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In-Silico and In-Vivo Evaluation of Luteolin and Imeglimin as Novel Therapeutic Agents in Reserpine-Induced Parkinsons Disease in Rats

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Abstract

Parkinson's disease (PD) is the second most prevalent late-life movement disorder, characterized as a hypokinetic condition involving selective and extensive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the striatum. This study investigated the neuroprotective effects of luteolin and imeglimin against reserpine-induced PD in rats, focusing on the dopamine transporter protein (DAT), α-synuclein aggregation, and proinflammatory cytokines using both in silico and in vivo approaches. In- silico molecular docking studies were followed by in vivo experiments. PD was induced in rats by administration of reserpine (1 mg/kg, s.c.) every other day for 3 days. Pretreatment with luteolin (50 mg/kg, p.o.) and imeglimin (100 mg/kg, p.o.) was given for 5 days. Behavioral assessments (orofacial dyskinesia, catalepsy, and rotarod) were performed on day 5, followed by biochemical and histopathological evaluations. Parameters measured included oxidative stress markers (CAT, GSH, SOD, and LPO), neurotransmitters (dopamine and nitric oxide), proinflammatory cytokines (TNF-α and IL-1β), and α-synuclein expression.

Reserpine-treated rats exhibited increased catalepsy duration, orofacial dyskinesia (tongue protrusions, vacuous chewing), and impaired motor coordination. Biochemical analysis showed reduced antioxidant levels (CAT, GSH, SOD), reduced dopamine levels, and elevated LPO, TNF-α, IL-1β, and α-synuclein. Combined treatment with luteolin and imeglimin significantly mitigated these behavioral and biochemical alterations—improving locomotor activity, reducing dyskinesia, and normalizing antioxidant and inflammatory markers. Dopamine levels were restored, and α-synuclein aggregation was notably reduced. Histopathology confirmed dopaminergic neuronal preservation and reduced Lewy body formation in the treatment group. These findings suggested that luteolin and imeglimin, particularly in combination, offer neuroprotective effects in PD by reducing oxidative stress, inflammation, and α-synuclein aggregation, thereby preserving dopaminergic neurons and brain function.

Keywords: α-Synuclein; DAT; Imeglimin; Luteolin; Parkinsons disease; Neuroinflammation.

1. Introduction

Parkinson's disease (PD) is a neurological condition that progresses with age. In the western world, the prevalence of parkinson's disease is 315 per 100,000 people of all ages, and it is anticipated to double by 2030, increasing death, morbidity, and socioeconomic hardship

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worldwide [1]. Motor symptoms include akinesia, stiffness, and tremor (known as cardinal symptoms), while axial symptoms include postural instability and gait problems. The degenerative process is currently well understood, although there is no definitive knowledge of the disease's genesis [2]. The major features of Parkinson's disease are progressive loss or destruction of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and dopamine (DA) depletion in the striatum (ST), which are related to the motor deficits of PD. Currently, levodopa (L-dopa) is the most effective therapy for the early-stage motor symptoms of parkinson's disease (PD); it is not considered a cure [3]. Reserpine is a vesicular monoamine inhibitor that can deplete brain catecholamines, leading to an akinetic state in experimental animals [4]. Natural phytochemicals and their derivatives have potential neuroprotective effects due to their multifaceted ability to regulate and modulate chronic inflammation, oxidative stress, and signaling across diverse pathways, all of which are characteristics of Parkinson's disease [5]. Luteolin, a flavonoid derived from *Cirsium japonicum*, has recently gained substantial attention due to its physiological functions, including antioxidant, anti-inflammatory, and neuroprotective effects [6]. Its antioxidant activity mainly involves reducing ROS levels and increasing SOD activity [7]. In addition, luteolin has been reported to have neuroprotective effects by preventing cell death via an antioxidant mechanism, upregulating ER/ERK/MAPK signaling, and acting as a novel activator of the dopamine transporter (DAT). Dopamine (DA) is primarily inactivated by reuptake through DAT, a monoamine transporter located on the presynaptic membrane of dopaminergic neurons, particularly in brain regions associated with dopaminergic signaling, and it is involved in several DA-related disorders, including attention-deficit hyperactivity disorder, bipolar disorder, clinical depression, and alcoholism, PD [8]. Luteolin can efficiently cross the blood–brain barrier (BBB) and enter the brain. Several studies have demonstrated its diverse health-promoting properties, including anti-apoptotic, antioxidant, anti-inflammatory, and neuroprotective effects [9]. Drug repurposing is an approach to discovering and developing medications. In general, repurposed drugs have an established safety profile, and this technique is associated with lower development costs and shorter timelines, as well as faster approval procedures [10]. Imeglimin is the first in a new class of oral antidiabetic agents, the glimins, which contain the tetrahydrotriazine moiety. It has been reported to act on the liver, muscle, and pancreatic β-cells to target the key defects of type 2 diabetes. It showed anti-inflammatory and antioxidant effects in CNS-related disorders [11].

However, despite reported antioxidant and neuroprotective activities, no major studies have been found on its neuroprotective potential in Parkinson's disease. Hence, the present study was designed to assess the utility of luteolin and imeglimin in combination for managing the prevalence of PD.

1. Materials and methods

2.1. Animals:

Male Albino Wistar rats (230-250 g) were housed in polypropylene cages and maintained under standard laboratory conditions at 25±2 °C, with a 12 h light/dark cycle and 50±5% relative humidity, and received food and water *ad libitum*. All the experiments were carried out during the light period. The studies were carried out in accordance with the guidelines given by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi (India). They were approved by the Institutional Animal Ethical Committee of M.V.P.S College of Pharmacy, Nashik, India (IAEC/Oct.2024/12).

2.2. Drugs and reagents:

Reserpine (Boehringer Ingelheim, Mumbai, India),) Luteolin (Dham tech Pharmaceuticals, Mumbai, India), Imeglimin (Zuventus Pharmaceuticals, India), Vitamin E (Merck Ltd., Goa, India).

2.3. Experimental design for reserpine-induced orofacial dyskinesia in rats

The animals were divided into six groups (n= 6). Group I: Normal saline solution Group II: Reserpine-treated group; reserpine (1 mg/kg, s.c.) in 0.1% acetic acid for 3 days, every other day. Group III: Luteolin (50 mg/kg, p.o.) for 5 days, followed by reserpine for 3 days, every other day. Group IV: Imeglimin (100 mg/kg, p.o.) for 5 days and reserpine for 3 days, every other day. Group VI:

Luteolin (50 mg/kg, p.o.) + Imeglimin (100 mg/kg, p.o.) for 5 days and reserpine for 3 days, every other day. Group VI: Vit. E (10 mg/kg, p.o.) for 5 days and reserpine for 3 days, every other day. Luteolin was prepared in 0.5% CMC in water, whereas imeglimin dissolved in 5% DMSO in water. The drugs were administered 30 min before reserpine. On the fifth day, 1 h after the last reserpine injection, all the rats were observed to quantify orofacial dyskinesia. Animals were sacrificed about 24 h after behavioral measurements for various biochemical and histopathological assays.

2.4. Computational In-silico docking studies

To elucidate the potential mechanisms underlying the neuroprotective effects of luteolin and imeglimin, computational LD_{50} prediction, in-silico target prediction, and molecular docking studies were conducted. Target proteins implicated in parkinson's disease pathology were α-synuclein, dopamine transporter (DAT), and key proinflammatory cytokines (TNF-α, IL-1β, and IL-6) for analysis. The Toxicity prediction was calculated using the "PROTOX-II" software. Swiss Target Prediction was used to identify potential biological targets for the compounds. Molecular docking was performed using "PYREX" for docking score analysis and "BIOVIA Discovery Studio" for visualization of molecular interactions and binding affinities.

2.5. Behavioral Assessment

2.5.1. Quantification of Orofacial Dyskinesia

Rats were individually placed in a small Plexiglass observation cage (30 × 20 × 20 cm) to score the frequency of vacuous chewing movements and tongue protrusions to measure the prevalence of orofacial dyskinesia on test day. Before behavioral evaluations were conducted, the animals were given 15 minutes to acclimate to the observation cage. When the animal was facing away from the observer, mirrors were positioned behind the cage's near wall and beneath the floor to allow for the observation of oral dyskinesia. The VCMs and TPs were described as a visible extension of the tongue beyond the mouth and a single mouth opening in the vertical plane that was not aimed at any physical material, respectively. VCMs and TPs that happened during a grooming session were not considered. Oral dyskinesia behavioral indicators were regularly assessed to identify the animals [12].

2.5.2. Assessment of catalepsy by the bar test

Six centimeters above the tabletop, a horizontal metal bar with a diameter of 2-5 mm was suspended. After carefully removing the rat from its home cage, the animal's body was held on the table by placing both forepaws over the bar. It was noted how long (in seconds) the animal stayed in this posture before lowering its forepaws to the tabletop. A cut-off time of 180 s was established as the maximum observation period. The test was conducted at various time points after treatment, including 0, 30, and 60 min. More severe motor impairment was indicated by a greater cumulative catalepsy score [13].

2.5.3. Assessment of motor activity by the rotarod test

The rotarod apparatus was a 75 cm-long, 3 cm-diameter, rubber-coated rod, divided into six sections, and raised 50 cm above the surface. It has a motor that turns it at 4 rpm and gradually increases the speed to 20 rpm. Only rats that stayed on the rod for at least a minute were included after they had undergone pre-screening. Test substances were given orally. Rats were put on the rod either 60 min after an oral dose. During a 1-minute trial, the number of falls and the latency to the first fall were recorded to evaluate balance and motor coordination [14].

2.6. Tissue homogenate preparation

Biochemical parameters were analyzed 24 h after the last reserpine injection on the last day of the trial, 1 h after behavioral assessments were completed. The animals were humanely killed using CO_2 euthanasia chamber. Medical-grade carbon dioxide gas was introduced at a flow rate of 20–30% of the chamber volume per minute (in accordance with AVMA Guidelines for the Euthanasia of Animals, 2020). This gradual fill rate minimizes distress and avoids sudden hypercapnia shock. Gas flow was maintained until 1 minute after cessation of respiratory movement to ensure death, followed by a secondary physical confirmation method

(e.g., cervical dislocation) before brain extraction. The brains were promptly extracted, cleaned with isotonic saline, and weighed. A tissue homogenate containing 10% (w/v) was made in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged in a Remi-C30 centrifuge (Remi Industries Ltd., Mumbai, India) at 1000 × g for 20 min at 4°C to perform the catalase test. The homogenate was centrifuged for 60 min at 4°C at 12,000 rpm for further enzyme tests. An Elico™ BL 200 bio spectrophotometer was used for all spectrophotometric analyses [15].

2.6.1. Estimation of catalase

The Luck (1971) method, which measures the breakdown of H_2O_2 at 240 nm, was used to test catalase activity. Three milliliters of 0.01 M H_2O_2 phosphate buffer (pH 7) and 0.05 ml of tissue homogenate supernatant (10%) make up the assay mixture. The absorbance change was measured at 240 nm after one minute. Using H_2O_2's millimolar extinction coefficient (0.071), enzyme activity was calculated per milligram of protein, and the results were reported as μmoles of H_2O_2 decomposed per min [15].

2.6.2. Estimation of reduced glutathione

The Ellman (1959) method was used to measure reduced glutathione (GSH) in the brain. 0.75 ml of 4% sulphosalicylic acid was used to precipitate a 0.75 ml sample of homogenate. The samples were centrifuged for 15 min at 4°C at 1200 × g. 4.5 ml of 0.01 M DTNB [5, 5'-dithiobis (2-nitrobenzoic acid)] in 0.1 M phosphate buffer (pH 8.0) and 0.5 ml of supernatant made up the assay mixture. At 412 nm, the developing yellow color was read. The findings were reported as μ moles of GSH per protein milligram [16].

2.6.3. Estimation of lipid peroxidation

Lipid peroxidation levels in the brain were quantitatively measured using the Wills (1966) method. The amount of malondialdehyde (MDA) generated was calculated using this approach by reacting with thiobarbituric acid at 532 nm. The reaction mixture consists of 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. Then, this combination was boiled for 1 h on a water bath at 95°C. It was then mixed with a 15:1 n-butanol:pyridine mixture to a final volume of 5 ml. After a vigorous shaking, the mixture was centrifuged for 5 min. The absorbance of the organic layer (upper) was measured at 532 nm. The molar extension coefficient of the chromophore was used to convert the values into n M of MDA per milligrams of protein ($1.56 \times 10^5 M^{-1}cm^{-1}$) [17].

2.6.4. Estimation of superoxide dismutase activity

The Kono (1978) method, which involved blocking the reduction of nitroblue tetrazolium chloride (NBT) and measuring it spectrophotometrically at 560 nm, was used to evaluate superoxide dismutase activity. 0.1 ml of 1 mM hydroxylamine hydrochloride was added to 0.1 ml of 0.1 mM ethylene diamine tetraacetic acid (EDTA), 0.1 ml of 24 μM NBT, 0.1 ml of 0.03% v/v Triton X 100 reagent, and 1 ml of post-nuclear fraction of brain homogenate to initiate the reaction. At 560 nm, the absorbance was measured following 20 min of incubation at 37°C. The findings were presented as a percentage inhibition of NBT lowering [18].

2.6.5. Estimation of striatal neuroinflammation

TNF-α and IL-1β were quantified using an immunoassay kit. Rat TNF-α, IL-1β levels are measured using the Quantikine® Rat VEGF ELISA Kit (TNF-α, IL-1β immunoassay, a 4.5-h solid phase ELISA). A microtitre plate reader was used in this solid-phase sandwich enzyme-linked immunosorbent assay. The concentrations of TNF-α and IL-1β were determined using standard curves.

2.7. Neurochemical analysis

2.7.1. Estimation of dopamine level

A homogenized supernatant liquid (1 ml) was mixed with 1 ml of ferric chloride (1.5 × 10−2 M) and 1 ml of potassium ferricyanide (1.5 × 10−2 M) in 25 ml of distilled water to quantify the amount of dopamine in the rat brain. After 30 min, it was set aside, and the UV-visible double-beam spectrophotometer was used to estimate the developed color at 735 nm [19].

2.7.2. Estimation of nitrite content

A colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylene diamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green et al. was used to measure the accumulation of nitrite in the striatum supernatant, an indicator of the production of nitric oxide (NO) (Green et al., 1982). After mixing equal amounts of supernatant and Greiss reagent, the mixture was allowed to sit at room temperature in the dark for ten minutes. Utilizing a Shimadzu spectrophotometer (Kyoto, Japan), absorbance was measured at 540 nm. Using the standard curve, the amount of nitrite in the supernatant was calculated and expressed as a percentage of the vehicle-treated group [20].

2.7.3. Estimation of protein

α-synuclein was quantified using an immunoassay kit. Rat α-synuclein levels were measured using the Quantikine® Rat VEGF ELISA Kit, an α-synuclein immunoassay, a 4.5-h solid-phase ELISA. A microlitre plate reader is used in this solid-phase sandwich enzyme-linked immunosorbent assay. α-synuclein concentrations were computed using the standard curves.

2.8. Histopathological analysis

The rat's brains were evaluated histopathologically. After being removed, the brains were promptly preserved in 10% buffered formalin. After being dried in alcohol, the brain was fixed in paraffin, and the striatum (SNpc) was removed. A microtome was used to cut 5 µm-thick serial histological sections from the paraffin blocks, which were then stained with hematoxylin and eosin (H and E). A 100X light microscope (Olympus, Japan) was used to view the slices, and photomicrographs were taken.

2.9. Statistical Analysis

All results were presented as mean ± S.E.M. (n=6). GraphPad Prism 10.4.1 software was used to evaluate all group data using one-way analysis of variance and Tukey's test. (GraphPad®, San Diego, US). *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared to vehicle group, all the treatment groups were compared to reserpine.

3. Results and Discussion

3.1. In-Silico docking studies

Toxicity predictions using the Protox-II platform indicated LD_{50} values of 3919 mg/kg for luteolin and 2000 mg/kg for imeglimin, placing both compounds in the low-toxicity category. These findings support the drug-likeness and relative safety of both luteolin and imeglimin for further *in vivo* evaluation.

Theoretical molecular docking studies were conducted to evaluate the binding affinities of luteolin and imeglimin with key proteins implicated in Parkinson's disease (PD) pathophysiology, including α-synuclein, the dopamine transporter (DAT), and proinflammatory cytokines (TNF-α, IL-1β, and IL-6). Luteolin exhibited notable binding affinities for α-synuclein, DAT, TNF-α, IL-1β, and IL-6, suggesting a potential multitarget neuroprotective effect. Imeglimin showed selective binding with α-synuclein, TNF-α, and IL-1β, suggesting its anti-inflammatory role in neurodegeneration.

Both compounds demonstrated binding affinities (kcal/mol) comparable to those of the standard antioxidant vitamin E, supporting their therapeutic potential in mitigating oxidative and inflammatory damage in PD **[Table 1]**.

Table 1. Calculated binding affinities for different target values (kcal/mol).

Targets	PDB ID	Calculated affinity of drugs (Kcal/mol)		
		Luteolin	**Imeglimin**	Vit-E
DAT	8Y2D	-8.4	-5.7	**-8.9**
IL-1β	7JWQ	-7.9	-4.8	**-6.8**
IL-6	8D82	-5.5	-4.5	**-5.7**
TNF-α	3BQ4	-8.6	-6	**-8.2**
α - synuclein	**8BQV**	**-9.2**	**-7.6**	**-9.7**

3D visualizations of binding interactions were demonstrated for each target protein, which were aligned with the drug mechanism using BIOVIA Discovery

Studio **[Figure S1]**. Docking against multiple Parkinson's disease–related targets was performed to assess the potential multitarget activity of luteolin and imeglimin, given the multifactorial nature of the disease. While high binding affinity to several targets may offer therapeutic advantages through polypharmacology, it may also indicate low selectivity, increasing the risk of off-target effects. Such in- silico predictions, therefore, require experimental validation to confirm specificity and safety. Therefore, behavioral, biochemical, and histopathological studies were carried out.

3.2. Behavioral Assessment:

3.2.1. Effect of luteolin and imeglimin on vacuous chewing movements (VCMs) and tongue protrusions (TPs)

The reserpine treated group had a significantly greater number of VCMs and TPs than the control group ($p < 0.001$). Pretreatment with luteolin (50 mg/kg, p.o.) and imeglimin (100 mg/kg, p.o.) resulted in a significant ($p<0.001$) reduction in the number of VCMs and TPs, both alone and in combination. Standard vitamin E also showed a significant decrease ($p<0.001$) in the number of VCMs and TPs compared with the reserpine-treated group **[Figure 1].**

3.2.2. Effect of luteolin and imeglimin on catalepsy

Catalepsy was assessed for 0, 30, and 60 min. Compared to the vehicle group, the reserpine-treated group showed a significantly higher incidence of catalepsy ($p<0.001$). There was a significant ($p<0.001$) reduction in catalepsy after treatment with luteolin and imeglimin, individually and in combination. Vitamin E significantly reduced catalepsy ($p<0.001$) as compared to reserpine treatment. **[Figure 2]**(1, 2, 3 denoted 0 min, 30 min, 60 min respectively).

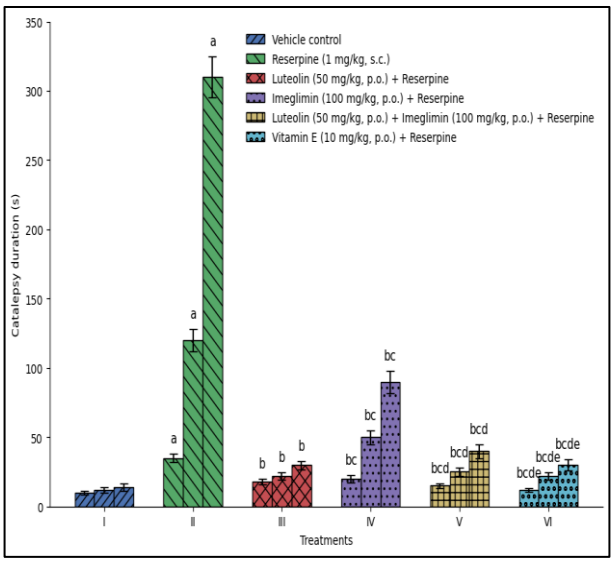
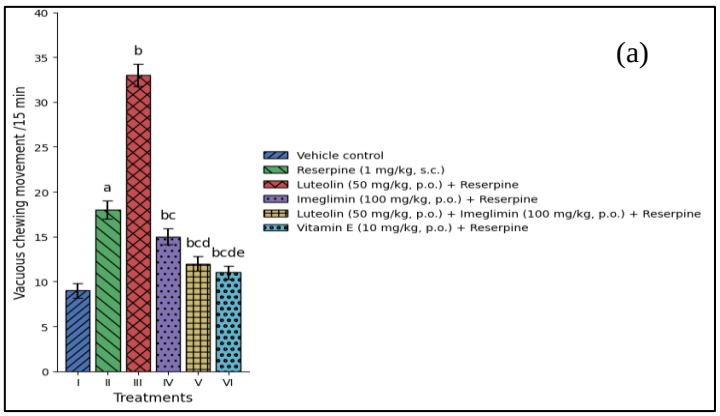


Figure 2. The effect of luteolin and imeglimin on catalepsy duration (s) is shown; all values are expressed as mean ± S.E.M. (n=6) and analyzed by One-way ANOVA followed by Tukey's test. *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared to vehicle group, all the treatment groups were compared to reserpine.



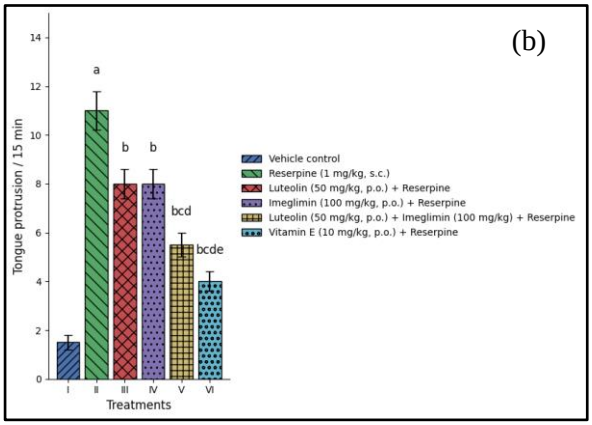


Figure 1. Effect of luteolin and imeglimin on a) Vacuous chewing movements and b) Tongue protrusions represents, all values are expressed as mean ± S.E.M. (n=6) and analyzed by One-way ANOVA followed by Tukey's test. *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared to vehicle group, whereas all the treatment groups were compared to reserpine.

3.2.3. Effect of luteolin and imeglimin on motor coordination

Falling latency was much lower in the reserpine group than the vehicle group ($p<0.001$). Pretreatment with imeglimin and luteolin alone and in combination showed a significant ($p<0.001$) increase in falling latency. Combined therapy showed more significant results. Vit E was significantly higher ($p<0.001$) than in the reserpine-treated group **[Figure 3]**.

3.3. Biochemical Estimation

3.3.1. Effect of luteolin and imeglimin on catalase levels

CAT levels were significantly reduced ($p<0.001$) in the reserpine-treated group compared with the vehicle group. In the rat brain, luteolin, imeglimin, and their combination significantly increased CAT levels ($p<0.001$) compared with the reserpine-treated group. CAT levels were significantly ($p<0.001$) higher in the vitamin E group **[Table 2]**.

3.3.2. Effect of luteolin and imeglimin on lipid peroxidation levels

LPO levels were significantly ($p<0.001$) higher in the reserpine treated group as compared to the vehicle group. LPO levels were significantly ($p<0.001$) decreased in the groups treated with luteolin, imeglimin, and combination as compared to reserpine group. LPO levels were significantly ($p<0.001$) lower in the vitamin E-treated group **[Table 2]**.

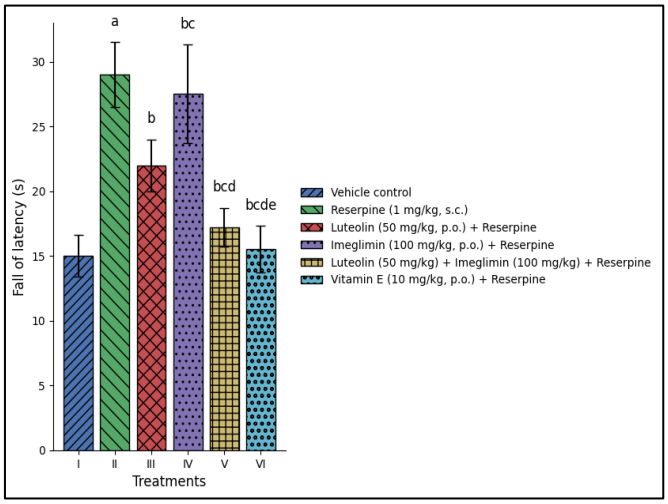


Figure 3. Effect of luteolin and imeglimin on motor coordination [fall of latency (s)] represents, all values are expressed as mean ± S.E.M. (n=6) and analyzed by one-way ANOVA followed by Tukey's test. *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared to vehicle group; all the treatment groups were compared to reserpine.

Table 2. Effect of luteolin and imeglimin on biochemical parameters.

Treatments	CAT (µ moles of H_2O_2 decomposed/ mg protein/min)	LPO (n moles of MDA/mg proteins)	SOD (% inhibition of reduction of NBT)	GSH (µ moles of GSH/mg proteins)
Vehicle	15±0.014	19±0.2	83±1.4	8±0.28
Reserpine (1mg/kg, s.c.)	4.9±0.0092[a]	58±0.19[a]	20±0.19[a]	3.5±0.14[a]
Luteolin (50 mg/kg, p.o.) + Reserpine	7±0.055[b]	36±0.062[b]	62±0.22[b]	6.4±0.41[b]
Imeglimin (100 mg/kg, p.o.)+ Reserpine	5.8±0.006[b]	44±0.062[b]	39±0.37[b]	5.1±0.24[b]
Luteolin (50 mg/kg,p.o.)+ Imeglimin (100 mg/kg, p.o.) + Reserpine	9.2±0.0098[bcd]	34±0.44[bcd]	65±0.59[bcd]	6.7±0.13[bcd]
Vitamin-E (10 mg/kg, p.o.) + Reserpine	9.5±0.041[bcde]	24±0.02[bcde]	75±0.61[bcde]	7.3±0.43[bcde]

3.3.3. Effect of luteolin and imeglimin on reduced glutathione levels

GSH levels were significantly reduced ($p<0.001$) in the reserpine group compared with the vehicle group. GSH levels were significantly higher in the groups treated with luteolin, imeglimin, and luteolin plus imeglimin ($p<0.001$) than in the reserpine group. GSH levels were also significantly ($p<0.001$) elevated by the standard medication, vitamin E **[Table 2]**.

3.3.4. Effect of luteolin and imeglimin on superoxide dismutase content

SOD levels in the resperpine group were significantly ($p<0.001$) lower than those in the vehicle group. Imeglimin, luteolin, and the combination of luteolin and imeglimin significantly raised SOD levels ($p<0.001$). Additionally, vitamin E significantly ($p<0.001$) raised SOD levels in comparison to the group treated with reserpine **[Table 2]**.

3.3.5. Effect of luteolin and imeglimin on nitric oxide

The reserpine-treated group showed a significant ($p<0.001$) increase in NO levels compared with the vehicle group, indicating neurodilation. Luteolin and imeglimin showed a significant decrease in NO levels ($p < 0.001$). The combination of luteolin and imeglimin was more effective in decreasing NO levels ($p<0.001$) than the reserpine-treated group, indicating a reduction in NO. Also, Vitamin E showed a significant decrease in NO levels ($p < 0.001$) compared with the reserpine-treated group **[Figure 4]**.

3.3.6. Effect of luteolin and imeglimin on brain dopamine content

Reserpine treatment resulted in significantly lower dopamine levels ($p<0.001$) compared with the vehicle group, indicating dopaminergic neuron degeneration. The combination of luteolin and imeglimin resulted in significantly higher dopamine levels ($p<0.001$) compared to the reserpine-treated group. Vitamin E significantly boosted dopamine levels ($p<0.001$) compared to the reserpine-treated group **[Figure 5]**.

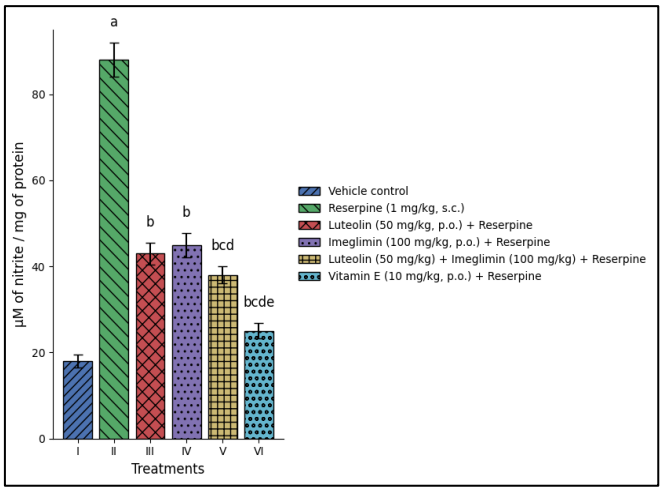


Figure 4. Effect of luteolin and imeglimin on nitric oxide levels in rat's brain. All values are expressed as mean ± S.E.M. (n=6) and analyzed by One-way ANOVA followed by Tukey's test. *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared with vehicle group, whereas all the treatment groups were compared to reserpine.

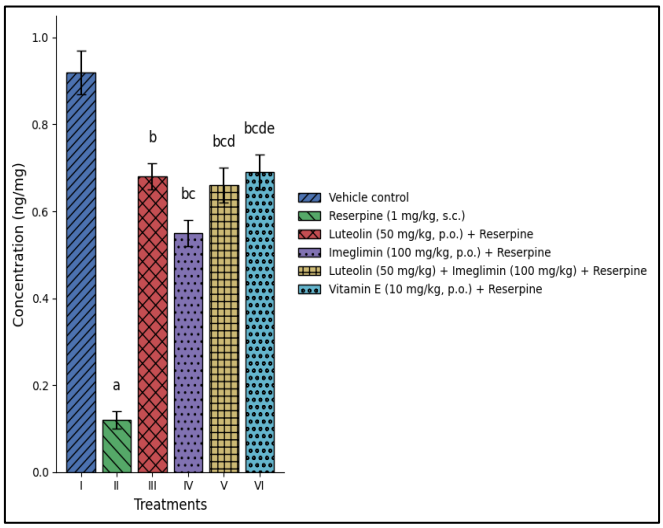


Figure 5. Effect of luteolin and imeglimin on dopamine levels in rat's brain. All values are expressed as mean ± S.E.M. (n=6) and analyzed by One-way ANOVA followed by Tukey's test. *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared with vehicle group, whereas all the treatment groups were compared to reserpine.

3.3.7. Effect of luteolin and imeglimin on TNF-α levels

The reserpine-treated group showed a significant ($p<0.001$) increase in TNF-α levels compared to the vehicle group, indicating the production of proinflammatory cytokines during neurotoxicity. Luteolin and imeglimin showed a significant decrease ($p<0.001$) in TNF-α levels; the combination of luteolin and imeglimin was more effective ($p<0.001$) in decreasing TNF-α levels than the reserpine-treated group, indicating a reduction in proinflammatory cytokines. Also, vitamin E showed a significant decrease in TNF-α levels ($p < 0.001$) compared to the reserpine-treated group **[Figure 6]**.

3.3.8. Effect of luteolin and imeglimin on IL-1β levels

The reserpine-treated group showed ($p<0.001$) increased levels of IL-1β compared to the vehicle group, indicating the production of proinflammatory cytokines during neurotoxicity. Luteolin and imeglimin significantly decreased IL-1β levels ($p<0.001$). The combination of luteolin and imeglimin was more effective than the reserpine-treated group in decreasing IL-1β levels ($p<0.001$), indicating a reduction in proinflammatory cytokines. Also, Vitamin E showed a significant decrease in IL-1β levels ($p<0.001$) compared with the reserpine-treated group **[Figure 7]**.

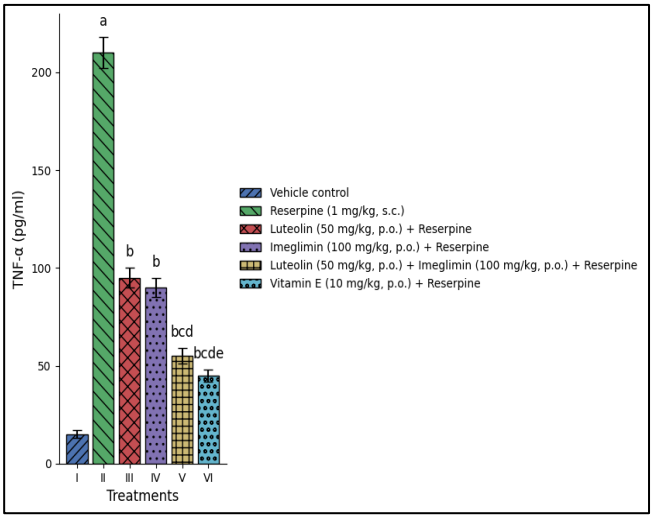


Figure 6. Effect of luteolin and imeglimin on TNF-α level in rat's brain. All values are expressed as mean ± S.E.M. (n=6) and analyzed by One-way ANOVA followed by Tukey's test. *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared with vehicle group, whereas all the treatment groups were compared to reserpine.

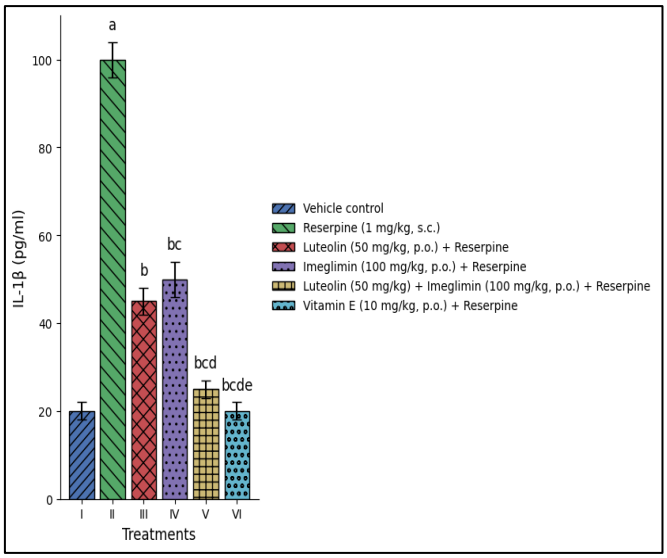


Figure 7. Effect of luteolin and imeglimin on IL-1β levels in rat's brain; all values are expressed as mean ± S.E.M. (n=6) and analyzed by One-way ANOVA followed by Tukey's test. *ns-non-significant*, [a]$p<0.001$ as compared to vehicle group, [b]$p<0.001$ as compared to reserpine-treated group, [c]$p<0.001$ as compared to luteolin, [d]$p<0.001$ as compared to imeglimin, [e]$p<0.001$ as compared to luteolin+ imeglimin (L+I), [f]$p<0.001$ as compared to vit E. Reserpine-treated group was compared with vehicle group, whereas all the treatment groups were compared to reserpine.

3.3.9. Effect of luteolin and imeglimin on α-Synuclein

Reserpine treatment significantly increased α-synuclein aggregation in the rat brain ($p<0.001$) compared with vehicle, suggesting abnormal protein accumulation. Luteolin ($p<0.001$), imeglimin ($p<0.001$), and their combination ($p<0.001$) all dramatically decreased α-synuclein levels, with the combination having the greatest effect. Vitamin E levels were significantly lower than in the reserpine treatment group ($p < 0.001$) **[Figure 8]**.

3.4. Histopathological examination

Representative photomicrographs (100X) of brain sections, histopathological analysis of H&E-stained sections from the substantia nigra pars compacta (SNpc) revealed marked dopaminergic neuronal loss, gliosis, and Lewy body–like inclusions in rotenone-treated rats, confirming dopaminergic neurotoxicity. Treatment with luteolin, imeglimin, and particularly their combination preserved neuronal morphology and reduced pathological inclusions, indicating substantial neuroprotection **[Figure 9]**.

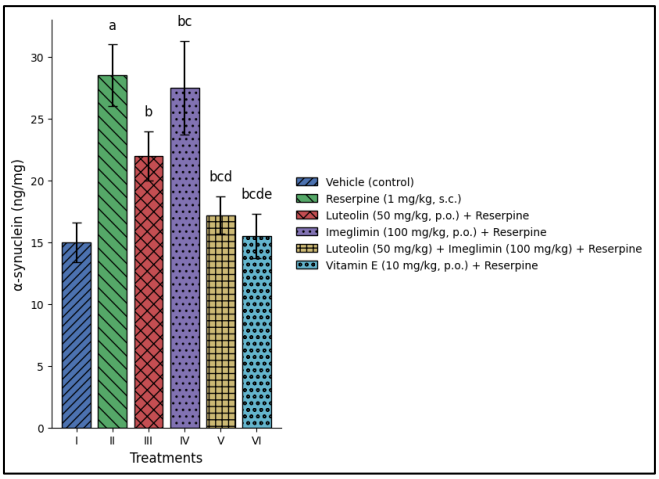


Figure 8. Effect of luteolin and imeglimin on α-Synuclein level in rat's brain.

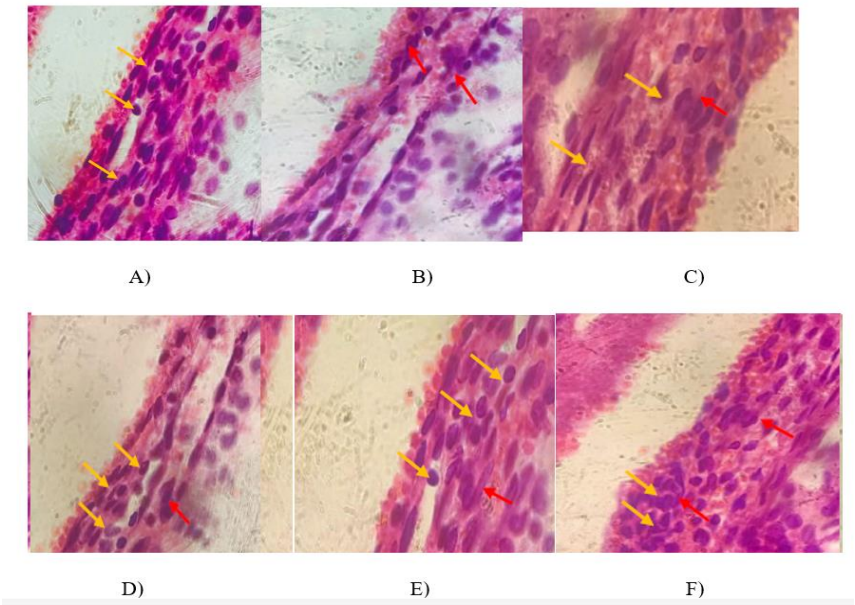


Figure 9. : Histopathological examination of reserpine induced parkinson's disease rats. All studied groups showed a closer view of the darkly pigmented dopaminergic neurons. (A) Vehicle group showed black cells are the neuromelanin- containing dopaminergic neurons (yellow arrows), (B) Reserpine group showed cluster of closely packed neurons (red arrows), severe loss of dopaminergic cells. Cells showed prominent aggregation of acidophilic plaques " Lewy bodies". (C) Luteolin (50 mg/kg,p.o.) + Reserpine showed mild cell dropout accompanied by moderate aggregation of Lewy bodies. (D) Imeglimin (100 mg/kg, p.o.) + Reserpine showed less loss of dopaminergic neurons and aggregation of lewy bodies. (E) L (50 mg/kg, p.o.+ I (100 mg/kg, p.o.) + Reserpine showed negligible or mild aggregation of closely packed neurons. (F) Vitamin E (10 mg/kg,p.o.) + Reserpine showed mild aggregation of Lewy bodies as similar to vehicle group.

3.5. Discussion

Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by dopaminergic neuronal loss in the substantia nigra pars compacta (SNpc), accompanied by oxidative stress, neuroinflammation, and α-synuclein aggregation [21, 22]. The reserpine model was used to induce Parkinson's in the rats. Reserpine significantly impaired motor function, as evidenced by increased catalepsy scores, vacuous chewing movements, and tongue protrusions, along with reduced latency in the rotarod test. These behavioral alterations mimic the extrapyramidal motor symptoms observed in human PD [23]. Reserpine was administered (1 mg/kg, s.c.) every other day for 3 days. Luteolin is a flavonoid obtained from the *Circium japonicum* plant and other sources, such as plant pigments, celery, and vegetables. Imeglimin is the

repurposed drug used in this study, an oral hypoglycemic drug that belongs to the class of triazines. In this study, the neuroprotective potential of luteolin and imeglimin was investigated in a reserpine-induced PD rat model, using both in silico docking and in vivo behavioral, biochemical, and histopathological evaluations.

In-silico docking studies were conducted to evaluate the binding potential of luteolin and imeglimin against PD-related targets. Luteolin showed strong binding to α-synuclein (–9.2 kcal/mol), TNF-α (–8.6 kcal/mol), IL-1β (–7.9 kcal/mol), IL-6 (-5.5 kcal/mol and DAT (–8.4 kcal/mol). Imeglimin exhibited moderate affinities, particularly to α-synuclein (–7.6 kcal/mol), TNF-α (–6.0 kcal/mol), IL-1β (–4.8 kcal/mol), IL-6 (-4.5 kcal/mol), and DAT (–5.7 kcal/mol). As vitamin E showed binding to α-synuclein (–9.7 kcal/mol), TNF-α (–8.2 kcal/mol), IL-1β (–6.8 kcal/mol), IL-6 (-5.7 kcal/mol), and DAT (–8.9 kcal/mol). These values were comparable to standard vitamin E. The docking confirmed their interaction with inflammatory, α-synuclein, and dopaminergic target (DAT). Toxicity predictions using Protox-II indicated LD_{50} values of 3919 mg/kg for luteolin and 2000 mg/kg for imeglimin, placing them in the low-toxicity category. The results supported their neuroprotective roles observed in vivo.

Pretreatment with luteolin and imeglimin, especially in combination, led to marked improvements in all parameters. This suggests that these agents can modulate motor deficits, potentially through dopaminergic restoration.

Oxidative stress plays a central role in PD pathology. The biochemical analysis showed that reserpine reduced the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), while increasing lipid peroxidation (LPO) and nitric oxide (NO) levels [24]. Treatment with luteolin and imeglimin significantly restored antioxidant enzyme levels and reduced oxidative markers, with the combined therapy showing synergistic effects. These findings are consistent with previous studies reporting the antioxidant and cytoprotective potential of flavonoids and mitochondrial enhancers in neurodegenerative conditions.

Neuroinflammation is another pathological contributor to PD. Luteolin, a plant-derived flavone, is known for its anti-inflammatory properties, which are mediated by inhibition of the NF-κB pathway. At the same time, imeglimin has been shown to modulate mitochondrial function and reduce inflammation in diabetic and neurological models. Elevated levels of proinflammatory cytokines, such as TNF-α and IL-1β, were observed in the reserpine group, consistent with the literature [25]. Both agents, particularly in combination, significantly reduced these inflammatory mediators.

Dopamine depletion, caused by the degeneration of dopaminergic neurons in the substantia nigra, is a core pathological feature of Parkinson's disease (PD). The dopamine transporter (DAT) regulates dopamine homeostasis, and its dysfunction contributes to PD severity. Luteolin, a natural flavonoid, has been reported to act as a DAT agonist, enhancing its activity and improving dopaminergic signaling [26]. In combination with other neuroprotective agents, luteolin upregulated DAT expression and demonstrated a synergistic effect with Imeglimin in dopamine recycling.

Importantly, α-synuclein aggregation, a hallmark of PD, was significantly upregulated by reserpine and attenuated by the treatments, which showed a synergistic effect of luteolin and imeglimin. In-silico docking studies corroborated these effects, demonstrating high binding affinity of both luteolin and imeglimin for α-synuclein and proinflammatory cytokines. These molecular interactions support the observed *in vivo* effects and indicate a potential multitarget mechanism of action.

Histopathological analysis confirmed neuronal preservation in the substantia nigra in the treated groups. Reserpine-induced neuronal loss and Lewy body-like inclusions were notably reduced in animals receiving combined therapy, indicating that luteolin and imeglimin not only alleviate behavioral and biochemical disturbances but also protect dopaminergic neurons structurally. Although immunohistochemical staining for dopaminergic markers such as tyrosine hydroxylase (TH) or dopamine transporter (DAT) was not performed, the observed preservation of neuromelanin-containing neurons, together with biochemical dopamine measurements and α-synuclein reduction, strongly supports dopaminergic neuroprotection.

The observed neuroprotective effects are likely attributable to the complementary mechanisms of action of luteolin and imeglimin. While luteolin exerts antioxidative and anti-inflammatory effects by

modulating ROS and cytokine levels, imeglimin targets mitochondrial dysfunction, a key factor in PD pathogenesis [3, 26]. This supports the hypothesis that a multi-modal approach is more effective than monotherapy for complex neurodegenerative disorders like PD.

Overall, the combination therapy showed effects comparable to or superior to those of monotherapy, highlighting its potential as an alternative or adjunctive strategy in PD management. The favorable toxicity profiles, oral bioavailability, and previous clinical use of both agents further support their translational relevance.

4. Conclusion

In this study, the neuroprotective effects of luteolin and imeglimin were evaluated in a reserpine-induced rat model of parkinson's disease. The combination of luteolin (50 mg/kg, p.o.) and imeglimin (100 mg/kg, p.o.) demonstrated significant improvements in behavioral and biochemical parameters, including reductions in vacuous chewing movements, tongue protrusions, and catalepsy, and a fall in latency. Additionally, the combination therapy showed superior efficacy in restoring dopaminergic function, reducing α-synuclein accumulation, oxidative stress, and proinflammatory cytokine levels, and improving dopaminergic neuron survival.

Histopathological analysis further confirmed the neuroprotective effects, with minimal Lewy body formation and preservation of dopaminergic neurons. These results highlight the potential of luteolin and imeglimin, particularly in combination, as effective therapeutic agents for the management of Parkinson's disease and related neurodegenerative disorders.

Overall, this study supports the clinical potential of repurposing existing drugs, such as imeglimin, and naturally derived compounds, such as luteolin, to develop novel therapeutic strategies to mitigate Parkinsonian symptoms and slow disease progression. Further studies are warranted to explore the underlying molecular mechanisms and potential clinical applications of these findings.

Ethical approval

The studies were carried out in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi (India), and were approved by the Institutional Animal Ethical Committee of M. V. P. S's College of Pharmacy (IAEC/Oct.2024/12).

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Conflict of interest

The authors declared that there is no conflict of interest regarding the publication of this paper.

Data availability

All data generated and analyzed during this study are included in the article. Any additional information supporting the findings is available from the corresponding author upon reasonable request.

Authors Contributions

Dr. Vandana S. Nade – Design, monitor, validation and preparation of manuscript
Study
Ms. Vaishanvi A, Ahire – Literature review, experimental conduct, analysis of data and preparation of manuscript
Dr. Laxman A. Kawale – Validation of data and manuscript editing
Dr. Balasaheb D. Siraskar – Experimental validation and analysis of data.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

REFRENCES

1. Balakris hnan R, Azam S, Cho DY, Su-Kim I, Choi DK. Natural phytochemicals novel therapeutic strategies to prevent and treat Parkinson's disease: current knowledge and future perspectives. Oxid Med Cell Longev (2021): 6680935.
2. Bonnet AM, Houeto JL. Pathophysiology of Parkinson's disease. Biomed Pharmacother (1999) 53(3):117-121.
3. Chagraoui A, Boulain M, Juvin L, Anouar Y, Barrière G, De Deurwaerdère P. L-DOPA in Parkinson's disease: Looking at the "false" neurotransmitters. Int J Mol Sci (2019); 21(1):294.
4. Li Y, Lou N, Liu X, Zhuang X, Chen S. Exploring new mechanisms of imeglimin in diabetes treatment: amelioration of mitochondrial dysfunction. Biomed Pharmacother (2024); 175:116755.
5. Nade VS, Ahire VA, Nikam VB, Kawale LA, Bombale PV, Patil DA, et al. Unlocking the power of phytochemicals in Parkinson's disease: bridging insights to future therapeutics. world j pharm sci (2024); 172:115195.
6. Jia Y, Liu T, Yu X, Zhang X, Wang K. Herbal medicines for Parkinson's disease: a systematic review of randomized controlled trials. Front Aging Neurosci (2012); 4:18.
7. Wang HR, Pei SY, Fan DX, Liu YH, Pan XF, Song FX, et al. Luteolin protects PC-12 cells against Aβ25-35-induced apoptosis through the ER/ERK/MAPK pathway. Evid Based Complement Alternat Med (2020): 2861978.
8. Sternberg Z, Chadha K, Lieberman A, Drake A, Hojnacki D, Weinstock-Guttman B, et al. Immunomodulatory responses of peripheral blood mononuclear cells from multiple sclerosis patients upon in vitro incubation with luteolin. J Neuroinflammation (2009); 6:28.
9. Jayawickreme DK, Ekwosi C, Anand A, Andres-Mach M, Wlaź P, Socała K, et al. Luteolin for neurodegenerative diseases: a review. Pharmacol Rep (2024); 76(4):644-68.
10. Parvathaneni V, Kulkarni NS, Muth A, Gupta V. Drug repurposing: a promising tool to accelerate drug discovery. Drug Discov Today (2019); 24(10):2076-85.
11. Pirags V, Lebovitz H, Fouqueray P. Imeglimin, a novel glimin oral antidiabetic, exhibits good efficacy and safety in type 2 diabetes. Diabetes Obes Metab (2012); 14(9):852-8.
12. Burger ME, Alves A, Callegari L, Athayde FR, Nogueira CW, Zeni G, et al. Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. Prog Neuropsychopharmacol Biol Psychiatry (2003); 27(1):135-40.
13. Kulkarni OS, Joshi SK, Thatte UM, Bhalerao S. Evaluation of toxicological and antioxidant potential of Nardostachys jatamansi in reversing haloperidol-induced catalepsy in rats. Indian J Physiol Pharmacol (2019); 63(3):236-41.
14. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. J Vis Exp (2015); (96):e52434.
15. Luck H. Methods of enzymatic analysis. Academic Press, New York (1971): 885-93.
16. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys (1959); 82(1):70-7.
17. Wills ED. Mechanism of lipid peroxide formation in animal tissues. Biochem J (1966); 99(3):667-76.
18. Kono Y. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys (1978); 186(1):189-95.
19. Gopi C, Sastry VG, Dhanaraju MD. Effect of novel phenothiazine derivatives on brain dopamine in Wistar rats. Beni Suef Univ J Basic Appl Sci (2019); 8(1):7.
20. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem (1982); 126(1):131-8.
21. Murakami H, Shiraishi T, Umehara T, Omoto S, Iguchi Y. Recent Advances in Drug Therapy for Parkinson's Disease. Internal Med (2023); 62(1):33-42.
22. Schonecker M. A strange syndrome in the oral area with application of chlorpromazine. Nervenarzt (1957); 28(12):526-8.
23. Patil RA. Piper nigrum mitigates reserpine-induced orofacial dyskinesia in Wistar rats. EC Pharmacol Toxicol (2022); 10:1-13.
24. Nade VS, Mardhekar VY, Bhoye UR, Kawale LA. Neuroprotective effect of linagliptin and celiprolol in reserpine-induced orofacial dyskinesia and rotenone neurodegeneration in rats. Int J Curr Pharm Res (2020); 12(4):63-71.
25. Naidu PS, Singh A, Kulkarni SK. Effect of Withania somnifera root extract on reserpine- induced orofacial dyskinesia and cognitive dysfunction. Phytother Res (2006); 20(2):140-6.
26. Zhang J, Liu X, Lei X, Wang L, Guo L, Zhao G, et al. Discovery and synthesis of novel luteolin derivatives as dopamine transporter agonists. Bioorg Med Chem (2010); 18(22):7842-8.

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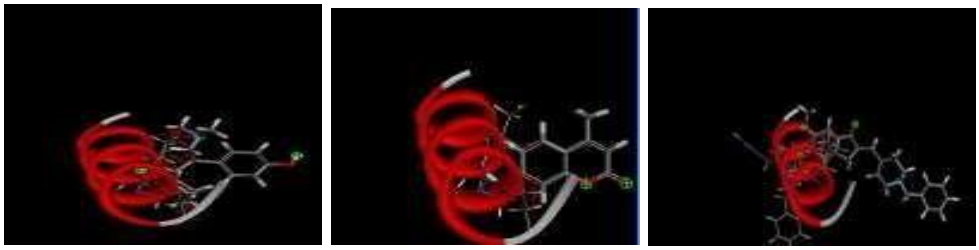

Figure S1 a) The binding interaction of luteolin, imeglimin, and vit E to the DAT protein with 3D structure respectively.

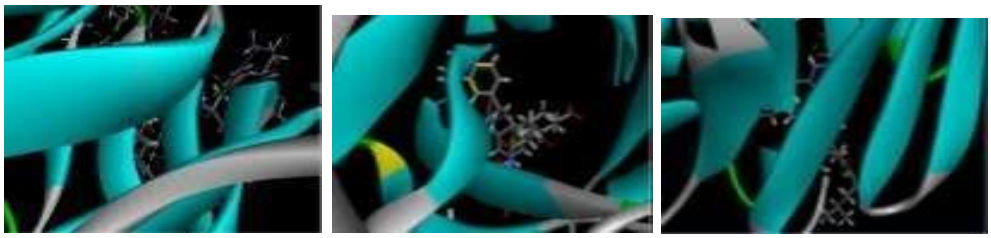

Figure S1 b) The binding interaction of luteolin, imeglimin, and vit E to the IL-1β with 3D structure respectively.

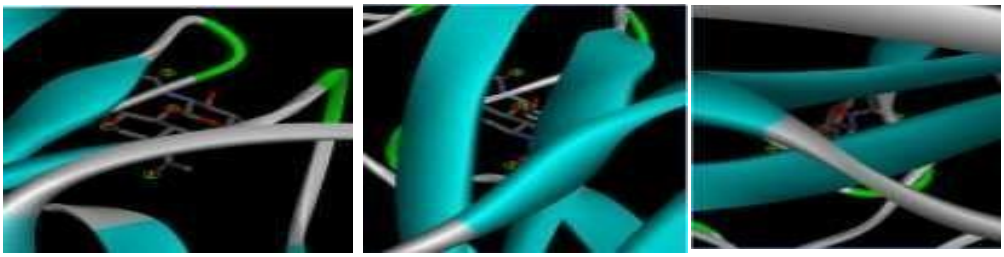

Figure S1 c) The binding interactions of luteolin, imeglimin, and vit E to the IL-6 with 3D structure respectively.

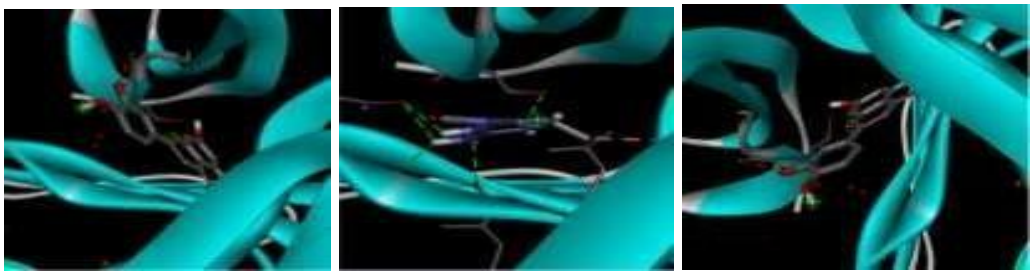

Figure S1 d) The binding interactions of luteolin, imeglimin, and vit E to the TNF-α with 3D structure respectively.

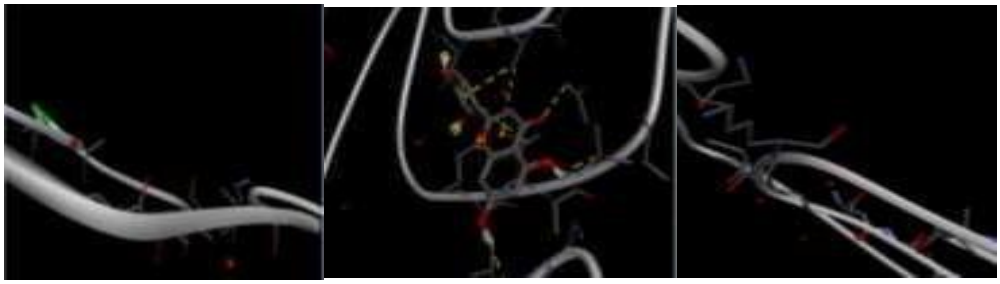

Figure S1 e) The binding interactions of luteolin, imeglimin, and vit E to the α-synuclein with 3D structure respectively.

Figure S1. The binding interactions of luteolin, imeglimin, and vit E to the targeted protein with 3D structure respectively