"Evaluation of neuroprotective effect of Gramine for the treatment of Parkinson's disease in rats."

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ABSTRACT:

Parkinson's disease (PD) is an extrapyramidal neurological disorder associated with loss of dopaminergic neurons in the substantia nigra pars compacta (SN) and accumulation of cytoplasmic inclusions known as lewy bodies. the rotenone model, have been used to investigate the underlying mechanisms of PD pathogenesis and try new neuroprotective medications. Gramine has attracted much attention due to its diverse antioxidant, antibacterial, anti-inflammatory, antitumor and insecticidal activities. This study investigates gramine potential to neuroprotective in parkinsonism disease induced by rotenone. Wistar rats were divided into six groups including controls, rotenone-only, and gramine at varying doses (13, 27.5, 55 mg/kg) with and without levodopa-carbidopa. Rotarod, hole board, actophotometer, open field, and grip strength tests were used to assess motor and exploratory behaviour. Levels of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), lipid peroxidation (MDA), nitric oxide (NO), and dopamine were measured. Molecular docking was performed against dopamine transporter (DAT), D3 receptor, TNF-α, and NF-κB to predict the potential targets of gramine. Brain tissues were examined for signs of neuronal degeneration. Gramine significantly improved motor coordination, exploratory behaviour, and grip strength compared to the rotenone group. Gramine restored antioxidant enzyme levels (CAT, SOD, GSH), reduced oxidative stress markers (MDA, NO), and increased dopamine levels. Gramine showed good binding affinity to DAT, D3 receptor, TNF-α, and NF-κB, suggesting multi-targeted neuroprotective mechanisms. Gramine reduced neuronal degeneration, showing fewer pyknotic nuclei and vacuolated cells in substantia nigra. Gramine exhibits significant neuroprotective effects against rotenone-induced Parkinsonism. The effects are likely due to its antioxidant, anti-inflammatory, and dopaminergic restorative actions. Gramine shows promise as a potential therapeutic candidate for managing Parkinson's disease.

KEYWORDS: Parkinson's disease, Gramine, Neuroprotection, Rotenone model, Oxidative stress

1. INTRODUCTION:

Parkinson's disease (PD) as a neurodegenerative disease is the most prevalent neurological ailment following Alzheimer's disease. In addition to the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc), pathological signs of Parkinson's disease (PD) include an increase in alpha-synuclein protein and the formation of Lewy bodies in the cytoplasm.[1] The prevalence of Parkinson's disease has been estimated nearby 1.51 cases per 1000 cases in all-age group. This prevalence increased from 1980 to 2023 and achieved its climax from 2010 to 2023.[2] The etiology Of PD is linked to the stimulation of microglia and astrocytes, in addition to neuroinflammation. Additionally, it was reported that downregulated mitochondrial activity, reactive oxygen species (ROS), and oxidative Stress produced by nitric oxide (NO) are participants in the pathogenesis Of Parkinson's disease. Neurodegeneration results from the activation of microglial cells which produce harmful substances including NO And some pro-inflammatory cytokines like TNF and interleukin-1 (IL-1). Activation of microglial cells leads to the development of cytotoxic factors such as NO, and some pro-inflammatory cytokines like TNF-αAnd interleukin-1β (IL-1β), and therefore give rise to neurodegeneration. [3]

Bradykinesia is the majority characteristic clinical feature of PD that manifests by troubles in initiation, execution and seize of movement. Rigidity, increased resistance to muscles stretches and relaxation due to tightness and stiffness of muscles and may occur proximally e.g. neck, shoulders, hips and distally e.g. wrists, ankles or both. Tremor at rest, mainly in distal part of the extremities, is in addition one of the most identifiable symptoms of PD. The later stage of PD is followed by freezing, akinesia. Particularly in patients with postural instability and flexed truncal posture, festination of gait (involuntary quickening of gait) may also occur.[4] One of the animal models used to investigate the basic mechanisms underlying the development of Parkinson's disease to test new neuroprotective medications is the rotenone model. Rotenone is a highly lipophilic insecticide that does not require a specific transporter to freely cross the blood-brain barrier and can cause neurotoxicity in experimental animals.[5]

One of the animal models used to investigate the basic mechanisms underlying the development of Parkinson's disease to test new neuroprotective medications is the rotenone model. Rotenone is a highly lipophilic insecticide that does not require a specific transporter to freely cross the blood-brain barrier and can cause neurotoxicity in experimental animals. [6] Gramine also known as *N*,*N*-dimethyl-1*H*-indole-3-methylamine, is an indole alkaloid initially isolated from *Arundo donax* L. and usually plays a defensive role in plants against herbivores. Gramine has attracted much attention due to its diverse antioxidant, antibacterial, anti-inflammatory, antitumor and insecticidal activities.[7] Gramine Inhibit triggering of microglia and promotes the axonal regeneration of neuron by NF-kB signaling pathway. It suppressed the JAK/STAT3 pathway which prevents neuro-inflammation and neuro-degeneration by suppressing the activation of immune response. Attenuation of these signalling pathways leads to down regulation of pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6).[8, 9]

Therefore, gramine may serve as a potential neuroprotective agent in Parkinson's disease by targeting inflammatory and oxidative stress-related pathways. Thus, the present study was designed to check the utility of gramine in the management of PD.

2. MATERIALS AND METHODS:

2.1. Chemicals:

Gramine (Yucca enterprises, Wadala, Mumbai); Rotenone (Dhamtec Pharma and Consultants, Navi Mumbai); levodopa – carbidopa (sun pharmaceutical, Gujrat). Every chemical utilized was of analytical quality and purchased from standard manufacturers.

2.2. Experimental Animals:

Wistar strain rats (230-250 g of 9 weeks old) of either sex was used for the study. Animals were procured and housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature 25 ± 2ºC, 12: 12 h L: D cycle and 50 ± 5% RH with unrestricted access to food and water. Animals were acclimatized to laboratory conditions before the test. All the experimental work was carried out during the light period (08:00-16:00 h). The study was carried out in harmony with the guidelines given by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), New Delhi (India). The Institutional Animal Ethical Committee of MVPS, College of Pharmacy, Nashik, India approved the protocol of the study (IAEC/Dec2023/02).

2.3. Computational in-silico docking studies: In order to find out, the exact mechanism of neuroprotection of gramine, theoretical binding studies of target prediction were performed using Swiss target prediction, Super- PRED, Software ProTox 3.0 for toxicity prediction, SwissADME for Log p value. Molecular docking is considered as significant method to validate the predictive neuroprotection mechanism of phytoconstituent as they highlight the interaction between ligand and protein molecule. For conducting molecular docking PyRx software were used and for visualization of active sites Biovia discovery studio software were used. Binding affinity of test

compound gramine were examined against various targets such as dat protein, D3 dopamine receptor, TNF-alpha, NF-kB and there binding affinity were compared with the standard drug levodopa.

2.4. Experimental design for Rotenone-induced neurodegeneration in rats

Animals were randomly assigned into 6 groups (n = 6 for each group). Group I –Vehicle (PEG:DMSO, 1:1 ratio), Group II – Rotenone (3 mg/kg, i.p. in PEG 200 and DMSO; 1:1 ratio for 21 days), Group III – Gramine (13 mg/kg, p.o.) + rotenone (3 mg/kg, i.p.), Group IV – Gramine (27.5 mg/kg, p.o) + rotenone (3 mg/kg, i.p.), Group V – Gramine (55 mg/kg, p.o) + rotenone (3 mg/kg, i.p.), Group VI – Levodopa- Carbidopa (30 mg/kg, p.o) + rotenone (3 mg/kg, i.p.). PEG- Polyethylene glycol; DMSO- Dimethyl sulfoxide. Gramine (13, 27.5 and 55 mg/kg, p.o.) were administered 30 minutes before the administration of rotenone for 21 days. Behavioural assessments were carried out on 21st day after 24 h of administration of last dose.

2.5. Behavioral test

2.5.1. Rotarod test: The rotarod test evaluates motor coordination and balance by measuring the time rats can stay on a rotating rod. The experiment utilized a rotating rod with a 3 cm diameter rubber coating, powered by a motor set to 2 revolutions per minute, positioned 50 cm above a tabletop. The 75 cm rod was divided into six sections with plastic discs, and cages below each section prevented the rats from falling too far if they lost their grip. The pre-test involved rats that could place on the rod for at least one minute. Treatment drugs were administered orally and rats were placed on the revolving rod after 60 minutes. The number of rats that fell off during a one-minute trial was recorded. At 35th day rats were tested on a rod spinning at 20 rpm, and the duration they could maintain their balance was measured to evaluate their motor skills and coordination. [10]

2.5.2. Hole Board Test: The hole board test is used to assess the behavioral aspects of the experimental animals. The device was a square with 16 evenly spaced holes that measured 30 cm in length (L) by 30 cm in width (W). Before being put in the middle of the hole board apparatus for the test evaluation, each animal was allowed to acclimate to the device for 30 minutes on the twentieth day of medication. During a 120-second session with each animal, focused (edge sniffing and head dipping), horizontal (walking and immobile sniffing), vertical (climbing and rearing), and immobility events were noted. [11]

2.5.3. Actophotometer: Actophotometer was used to measure locomotor activity of the animals. It's a rectangular closed apparatus with horizontally running beams of light. These beams get cut with the movement of inserted animals which are further counted by automatic tracking system. Animal is placed in a designated area of the arena and allowed to move freely for 5 min, while being monitored by an automatic tracking system. Trial begins immediately and ends when defined duration has done. The animal is then placed back into their cage. Recorded parameter was no. of beam cutting by specific animal. [12]

2.5.4. Open Field Test: A white acrylic open field apparatus measuring 50 cm in length, 50 cm in breadth, and 38 cm in height was used to measure locomotor activity. The field was lit by ambient, low-level room lights. The animal's time spent in the central and periphery zones was recorded by an aerial video camera, and video-tracking software was used to evaluate the data. To rule out the possibility that the rats' performance was impacted by differentiation in their baseline activity levels, their locomotor activity was assessed. In the open field arena, the rat was put in the middle of the trial chamber and given ten minutes to explore. The test lasted for ten minutes. [13]

2.5.5. Grip strength: We utilized a grip strength meter manufactured by Olympus Instruments to gauge the forelimb grip strength of rats. To assess neuromuscular function and detect motor neurotoxicity, this standardized test is widely used. The rat was placed on a metallic grid or pull bar attached to the apparatus for gripping during the assessment. The rat was then gently drawn rearward on a horizontal plane, which caused it to use all of its might to hold on. The rat's applied force was recorded by the Newton measurement. We were able to assess the strength and muscle function of the rats' forelimbs by measuring the grip force. This evaluation gives us important details about their motor skills and allows us to monitor any alterations in neuromuscular function that might take place over the trial. [14]

2.6. Biochemical estimation:

2.6.1. Tissue homogenate preparation: The animals were sacrificed using a CO2 euthanasia chamber on the last day, 1 h after all behavioral tests, and brains were removed. After being cleaned in an isotonic saline solution, the brains were separated and weighed. The tissue homogenate (10% w/v), with 0.1M phosphate buffer (pH 7.4) was made. Centrifugation of the homogenate at 1000 g for 20 minutes at 4 °C was done and post nuclear fraction was collected for catalase assay (Remi - C30, Remi Industries Ltd. Mumbai, India); for other enzyme assays, centrifugation was at 12000 g for 60 min at 4ºC. A was used for subsequent assays. Following biochemical parameters were performed using Bio spectrophotometer (Elico BL-200). [15]

2.6.2. Estimation of catalase: The Luck (1971) approach was employed to evaluate catalase activity. H_2O_2 breakdown was assessed at 240 nm. The assay combination included 3 ml of 0.01 M H_2O_2- phosphate buffer (pH 7) and 0.05 ml of tissue homogenate supernatant (10% w/v). At 240 nm, the change in absorbance was seen after 1 minute. In order to determine enzyme activity, the millimolar extinction coefficient of H_2O_2 (0.071) was utilized. The findings were displayed as milligrams of protein per minute of H_2O_2 decomposition. [16]

2.6.3. Estimation of malondialdehyde (Lipid peroxidation assay): The method developed by Wills (1966) was used to measure the amount of lipid peroxidation in the brain quantitatively. By reacting with thiobarbituric acid at 532 nm, this method evaluated the amount of malondialdehyde (MDA) produced. 1.5 ml of 20% acetic acid, 0.1 ml of tissue homogenate, 0.2 ml of 8% sodium lauryl sulphate (SLS), and 1.5 ml of a 0.8% thiobarbituric acid (TBA) solution make up the reaction mixture. Then, the mixture was heated for one hour in a water bath at 95°C. Further, 5 ml of 15:1 n- butanol and pyridine mixture were added. After a vigorous shaking, the mixture was centrifuged for 5 min. The organic layer's upper layer's absorbance was measured at 532 nm. The molar extension coefficient of the chromophore was used to convert the values into nM of MDA per milligrams of protein ($1.56x10^5M^{-1}cm^{-1}$). [17]

2.6.4. Estimation of superoxide dismutase (SOD): The activity of superoxide dismutase was measured using the Kono method, which involved inhibiting the reduction of nitro blue tetrazolium chloride (NBT) and was determined at 560 nm spectrophotometrically. The reaction was started by adding 0.1ml of 1 mM hydroxylamine hydrochloride to a reaction mixture containing 0.1ml of 0.1 mM ethylene diamine tetra acetic 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. After 20 min of incubation at 37°C, the absorbance was measured at 560 nm. The results were expressed at percentage inhibition of reduction of NBT. [18]

2.6.5. Estimation of reduced glutathione: The amount of reduced glutathione (GSH) in the brain was calculated using Ellman's method. With 0.75 ml of 4% sulphosalicylic acid, a 0.75 ml homogenate sample was precipitated. The samples were centrifuged for 15 min at 4°C. In 0.1 M phosphate buffer (pH 8.0), the assay mixture contained 0.5 ml supernatant and 0.4 ml of 0.01 M DTNB [5,5'- Dithiobis (2-nitrobenzoic acid)]. At 412 nm, the yellow color was produced that was measured quickly. The result was represented in µM of GSH per milligram of protein. [19]

2.6.6. Estimation of NO level: The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid). Equal volumes of supernatant and Greiss reagent were mixed; the mixture was incubated for 10 min at room temperature in the dark and the absorbance at 543 nm was determined spectrophotometrically. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as µmol of nitrite per ml of homogenate. [20]

2.6.7. Estimation of dopamine: A UV-visible spectrophotometer was used to measure the amount of dopamine in the rat brain following repeated administration of the synthesized compounds, either by themselves or in combination with amphetamine. A homogenized supernatant liquid (1 ml) was mixed with 1 ml of potassium ferricyanide (1.5 × 10−2 M) and 1 ml of ferric chloride (1.5 × 10−2 M) in 25 ml of distilled water to quantify the amount of dopamine in rat brain. The UV visible double beam spectrophotometer at 735 nm was used to estimate the developed color after it had been set aside for 30 minutes.[21]

2.7. Histopathological analysis: The rat brains were examined for histopathological evaluation. The brains were removed and immediately fixed in 10% buffered formalin. The brain, which had been alcohol-dehydrated and embedded in paraffin, was sectioned to reveal the substantia nigra. Using a microtome, five-micrometer thick serial histological slices were cut from paraffin blocks and stained with hematoxylin and eosin (H&E). Digital microscope (Olympus, Japan), was used to analyses the sections under a light microscope while taking photomicrographs.

2.8. Statistical analysis: All results were represented as mean ± S.E.M. Graph Pad Prism 10.4.1 software was used to evaluate all the group data using one-way analysis of variance and Dunnett's test. (GraphPad®, San Diego, CA, USA). Data of behavioral test were statistically analyzed using one – way analysis of variance (ANOVA) followed by Dunnett's test, while data of biochemical parameter were also analyzed using one- way analysis of variance (ANOVA) followed by dunnett's test. A value of $P < 0.05$ was considered to be statistically significant.

3. RESULT:

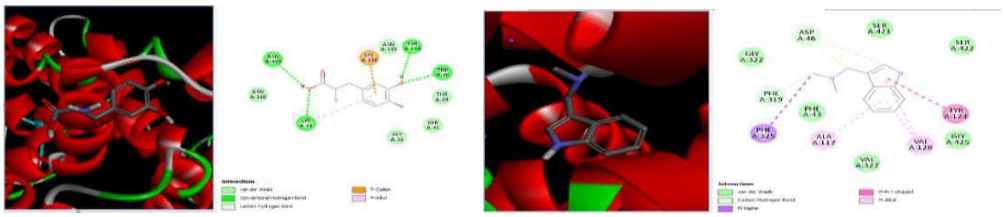
3.1. In-silico docking studies:

To predict the therapeutic targets and mechanism of neuroprotective of gramine, theoretical binding studies were performed on DAT (Dopamine Transporter), TNF- alpha, nuclear factor kappa B (NF-κB), D3 receptor. According to binding affinity scores given byv Pyrex software gramine can be capable to bind the selected targets. The results showed that values for calculated binding affinity of gramine (Kcal/mol) are comparable to the standard drug levodopa. (table 1)

Table 1: calculate binding affinities for different targets.

Targets	Calculated affinity of drugs (Kcal/mol)	
	Gramine	Levodopa
DAT	-5.9	-6.4
TNF-alpha	-5.9	-6.6
NF-kB	-5	-5.8
D3 receptor	-4.9	-5.9

A.



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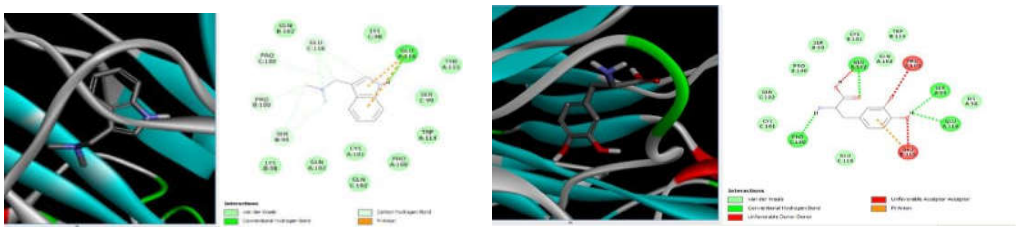

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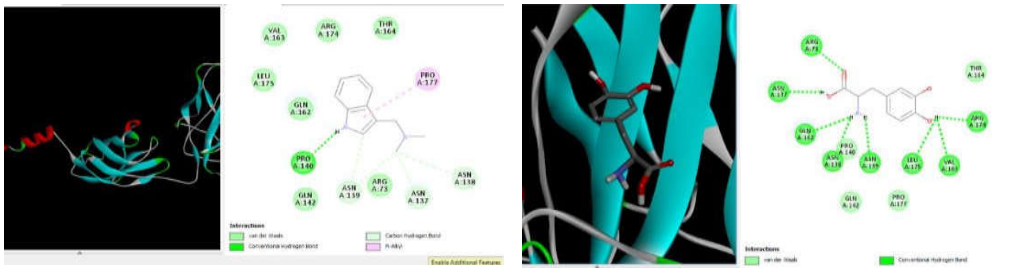

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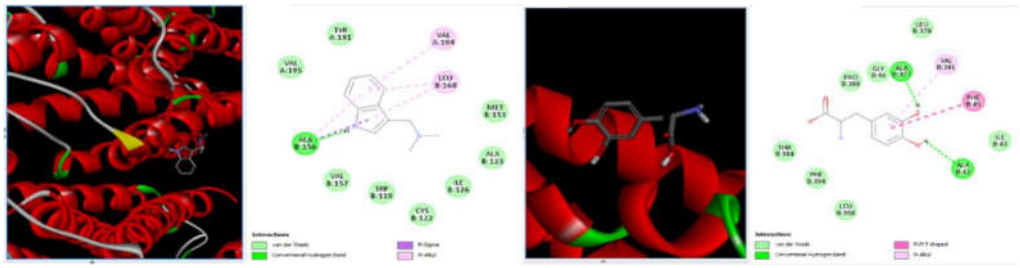

Figure 1. shows binding of protein with ligand in 3D and 2D views. A. DAT protein by gramine and levodopa. B. TNF -alpha by gramine and levodopa. C. NF-kB by Gramine and levodopa. D. D3 Dopamine receptor by Gramine and levodopa

3.2. Effect of Gramine on Behavioural parameters in Rotenone-induced rats:

Rotenone significantly ($p < 0.001$) reduced latency to fall in the rotarod test compared to the vehicle group. Gramine (13, 55 mg/kg) ($p < 0.001$) and 27.5 mg/kg ($p < 0.05$) significantly increased latency, similar to levodopa-

carbidopa (30 mg/kg; $p < 0.001$). In the hole board test, rotenone reduced the number of pokings ($p < 0.001$), while gramine (13, 27.5, 55 mg/kg) and levodopa-carbidopa significantly increased them ($p < 0.001$). In the actophotometer test, rotenone reduced total locomotor activity ($p < 0.001$); gramine and levodopa-carbidopa reversed this effect ($p < 0.001$). In the open field test, rotenone reduced square crossings and rearings ($p < 0.001$), which were significantly restored by gramine and levodopa-carbidopa ($p < 0.001$). Lastly, grip strength was reduced by rotenone ($p < 0.001$) but significantly improved by all doses of gramine and levodopa-carbidopa ($p < 0.001$).

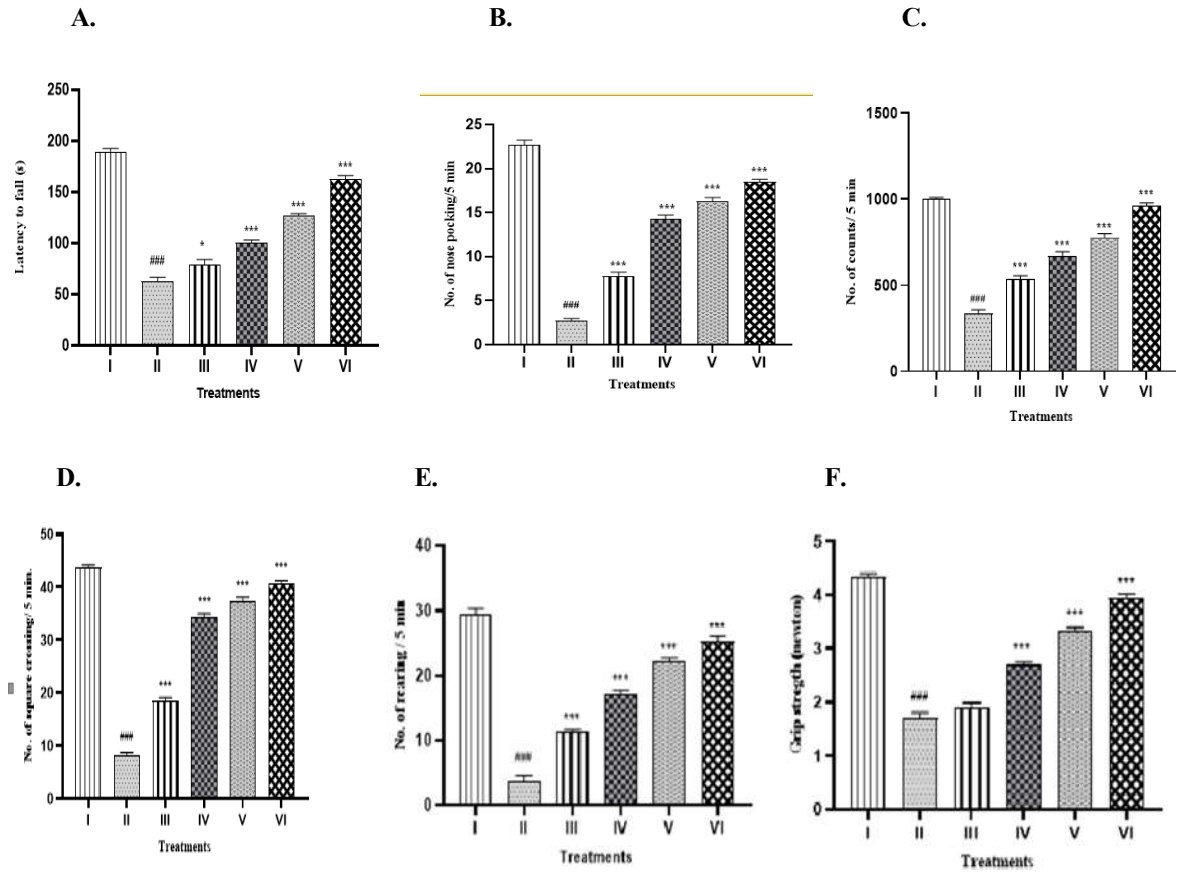

Figure 2. Data are expressed as mean ± SEM (n = 6) and analysed by one – way analysis of variance (ANOVA) followed by Dunnett's test. $^{###}P < 0.001$ as compared to vehicle treated animals, $^{***}P < 0.001$ as compared to Rotenone treated animal. Effect of gramine on A. fall of latency in rota rod test. B. no. of nose pocking in hole and board test. C. total locomotor activity in actophotometer test. D. no. of square crossing in open field test. E. no. of rearing in open field test. F. grip strength test.

3.3. Effect of gramine on Biochemical parameters:

Rotenone significantly ($p < 0.001$) decreased levels of CAT, SOD, and GSH, while significantly increasing LPO and NO levels, indicating oxidative damage in rats. Gramine (13, 27.5, and 55 mg/kg) significantly increased CAT levels and reduced LPO and NO levels ($p < 0.001$). SOD and GSH levels were significantly restored at 27.5 and 55 mg/kg, with the 13 mg/kg dose showing mild or non-significant effects. Levodopa-carbidopa showed a similar protective effect, significantly ($p < 0.001$) improving all oxidative stress parameters compared to the rotenone group.

Table 2: Effect of gramine 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)	NO (µ moles of nitrite/mg proteins)
Control	14.2 ± 0.292	9.56 ± 0.42	82.7 ± 0.2.11	9.15 ± 0.414	22 ± 0.97

Rotenone (3mg/Kg i.p.)	5.35±0.382$^{###}$	14.98± 0.24$^{###}$	35.9 ± 1.9$^{###}$	3.16 ± 0.232$^{####}$	69 ± 0.8$^{####}$
Gramine (13 mg/kg p.o.) + Rotenone	7.13 ± 0.332***	6.97 ± 0.2***	44.6 ± 1.16*	4.25 ± 0.26	55 ± 0.7***
Gramine (27.5 mg/kg p.o.) + Rotenone	7.9 ± 0.266**	8.68 ± 0.4***	54.8 ± 1.2***	4.77 ± 0.27**	53 ± 0.38***
Gramine (55 mg/kg p.o.) + Rotenone	8.94 ± 0.318***	8.45 ± 0.3***	64 ± 19.4***	5.44 ± 0.29***	50 ± 0.4***
Levodopa-carbidopa (30mg/ kg p.o.) + Rotenone	9.28 ± 0.401***	9.91 ± 0.2***	71.4± 1.94***	6.52 ± 0.235***	44 ±1.1***

3.4. Effect of gramine dopamine levels:

Significant ($p < 0.001$) decrease in levels of dopamine were observed after rotenone administration as compared to vehicle group. Administration of gramine (27.5 and 55 mg/kg) ($p < 0.001$) and gramine (13mg/kg) ($p < 0.01$) showed significant rise in levels of dopamine as compared to rats treated with rotenone. Levodopa carbidopa also showed significant ($p<0.001$) increase in dopamine levels as compared to rotenone treated group ($p < 0.001$).

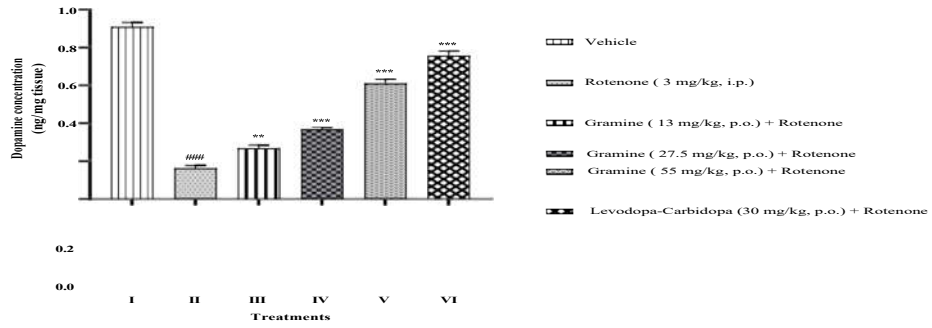

Figure.3. Effect of gramine on dopamine level. Data are expressed as mean ± SEM (n = 6) and analysed by one – way analysis of variance (ANOVA) followed by Dunnett's test. $^{###}P < 0.001$ as compared to vehicle treated animals, $^{***}P < 0.001$ as compared to Rotenone treated animal.

4. Histopathological analysis: Histopathological analysis of rat's SNPc showed presence of normal neuronal cells with prominent nucleus (black arrow) in control animal. In Rotenone (3 mg/kg, i.p.) treated group there was more evidence cytoplasmic inclusion of Lewy bodies (white arrow) are seen. more evidence of pyknotic nuclei (blue arrow), vacuolated cytoplasm (Vc) (yellow arrow) and severe loss of dopaminergic cells. Rotenone with gramine (13 mg/kg) group indicates less evidence of pyknotic nuclei and neuronal degeneration and few cytoplasmic inclusions of Lewy bodies. Rotenone with gramine (27.5 mg/kg) group indicated the vacuolated cytoplasm and pyknotic nuclei was less noticed, less number of cytoplasmic inclusions of Lewy bodies. Rotenone with gramine (55 mg/kg) group very less pyknotic nuclei and greater number of prominent nuclei neurons indicating less brain tissue damage. Rotenone with levodopa-carbidopa showed optimal sized cells with defined nucleus (black arrow).

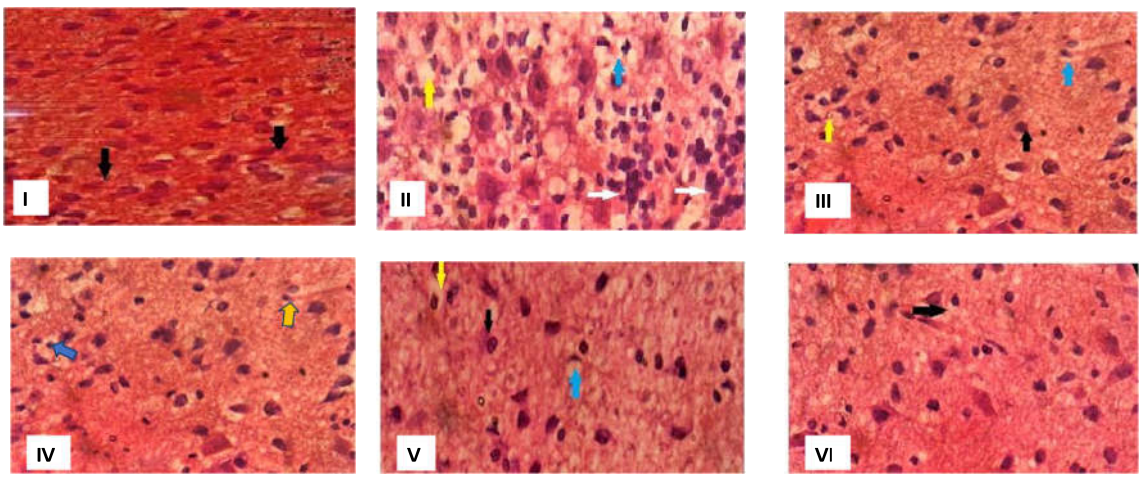

Figure.4. Histopathological changes of substantia nigra pars compacta (SNPc) region of rat's brain.

5. DISCUSSION:

With a frequency of about 14 per 100,000 people worldwide and about 50,000 new cases each year, Parkinson's disease is the second most prevalent neurological illness. According to epidemiological research, pesticide exposure may raise the chance of Parkinson's disease. The etiopathology of Parkinson's disease is yet unknown. Nonetheless, the oldest and most widely recognized theory about the etiopathology of Parkinson's disease is that selective oxidative stress occurs in the SN.[22] Numerous characteristics of Parkinson's disease (PD), such as selective dopaminergic degeneration, enhanced oxidative damage, and a-synuclein aggregation, are replicated when complex I is systemically inhibited by the insecticide rotenone (ROT). [23] A growing body of evidence points to complex I as a potential ROS source. Complex I contains an electron leakage site upstream of the rotenone-binding site; partial inhibition of complex I, as rotenone produces it or as observed in Parkinson's disease, can increase the production of ROS. [24]

The goal of the current study was to assess Gramine's neuroprotective effects in a rat model of rotenone-induced parkinsonism. By improving behavioral, biochemical, neurotransmitter, and histological parameters, the results showed that gramine had strong neuroprotective effects. To predict mechanism of neuroprotection, the in-silico docking studies were performed to identify targets for gramine. The outcome showed that.calculated affinities (Kcal/mol) of gramine was found to be for Dopamine transporter (-5.9), TNF-alpha (-5.9), NF-kB (-5), D3 receptor (-4.9). the values exhibited good affinities for the targets, suggesting gramine as potential candidate for the parkinsonism disease. this target such dopamine transporter, D3 receptor were confirmed by estimating neurotransmitter dopamine level in rat’s brain. The result of in-vivo study indicated that gramine demonstrate notable neuroprotective effect in parkinsonism illness brought on by rotenone.

In the current investigation, rotenone (3 mg/kg, i.p.) was given for 21 days to cause neurodegeneration and Parkinson's disease symptoms. affected hypokinetic movements in the hole board test and decreased grip strength activity in the grip strength test and rotarod demonstrated that rotenone treatment dramatically affected motor coordination. When comparing the rotenone-treated group to the vehicle-treated group, behavioural parameters like the rotarod-test revealed a lower latency to fall off and fewer counts in the actophotometer. The results of the present study demonstrated that treatment with gramine for 21 days in rotenone treated animals significantly attenuated the impairment of motor skills, muscular rigidity, and exploratory behavior and improved the grip strength. Levodopa-Carbidopa (30mg/kg) was employed as the standard drug which showed significant results in behavioural parameters as compared to rotenone treated group. Rotenone treated animals showed elevated levels of lipid peroxidation, nitrite along with significant reduction in antioxidant enzymes such as superoxide dismutase, catalase and reduced glutathione indicating generation of free radicals which might be responsible for neurodegeneration. On a biochemical level, gramine significantly restored antioxidant defences (increased CAT, SOD and GSH level) and attenuate oxidative stress marker (reduced LPO and NO level), indicating its potent antioxidative potential. Additionally, gramine elevated levels of key neurotransmitter- dopamine which is known to depleted in parkinsonism disease. this neurotransmitter improvement could be connected to gramine observed binding affinity to dopamine transporter and D3 receptor, as shown in the in silico docking studies.

In histopathological analysis, rotenone treated group showed the presence of pyknotic nuclei, vacuolated cytoplasm (Vc) and severe loss of dopaminergic cells compared to vehicle treated group. Whereas, gramine treated group showed fewer number of pyknotic nuclei and vacuolated cytoplasm as compared with rotenone treated group. Showing structural recovery in the SNPc and reduced sign of neurodegeneration in gramine treated group.

6. CONCLUSION:

From the findings of the current investigation, we infer that because of its anti-inflammatory and antioxidant properties, gramine showed a protective effect against rotenone-induced parkinsonism illness. Furthermore, according to neuroprotective research, gramine appears to be a unique and promising pharmacological agent.

7. ACKNOWLEDGEMENT:

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