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RESEARCH ARTICLE

Neuroprotective Effect of *Ocimum sanctum Linn* on Rotenone Induced Parkinsonism in Rats

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder characterised by a loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) manifesting in motor, cognitive and behavioural anomalies. Experimental PD was induced by administration of rotenone, a neurotoxin which developed all the essential features of PD. PD is attributed to oxidative and inflammatory stress and hence drugs targeting these pathways hold promise as neuro-therapeutics. *Ocimum sanctum Linn* (OS*)* has been shown to have antioxidant, antianxiety and anti-inflammatory properties and thus was tested for its neuroprotective effects. Twenty four male wistar rats were taken for the study and divided into four groups of six rats each. Group I is the vehicle treated. Group II, III and IV were treated with rotenone (1.5 mg/kg/48hr/s.c) for 11 days. Group III & IV were treated with low (100mg/kg/p.o) and high (200 mg/kg/p.o) doses of OS daily for 11 days. The behavioural alterations were evaluated by the open field test, pole test and rotarod test. Biochemical changes were assayed by estimating MDA, GSH and SOD. Histopathological study of the substantia nigra (SN) was also done. Treatment with lower and high dose of OS reversed the locomotor deficits and biochemical alterations due to rotenone which were supported by histopathological studies. The present study exhibited neuroprotective activity in rotenone induced PD. Hence, *Ocimum sanctum* Linn. extract may be considered as possible candidate in the treatment of PD.

KEYWORDS

Ocimum Sanctum Linn., Neuroprotective Effect, Rotenone, Parkinson's Disease

INTRODUCTION

Parkinson's Disease is a second most common, chronic, progressive neurodegenerative disorder. It presents with four cardinal motor manifestations tremor at rest, rigidity, bradykinesia and postural instability. The dopaminergic deficit results in motor disabilities as well as cognitive disturbances.[1]

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Research has found that idiopathic PD begins most often between 45 and 65 years of age and approximately 1% of individuals over 60 years have PD.[2,3] PD is one of the most common neurological diseases in the United States.[4] The prevalence in the United States and Western Europe is approximately 1-2 per 1000. Numerous factors such as reactive oxygen species induced damage, excitotoxicity, mitochondrial dysfunction and inflammation mediated cell injury have been implicated in the etiology of this disorder.[1] The pathogenesis of PD is the degeneration of neural connection

between the substantia nigra and the striatum, two brain regions essential for motor function. Striatal dopamine deficiency and the presence of cytoplasmic inclusions called lewy bodies are the pathological hallmark of PD.[5] Neuronal toxins such as 6-hydroxydopamine (6-OHDA),1-methy,4-phenyl, 1,2,3,4-tetrahydropyridine (MPTP) and rotenone are commonly employed to induce parkinsonism in rats.[1]

Rotenone, a naturally occurring compound is commonly used as an "organic" insecticide to kill nuisance fish in lakes. Additionally, rotenone is a lipophilic compound that easily crosses the blood-brain barrier. Rotenone induced neurotoxicity is mediated by its ability to inhibit complex I leading to generation of reactive oxygen species. The rotenone model appears to be an accurate model in that systemic complex I inhibition results in specific, progressive and chronic degeneration of the nigrostriatal pathway similar to that observed in human PD.

It also reproduces the neuronal inclusions and oxidative damage seen in PD. Thus, the rotenone model recapitulates most of the mechanisms thought to be important in PD pathogenesis. For this reason, neuroprotective drug treatment trials in this model may be more relevant to PD than other, more acute model systems.[5]

Holy Basil (scientific name is *Ocimum Sanctum*) or Tulsi is undoubtedly the best medicinal herb ever known. It has endless miraculous and medicinal values and is being worshipped in India since thousands of years.[6] The leaves of *Ocimum sanctum* Linn. is a potent antioxidant and is believed to have beneficial effects.

The leaves of *Ocimum sanctum* (linn.) contains 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene. Fresh leaves and stem of *Ocimum Sanctum* extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol. Two flavonoids, viz., orientin and vicenin from aqueous leaf extract of *OS* have been isolated. Ursolic acid, apigenin, luteolin, apigenin- 7-O-glucuronide, luteolin-7-O glucuronide, orientin and molludistin have also been isolated from the leaf extract. *Ocimum Sanctum* also contains a number of sesquiterpenes and monoterpenes viz., bornyl acetate, β-elemene, neral, α- and β-pinenes, camphene, campesterol, cholesterol, stigmasterol and β-sitosterol.[7,8,9,10,11] The health benefits of holy basil include oral care, relief from respiratory disorders, fever, asthma, lung disorders, heart diseases and stress. A few leaves dropped in drinking water or foodstuff can purify it and can kill germs in it. Even smelling it or keeping it planted in a pot indoors can protect the whole family from infections, cough and cold and other viral infections.[6]

The present study has been undertaken to evaluate the neuroprotective effect of *Ocimum sanctum* Linn. on rotenone induced Parkinsonism in rats.

MATERIALS AND METHOD

Drugs and Chemicals

Rotenone was procured from sigma Aldrich, USA and Dimethylsulfoxide (DMSO), PEG-300 (polyethylene glycol) from S.D Fine Chemicals, Mumbai. All the chemicals used were of analytical grade.

Collection of Plant Material

Leaves of *Ocimum sanctum* (Linn.) were collected in the month of Jan 2013, from the agricultural fields of Hyderabad, Andhra Pradesh. The leaves were shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation of Hydro Alcoholic Extract by Maceration Process

Fresh leaves of *Ocimum sanctum* linn. were collected from fields. Leaves were shade dried and were powdered using mechanical grinder. 350 g of powdered material was then subjected to simple maceration with 50%

alcohol for 7 days with intermittent shaking, filtered, evaporated and vacuum dried. A brownish residue weighing 11% (w/w) was obtained and kept in air tight bottles until use.

Experimental Animals

Male wistar rats were obtained from National Institute of Nutrition Hyderabad. They were acclimatized to laboratory conditions (25±3^{o}C of temperature, 12-h light/dark cycle), food and water was given ad libitium. After an acclimatization period of 1 week, they were randomly divided into 4 experimental groups.

All the experimental procedures were carried out in accordance with committee for the purpose of control and supervision of experiments on animal (320/CPCSCEA dated 03-01-2001) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee (GPRCP/IAEC/11/13/3/PCL/AE-6-Rats-M-24), G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad, India.

Experimental Design

Male wistar rats weighing between 150-200g were divided into following four groups of six rats each, in which two groups received extract of OS as low (100 mg/kg) and high doses (200 mg/kg).[12]

Group 1: Normal control (received six subcutaneous injections of the vehicle (DMSO+PEG-300, 1:1 v/v) every 48h for 11days).

Group 2: Disease control (received six subcutaneous injections of rotenone (1.5mg/kg, in a max volume of 5ml/kg) every 48h for 11 days).

Group 3: Received rotenone + Low dose of *Ocimum sanctum* Linn. extract (Low dose of OS) p.o daily for 11 days.

Group 4: Received rotenone + High dose of *Ocimum sanctum* Linn. extract (High dose of OS) p.o daily for 11 days.

On the 13th day behavioral tests were conducted like open field test, pole test and rotarod test. Further animals were sacrificed and the brain was dissected out for biochemical estimations of malondialdehyde (MDA), reduced glutathione (GSH), super oxide dismutase (SOD) and histopathology test.

Behavioral Tests

Open Field Test

Exploratory behavior was evaluated in an open-field paradigm (Lister, 1990).[13] The open field was made of plywood and consisted of a floor (96X96 cm) with high walls. The entire apparatus was painted black except for 6-mm-thick white lines that divided the floor into 16 squares. Each animal was placed at one corner of the apparatus and for next 5 min, it was observed for the ambulations (number of squares crossed) and number of rearings.[14]

Pole Test[15]

The pole test has been used previously to assess basal ganglia-related movement disorders in rodents. The rats were placed head-up on top of a vertical wooden pole 50 cm long (1 cm in diameter). The base of the pole was placed in the home cage.

When placed on the pole, animals orient themselves downward and descend the length of the pole back into their home cage. The rats received 2 days of training that consisted of 5 trials for each session. On the test day, the animals received 5 trials, total time to descend (t-total) was measured. The mean of the 5 trials was used and compared.

Rotarod Test for Motor Co-Ordination[16]

The rotarod test utilizes a rotating rod maintained at a speed of 40 RPM which rats were placed individually on rotating rod and the time taken by each animal to maintain its balance on the rotarod was observed.

Biochemical Estimations

Determination of Malondialdehyde (MDA) Concentration

Level of Malondialdehyde was determined by the procedure described by Ohkawa *et al*.[17]

Determination of Reduced Glutathione (GSH) Level

Glutathione GSH was analyzed according to Ellman's method.[18]

Determination of Super Oxide Dismutase (SOD)

The SOD level was determined by the procedure described by Mishra *et al*.[19]

Histopathology

After performing the behavioral tests, animals were deeply anesthetized with ether, their brains were quickly removed and washed with ice-cold saline. One hemisphere from each brain was perfused with 10% paraformaldehyde solution and serial coronal sections (H and E). After staining, the sections were examined using a bright-field microscope.[16]

Statistical Analysis

Data expressed as mean ± SEM. All data were analyzed by one way analysis of variance (ANOVA) followed by Bonferroni's post hoc test by using Graph Pad prism software (5.03 version).P values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

In the present study, repeated systemic administration of rotenone to rats (1.5 mg/kg/48 h/6 doses, s.c) produced motor impairment, histopathological changes and biochemical deficit.

Effect of *Ocimum sanctum* Linn. Extract, Rotenone, Normal Control on Behavioral Activity in Rotenone Induced Parkinsonism in Rats

Open Field Test

In the open field test, all animals in each group showed variable number of ambulations and rearings. There was significant decrease in number of ambulation and rearings in rotenone treated animals i.e 7.6±1.208 ($p<0.001$) and 4±1.000 ($P<0.01$), when compared with normal control animals i.e, 21.8±2.538 and 18.6±6.465.

There was significant increase in number of ambulations in Low dose OS treated animals i.e., 14.2±1.855 ($P<0.05$) and there was increase in no. of rearings, but was not significant 12±6.403 ($P>0.05$). In high dose of OS treated animals there was significant increase in number of ambulation 20.4±1.503 ($P<0.001$) and rearings 18.2±6.907 ($P<0.001$), when compared with rotenone treated animals i.e, (7.6±1.208 and 4±1.000) (Table 1) (Fig 1) (Fig 2).

Pole Test

In the pole test, all animals in each group showed variable time to reach bottom of pole. There was extremely significant decrease in time taken by rotenone treated animals to reach the bottom of the pole i.e, 4±0.790 ($p<0.001$), when compared with normal control animals i.e, 12.88±2.756.

In low dose of OS treated animals there was increase in time taken by animals to reach bottom of the pole i.e, 7.14±1.936 ($p>0.05$) but was not significant, when compared with normal control animals. i.e, 4±0.790. In high dose of OS treated animals there was extremely significant increase in time taken by the animals to reach the bottom of the pole. i.e, 11.7±2.214 ($p<0.001$), when compared to rotenone treated animals i.e, (4±0.790) (Table 1) (Fig 3).

Rotarod Test

In the rotarod test, all animals in each group showed variable latency to fall down. There was extremely significant decrease in latency to fall down from the rotating rod in rotenone treated animals i.e, 5.54±1.566 ($p<0.001$), when compared with normal control animals i.e, 32.52±9.250.

In low dose of OS treated animals there was increase in latency of fall from the rotating rod 9.52±4.625 ($p>0.05$) but was not significant, when compared with rotenone treated animals. i.e. 5.54±1.566. In high dose of OS treated animals there was very significant increase in latency of fall from from the rotating rod i.e 21.452±6.840 ($p<0.01$), when compared to rotenone treated animals i.e, (5.54±1.566) (Table 1) (Fig 4).

Table 1: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on behavioral activity in rotenone induced Parkinsonism in rats.

Treatment	Open field test		Pole test (sec)	Rotarod test (sec)
	Ambulation frequency (No. of squares crossed)	No. of Rearings		
Normal Ve Vehicle	21.8±2.538	18.6±6.465	12.88±2.756	32.52±9.250
Rotenone	7.6±1.208[a]	4±1.000[b]	4±0.790[a]	5.54±1.566[a]
Low dose of OS (100 mg/kg)	14.2±1.855[γ]	12±6.403	7.14±1.936	9.52±4.625
High dose of OS (200 mg/kg)	20.4±1.503[α]	18.2±6.907[β]	11.7±2.214[α]	21.452±6.840[β]

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test. *p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Open Field Test

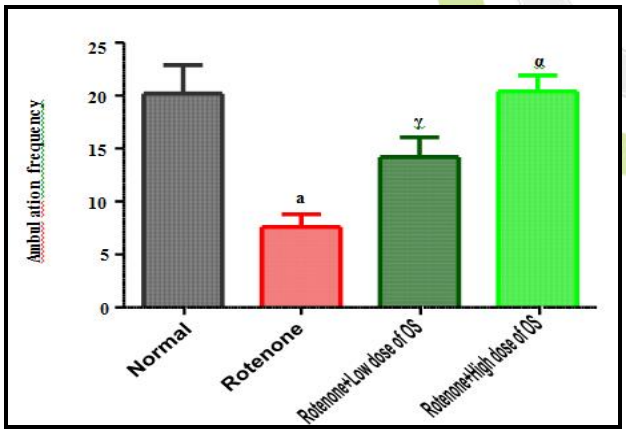

Figure 1: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on ambulation frequency (open field test) in rotenone induced Parkinsonism in rats

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.0001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Open Field Test

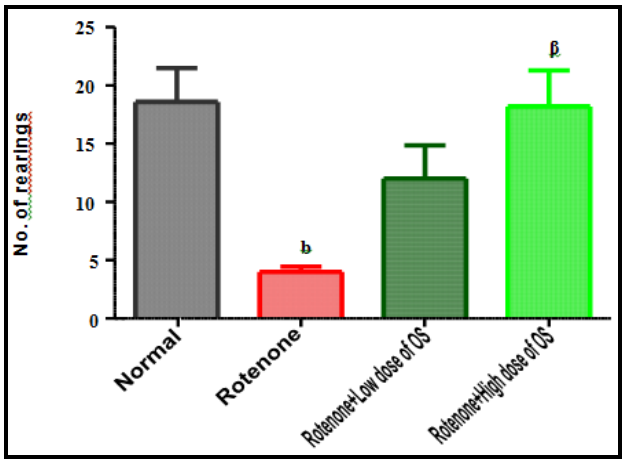

Figure 2: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on no. of rearings (open field test) in rotenone induced Parkinsonism in rats

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.0001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Pole Test

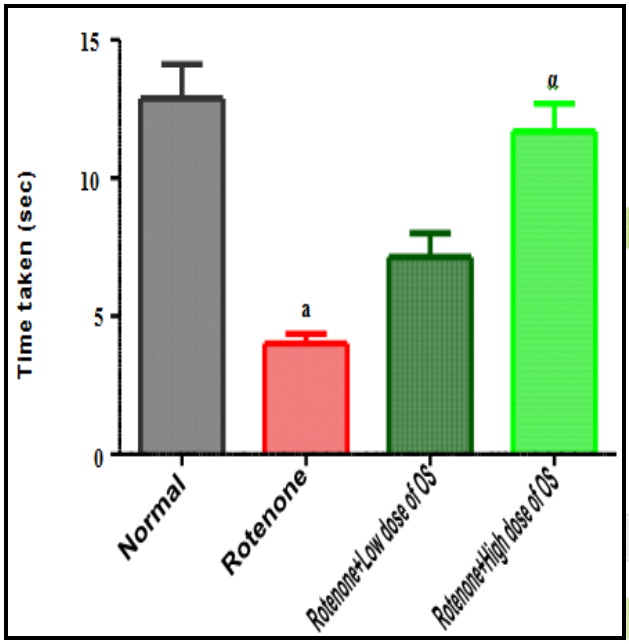

Figure 3: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on behavioral activity (pole test) in rotenone induced Parkinsonism in rats

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.0001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Rotarod Test

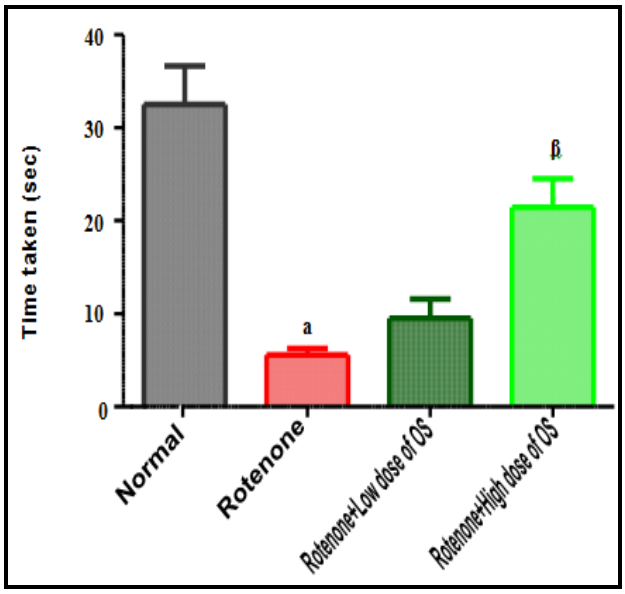

Figure 4: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on behavioral activity (rotarod test) in rotenone induced Parkinsonism in rats

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.0001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Effect of *Ocimum sanctum* Linn. Extract, Rotenone and Normal Control on Biochemical Parameters in Rotenone Induced Parkinsonism in Rats

Malondialdehyde Level

In the estimation of MDA levels, all animals in each group showed variable levels of MDA. There was significant increase in levels of MDA in rotenone treated animals i.e 185.78±58.956 (p<0.001), when compared with normal control animals i.e, 26.76±2.756.

There was significant decrease in MDA levels in low and high doses of OS treated

animals i.e, 61.36±8.739 (P<0.001) and 37.4±11.918 (P<0.001), when compared with rotenone treated animals i.e, (185.78±58.956) (Table 2) (Fig 5).

Glutathione Concentration

In the estimation of reduced glutathione (GSH) levels, all animals in each group showed variable levels of GSH. There was significant decrease in the levels of GSH in rotenone treated animals i.e 21.16±3.356 (p<0.001), when compared with normal control animals i.e, 77.76±8.041. There was increase in GSH levels in low dose of OS treated animals i.e, 30.2±9.208 (P<0.001) but was not significant when compared with rotenone treated animals i.e, 21.16±3.356. There was significant increase in GSH levels in high doses of OS treated animals i.e, 58.88±7.169 when compared with rotenone treated animals i.e, (21.16±3.356) (Table 2)(Fig 6).

Superoxide Dismutase

In the estimation of superoxide dismutase (SOD) levels, all animals in each group showed variable levels of SOD. There was significant decrease in the levels of SOD in rotenone treated animals i.e 41.6±3.647 (p<0.001), when compared with normal control animals i.e,119.6±10.877. There was increase in SOD levels in low dose of OS treated animals i.e, 48.2±9.654 (P<0.001) but was not significant when compared with rotenone treated animals i.e, 41.6±3.647. There was significant increase in SOD levels in high doses of OS treated animals i.e, 91.4±14.690 when compared with rotenone treated animals i.e, (41.6±3.647) (Table 2) (Fig 7).

Table 2: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on biochemical parameters in rotenone induced Parkinsonism in rats

Treatment	MDA	GSH	SOD
Vehicle	26.76±2.756	77.76±8.041	119.6±10.877
Rotenone	185.78±58.956**a**	21.16±3.356a	41.6±3.647**a**
Low dose of OS	61.36±8.739α	30.2±9.208	48.2±9.654
High dose of OS	37.4±11.918α	58.88±7.169α	91.4±14.690α

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's Post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P <0.0001), b (P<0.01) when compared to normal control.

α (P<0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Concentration of MDA in SN

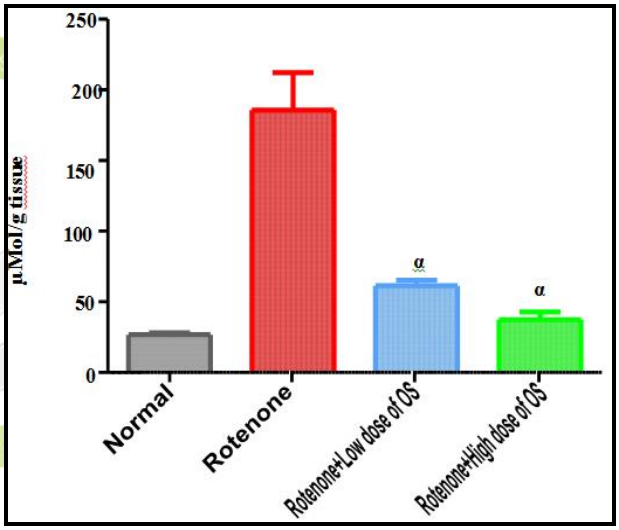

Figure 5: Effect of *Ocimum sanctum* Linn. Extract, rotenone and normal control on MDA level in SN in rotenone induced Parkinsonism in rats

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.0001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group

Concentration of GSH in SN

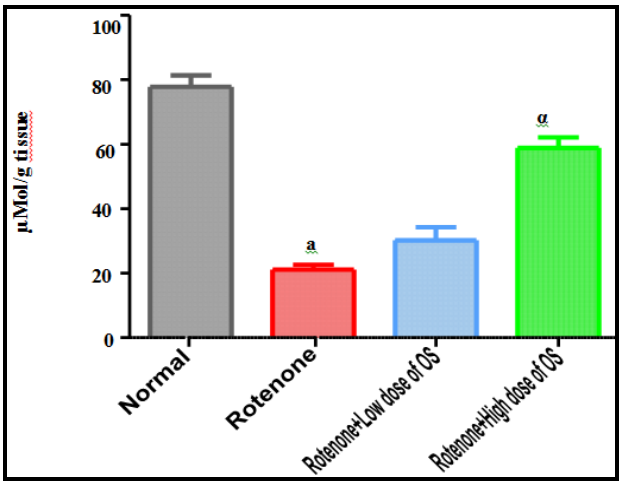

Figure 6: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on GSH level in SN in rotenone induced Parkinsonism in rats

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.0001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Concentration of SOD in SN

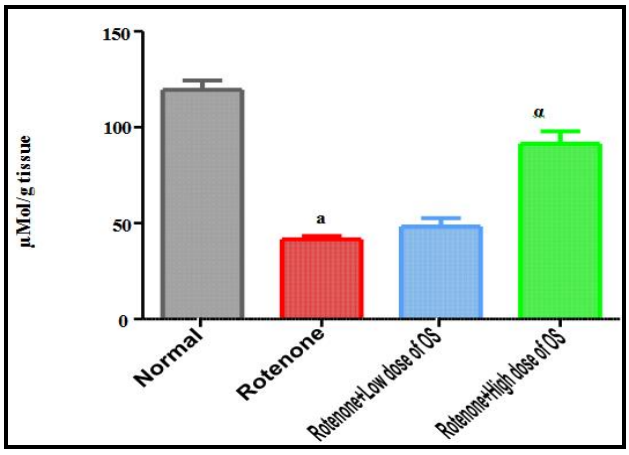

Figure 7: Effect of *Ocimum sanctum* Linn. extract, rotenone and normal control on SOD level in SN in rotenone induced Parkinsonism in rats

Values are Mean ± SEM, n= 6, results are analysed using ANOVA followed by Bonferroni's post hoc test.

*p<0.05 considered significant, **p<0.01 considered very significant, ***p<0.001 considered extremely significant.

a (P < 0.0001), b (P<0.01) when compared to normal control.

α (P < 0.001), β (P<0.01), γ (P<0.05) when compared to rotenone treated group.

Histopathology

Pathological changes during PD include neuronal cell loss, presence of lewy bodies and and undamaged striatum.

In the normal control group animals showed normal neuronal density and there was no presence of lewy bodies in the substantia nigra and there was no structural damage in substantia nigra (Fig.8).

In rotenone treated group there was neuronal cell death, slight structural damage to the SN and there was huge distribution of lewy bodies near the substantia nigra (Fig.9).

In the low and high dose of OS there was decrease in the neuronal cell death when compared to the rotenone treated group (Fig. 10, 11).

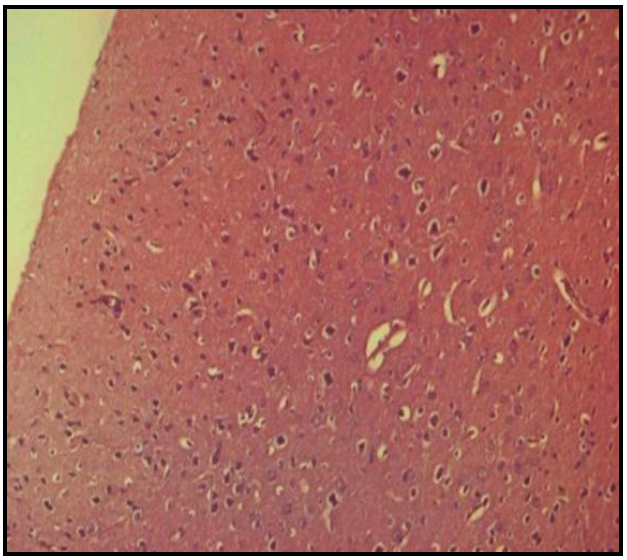

Figure 8: Histopathology of Normal control

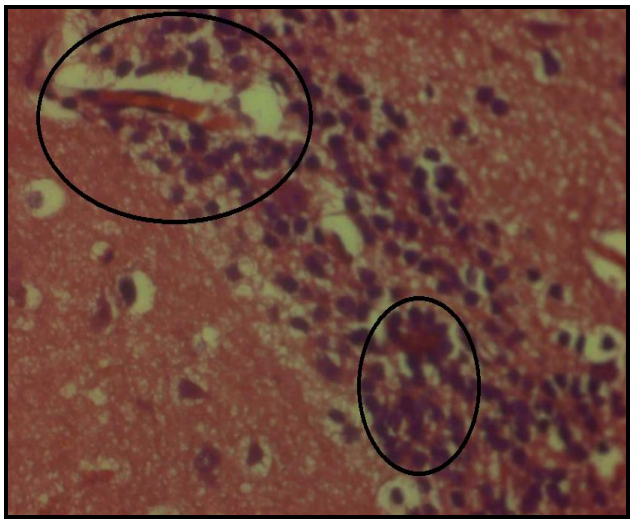

Figure 9: Histopathology of Rotenone Treated Animals

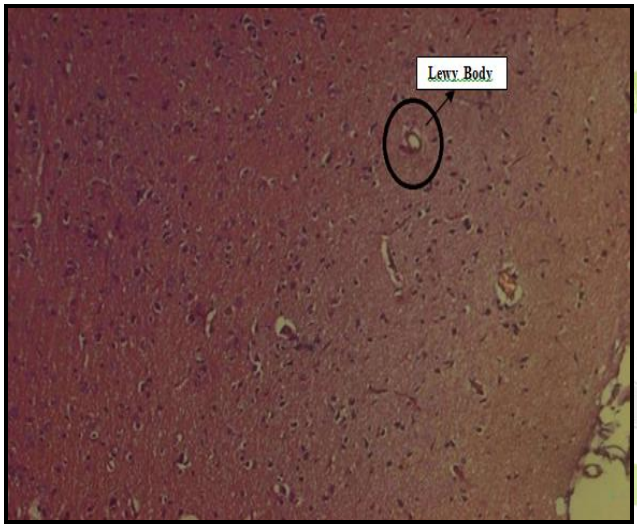

Figure 10: Histopathology of Low dose of OS treated animals

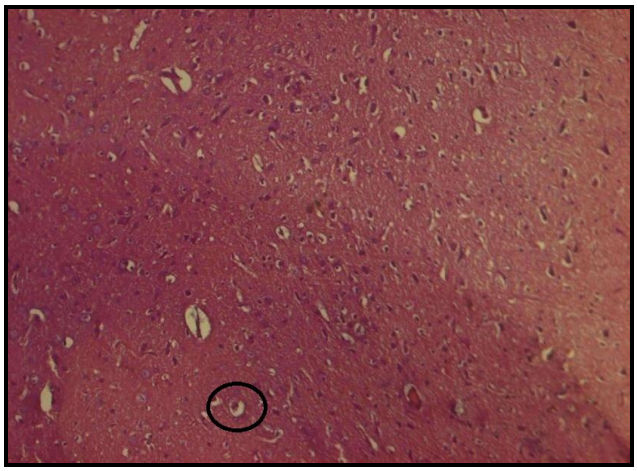

Figure 11: Histopathology of High dose of OS treated animals

The present study demonstrated the protective effect of *Ocimum sanctum* (Linn.) on rotenone induced Parkinsonism in rats. Exposure to rotenone for continuously 11 days induced symptoms like bradykinesia, rigidity, resting tremor, abnormalities of balance and posture.

The Open field test is commonly used to assess locomotor, exploratory and anxiety-like behavior. This test is particularly useful in evaluating the locomotor responses and as well as behavioral responses to novelty. In the open field test, rotenone caused a significant reduction in behavioral alteration as by decreasing the number of ambulation and rearings. Our results were supported by earlier reported studies that there would be a decrease in the locomotor activity in the rotenone treated animals.15 Treatment with *Ocimum sanctum* normalised ambulation and no. of rearings in open field which are used as a measure of locomotor activity. According to earlier reported studies treatment with *Ocimum sanctum* would improve the locomotor activity. These behavioral changes were because of its anti-anxiety effect.[20]

Rotarod test is used to assess motor coordination, skill learning and balance in rodents, which are the basic indicators of normal motor function.[21] The rotarod performance test evaluates balance and sensory motor coordination in animals. There was a significant deterioration of the rotarod performance in rotenone-treated animals, compared to the control animals. Treatment with *Ocimum sanctum* normalised rotarod performance when compared with rotenone treated animals. This change suggests that there was a significant impact of *Ocimum sanctum* in improving the performance of the animals.[22]

Pole test is a useful method in evaluating the movement disorders caused by striatum dopamine depletion. In the rotenone treated animals there was significant decrease in the time taken to reach the bottom of the pole when compared with normal control animals.

Pole test is the hallmark to evaluate the level of depletion of dopaminergic neurons in striatum.[23]

There was significant improvement in the performance of Low dose of OS and High dose of OS treated animals when compared with rotenone treated animals. These results were supported by earlier studies that, treatment with *Ocimum sanctum* could improve the performance in pole test and also decrease the dopaminergic depletion in striatum. Thus, rotenone exposure in rodents provides a valuable model for studying mechanisms of oxidative stress induced dopaminergic damage.

It is well known that the disabling symptoms in PD are primarily due a profound deficit in striatal dopamine (DA) content that results from the degeneration of dopaminergic neurons. Probably, there is not a single factor responsible for neurodegeneration, it appears that several factors are acting in concert. Therefore, there is a need to use multiple agents to protect damaging dopaminergic neurons from multiple pathologies in PD.[24, 25]

Oxidative stress generated as a result of mitochondrial dysfunction and oxidative metabolism of DA plays an important role in the PD pathogenesis.[26.27] Free radicals produced due to mitochondrial complex-1 defect could be responsible for the oxidative damage generated in dopamine metabolism,[28] which further yield reactive oxygen species (ROS)[29,30] resulting in a vicious cycle. Oxidative stress increases with age leading to various kinds of neurodegenerative diseases.[31] The reduced levels of endogenous antioxidant molecules such as glutathione (GSH), antioxidant enzymes such as superoxide dismutase (SOD), and lipid peroxidation product malondialdehyde (MDA) in the brain could contribute to neuronal death. Indeed, postmortem studies in PD brains demonstrate increased iron, decreased GSH, and oxidative damage to lipids, proteins, and DNA, suggesting that the SN is in a state of oxidative stress.[32] These findings introduced the requirement of using antioxidants as a therapeutic intervention in PD in addition to other protective agents.

In the present study rotenone treated animals showed increased levels of MDA and decreased levels of GSH and SOD, when compared with normal control animals which supports the earlier finding that rotenone alters the levels of GSH, SOD and MDA.[15]

Treatment with *ocimum sanctum* significantly reversed the altered oxidative stress markers due to its antioxidant properties in rat brain.[12] In histopathological findings there was maximum neuronal cell loss in the substantia nigra (SN) and presence of lewy bodies at SN was identified that indicates the damage of neurons at SN in rotenone treated animals. In accordance with earlier findings it has emerged that SN is more vulnerable to damage by rotenone that may be attributed to higher susceptibility of SN for free radicals. Histological findings in SN were more correlated with impaired motor coordination responses along with biochemical evidences. This indicates that brain areas may respond differently to oxidative stress caused by rotenone. Variable vulnerability of brain areas to rotenone induced oxidative stress has also been demonstrated in the in vitro study. In the present study treatment with *Ocimum sanctum* has decreased the histopathological changes by rotenone. It also increased the motor coordination among the animals which supports our study.[33]

On the basis of these finding, we conclude the neuroprotective effect of *Ocimum sanctum* (Linn.) on rotenone induced parkinsonism in rats, as it improves the behavioural deficits, altered stress markers and histological changes due to its anti-anxiety, antioxidant and neuroprotective effect.

CONCLUSION

In the present study administration of rotenone for 11 days have significantly induced Parkinsonism manifested by behavioral alterations and biochemical changes also evidenced by histopathological findings. Treatment with *Ocimum sanctum* significantly improved behavioral alterations and inhibited oxidative stress markers. In conclusion, the present study suggests that hydroalcoholic extract of *Ocimum sanctum* may show

beneficial effects in relieving symptoms of Parkinsonism. The protective effect of *Ocimum sanctum* (Linn.) may be attributed to the antioxidant and antianxiety effects and neuroprotective effect.

The exact mechanism and active constituent by which Ocimum sanctum showed the neuroprotective effect in rotenone treated animals is not fully elucidated, further investigation is needed.

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