as tremor, rigidity, and bradykinesia.

The present study demonstrates the anti-Parkinsonian and neuroprotective potential of Trimyristin and Dihydroberberine using haloperidol and reserpine animal models of Parkinson's disease. It has been examined in rats with haloperidol-induced catalepsy and reserpine -induced neurotoxicity by assessing its behavioral, biochemical, histopathological parameters. Levodopa in combination with carbidopa served as the standard treatment in the present study. Levodopa replenishes depleted dopamine levels in the brain, while carbidopa inhibits peripheral aromatic L-amino acid decarboxylase, thereby enhancing the central bioavailability of levodopa and reducing its peripheral metabolism. This combination effectively improves motor symptoms associated with Parkinson's disease and is considered the gold-standard therapy.

Haloperidol (0.5 mg/kg, i.p.) produced marked Parkinsonian-like behavioral deficits, including catalepsy, vacuous chewing movements, tongue protrusions, muscular rigidity, postural instability, reduced exploratory behavior, and impaired locomotor activity. These behavioral alterations, observed during the assessment period, are attributable to haloperidol-induced blockade of dopaminergic transmission in the nigrostriatal pathway and validate the successful induction of experimental Parkinsonism[18]. Haloperidol-induced catalepsy is widely used as a pharmacological model of Parkinsonian motor dysfunction due to its ability to block striatal dopamine D_2 receptors, leading to impaired dopaminergic neurotransmission and motor rigidity[19]. In the current study, the tested Trimyristin (30, 100 mg per kg) and Dihydroberberine (50,100 mg per kg) significantly improvement in behavioral parameters compared with the haloperidol-treated group. These findings suggest that the Trimyristin (30, 100 mg per kg) and Dihydroberberine (50,100 mg per kg) exert antiparkinsonian activity, possibly by restoring dopaminergic neurotransmission or modulating dopamine receptor function within the nigrostriatal pathway. The observed improvement in motor performance supports their potential as neuroprotective agents for the management of Parkinson's disease.

In this study reserpine animal model are used to induce parkinsonism in rats. Reserpine, a selective inhibitor of the vesicular monoamine transporter (VMAT), causes profound depletion of dopamine (DA), norepinephrine (NA), and serotonin (5-HT), thereby producing Parkinsonian features such as muscular rigidity, tremors, and akinesia[20,21]. In the present study, reserpine (1 mg per kg, s.c.) induced marked motor deficits, including impaired locomotor activity, reduced exploratory behavior, and diminished grip strength. Treatment with Trimyristin,

Dihydroberberine, and the standard drug levodopa–carbidopa significantly attenuated these behavioral abnormalities. Furthermore, Trimyristin and Dihydroberberine effectively increase locomotor activity, exploratory behavior, curiosity, and grip strength, demonstrating a significant reversal of reserpine-induced motor impairments in rats. Amelioration of symptoms of reserpine by Trimyristin and Dihydroberberine demonstrates anti-parkinsons activity.

Oxidative stress is a major pathological mechanism underlying dopaminergic neuronal degeneration in Parkinson's disease (PD) [22]. Excessive generation of reactive oxygen species (ROS) disrupts cellular redox homeostasis, resulting in oxidative damage to lipids, proteins, and nucleic acids, thereby contributing to neuronal dysfunction and death. Dopamine metabolism by monoamine oxidase-B produces hydrogen peroxide, which, in the presence of iron-rich regions such as the basal ganglia, generates highly reactive hydroxyl radicals through Fenton chemistry[23]. This oxidative environment also promotes α-synuclein aggregation and the formation of Lewy bodies, characteristic pathological features of PD. To evaluate oxidative stress, the activities of endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT), along with lipid peroxidation (LPO), were assessed in brain tissue. SOD constitutes the first line of antioxidant defense by catalyzing the conversion of superoxide radicals into hydrogen peroxide, whereas CAT detoxifies hydrogen peroxide into water and oxygen, thereby preventing the formation of highly reactive hydroxyl radicals. In contrast, elevated LPO reflects increased membrane lipid damage caused by ROS and serves as a reliable marker of oxidative injury. In the present study, reserpine- and haloperidol-treated rats exhibited a significant decline in SOD and CAT activities accompanied by a marked increase in LPO levels, confirming enhanced oxidative stress and impaired antioxidant defense. Administration of Trimyristin and Dihydroberberine significantly restored SOD and CAT activities while reducing LPO levels, indicating attenuation of oxidative damage. Furthermore, treatment also improved brain dopamine levels, suggesting preservation of dopaminergic neurons. These findings demonstrate that the neuroprotective effects of Trimyristin and Dihydroberberine are, at least in part, mediated through reinforcement of the endogenous antioxidant defense system and suppression of oxidative stress-induced neuronal injury.

Neuroinflammation is a hallmark of Parkinson's disease (PD) and other neurodegenerative disorders. Activated microglia play a central role in mediating neuroinflammation by releasing reactive oxygen and nitrogen species, including superoxide and nitric oxide (NO), which contribute to oxidative and nitrosative stress[24].

Previous study has found that Dihydroberberine, an isoquinoline alkaloid has anti- inflammatory, anti-tumour and hypolipidemic bioactivities. Dihydroberberine was proved to be one of the anti-inflammatory ingredients. The anti-inflammatory mechanism might be associated with dual modulation of NF-κB and MAPK signaling pathways and regulation of inflammatory mediators[25]. Trimyristin possesses neuro-protective potentials against Sodium arsenite-induced neurotoxicity in rats. In the present study, treatment with Trimyristin (30 and 100 mg per kg, p.o.) and Dihydroberberine (50 and 100 mg per kg, p.o.) significantly reduced lipid peroxidation (LPO) and NO levels, indicating attenuation of oxidative stress and neuroinflammation associated with Parkinsonian pathology.

Histopathological analysis of the reserpine-treated group demonstrated pyknotic nuclei, cytoplasmic vacuolation, and marked loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), indicating severe neurodegeneration. These alterations are attributed to VMAT2 inhibition by reserpine, resulting in cytosolic dopamine accumulation, oxidative stress, mitochondrial dysfunction, and activation of apoptotic pathways. Additionally, oxidative stress-induced neuroinflammation further contributes to dopaminergic neuronal loss. Treatment with Trimyristin (30 and 100 mg/kg, p.o.) and Dihydroberberine (50, and 100 mg/kg, p.o.) markedly attenuated these histopathological changes, as evidenced by reduced pyknosis, decreased cytoplasmic vacuolation, and preservation of SNpc neuronal architecture. These protective effects are likely mediated through their antioxidant and anti-inflammatory activities, which limit oxidative damage, maintain mitochondrial integrity, and inhibit apoptosis. The histological improvements are consistent with the observed restoration of antioxidant status and behavioral recovery, supporting the neuroprotective efficacy of Trimyristin and Dihydroberberine against reserpine-induced Parkinsonian neurodegeneration.

Overall, the histopathological findings support the behavioral and biochemical results, demonstrating that Trimyristin and Dihydroberberine protect dopaminergic neurons by reducing oxidative stress, suppressing neuroinflammation, and preventing neuronal degeneration in experimental models of Parkinson's disease.
The *in silico* molecular docking analysis demonstrated that the Dihydroberberine and Trimyristin exhibited favorable binding interactions with the Dopamine (6CM4) receptor, indicating their potential therapeutic

relevance in Parkinson's disease. These results were corroborated by the *in silico* molecular docking study, which showed that Dihydroberberine and Trimyristin had a high binding affinity for the dopamine receptor, comparable to that of the standard therapy. The docking findings support the hypothesis that the Dihydroberberine and Trimyristin can effectively bind to the target protein and may contribute to their neuroprotective effects. Collectively, these results highlight the potential of as promising candidates for the management of Parkinson's disease.

CONCLUSION:

The present study demonstrated that Trimyristin and Dihydroberberine significantly attenuated behavioral deficits, restored dopamine and endogenous antioxidant enzyme levels, reduced oxidative stress, and preserved neuronal architecture in the substantia nigra in experimental models of Parkinson's disease. These findings indicate that the neuroprotective effects of both compounds are mediated through antioxidant and dopaminergic mechanisms, resulting in reduced neurodegeneration.

Overall, the behavioral, biochemical, and histopathological evidence supports the therapeutic potential of Trimyristin and Dihydroberberine as promising neuroprotective agents for Parkinson's disease. Nevertheless, further mechanistic investigations, pharmacokinetic studies, and well-designed clinical trials are required to establish their safety, efficacy, and translational potential for clinical use.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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