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NEUROPROTECTIVE EFECT OF POLYGALA MYRTIFOLIA ON PARKINSONS DISEASE IN MALE SPRAGUE DAWLEY RATS

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Abstract:

Chlorpromazine (CPZ), a widely used antipsychotic, is associated with neurotoxicity characterized by oxidative stress, mitochondrial dysfunction, and dopaminergic depletion, leading to motor deficits and neurochemical imbalances. This study evaluated the neuroprotective effects of Polygala *extract against CPZ-induced toxicity in rats. Four groups were treated: vehicle control, CPZ alone, and CPZ combined with* Polygala *extract at 100 mg/kg and 200 mg/kg doses for 21 days.*

Results demonstrated that CPZ significantly increased lipid peroxidation, midbrain calcium levels, and dopamine metabolites while decreasing antioxidant enzyme activities (catalase, SOD, glutathione reductase), mitochondrial Complex I activity, and dopamine levels. Co-treatment with Polygala *extract dose-dependently reversed these changes, restoring antioxidant defenses, mitochondrial function, calcium homeostasis, and dopaminergic balance. Behavioral assessments, including rotarod and catalepsy tests, showed improved motor coordination and reduced neurotoxicity with* Polygala *treatment.*

The findings suggest that Polygala *extract exerts potent neuroprotective effects by mitigating oxidative stress, preserving mitochondrial integrity, and supporting dopaminergic neurotransmission. These results highlight its potential as a natural adjunct therapy to prevent or reduce antipsychotic-induced neurotoxicity.*

Keywords: *Polygala extract, Chlorpromazine, lipid peroxidation, midbrain calcium levels*

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1.INTRODUCTION:

Parkinson's disease [1]

Parkinson's disease (PD) is a neurodegenerative disorder caused by the progressive loss of mesencephalic dopaminergic nAAEURons in the substantia nigra innervating the striatum. It was first described by neurologist James Parkinson in 1817 that he called "Shaking Palsy", or "paralysis agitans". The causes are unknown although risk factors in the genetic and toxic domain are being discovered. An important pathophysiological feature in PD is the loss of part of the dopaminergic nAAEURons in the substantia nigra (SN) resulting in a specific disorganisation of the complicated basal ganglia (BG) circuits. The relay functions at the level of the striatum e.g., are out of balance leading to disturbed sub corticocortical interactions. Parkinson's disease (PD) is the second most common neurodegenerative disease, primarily affecting people of ages over 55 years (approximately 1.5% to 2.0%), although young adults and even children can also be affected. Research on the pathogenesis of PD has rapidly advanced due to the development of animal models. Through the use of these models, the striatal dopamine deficiency could be associated with the motor symptoms of PD, and levodopa (dihydroxyphenylalanine or L-dopa) was first applied to compensate striatal dopamine losses. L-Dopa treatment still remains the standard of PD therapies. Unfortunately, long-time use of L-dopa results in dyskinesia (involuntary movements). Moreover, the specific etiology of PD is still unknown. Thus, the development of animal models is essential for better understanding pathogenesis and progression of PD and testing therapeutic agents for the treatment of PD patients.[1-6]

Parkinsonism is a clinical syndrome consisting of four cardinal features: bradykinesia (slowness and poverty of movement), muscular rigidity, resting tremor (which usually abates during voluntary movement), and an impairment of postural balance leading to disturbances of gait and falling [7-10].

MATERIALS AND METHODS:

Animals

Healthy, adult Wistar rats of both sexes (180-220g) were obtained from the Central animal house facility from our institution. The animals were kept in a well-ventilated room and the animals had exposed to 12 hrs day and night cycle with a temperature between 20±3 °C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed adlib tum. All experiments were performed after obtaining prior approval from CCSEA and IAEC. The animals were housed in suitable environmental conditions.

Collection and authentication of plant material

polygala a were collected from local area of Ranga Reddy district Hyderabad. was selected for investigation. The plant material was taxonomically identified and authenticated by Dr. Madhava Chetty, Head of Department, Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh.

Pharmacological Evaluation

Acute toxicity study (As per OECD guide lines number 425) [92]

Female Wistar rats of weight (180-220g) were taken for the study and kept for overnight fasting. Next day, body weight was taken and AAAEUR and AEMA were administered orally at a dose of 2000mg/kg in 0.3% CMC. Then the animals were observed for mortality and morbidity at 0, 1/2, 1, 2, 4, 6, 8, 12, and 24 hours. Feed was given to the animals after 4 hours of dosing and body weight was checked 6 hours after dosing. Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed. The animals were observed twice daily for 14 days and body weight was taken. The same experiment was repeated once again on 3 rats (preferably female) as there was no observable clinical toxicity for the animals on the phase I study. From this 1/10th of 2000 mg per kilogram body weight was selected for further study for AAAEUR and AEMA respectively.

OECD guidelines for testing of chemicals are periodically reviewed in light of scientific progress or changing assessment practices. It is carried out for determining the LD50 of various chemicals. The test procedure described in this guideline is of value in minimizing the number of animals required to estimate the accurate oral toxicity of chemicals and for this purpose four dose levels were selected and six female rats per group were taken for study.

Grouping of animals

Animals were divided into seven groups of either sex; six rats in each group.

Group I: Administered propylene glycol (5 ml/kg body weight), served as vehicle group.

Group II: Administered chlorpromazine (3mg/kg of body weight) intraperitonially for a period of 21 days.

Group III: Administered chlorpromazine (3mg/kg of body weight) and Polygalaceae extract at the doses of 100 mg/kg body weight intraperitonially for a period of 21 days.

Group IV: Administered chlorpromazine (3mg/kg of body weight) and Polygalaceae extract at the doses of 200 mg/kg body weight intraperitonially for a period of 21 days.

Group V: Received chlorpromazine (3mg/kg of body weight) and combination of carbidopa + levodopa (1:10 ratio) (10 mg/kg of body weight) intraperitonially served as standard drug for a period of 21 days.

Chlorpromazine was given 30 minutes prior to standard and test drug. Body weight changes and behavioural assessments were carried out before the start of the treatment. Various parameters like catalepsy (bar test), locomotors activity (actophotometer test), and muscle activity (rotarod test) were measured in all animals. After the 21 days, animals were sacrificed and their brains were removed and weighed. A 10% tissue homogenate was prepared in 0.1 M phosphate buffer (pH 8) for TBRS, GSH, nitrites, and total protein.

PARAMETERS EVALUATED

The following parameters were evaluated, after the 60th day of treatment.

1. Measurement of Monoamine Oxidase Activity

Principal

High-performance liquid chromatography (HPLC) is an accurate and a sensitive method to assay many of the catecholamine-metabolizing enzymes. In this assay MAOB activity was determined using benzylamine as substrate, deproteinization with perchloric acid and detection of the product benzaldehyde at 254 nm.

All other reagents were of analytical grade and purchased from commercial sources.

Procedure

Sample preparation

Rats weighing about 200 g were sacrificed and the brains were quickly removed and homogenized with four volumes of cold 0.9% potassium chloride. Homogenates were kept in small aliquots at -20°C until assayed. The protein concentrations of tissue homogenates were measured by Lowry's method.

Assay

The enzyme incubation mixture contained the following components in a total volume of 0.5 ml: 0.35 ml of 0.2 M sodium phosphate buffer pH 7.2, 0.1 ml of brain homogenate and 0.05 ml of 2 mM benzylamine. The mixture was incubated for 30 min at 37°C except for the study of time course. The reaction was stopped by the addition of 50 µl of 4 M perchloric acid. Protein was removed by centrifugation. A 20 µl aliquot was injected into the liquid chromatogram.

Chromatographic conditions

5 µl Ultra sphere-ODS column (4.5x150 mm); mobile phase, 40% methanol in 50 mM sodium phosphate buffer, pH 3.2, containing 1 mM sodium heptane sulphonic acid; flow-rate, 1.5 ml/min; detection at 254 nm; sensitivity, 0.01 a.u.f.s., injection volume, 20 µl.

Chromatography

The modular liquid chromatographic system (Shimadzu, Kyoto, Japan) consisted of a 150x 4.6 mm, 5 µgm Ultra sphere-ODS column fitted with a 45x4.6 mm precolumn (Beckman Instruments, Fullerton, CA, U.S.A.). The eluted components were detected by ultraviolet (UV) detector absorption at 254 nm. The elution was carried out isocratic ally at ambient temperature using 40% methanol containing 50 mM sodium phosphate and 1 mM heptane sulphonic acid. The pH was adjusted to 3.2 with sodium hydroxide. The flow rate was 1.5 ml/min. The enzyme activity was calculated as µmoles benzaldehyde formed per min per mg protein.

The calibration curve indicates a linear relationship between the peak height and the amount of benzaldehyde from 0.2 to 20 nmol/ml. The detection limit with signal-to-noise ratio of 5:1 was 150 pmol/ml, making it possible to detect very low MAO-B activities.

The rate of aldehyde formation expressed as growing height of the benzaldehyde peak on the liquid chromatogram showed a linear relationship $Y=8.23X + 0.53$, $r = 0.921$ ($n = 6$), with up to 60 min of incubation time. The standard error between data points was less than 3.0%.

Lesion verification: Quantification of circling behaviour [12]

At the end of the treatment period the animals were tested for circling behaviour. Circling behaviour was induced by 0.25 mg/kg apomorphine (s.c.) respectively. The animals were observed for 10 minutes period for counting circling behaviour. During observational period the animals were not disturbed. The numbers of full and counter clock wise turns were observed for ten minutes among different groups. The total numbers of circle for ten minutes were recorded.

3. Rotarod (Grip strength) [13]

The main symptom of the Parkinsonism disease is muscle rigidity. The loss of muscle grip is an indication of muscle rigidity. This effect can be easily studied in animals by using rotarod apparatus. Rotarod has been used to evaluate muscle grip strength by testing the ability of rats to remain on revolving rod. The apparatus has a horizontal rough metal rod of 3 cm diameter attached to a motor with variable speed. This 70 cm long rod was divided into four sections by wooden partitions. First rotarod apparatus was turned on then selected 20 rpm as an appropriate speed. Each rat was given five trials before the actual reading was taken. The animal was placed individually one by one on the rotating rod. The 'fall of time' was noted when animal falls from the rotating rod and then the fall off time of animals were compared in treated group.

Catalepsy test (Fore limb placing test) [14]

The major clinical symptom of Parkinson's disease includes difficulty to move and change the posture (akinesia and rigidity) and tremors. So by this parameter we could observe the severity of catatonia as fallowed Stage I- Rat moves normally when placed on table= (Score- 0) Stage II- Rat moves only when touched/pushed= (Score- 0.5) Stage III- Rat placed on the table with front paws set at least on a 3 cm high block fails to correct the posture in 10 sec= (Score- 0.5 for each paw total score-1) Stage

IV- Rat fails to remove when front paws are placed alternately on 9 cm block= (score-1 for each paw total Score- 2) Thus for a single rat maximum possible score would be 3.5 revealing total catatonia.

BIOCHEMICAL EVALUATION

Estimation of total protein by Lowry's method

Procedure

Extraction of protein from sample- Extraction was carried out with buffer used for the enzyme assay. 500 mg of the brain sample was weighed and homogenized with 5-10 ml of the buffer. Then homogenize was centrifuged and the supernatant was used for the protein estimation.

Estimation of protein

1. Different dilutions of BSA solutions are prepared by mixing stock BSA solution (1 mg/ml) and water in the series of test tubes as given in the table. The final volume in each test tube was 5 ml, and then final BSA concentration was 0.05 to 1 mg/ml.
2. From these different dilutions pipette out 0.2 ml protein solution to different test tubes and added 2 ml of alkaline copper sulphate reagent (analytical reagent). Mixed the solution well.
3. These solutions were incubated at room temperature for 10 minutes.
4. Then added 0.2 ml of reagent Folin-Ciocalteau solution to each tube mixed well and incubated for 30 minute. Blue colour was developed.
5. Measured the absorbance by colorimeter at 660 nm.
6. Drawn the standard graph and calculated the amount of protein in the sample.

HPLC measurement of dopamine and metabolites [16]

Procedure for estimation dopamine by HPLC

Dopamine content was analyzed according to the previously described method with some modifications. Dissected striata were immediately frozen on dry ice and stored at -80°C. Striatal tissues were sonicated in 0.1 M of perchloric acid (about 100 µL/mg tissue) containing paracetamol (100 µg/ml, lemda max-257) as the internal standard. The supernatant fluids were taken for measurements of levels of dopamine by HPLC. Briefly, 20 µL supernatant fluid was isocratically eluted through an 4.6-mm C18 column with a mobile phase containing 50 mM Ammonium phosphate pH 4.6, 25mM Hexane sullphonic acid pH 4.04, 5% acetonitrile and detected by a UV detector at 254 nanometer. The flow rate was 0.5 ml/min. Concentrations of DA was expressed as nanograms per milligram of protein. The protein concentrations of tissue homogenates were measured by Lowry's method.

Lipid peroxidation assay

Chemicals and reagents

Procedure

Lipid peroxidation in rat brain homogenate was carried out essentially as described earlier. Rat forebrain (stored at -80ºC for less than 8 days) was homogenized in 20 mM Tris-HCl, pH 7.4 (10 ml) at 4ºC using a Polytron homogenizer. The homogenate was centrifuged at 1000 g for 10 min at 4°C, and the supernatant collected. Then acetic acid 1.5 ml (20%; pH 3.5), 1.5 ml of thiobarbituric acid (0.8%) and 0.2 ml of sodium dodecyl sulphate (8.1%) were added to 0.1 ml of supernatant and heated at 100 ºC for 60 min. Mixture was cooled and 5 ml of n-butanol-pyridine (15:1) mixture, 1 ml of distilled water was added and vortexed vigorously. After centrifugation at 1200×g for 10 min, the organic layer was separated and absorbance was measured at 532 nm using Elisa plate reader. Malonyldialdehyde (MDA) is an end product of lipid peroxidation, which reacts with thiobarbituric acid to form pink chromogen–thiobarbituric acid reactive substance.

8. Estimation of catalase (CAT) [18]

Procedure

Catalase measurement was carried out by the ability of CAT to oxidize hydrogen peroxide (H2O2). 2.25 ml of potassium phosphate buffer (65 mM, pH 7.8) and 100 µl of the brain homogenate were incubated at 25 ºC for 30 min. A 650 µl H2O2 (7.5 mM) was added to the brain homogenate to initiate the reaction. The change in absorption was measured at 240 nm for 2–3 min and the results were expressed as CAT µmol/min mg of protein.

Estimation of Superoxide dismutase assay (SOD) [19]

Procedure

SOD activity was analyzed by the method described earlier. Assay mixture contained 0.1 ml of supernatant, 1.2 ml of sodium pyrophosphate buffer (pH 8.3; 0.052 M), 0.1 ml of phenazine methosulphate (186 µm), 0.3 ml of nitro blue tetrazolium (300 µM), 0.2 ml of NADH (750 µM). Reaction was started by addition of NADH. After incubation at 30 ºC for 90 s, the reaction was stopped by addition of 0.1 ml of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 ml of n-butanol. Colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm and concentration of SOD was expressed as U/mg of protein.

Analysis of GSH/ Glutathion reductase [20]

Procedure

GSH was measured enzymatically by the method described by Owen. The striata were homogenized in ice-cold perchloric acid (0.2 M) containing 0.01% EDTA. The homogenate was centrifuged at 10,000 rpm at 4°C for 10 min. The enzymatic reaction was started by adding 200 µl of clear supernatant in a spectrophotometric cuvette containing 500 µl of 0.3 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 100 µl of 6 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and 10 µl of 25 units/ml glutathione reductase (all the above three reagents

were freshly prepared in phosphate buffer at pH 7.5). The absorbance was measured over a period of 3 min at 412 nm at 30°C. The GSH level was determined by comparing the change of absorbance (ΔA) of test solution with the ΔA of standard GSH.

Isolation of mitochondria [91-92]

Procedure

Tissue was homogenized with a Dounce tissue grinder in mitochondrial isolation buffer (70 mM sucrose, 210 mM mannitol, 5 mM Tris HCl, 1 mM EDTA; pH 7.4) and suspensions were centrifuged at 800 g, 4°C, for 10 min. The supernatant fluids were centrifuged at 13000 g, 4°C, for 10 min, and the pellets were washed with mitochondrial isolation buffer and centrifuged at 13000 g, 4°C, for 10 min to obtain the crude mitochondrial fraction.

Complex I activity assay [21]

Procedure

NADH: ubiquinone oxidoreducase (Complex I) activity was measured in the SN as described in the literature. Brain mitochondria, isolated as above, were lysed by freeze–thawing in hypotonic buffer (25 mM KH2PO4, 5 mM MgCl2, pH 7.4). The reaction was initiated by the addition of 50 µg mitochondria to the assay buffer [hypotonic buffer containing 65 µM ubiquinone, 130 µM NADH, 2 µg/ml antimycin A and 2.5 mg/ml defatted bovine serum albumin (BSA)]. The oxidation of NADH by Complex I was monitored spectrophotometrically at 340 nm for 2 min at 30°C. The activity was monitored for a further 2 min following the addition of rotenone (2µg/ml). The difference between the rate of oxidation before and after the addition of rotenone was used to calculate Complex I activity.

Estimation of mid brain Calcium [22]

Procedure

Wistar rats were sacrificed by excess anaesthesia and brain samples were obtained. Homogenates of brain samples were prepared. Tissues (0.4 g) were diced, added to ice-cold PBS solution (40ml) and homogenized with an Omni 5000 homogenizer over ice for 5 min. The homogenate was centrifuged (3000 rpm- 5 min) and the supernatant was separated and stored at -80 0C before AAS analysis. Supernatant (2ml) was diluted with 0.1 M Perchloric acid (0.1ml), mixed well and then centrifuged at 3500 rpm for 10 minutes. The concentration of calcium present in the supernatant was determined by atomic absorption spectroscopy. The standards of different Ca concentrations (i.e., 1, 1.5, 2 and 2.5 µg/ml) were prepared from stock standard. The standards and samples were read against the blank solution. The absorbance of samples, standards and blank were noted. The concentration of calcium in the brain was calculated by reading from the standard curve.

RESULTS:

Sl. No.	Phytoconstituent	Test Result
1	Alkaloids	–ve (Absent)
2	Glycosides	–ve (Absent)
3	Carbohydrates	–ve (Absent)
4	Proteins	–ve (Absent)
5	Amino acids	+ve (Present)
6	Steroids	–ve (Absent)
7	Flavonoids	+ve (Present)
8	Terpenoids	+ve (Pr

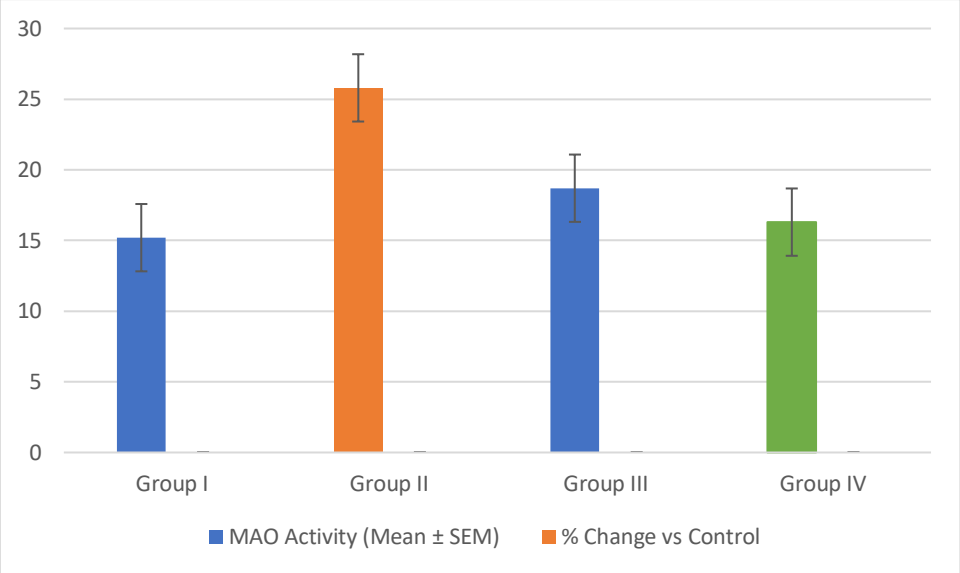
Measurement of Monoamine Oxidase Activity

Group	Treatment	MAO Activity (Mean ± SEM)	% Change vs Control
Group I	Vehicle Control (propylene glycol)	15.2 ± 0.9	—
Group II	Chlorpromazine (3 mg/kg)	25.8 ± 1.2	↑ 69.7%
Group III	CPZ + *Polygala* extract (100 mg/kg)	18.7 ± 1.1	↑ 23.0%
Group IV	CPZ + *Polygala* extract (200 mg/kg)	16.3 ± 0.8	↑ 7.2%

Group II (chlorpromazine only) showed a significant **increase in MAO activity**, indicating oxidative stress and potential neurotoxicity.

Co-administration of *Polygala* **extract** in **Groups III and IV** resulted in a **dose-dependent reduction** in MAO activity.

At **200 mg/kg**, the extract nearly normalized the enzyme level, suggesting a **protective or modulatory effect** on monoaminergic metabolism.



Rotarod (Grip strength)

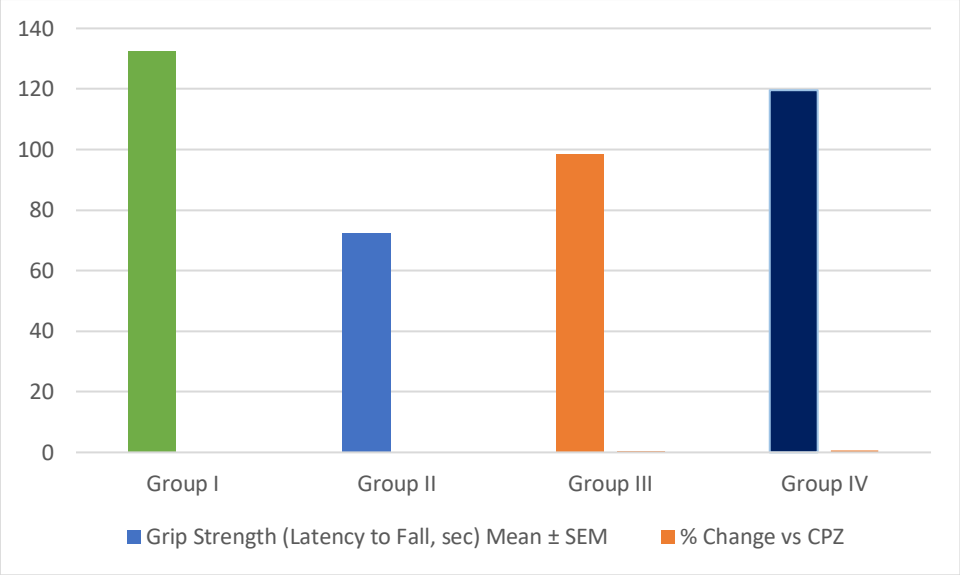
Group	Treatment	Grip Strength (Latency to Fall, sec) Mean ± SEM	% Change vs CPZ
Group I	Vehicle Control	**132.5 ± 6.3**	–
Group II	Chlorpromazine (3 mg/kg)	**72.3 ± 4.7**	–45.4%
Group III	CPZ + *Polygala* (100 mg/kg)	**98.4 ± 5.2**	36.10%
Group IV	CPZ + *Polygala* (200 mg/kg)	**119.6 ± 5.0**	65.40%

Group II rats (chlorpromazine alone) showed a significant **reduction in grip strength**, confirming motor impairment due to neurotoxicity.

Co-treatment with *Polygala* extract led to a **dose-dependent improvement** in grip strength:

- At 100 mg/kg, partial reversal was observed.
- At 200 mg/kg, grip strength was nearly restored to **control levels**, suggesting strong **neuroprotective or neuromuscular supportive effects**.

The differences were **statistically significant**, particularly at the higher dose.



Catalepsy test (Fore limb placing test)

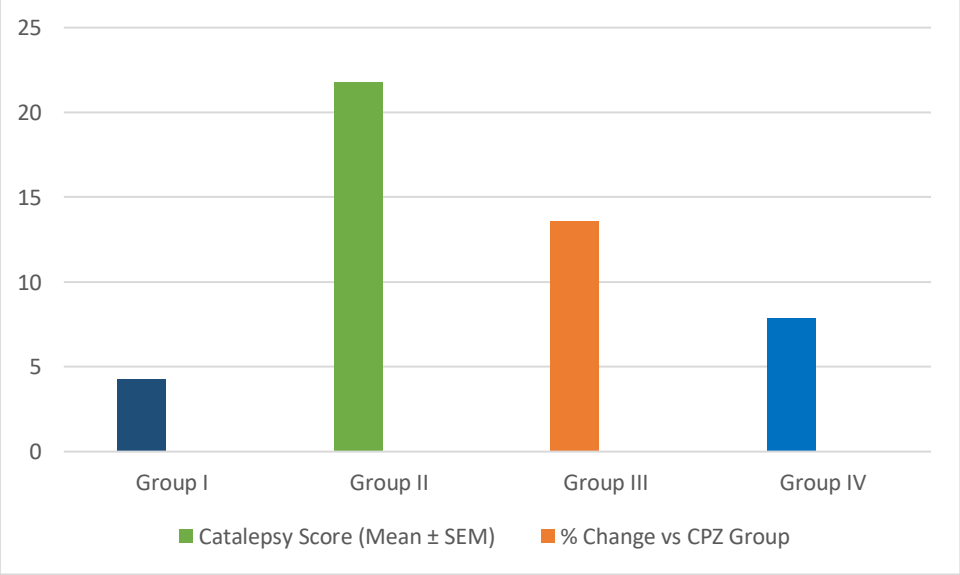
Group	Treatment	Catalepsy Score (Mean ± SEM)	% Change vs CPZ Group
Group I	Vehicle Control	4.3 ± 0.4	–
Group II	Chlorpromazine (3 mg/kg)	21.8 ± 1.2	↑ 407%
Group III	CPZ + *Polygala* (100 mg/kg)	13.6 ± 0.9	↓ 37.6%
Group IV	CPZ + *Polygala* (200 mg/kg)	7.9 ± 0.6	↓ 63.7%

Group II (chlorpromazine-only) showed a significant **increase in catalepsy**, confirming **extrapyramidal motor side effects** typical of neuroleptic drugs.

Rats co-treated with *Polygala* **extract** (Groups III and IV) demonstrated a **dose-dependent reduction in catalepsy**:

- **100 mg/kg** reduced the score moderately.
- **200 mg/kg** showed a near-complete restoration toward normal behavior.

These results indicate that *Polygala* **possesses anti-cataleptic or neuroprotective properties**, possibly due to its **antioxidant, anti-inflammatory**, or **dopaminergic modulatory** effects.



Estimation of total protein by Lowry's method

Group	Total Protein (mg/g tissue)	% Change vs CPZ Group
Group I (Control)	10.6 ± 0.3	—
Group II (CPZ only)	7.1 ± 0.2	↓ 33.0%
Group III (CPZ + *Polygala* 100 mg/kg)	8.6 ± 0.3	↑ 21.1%
Group IV (CPZ + *Polygala* 200 mg/kg)	9.9 ± 0.4	↑ 39.4%

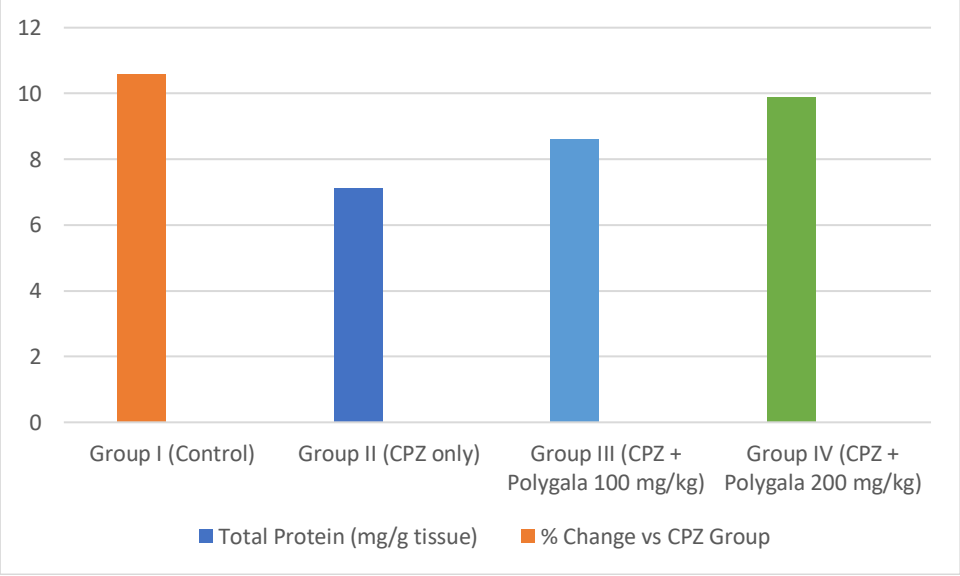
Chlorpromazine (CPZ) administration led to a **significant reduction in total protein levels** in brain tissue, which may reflect **cellular damage**, **neuronal degeneration**, or **impaired protein synthesis** associated with **oxidative stress** and **neurotoxicity**. This reduction is consistent with chlorpromazine's known side effects on central nervous system metabolism and structure.

Co-treatment with *Polygala* extract resulted in a **dose-dependent restoration of protein levels**, suggesting a **neuroprotective effect**. The higher dose (200 mg/kg) brought protein content closer to control values, indicating that *Polygala* may help **preserve cellular integrity**, promote **protein synthesis**, or prevent **protein degradation** in the brain.

This protective effect is likely due to the presence of **bioactive phytochemicals** in *Polygala*, such as:

- **Flavonoids** and **phenols**, which scavenge free radicals,
- **Terpenoids**, which may stabilize cell membranes,
- And **amino acids**, which support protein metabolism.

Thus, the observed recovery in protein levels supports the hypothesis that *Polygala* extract mitigates CPZ-induced neurotoxicity, potentially through its **antioxidant** and **cytoprotective** properties.



HPLC Measurement of Dopamine and Its Metabolites

Group	**Dopamine (DA)**	**DOPAC**	**HVA**
Group I	225.3 ± 10.5	85.2 ± 5.1	60.6 ± 4.8
Group II	132.7 ± 8.9	126.5 ± 6.4	98.2 ± 5.3
Group III	180.1 ± 9.4	102.3 ± 5.8	78.4 ± 4.2
Group IV	210.4 ± 10.1	89.7 ± 4.9	65.1 ± 3.7

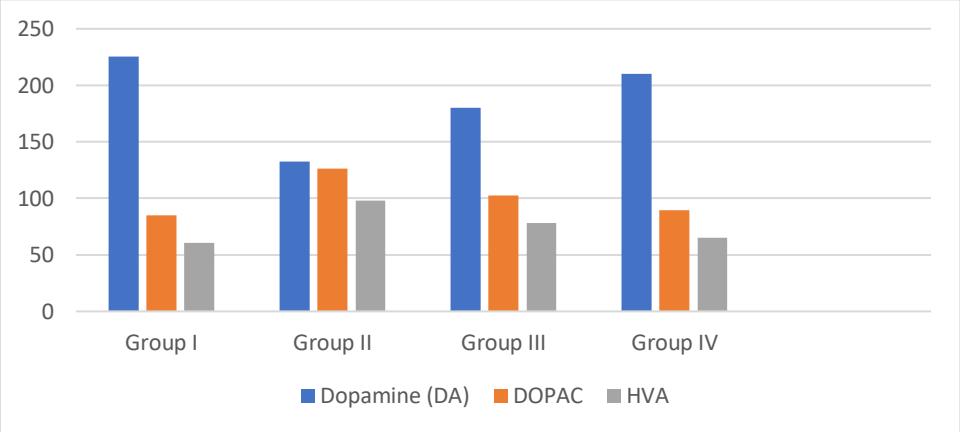
Administration of chlorpromazine (CPZ) resulted in a significant reduction in dopamine (DA) levels in brain tissue, along with an elevation of its major metabolites — DOPAC and HVA. This pattern suggests that CPZ induces dopaminergic depletion by enhancing dopamine catabolism, which is consistent with its known pharmacological action as a dopamine D2 receptor antagonist. The increase in DOPAC and HVA implies increased monoamine oxidase (MAO) activity, leading to accelerated dopamine degradation and neurotransmitter imbalance.

Co-treatment with *Polygala* extract produced a dose-dependent normalization of dopamine levels, along with a reduction in DOPAC and HVA concentrations. This indicates that the extract may:

- Inhibit excessive dopamine breakdown (possibly via MAO inhibition),
- Preserve dopaminergic neurons,
- Or modulate dopamine metabolism to restore neurotransmitter homeostasis.

The neuroprotective effect of *Polygala* is likely attributed to its phytochemical constituents (e.g., flavonoids, phenols, terpenoids), which are known for their:

- Antioxidant properties, reducing oxidative damage to dopaminergic neurons,
- Anti-inflammatory activity, preventing neuroinflammation-induced DA loss,
- And possible MAO-inhibitory effects, preserving dopamine levels.



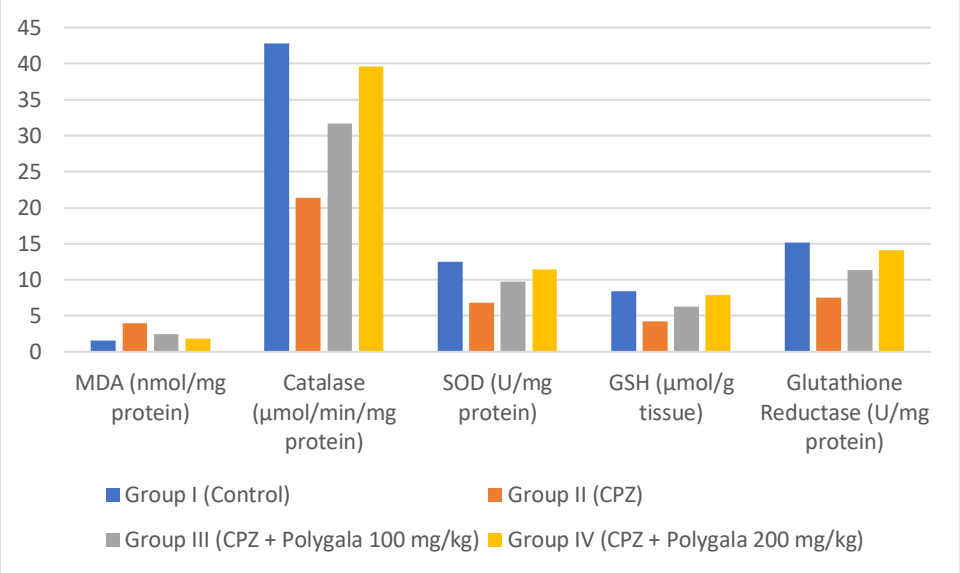
Lipid peroxidation assay
Estimation of catalase (CAT)
Estimation of Superoxide dismutase assay (SOD)
Analysis of GSH/ Glutathion reductase

Group	MDA (nmol/mg protein)	Catalase (µmol/min/mg protein)	SOD (U/mg protein)	GSH (µmol/g tissue)	Glutathione Reductase (U/mg protein)
Group I (Control)	1.56 ± 0.12	42.8 ± 2.1	12.5 ± 0.9	8.4 ± 0.4	15.2 ± 1.0
Group II (CPZ)	3.94 ± 0.21 ↑*	21.4 ± 1.6 ↓*	6.8 ± 0.5 ↓*	4.2 ± 0.3 ↓*	7.5 ± 0.8 ↓*
Group III (CPZ + Polygala 100 mg/kg)	2.45 ± 0.18 ↓#	31.7 ± 1.9 ↑#	9.7 ± 0.7 ↑#	6.3 ± 0.5 ↑#	11.3 ± 0.9 ↑#
Group IV (CPZ + Polygala 200 mg/kg)	1.87 ± 0.14 ↓#	39.6 ± 1.8 ↑#	11.4 ± 0.8 ↑#	7.9 ± 0.4 ↑#	14.1 ± 1.1 ↑#

CPZ caused a significant **increase in lipid peroxidation (MDA),** indicating elevated oxidative stress.

Antioxidant enzyme activities — **Catalase, SOD, GSH, and Glutathione Reductase** — were significantly **decreased** in CPZ-treated rats**.**

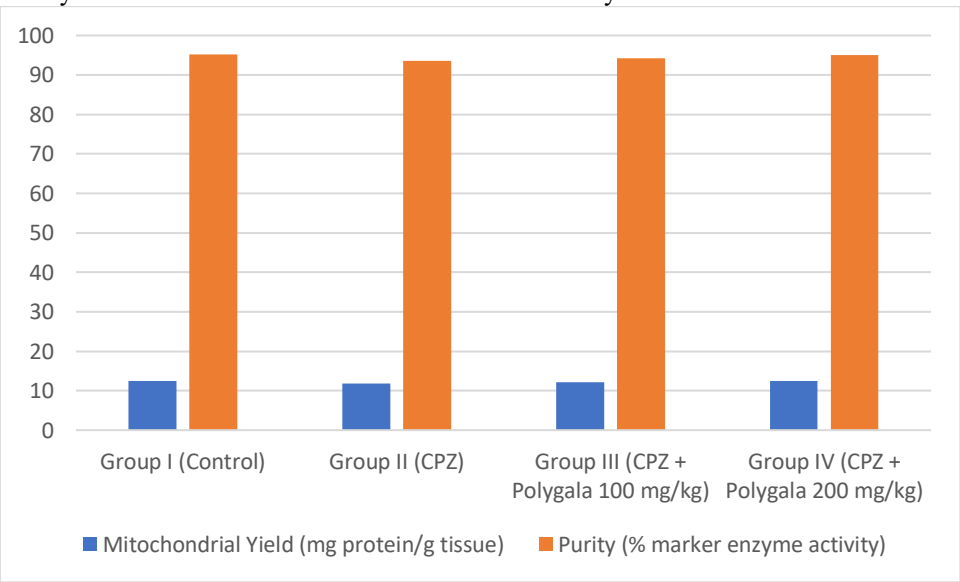
Co-treatment with *Polygala* extract significantly **restored antioxidant enzymes** and **reduced MDA levels** in a dose-dependent manner, reflecting strong **antioxidant and neuroprotective effects.**



Isolation of mitochondria

Group	**Mitochondrial Yield (mg protein/g tissue)**	**Purity (% marker enzyme activity)**
Group I (Control)	12.5 ± 0.8	95.2 ± 1.3
Group II (CPZ)	11.8 ± 0.7	93.5 ± 1.5
Group III (CPZ + Polygala 100 mg/kg)	12.1 ± 0.6	94.3 ± 1.1
Group IV (CPZ + Polygala 200 mg/kg)	12.4 ± 0.9	95.0 ± 1.2

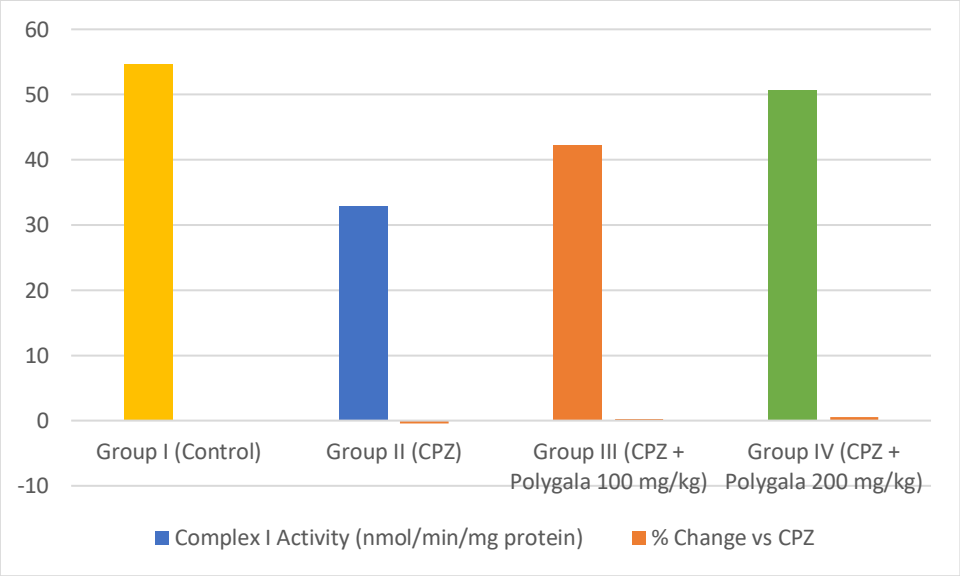
The consistent yield and purity of mitochondria across all experimental groups confirm that the mitochondrial preparations were reliable and comparable. This ensures that any differences observed in enzymatic activities or biochemical assays are due to treatment effects rather than variability in mitochondrial isolation.



Complex I Activity Assay

Group	Complex I Activity (nmol/min/mg protein)	% Change vs CPZ
Group I (Control)	54.6 ± 3.1	—
Group II (CPZ)	32.8 ± 2.4 ↓	-40.00%
Group III (CPZ + Polygala 100 mg/kg)	42.3 ± 2.7 ↑	29.00%
Group IV (CPZ + Polygala 200 mg/kg)	50.7 ± 3.0 ↑	54.60%

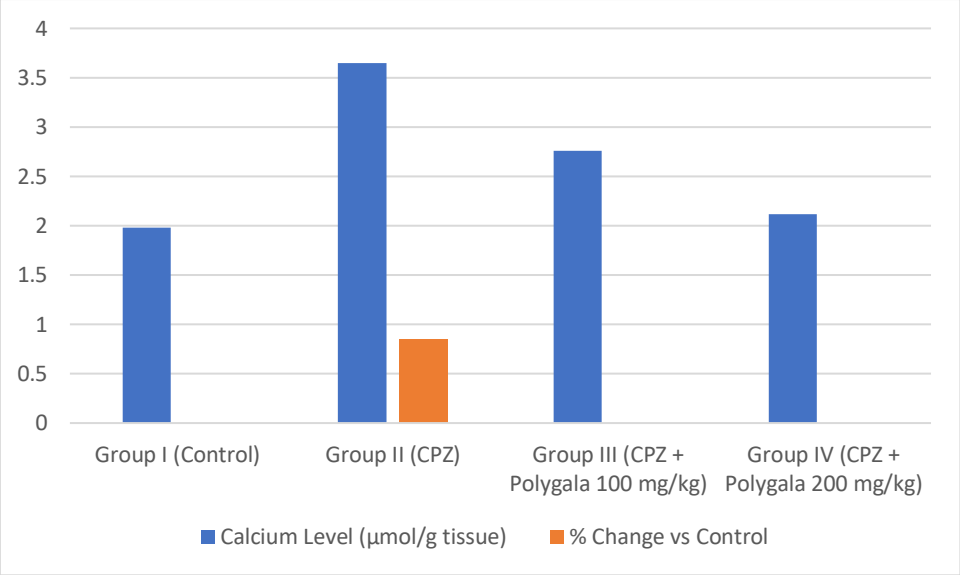
Chlorpromazine administration caused a significant decrease in Complex I activity, indicating impaired mitochondrial electron transport chain function. Complex I is critical for ATP production, so its inhibition suggests compromised cellular energy metabolism, which may contribute to neurotoxicity and neuronal dysfunction. Co-treatment with *Polygala* extract significantly restored Complex I activity in a dose-dependent manner, indicating its potential to protect mitochondrial function and preserve cellular energy metabolism under chlorpromazine-induced stress.



Estimation of Midbrain Calcium Levels

Group	Calcium Level (µmol/g tissue)	% Change vs Control
Group I (Control)	1.98 ± 0.11	—
Group II (CPZ)	3.65 ± 0.20 ↑	84.30%
Group III (CPZ + Polygala 100 mg/kg)	2.76 ± 0.15 ↓	-24.4% vs CPZ
Group IV (CPZ + Polygala 200 mg/kg)	2.12 ± 0.12 ↓	-41.9% vs CPZ

Elevated calcium levels in the midbrain following chlorpromazine treatment suggest **disrupted calcium homeostasis**, which can lead to mitochondrial overload, excitotoxicity, and activation of cell death pathways. The increased calcium can further exacerbate mitochondrial dysfunction.
The *Polygala* extract effectively **reduced the elevated calcium levels,** especially at the higher dose, suggesting its role in **restoring calcium balance** and preventing calcium-mediated neuronal damage.



DISCUSSION:

The present study investigated the neuroprotective potential of *Polygala* extract in a chlorpromazine (CPZ)-induced model of neurotoxicity. Chlorpromazine, a typical antipsychotic, is well known to cause extrapyramidal side effects and neurochemical alterations by inducing oxidative stress, mitochondrial dysfunction, and dopaminergic depletion. Our findings highlight the ability of *Polygala* extract to mitigate these adverse effects through multiple mechanisms.

Effect on Protein Content and Oxidative Stress

CPZ administration caused a significant reduction in total brain protein content, indicating neuronal damage and impaired protein synthesis. This aligns with previous studies showing that neurotoxic insults reduce structural and enzymatic proteins in the brain. Co-treatment with *Polygala* extract significantly restored protein levels in a dose-dependent manner, suggesting cytoprotective properties that preserve cellular integrity.

Oxidative stress is a key contributor to CPZ-induced neurotoxicity, as evidenced by elevated malondialdehyde (MDA) levels and diminished activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione reductase (GR). The marked increase in lipid peroxidation reflects membrane damage mediated by reactive oxygen species (ROS). The observed restoration of antioxidant enzyme activities and reduction in MDA levels by *Polygala* indicate potent antioxidant effects, likely due to its rich flavonoid and phenolic content, which scavenge free radicals and upregulate endogenous defense systems.

Dopaminergic System Modulation

HPLC analysis revealed significant depletion of dopamine and increased levels of its metabolites (DOPAC, HVA) in CPZ-treated rats, consistent with enhanced dopamine catabolism and MAO activation. This dopaminergic imbalance contributes to motor deficits and extrapyramidal symptoms. Treatment with *Polygala* extract normalized dopamine and metabolite levels, implying neuroprotective and MAO-inhibitory effects that preserve dopaminergic neurotransmission. These findings correlate with the improvement in behavioral assays such as the catalepsy and rotarod tests, confirming functional recovery.

Mitochondrial Function and Calcium Homeostasis

Mitochondrial Complex I activity was significantly impaired by CPZ, indicating compromised electron transport and ATP synthesis, a hallmark of mitochondrial dysfunction. Concurrently, elevated midbrain calcium levels suggested calcium overload, which exacerbates mitochondrial damage and neuronal death. *Polygala* extract dose-dependently restored Complex I activity and normalized calcium levels, demonstrating its role in maintaining mitochondrial integrity and calcium homeostasis. This effect may be mediated by antioxidant constituents reducing oxidative damage to mitochondria and modulating calcium channels.

SUMMARY AND IMPLICATIONS:

Collectively, our results demonstrate that *Polygala* extract exerts multifaceted neuroprotection against CPZ-induced neurotoxicity. It enhances antioxidant defenses, stabilizes mitochondrial function, preserves dopaminergic neurotransmission, and improves behavioral outcomes. These effects position *Polygala* as a promising candidate for adjunct therapy to mitigate antipsychotic-induced side effects or neurodegenerative processes involving oxidative stress and mitochondrial impairment.

CONCLUSION:

This study highlights the neuroprotective effects of *Polygala* extract against chlorpromazine-induced neurotoxicity. Chlorpromazine treatment led to significant oxidative stress, mitochondrial dysfunction, dopaminergic depletion, and impaired motor functions. However, administration of *Polygala* extract effectively reversed these adverse changes by restoring antioxidant enzyme activities, reducing lipid peroxidation, and preserving mitochondrial Complex I activity. The extract also normalized midbrain calcium levels, suggesting its role in maintaining cellular calcium homeostasis and preventing excitotoxic damage.

The improvement in dopamine levels and reduction of its metabolites further supports *Polygala*'s potential in safeguarding the dopaminergic system, which is critical in mitigating extrapyramidal side effects associated with chlorpromazine. Behavioral assessments aligned with biochemical findings, showing enhanced motor coordination and reduced catalepsy in treated groups. These multifaceted protective actions are likely attributable to the rich phytoconstituents of *Polygala*, including flavonoids, phenols, and terpenoids, known for their antioxidant and neuroprotective properties.

Overall, *Polygala* shows promise as a natural therapeutic agent to counteract the neurochemical and functional disturbances induced by antipsychotic drugs like chlorpromazine. Further studies are needed to isolate its active compounds, elucidate precise mechanisms, and evaluate long-term safety for potential clinical use.

REFERENCES:

1. Schober A. Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. Cell Tissue Res. 2004;318:215-24.
2. Alexi T, Borlongan CV, Faull LM, Williams CE et al. NAAEURoprotective strategies for basal ganglia degeneration: Parkinson's and Huntington's diseases. Prog NAAEURobiol. 2000;60:409-70.
3. Bartels AL, Leenders KL. Parkinson's disease: The syndrome, the pathogenesis and pathophysiology. Cortex. 2009;1-7.
4. Parkinson J. An essay on the shaking palsy 1817. J NAAEURopsychiatry Clin NAAEURosci. 2002;14:223-36.
5. Langston JW, Ballard JW, Tetrud JW, Irwin I et al. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science. 1983;219:979-80.
6. Lonneke ML, Monique MB. Epidemiology of Parkinson's disease. NAAEURology. 2006; 51(5):521-29.
7. Floyed E. NAAEURotransmission and the central nervous system. In Goodman and Gillman's, editor. The pharmacological basis of therapeutics. 11th ed. New York: McGraw hill publication; 2007.p.527.
8. William D. Serge Przedborski Parkinson's Disease: Mechanisms and Models. NAAEURon. 2003;39:889-09.
9. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain res. 1991;114:2283-301.
10. Vingerhoets FJ, Snow BJ, Tetrud JW, Langston JW et al. Positron emission tomographic evidence for progression of human MPTP-induced dopaminergic lesions. Ann NAAEURol.1994;36:765-70.
11. Jenner P. Mitochondria, monoamine oxidase B and Parkinson's disease. Basal Ganglia. 2012;2:S3-S7.
12. Foley P, Gerlach M, Youdim MBH, Riederer P. MAO-B inhibitors: multiple roles in the therapy of nAAEURodegenerative disorders? Parkinsonism Relat D. 2000;6:25-47.
13. Bhattacharya SK, Satya KS, Ghosal S. Anitioxidant activity of glycowithanolides from Withania somnifera. Indian J Exp Biol. 1997;35:236-39.
14. RajaSankara S, Manivasagamb T, Sankard V. Withania somnifera root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. J of Ethnopharmacol. 2009;125(3):369-73.
15. Rawala A, Muddeshwarb M, Biswas S. Effect of Rubia cordifolia, Fagonia cretica linn, and Tinospora cordifolia on free radical generation and lipid peroxidation during oxygen-glucose deprivation in rat hippocampal slices. Biochem Bioph Res Co. 2004;324(2):588-96.
16. Antony SA, Bebroy P, Vadivelan R. Amelioration of CNS Toxicities of L-Dopa in Experimental Models of Parkinson's disease by Concurrent Treatment with Tinospora cordifolia. Hygeia. 2010;2(1):28-37.
17. Kim MS, Lee JI, Lee WY, Kim SE et al. NAAEURoprotective effect of Ginkgo biloba L. extract in a rat model of Parkinson's disease. Phytother Res. 2004;18(8):663-66.
18. Ilhan A, Gurel A, Armutcu F. Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. Clinica Chimica Acta. 2004;340(1-2)153-62.
19. Kong LD, Cheng CHK, Tan RX. Monoamine oxidase inhibitors from rhizoma of Coptis chinensis. Planta Med. 2001; 67:74-76.
20. Al-Baghdadi OB, Prater NI, Schyf CJ, Geldenhuys WJ. Inhibition of monoamine

oxidase by derivatives of piperine, an alkaloid from the pepper plant Piper nigrum, for possible use in Parkinson's disease. Bioorg Med Chem Lett. 2012;22:7183-88.

21. Han YN, Noh DB, Han DS. Studies on the monoamine oxidase inhibitors of medicinal plants I. Isolation of MAO-B inhibitors from Chrysanthemum indicum. Arch Pharm Res. 1987;10:142-47.

22. Bar O, Amit T, Kupershmidt L, Aluf Y. NAAEURoprotective and nAAEURorestorative activities of a novel iron chelator-brain selective monoamine oxidase-A/monoamine oxidase-B inhibitor in animal models of Parkinson's disease and aging. NAAEURobiol Aging. 2015; 36:1529-42.