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Research Article

NEURORESTORATIVE, ANTIOXIDANT, AND NEUROPROTECTIVE ACTIVITY OF CUCUMIS MELO L. VAR RETICULATUS PEEL AGAINST DOPAMINERGIC NEURODEGENERATION IN CHLORPROMAZINE INDUCED ANIMAL MODEL OF PARKINSON'S DISEASE

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Abstract:

Parkinson's disease is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to motor impairments such as tremors, rigidity, and bradykinesia. Oxidative stress and depletion of endogenous antioxidants play a major role in the pathogenesis of this disorder. The present study was undertaken to evaluate the neurorestorative, antioxidant, and neuroprotective activity of the methanolic peel extract of *Cucumis melo* L. var. *reticulatus* against dopaminergic neurodegeneration in a Chlorpromazine-induced animal model of Parkinsonism. Phytochemical screening of the extract revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, and polyphenols. Acute toxicity studies conducted according to OECD guidelines indicated that the extract was safe up to 2000 mg/kg. Parkinsonian symptoms were induced in rats by chronic administration of chlorpromazine for 21 days. Behavioral parameters including catalepsy (bar test), locomotor activity (Actophotometer test), and motor coordination (rotarod test) were evaluated to assess the antiparkinsonian activity of the extract. In addition, biochemical parameters such as thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and nitrite levels were measured to determine oxidative stress in brain tissue. The results demonstrated that chlorpromazine administration significantly increased catalepsy and oxidative stress while decreasing locomotor and muscular activity. Treatment with methanolic extract of *Cucumis melo* var. *reticulatus* peel at doses of 100 mg/kg and 200 mg/kg significantly reduced cataleptic behavior, improved locomotor and motor coordination, and restored antioxidant levels by decreasing lipid peroxidation and increasing glutathione levels in brain tissue. The methanolic peel extract of *Cucumis melo* var. *reticulatus* exhibited significant neuroprotective and antioxidant activity against chlorpromazine-induced Parkinsonism in rats. The observed effects may be attributed to the presence of flavonoids and polyphenolic compounds that help reduce oxidative stress and protect dopaminergic neurons. These findings suggest that *Cucumis melo* peel may serve as a promising natural source for the development of therapeutic agents for the management of Parkinson's disease.

Keywords: Parkinson's disease, *Cucumis melo*, chlorpromazine, neurodegeneration

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INTRODUCTION:

Parkinson's disease (PD) or paralysis agitans is first described by James Parkinson (1817) as 'shaking palsy'. It is a multicentric neurodegenerative disorder, characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and degeneration of nondopaminergic systems such as noradrenergic, serotonergic, and cholinergic systems [1]. The cardinal motor symptoms include bradykinesia (slowness of movement), rigidity, resting tremors (stiffness in the muscle), and dyskinesia (impaired movement), whereas non-motor symptoms such as depression, dementia, sleep deprivation, and autonomic failure appear in advanced stages of PD [2]. PD is recognized as the second most common neurodegenerative disorder after Alzheimer's disease with a prevalence of 0.1% of the global population (WHO 2004). The major concern is due to the lack of early diagnosis and prognosis techniques, the cardinal clinical symptoms of PD only appear after the loss of at least 50% of dopaminergic neurons in the substantia nigra (SN) pars compacta and concomitant reduction of $\approx 80\%$ dopamine in the striatum [3,4] which permits very limited treatment options against irreversible dopaminergic neuronal damage.

Furthermore, the etiology and pathogenesis of PD were suggested to be multi-factorial with an intricate combination of genetic and environmental components. In this context, a better understanding of the convoluted mechanisms involved in PD progression has come from 1- methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (CHLORPROMAZINE) neurotoxin which has become the gold standard model in PD research [5]. Extensive pre-clinical and clinical research has revealed that there is an intriguing association between neurotransmitter dysfunction, inflammation, glutamatergic toxicity, mitochondrial impairment, elevation of iron as well as nitric oxide levels and imbalance in antioxidants/pro-oxidants status in the disease progression [6, - 8]. Among the various proposed hypotheses for the etiology of PD, oxidative stress remains the strongest leading theory for PD progression and several pre-clinical and clinical studies have demonstrated the direct impact of oxidative stress on PD pathogenesis [9]. Dopaminergic neuronal cells of SNpc are particularly more vulnerable to oxidative damage due to the presence of high concentrations of iron and low concentrations of endogenous antioxidants relative to the other brain regions [10].

Moreover, the dual production of reactive oxygen species (ROS) derived from impaired mitochondria and dopamine (DA) metabolism, further exacerbates the oxidative stress-mediated cellular damage and thus DA degeneration [11]. Furthermore, ROS

generation through dopamine metabolism leads to the auto-oxidation of DA with subsequent production of di-hydroxy phenylacetic acid, DA quinone, and H_2O_2 [12]. These metabolites further disrupt redox homeostasis through glutathione depletion and highly destructive hydroxyl radicals which are produced from excessive hydrogen peroxide (H_2O_2) via Fenton reaction [13,14] resulting in oxidative modifications of almost all cellular macromolecules and thus leading to dopaminergic cell death [13-17]. Meanwhile, DA metabolism is governed by the monoamine oxidase B (MAO-B) enzyme, which catalyzes the oxidative deamination of DA in the substantia nigra and striatum and forms H_2O_2 as the major by-product. In addition, MAO-B is also involved in the conversion of CHLORPROMAZINE to MPP⁺ (1-methyl-4-phenylpyridinium) neurotoxic metabolite and it is quite evident that H_2O_2 and MPP⁺ exert cytotoxicity over dopaminergic neurons. Henceforth, the disparity in MAO-B activity also plays a significant role in neurochemical alteration in the PD brain [18,19].

Concomitantly, mounting evidence suggests that PD is a brain wide mitochondrial disease, in which reduced activity of mitochondrial complex I in substantia nigra [20] and frontal cortex were reported in PD patients. Moreover, analysis in peripheral tissue and skeletal tissues suggests a global reduction in mitochondrial complex I activity in PD cases. Furthermore, it is reported that MPP⁺ binds with mitochondrial complex-I which in turn promotes uncoupling of oxidative phosphorylation and triggers excessive ROS generation. Excessive ROS subsequently generates highly reactive hydroxyl radicals and peroxynitrite; which collectively exacerbate DA degeneration, either through direct production of superoxide anions from increased levels of free electrons and/or indirectly through dysregulation of iron. Concurrently, impaired complex I activity leads to disruption of the electron transport chain (ETC) which further exacerbates mitochondrial dysfunction and ATP depletion, which may in part cause dopaminergic cell death in the SNpc and striatum. Moreover, the dysfunction of the remaining complexes appears to be the secondary consequence of ETC disruption through complex I-mediated excessive ROS generation. Complex I and III inhibition causes an increase in electron release from the ETC into the mitochondrial matrix, which subsequently reacts with oxygen to form deleterious free radicals such as superoxide (O_2^-), hydroxyl ions (OH^-), and peroxynitrite ($ONOO^-$). Oxidative and nitrosative stress along with mitochondrial impairment leading to dopaminergic degeneration of the substantia nigra is believed to be the central cascade of PD progression. The elevated $ONOO^-$ level acts as a powerful oxidant, which further damages

mitochondrial complexes. Selective damage to Complex I activity by ONOO⁻ appears to be the result of protein modifications in the form of S-nitrosation, whereas inhibition of Complex IV activity may be due to irreversible binding of ONOO⁻ in competition with molecular oxygen. Subsequently, excessive ONOO⁻ reacts predominately with tyrosine and cysteine residues of proteins, which are the major component of other associated mitochondrial complexes leading to mitochondrial dysfunction through oxidative phosphorylation and further inhibiting mitochondrial complex activity [21].

Along with oxidative stress and mitochondrial impairment, exacerbated inflammatory response is also reported as a primary intrinsic factor in PD progression. Inflammatory responses mainly involve microglial activation, overproduction of cytokines and other pro-inflammatory mediators as well as the release of ROS and RNS in the PD brain. Moreover, up-regulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were also observed in the SN of PD patients [22]. These inflammatory responses together with other factors released from the dying dopaminergic neurons seem to amplify the impairment in immune responses resulting in irreversible destruction of dopaminergic neurons [23].

Taken together it is evident that multiple factors are associated with PD pathogenesis; henceforth it is apparent that the treatment strategy requires therapeutic candidates with multitargeted pharmacological action. In this context, despite major therapeutic advancements, existing conventional drugs only provide symptomatic relief with severe side effects in long-term use. Current pharmacotherapy for PD includes L-DOPA, dopamine agonists, monoamine oxidase (MAO) inhibitors, and anti-cholinergic and catechol-O-methyltransferase (COMT) inhibitors. Because no adequate treatment interventions exist for PD cure, neuroprotective strategies that could prevent or delay the disease progression may provide promising therapeutic modalities for the prevention and treatment of PD. About it, medicinal plants have immense potential as therapeutic candidates, since they possess potent pharmacological activities, low toxicity, and economic viability. Several Indian medicinal plants have been used for thousands of years in the Indian System of Medicine (Ayurveda) for the management of neurodegenerative diseases including Parkinson's, Alzheimer's, loss of memory, degeneration of nerves, and other neuronal disorders. In Ayurveda, PD is defined as "Kampavata" and several plant-based formulations have been mentioned in Ayurvedic literature and recommended for the treatment and management of PD from time immemorial. Among several

formulations, *Mucuna pruriens* seed preparations (a rich source of L-dopa) are in contemporary

use for the treatment of PD in India as well as other countries. Various preclinical and clinical reports suggest that *Mucuna pruriens* seeds contain L-dopa and provide long-term amelioration of Parkinson's symptoms with reduced risk for dyskinesia in 6-OHDA rat model of PD [24] and possessed higher anti-Parkinsonian activity when compared with synthetic levodopa [25]. However, current pre-clinical and clinical evidence is insufficient to evaluate the efficacy and safety of various herbal drugs used in PD management. Therefore, the scientific validation of ancient wisdom of science warrants well-designed preclinical studies to validate their efficacy and mode of action to justify the clinical observations. With this pre-context, the present research work has been designed to investigate the neuroprotective effect of bioactive fractions of two revered medicinal plants of the Indian System of Medicine (ISM) viz. *Withania somnifera* (L.) and *Curcuma longa* (L.) in the chlorpromazine model.

MATERIALS AND METHODS:

Chemicals and reagents

Chlorpromazine, levodopa, and carbidopa were procured as gift samples from Sun Pharmaceuticals (Baddi, India). All chemicals are analytical grade and purchased from Vijaya Enterprises (Hyderabad, India).

Collection of Plant Materials

The collected plant materials were identified and authenticated based on macroscopic and microscopic characters at the Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh.

Preparation of methanolic extract

Cucumis melo L. peel powder was extracted with methanol by Soxhlet apparatus. The drug (25g) to be is packed in a paper cylinder made from a filter paper and it is placed in the body of Soxhlet extractor. The alcohol (200ml) is placed in the flask. The apparatus is fitted. The process of filling and emptying of the extractor is repeated until the drug is exhausted.

Pharmacological studies

Animal Experiments were carried out on Wistar rats (150–200 g) of either sex and were obtained from the animal house of our institution. They were housed under standard light/dark cycle and fed with standard pellet diet and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA), New Delhi, India.

Determination of LD50.

LD50 of the extract was determined following the Organizations for Economic Cooperation and Development (OECD) guidelines. Acute toxicity class method (OECD guideline no. 423) and revised up-and down method (OECD guideline no. 425) were followed for the testing of chemicals. Animals were observed for 24 hours. The optimum dosage of Cucumis melo l. var at (2000mg/kg body weight) were recorded. For the experimental purpose we selected two different doses 100 mg/kg and 200 mg/kg.

Body weight and general observations

The body weight of the animals was recorded in an interval of 3 days by using digital weighing balance. The general observations included- reduced activity, freeze movement, salivation and increased respiratory rate after every chlorpromazine injection.

Determination of muscular coordination by Rota-rod Test [26]

The motor coordination activity was assessed by Rota-rod apparatus (Lark, Pvt. Ltd. India) as described by Kelly et al¹¹⁷ (1998). The instrument has a rotating rod (30mm diameter) and was divided into four compartments, thus permitting test of four rat at a time. The rotation speed of the rod was fixed at 20 rpm and rat were tested for the total duration of 180 s. The apparatus automatically records time in 0.1 s when a mouse falls off from the rotating shaft. The time (seconds) spent on the rod (fall latency) was recorded for each rat and used as a measure of motor function.

Assessment of gross behavioral activity by Open Field Test

The open field test was performed as described elsewhere¹¹⁸. The open-field apparatus consists of a box (26x26x39 cm) with clear plexi-glass walls and floor. Briefly, the animals were placed in the center of a box with the floor lined by equal segments, and the motor (the number of crossed segments) activity was monitored for 5 min. An automated sensor system, consisting of 16 photo beams per side measured the fine movement, expressed as total ambulation time.

Assessment of grip strength by Hang time test

The grid hang test¹¹⁹ measured neuromuscular strength. The rats were hoisted by their tails, gradually put on a horizontal grid (grid 12 cm², opening 0.5 cm²), and sustained until they clutched the grid with their fore and rear paws. The grid was then flipped, allowing the rats to hang upside down. The grid was set 20 cm above a hard surface to deter falling while also preventing injuries in the event of a fall. The equipment was designed with a 3-inch wall to keep the animals from crossing to the upper side of the grid. The animals were obliged to stay on the grid for 30 seconds and were allowed ten attempts with one-minute intervals with the maximum hanging. The calculation was made as percentage of hanging time:

$$= \text{Maximum hanging time} / 30 \text{ sec} \times 100$$

Assessment of depression by Tail suspension test

The depressive behavior of the rat was analyzed by the tail-suspension test. One hour after the drug administration, rat tail was attached to the cord with adhesive tape (20 mm apart from the extremity of the tail end) over a nylon cord of about 30 cm in length, stretched between two metal tripods at a height of 50 cm. After the initial period of 1 min. hyper motor activity (agitation) was slowed down and the immobility time was measured with a stopwatch, for a total duration of 04 minutes¹²⁰. The mouse was 150 mm away from the nearest object and was both acoustically and visually isolated. Rat was considered immobile when they hung passively and completely motionless.

Quantitative assay of neurotransmitters content Quantification of dopamine

The quantitative assay of dopamine (DA) was performed by using HPLC-fluorescence detection as described earlier¹²¹. Briefly, striatum was homogenized in 0.1 M perchloric acid and the homogenate was centrifuged at 14,000 g (4°C) for 20 min. The supernatant was filtered

through 0.45µm filter membrane and 20µL samples were injected on the column. The mobile phase was a buffered solution containing 0.02 mol/L sodium citrate, 0.05 mol/L sodium dihydrate phosphate (pH 4.5), and 5% methanol (v/v). The mobile phase flow rate was fixed at 1 mL/min. The DA signals were detected at the excitation wavelength of 280 nm and an emission wavelength of 320 nm. The quantification of DA was carried out by measuring the chromatographic peak areas using external standards. Results were expressed as micrograms per gram wet tissue.

Quantification of oxidative stress markers

Quantitative assay of Lipid Peroxidation (LPO)

Lipid peroxidation was assessed by determining the concentrations of thiobarbituric acid reactive substances (TBARS). The TBARS determination was performed spectrophotometrically and the levels of lipid peroxidation in brain tissues were quantified by the method described by Okhawa et al¹²² (1979). The sample of brain tissue (0.1 ml) and 1.5 ml of TCA (1.22M) was mixed and allowed to stand for 15 min at room temperature. The tube was centrifuged and to the supernatant 1.5 mL of thiobarbituric acid solution was added and kept in boiling water bath for 15 minutes. After cooling, 3.0 mL of chloroform was added and the tubes were shaken vigorously and centrifuged at 2000 g for 10 minutes. The absorbance of the chromophore formed was measured at 530 nm. A series of standard solution in the range 2- 10 nM /mL were treated in a similar manner and the values were expressed as nM of TBARS/mg protein.

Quantitative assay of total glutathione (GSH)

Total glutathione content was estimated by the method of Ellman (1959).¹²³ 0.1 ml of sample was

mixed thoroughly with 1.8 ml of precipitating reagent (containing metaphosphoric acid, EDTA and NaCl) and allowed to stand for 10 minutes and centrifuged at 2000g for 15 min. To

1.0 ml of supernatant, 4.0 mL of phosphate buffer (0.2M) and 0.5 mL of dithionitrobenzoic acid (0.6mM) were added. Standard glutathione in the range of 20-100 µg were also processed similar to the samples. The color developed was read at 412 nm and the total glutathione level was expressed as mg/100 mg of wet tissue.

Quantitative assay of total nitrite content

Nitrite are the primary stable end products of NO which can be measured by the absorbance of the magenta-colored azo dye formed from NO₂⁻ and Griess reagent as described by Gilchrist et al (2002). 124 50 µL of sample homogenate was added to 96 well plates containing each 50 µL of 1% sulphanilamide and 0.1 % naphthyl ethylenediamine dihydrochloride per well. The reaction mixture was mixed gently and kept for incubation for 10 min and the absorbance was measured at 543 nm against a control containing Griess reagent only. Sodium nitrite was used as a standard.

Quantitative assay of protein carbonyls

Protein carbonyls were determined by the method of Reznick and Packer¹²⁵ (1994) with little modifications. Briefly, 0.5mL of brain homogenate was mixed with 10% TCA and centrifuged at 5000g for 10 minutes. The precipitated protein was suspended in 0.1 mL of 0.2% 2,4- dinitrophenyl hydrazine and incubated for 1 hr at 37°C and precipitated again with TCA. The reaction mixture was centrifuged again and washed with ethanol: ethyl acetate (50:50) and dissolved in 6mM guanidine hydrochloride. The absorbance was determined at 370nm (molar extinction coefficient= 20mM⁻¹ cm⁻¹). Protein carbonyls were expressed as pM/mg protein.

Histopathological analysis

The histopathological analysis of the ventral midbrain was done to examine the alterations in the substantia nigra region of the experimental groups. Briefly, the ventral midbrain was fixed in 10% buffered neutral formalin and embedded in paraffin. Coronal sections of 5 µm thickness were fixed on

slides followed by haematoxylin and eosin staining and examined under light microscope.

Statistical analysis

All results are presented as mean ± SD. The inter group variation was measured by one way analysis of variance (ANOVA), followed by Posthoc LSD analyzed by SPSS software (version 17). The level of significance was considered at P<0.05. The determination of correlation coefficient was obtained by two-tailed Pearson's correlation test and linear regression plots were performed by Graphpad Prism 5.0.1 software (Graphpad Software Inc., CA, USA).

RESULTS:

Preliminary phytochemical screening

After extractions the extracts were subjected to a vacuum rotary evaporator and concentrated extracts were obtained along with solvent recovery and the preliminary phytochemical screening was carried out the result was shown in table 5.1.

Acute Toxicity (LD50).

The methanolic peel extract of Cucumis melo var. L did not produce any mortality up to a dose level of 2000mg/kg body weight. So, the two-dose ranges 100mg/kg and 200mg/kg were selected.

PHARMACOLOGICAL STUDIES

Anti-Parkinson activity.

Catalepsy study.

All the animals were evaluated for catalepsy using a bar test for 2 – 4 sec weekly observation and at last on the 21st day of treatment. The cataleptic behaviour (inability to correct abnormal posture) of Chlorpromazine treated animals was found to increase significantly every week compared to vehicle-treated normal control group animals. The animals were pre-treated with the methanolic extract of Cucumis melo var. L. peel showed a significant decrease in catalepsy score compared to Chlorpromazine treated animals (Table 6.22 and Fig 6.15). The animals treated with levodopa and carbidopa group significantly prevented the increase in catalepsy compared to Chlorpromazine treated animals on the 21st day.

Table : Effect of Cucumis melo var. L on chlorpromazine induced catalepsy in rat.

Treatment	Time (sec)			
	0 Day	7 Day	14 Day	21 Day
Normal propylene glycol (5 ml/kg)	1.9±0.14	2.2±0.35	2.1±0.19	2.3±0.47
Chlorpromazine (3 mg/kg)	3.4±0.43	9.8±0.18*	15.7±0.91*	19.2±0.57*
Methanolic extract (100 mg/kg)	2.8±0.81	5.3±0.25 ^a	8.6±0.47 ^a	9.5±0.61 ^a
Methanolic extract (200 mg/kg)	2.5±0.52	4.9±0.46 ^a	7.2±0.64 ^a	7.9±0.49 ^a
Carbidopa + levodopa (10 mg/kg)	2.2±0.36	4.1±0.68 ^a	6.1±0.24 ^a	6.5±0.73 ^a

Values are expressed as mean $\pm$ SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with the normal control group; significantly different at a P<0.05 when compared with Chlorpromazine treated group.

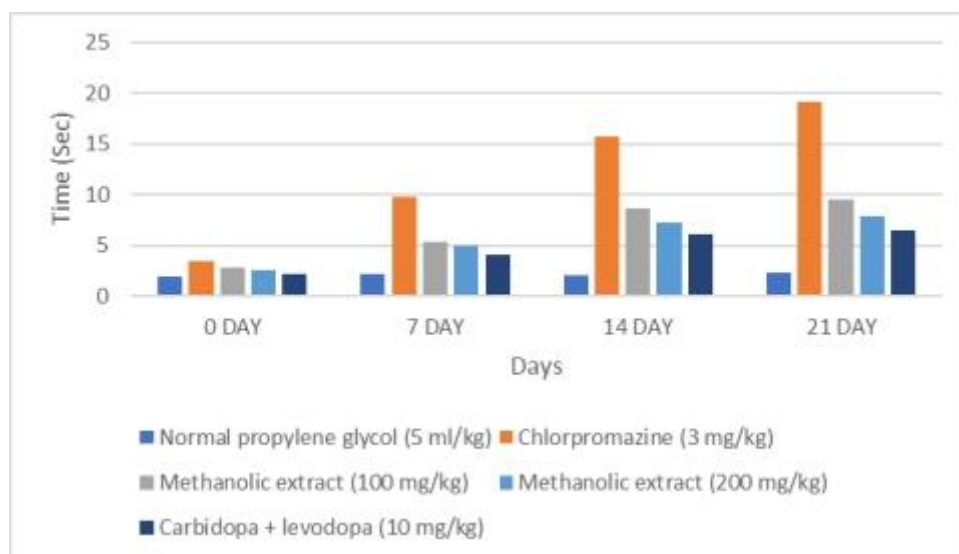


Figure 1: Effect of Cucumis melo var. L on chlorpromazine induced catalepsy in rats.

Effect of Cucumis melo var. L on motor coordination

The locomotor activity of animals was evaluated using an Actophotometer. Locomotor activity of vehicle treated normal control group was found to be 72.76 for all four weeks of treatment. The administration of Chlorpromazine to animals demonstrated a significant decrease in locomotor activity compared to vehicle-treated normal control group animals. The animals were pre-treated with the ethanolic extract of Cucumis melo var. L showed significant changes in locomotor activity compared to Chlorpromazine treated animals (Table 5.3 and Fig 6.16) after the first week. The animals treated with levodopa and carbidopa group significantly increased their locomotor activity compared to Chlorpromazine treated animals on the 21st day.

Treatment	Locomotor activity (counts/min)			
	0 Day	7 Day	14 Day	21 Day
Normal propylene glycol (5 ml/kg)	73.1 $\pm$ 1.02	75.6 $\pm$ 0.84	72.3 $\pm$ 0.147	76.5 $\pm$ 1.53
Chlorpromazine (3 mg/kg)	70.6 $\pm$ 1.35	51.2 $\pm$ 1.09*	39.1 $\pm$ 0.81*	28.4 $\pm$ 1.36*
Methanolic extract (100 mg/kg)	74.8 $\pm$ 0.73	65.1 $\pm$ 0.64	55.8 $\pm$ 0.43 ^a	51.7 $\pm$ 1.28 ^a
Methanolic extract (200 mg/kg)	71.5 $\pm$ 1.42	68.2 $\pm$ 1.38 ^a	61.2 $\pm$ 0.69 ^a	59.4 $\pm$ 1.61 ^a
Carbidopa + levodopa (10 mg/kg)	72.9 $\pm$ 0.59	70.4 $\pm$ 1.21 ^a	68.7 $\pm$ 1.39 ^a	65.2 $\pm$ 1.74 ^a

Values are expressed as mean $\pm$ SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group; significantly different at aP<0.05 when compared with Chlorpromazine treated group.

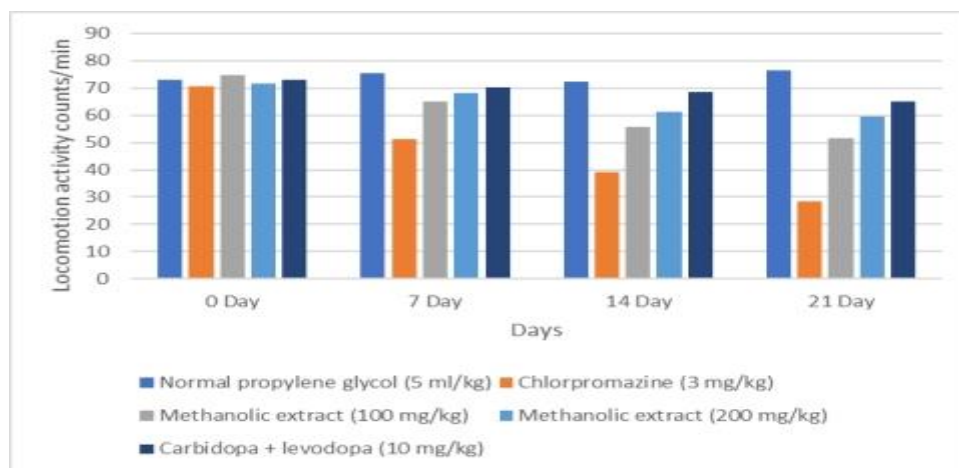


Figure 2: Effect of Cucumis melo var. L on chlorpromazine induced hypolocomotion in rat. Muscle activity (Rotarod test).

Muscle rigidity of animals was evaluated by using the rotarod apparatus. The mean fall-off time of vehicle treated normal control group animals from the rotarod was found to be 119.121 seconds during weekly observation of the treatment. The administration of Chlorpromazine to animal demonstrated a significant decrease in the rotarod readings (muscle rigidity) compared to vehicle treated normal control group animals. The animals pre-treated with the methanolic extract of Cucumis melo var. L showed a significant increase in rotarod readings compared to chlorpromazine treated animals (Table 5.4 and Fig 6.17) after first week. The animals treated with levodopa and carbidopa group significantly increased the decrease in rotarod readings compared to Chlorpromazine treated animals on the 21st day.

Table 5.4: Effect of Cucumis melo var. L on chlorpromazine induced muscle rigidity in rat.

Treatment	Mean fall-off time (sec)			
	0 Day	7 Day	14 Day	21 Day
Normal propylene glycol (5 ml/kg)	121.4±1.63	119.7±1.08	121.2±1.86	120.7±1.54
Chlorpromazine (3 mg/kg)	114.3±1.28	88.5±1.86*	71.1±1.42*	48.4±1.53*
Methanolic extract (100 mg/kg)	116.7±1.43	97.9±1.38 ^a	91.2±1.63 ^a	82.7±1.25 ^a
Methanolic extract (200 mg/kg)	119.4±1.62	101.2±1.39 ^a	95.8±1.79 ^a	89.4±1.08 ^a
Carbidopa + levodopa (10 mg/kg)	118.3±0.85	108.7±1.49 ^a	97.1±1.31 ^a	92.5±1.57 ^a

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group; significantly different at aP<0.05 when compared with Chlorpromazine treated group.

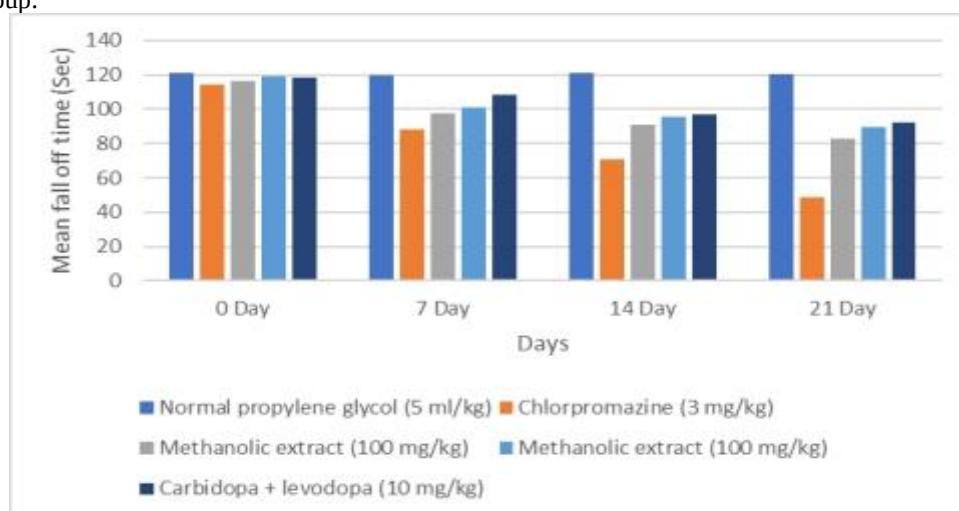


Figure 3: Effect of Cucumis melo var. L on chlorpromazine induced muscle rigidity in rat.

Neuroprotective effect of Cucumis melo var. L on chlorpromazine intoxication**➤ Effect of Cucumis melo var. L on dopaminergic neurotransmission**

Table 5.5 demonstrates the striatal dopamine (DA) and its metabolite content in chlorpromazine-induced rats and its prevention on Cucumis melo var. L treatment. Chlorpromazine intoxication significantly ($P < 0.05$) reduces DA content up to 4.53 $\mu\text{g/gm}$ of

Tissue which was significantly prevented on treatment with Cucumis melo var. L. The dopamine content was found to be 9.65 and 12.71 $\mu\text{g/gm}$ of tissue in Cucumis melo var. L. peel extract 100 and 200 mg/kg of body weight treated groups respectively ($P < 0.05$). Similarly, the metabolites of DA, DOPAC, and HVA were also concomitantly reduced in chlorpromazine-treated rats, whereas DA turnover increased in the diseased group. Thus, the pre-treatment with Cucumis melo var. L peel extract has protected the DA and its metabolite depletion in the treated groups.

Treatment	DOPAMINE ($\mu\text{g/gm}$ of tissue)	DOPAC ($\mu\text{g/gm}$ of tissue)	HVA ($\mu\text{g/gm}$ of tissue)	DA turnover (DOPAC+HVA/DA)
Normal propylene glycol (5 ml/kg)	15.14 $\pm$ 1.46	2.64 $\pm$ 0.12	1.68 $\pm$ 0.41	0.28 $\pm$ 0.04
Chlorpromazine (3 mg/kg)	4.53 $\pm$ 0.37*	1.29 $\pm$ 0.13*	0.44 $\pm$.04*	0.38 $\pm$ 0.06*
Methanolic extract (100 mg/kg)	11.26 $\pm$ 0.41*	1.86 $\pm$ 0.17*	0.63 $\pm$.07*	0.22 $\pm$ 0.03*
Methanolic extract (200 mg/kg)	12.71 $\pm$ 1.54*	1.98 $\pm$ 0.18*	0.66 $\pm$ 0.08*	0.21 $\pm$ 0.03*
Carbidopa + levodopa (10 mg/kg)	9.65 $\pm$ 0.38*	1.73 $\pm$ 0.15*	0.52 $\pm$ 0.06*	0.23 $\pm$ 0.03*

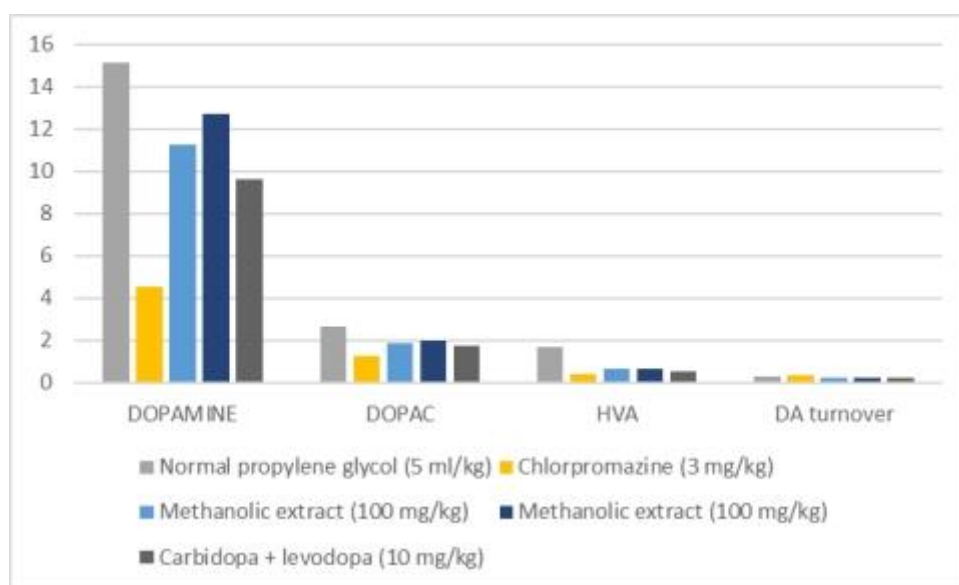


Figure 4: Effect of Cucumis melo var. L on dopamine and its metabolites

Protective effect of Cucumis melo var. L peel against chlorpromazine-induced dopaminergic neurotoxicity. Administration of Cucumis melo var. L peel extract or carbidopa + levodopa significantly protected the depletion of dopamine and its metabolites with concomitant elevation in DA turnover in chlorpromazine-induced rat brain. Values are expressed as mean $\pm$ S.D and statistically significant ($P < 0.05$) for normal control vs

chlorpromazine and ($P < 0.05$) MPTP vs treated group (n=6).

➤ **Effect of Cucumis melo var. L on oxidative stress markers**

➤ **Lipid Peroxidation Assay (TBARS).**

The animals treated with Chlorpromazine showed significant increases in TBARS level in brain compared to normal control group animals. The TBARS level in animals treated

with methanolic extract of *Cucumis melo* var. L and standard drug was significantly decreased compared to Chlorpromazine treated animals (Table 5.6 and Fig 6.18).

➤ **Estimation of Reduced Glutathione (GSH).**

The animals treated with chlorpromazine showed a significant decrease in GSH levels in brain compared to normal control group animals. The GSH level in animals treated with methanolic extract of *Cucumis melo* var. L and standard drug was significantly increased compared to Chlorpromazine treated animals (Table 5.6 and Fig 6.18).

➤ **Estimation of Nitrite.**

The animals treated with Chlorpromazine showed significant increases in nitrite level in

brain compared to normal control group animals. The nitrite level in animals treated with methanolic extract of *Cucumis melo* var. L and standard drug was significantly decreased compared to Chlorpromazine treated animals (Table 5.6 and Fig 6.18).

➤ **Estimation of Protein.**

The animals treated with chlorpromazine showed a significant decrease in protein levels in brain compared to normal control group animals. The protein level in animals treated with methanolic extract of *Cucumis melo* var. L and standard drug was significantly increased compared to chlorpromazine treated animals (Table 5.6 and Fig 6.19).

Table 5.6: Effect of *Cucumis melo* var. L on the different parameters in rat brain.

Treatment	GSH level (nM/mg protein)	TBARS level (nM/mg protein)	Nitrite level (nM/mg of protein)	Total protein level (mg/mL)
Normal propylene glycol (5 ml/kg)	6.28±0.63	1.24±0.19	2.05±0.18	14.29±0.51
Chlorpromazine (3 mg/kg)	1.15±0.42*	8.63±0.38*	9.47±0.61*	4.58±0.41*
Methanolic extract (100 mg/kg)	5.02±0.31a	2.38±0.12a	3.21±0.17a	11.18±0.38a
Methanolic extract (200 mg/kg)	5.68±0.49a	2.01±0.96a	2.96±0.57a	12.42±0.65a
Carbidopa + levodopa (10 mg/kg)	6.72±0.68a	1.92±0.11a	2.83±0.43a	13.63±0.83a

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at *P<0.05 when compared with normal control group; significantly different at a P<0.05 when compared with Chlorpromazine treated group.

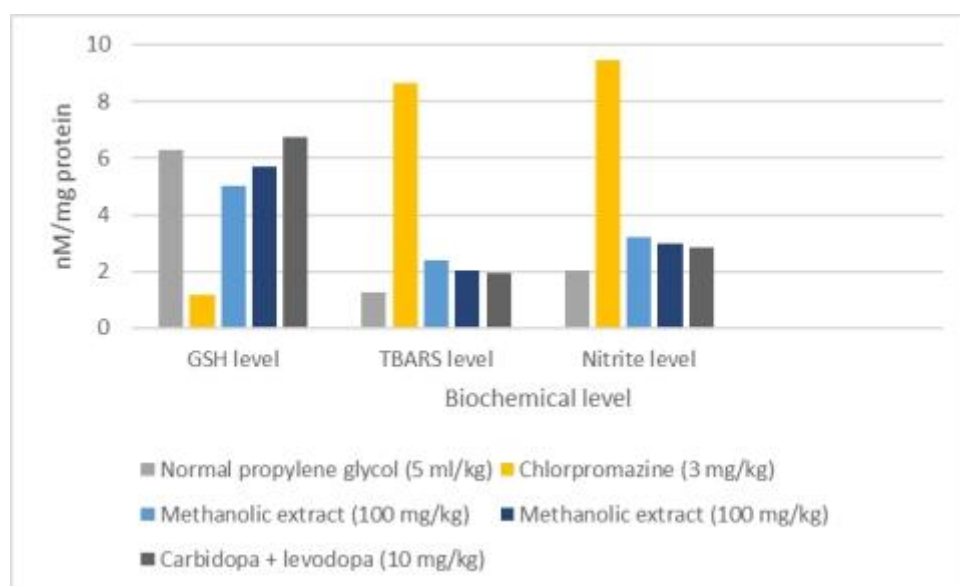


Figure 5: Effect of *Cucumis melo* var. L on the GSH, Nitrite and TBARS level in rat brain.

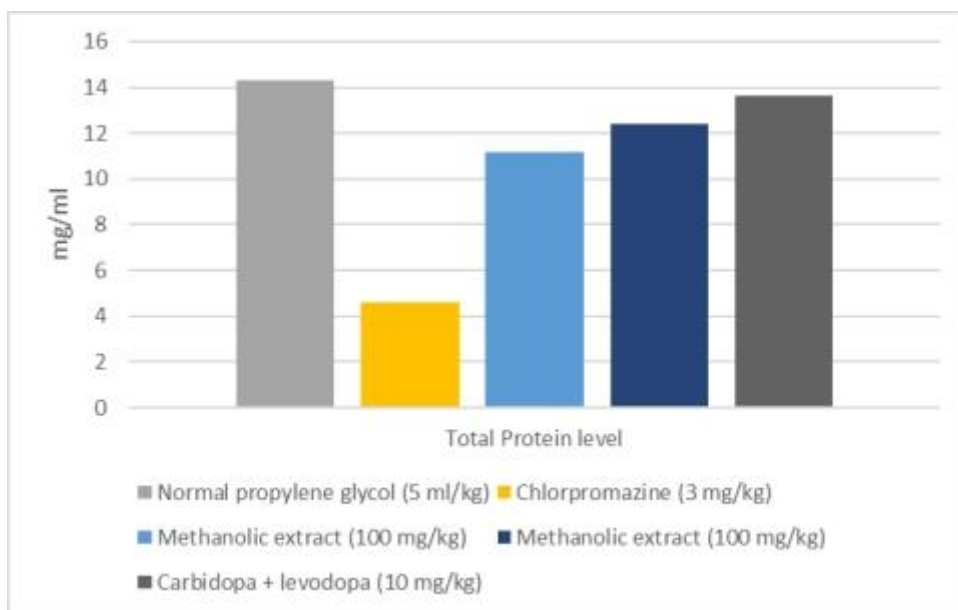


Figure 6: Effect of Cucumis melo var. L on the total protein level in rat brain.

Histological observations.

Histological analysis revealed that there is no significant pathological change observed in the substantia nigra of the control rat brain section (A). Diseased control group (B) reveals blood vessels haemorrhages in the substantia nigra (figure 6.13).

The methanolic extract (200 mg/kg) treated group reveals the presence of a single focus of moderate gliosis and mild loss of neuropil while no hemorrhages were observed. Methanolic extract (100 mg/kg) treated rat brain sections reveals loss of neuropil and mild foci of congestion. Effect of Methanolic extract (100 mg/kg and 200 mg/kg) on neurons of substantia nigra in the chlorpromazine-induced and treated groups. Effect of Methanolic extract (100 mg/kg) (D) and Methanolic extract (200 mg/kg) (C) on substantia nigra in the chlorpromazine-induced rat (B). Methanolic extract (200 mg/kg) administration prevented the microvasculature damage in the substantia nigra as observed in H&E staining of the mid-brain sections. Magnification was 40x; (n = 4).

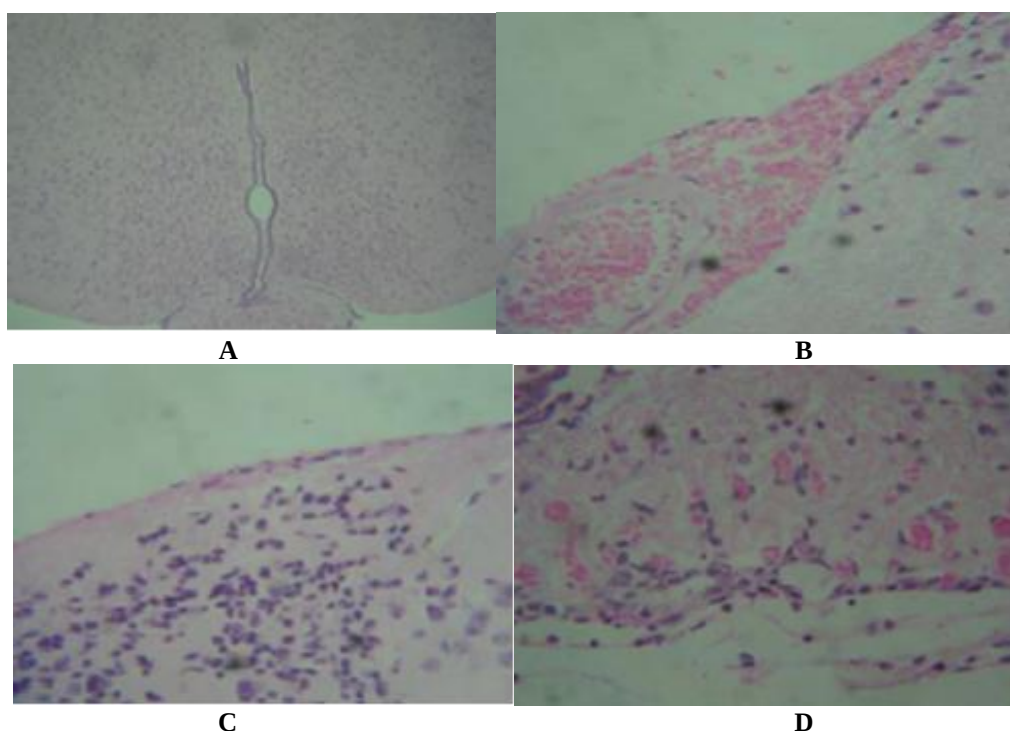


Figure 7: Protective effect of Cucumis melo var. L on vascular haemorrhage.

6. DISCUSSION:

Parkinson's disease is a chronic neurodegenerative disorder characterized by loss of dopamine neurone of SNpc. The pathogenesis of Parkinson's disease includes oxidative stress, protein accumulation like α -synuclein, mitochondrial dysfunction, apoptosis, and neuronal excitotoxicity. Among all, oxidative stress is a crucial pathological mechanism for Parkinson's disease. SNpc is more vulnerable to reactive oxygen species as it contains more amount of dopamine.

Chlorpromazine is one of the antipsychotic drugs listed as essential drugs by WHO in 2003 to treat both acute psychosis and chronic psychosis. It has been associated with various side effects. Chronic treatments with Chlorpromazine increase the dopamine receptor binding site in neostriatum and in mesolimbic region, which could account for dopamine hypersensitivity that induced tardive dyskinesia. Chlorpromazine induced Parkinsonism by interfering with the storage of catecholamines in intracellular granules which may cause monoamine depletion in nerve terminals and in the induction of hypolocomotion and muscular rigidity¹²⁶⁻¹²⁹; thus, Chlorpromazine was produced by the Parkinson disease-like symptoms followed by chronic treatments of rats for 21 days.

It results in an increase level of oxidative stress that may cause the reduction in antioxidant enzymes which were also seen with the atypical agent's ziprasidone, risperidone, and olanzapine. Except olanzapine both typical and atypical agents increase lipid peroxidation after chronic dosing. There was a significant increase in catalepsy, a decrease in movements, and a decrease in body weight following chlorpromazine administration to rat.

The present study suggested that chlorpromazine developed Parkinson's like behavioral symptoms in rat. The oxidative stress was measured through determination of levels of TBARS, reduced glutathione, and nitrite level in brain tissue. Lipid peroxidation is a sensitive marker of oxidative stress. Lipid peroxidation occurs due to attack by radicals on double bond of unsaturated fatty acid and arachidonic acid which generate lipid peroxy radicals. These radicals have further attacks on other unsaturated fatty acids. Increased levels of the lipid peroxidation product have been found in the substantia nigra of Parkinson's disease patients. In the present study the same result was observed in the brain homogenates of chlorpromazine treated control animals. Brain protects against oxidative stress by SOD, catalase, and glutathione peroxidase and thus these antioxidant enzymes protect brain from neurodegeneration. Glutathione peroxidase protects brain from neurodegeneration by

scavenging H_2O_2 generated by cellular metabolism and balance formation and decomposition of H_2O_2 in normal condition¹³⁰⁻⁻¹³². It is obvious that reduced glutathione is the limiting factor in the removal of H_2O_2 .

Neuronal cell loss may cause the depletion of reduced glutathione in the substantia nigra in Parkinson's disease. Nitric oxide production can be determined by nitrite determinations in biological material. Nitric oxide has been involved in the cytotoxicities by activation of macrophages or excess stimulation of neurons by glutamate. In further study on glutamate stimulation causes neurotoxicity in primary cultures of rat fetal cortical, striatal, and hippocampal neurons¹³³⁻¹³⁵. Chlorpromazine group showed a significant increase in the level of TBARS and gradual decrease in GSH levels in brain as compared control group. All observations showed that chlorpromazine increases the oxidative stress in the brain of animals. The administration of methanolic extract of cucumis melo var. L peel were used in the chlorpromazine model in rat; both doses were found to be significant in reducing the catalepsy, increasing the locomotor activity (actophotometer), and increasing the muscle activity (rotarod test) in a chlorpromazine model of parkinson in rat which indicates methanolic extract of cucumis melo var. L peel has potential effects against Parkinson's -like symptoms produced in various experimental models.

The antioxidative properties of methanolic extract of Cucumis melo var. L peel reduced the duration of the catalepsy which decreased the elevated levels of lipid peroxidation in the chlorpromazine treated animals. The findings of in vivo antioxidant (oxidative stress marker) confirmed the neuroprotective effect of the methanolic extract of Cucumis melo var. L is due to the presence of high triterpenoids and flavonoid content.

SUMMARY AND CONCLUSION:

Cucumis melo var. L are rich in secondary metabolites such as alkaloids, tannins, carbohydrates, flavonoids, saponins, proteins, glycosides, steroids, and triterpenoids. Since the flavonoids and polyphenol compounds were found to be active against various pharmacological activity. In the present study, Cucumis melo var. L was selected for the screening of neuroprotective and antioxidant activity against chlorpromazine-induced Parkinson's. The findings of anti-Parkinson activity of the methanolic extract of Cucumis melo var. L peel demonstrated that this plant has neuroprotective properties.

Neurodegenerative disorders are usually characterised by the accumulation of abnormal

protein aggregation that leads to inflammation as well as oxidative stress in the central nervous system (CNS). These leads to the development of many neuropsychiatric and neurodegenerative disorders including depression and anxiety, AD, and PD. The PD and AD are the most common disorders of nervous system caused by environmental and genetic influences. It has been observed that various types of biological mechanisms are associated with neurodegenerative disorders such as oxidative stress, aggregates of proteins in neurons, depletion or in sufficient synthesis of neurotransmitters, degradation of neurotransmitters in the synaptic cleft due to the higher activity of enzymes, abnormal ubiquitination, mitochondrial dysfunction, and excitotoxicity of neurons as well as disarrangement or damage of the blood brain barrier. Although a multitude of pharmaceutical agents are available for the treatment of mental disorders, patients eventually cannot tolerate the side effects, do not respond adequately, or lose their response.

In comparison, many therapeutic herbs have far fewer side effects. They can provide an alternative treatment or be used to enhance the effect of prescription medications.

Further, the development of novel potential anti-parkinson activity agents with a fewer side effect is a great of interest.

It has been documented in previously reported scientific data that the several plants with antioxidant activity have been found to be effective in the treatment of Parkinson's disease. The extensive literature review demonstrate that Cucumis melo var. L contains flavonoids and polyphenol as major chemical constituents. Further, traditionally Cucumis melo var. L is used in the treatment of kidney stones, flatulence, leprosy, fever, jaundice, diabetes, obesity, cough, bronchitis, ascites, anaemia, constipation, other abdominal disorders, reduced blood pressure, dyspepsia, flatulence, leprosy, fever, jaundice, diabetes, obesity, cough, bronchitis, ascites, anaemia, constipation, other abdominal disorders, amentia and menorrhagia

The anti-parkinson activity of Cucumis melo var. L peel extract has not been scientifically reported. Hence, we planned to evaluate the neuroprotective and antioxidant activity activity of Cucumis melo var. L peel methanolic extract.

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