Evaluation The Antiparkinsonian Effect of Salvia Officinalis on Animal Model of Parkinson's Disease

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Abstract
Parkinson's disease (PD) is an incurable condition that causes the dopaminergic neurons in the substantia nigra pars compact (SNc) to degenerate locally. The formation of Lewy bodies, whose main component is aggregated -synuclein, in the surviving neurons and the specific degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), are typically considered to be the two key pathological characteristics of Parkinson's disease (PD). The purpose of this study was to assess the effects of salvia officinalis extract on the brain of animal model of Parkinson disease, and if it can show better results when given synergistically with other parkinson's treatments due to knowledge provided from previous studies about anti-inflammatory and anti oxidant properties of salvia officinalis. Fifty male rat where divided in to five groups, group 1 (control) does not exposed to rotenone nor receive any treatment only given normal saline for 30 days, group 2 was injected by rotenone (2.5 mg/kg) IP for 30 days without any treatment, group 3 where injected with rotenone(2.5 mg/kg) and received sinemet tablet by gavage after dissolving it with water on day 15 for 30 days also, group 4 where injected with rotenone(2.5 mg/kg) and received extract of S. Officinalis (500 mg/kg)orally by gavage on day 15 for 30 days, group 5 where injected with rotenone(2.5 mg/kg) and received extract of S. Officinalis (500 mg/kg)and sinemet tablet orally by gavage on day 15 for 30 days, the antiparkinson effect of salvia officinalis and sinemet were evaluated by using open field test, force gripping test, and rotarod test. At the end of the experiments, animals were sacrificed by decapitation, the malondialdehyde(MDA) was determined by enzyme linked immunosorbent assays kits (Elisa) in rats blood. and number of neurons cells preserved with tyrosine hyrdroxylase(TH) enzyme and it's intensity were determined by immunohistochemical studies on rats brain tissues. In group 2, 4, 5 there was significant decrease in weight comparing with group 1 and in group 2,3,4 there was significant decrease in no. Of rotations, rotation distance, time of rotation comparing with group1. While in group3,4,5 there was significant increase as compared with group2 in term of rotarod test (P-value <0.05). In group 2, 3, 4 there was significant decrease in crossing, rearing, grooming time, no. Of visits to central area comparing with group1. While in group3,4,5 there was significant increase as compared with group2. in term of open field test (P-value <0.05). Furthermore, In group 2, 3, 4 there was significant decrease in force gripping as compared with group1 . While in group3,4,5 there was significant increase as compared with group2 In force gripping test (P-value <0.05). In biochemical test there was significant increase in MDA level in group 2 as compared with group 1. And significant decrease in its level in group 3, 4, 5 as compared with group 2 (P-value <0.05). In immunohistochemical studies the no. Of dopaminergic neurons with +ve tyrosine hydroxylase enzyme was significantly decreased in group 2, 3, 4, 5 as compared with group 1. While there was significant increase in group 3, 4, 5 as compared with group 2,(P-value <0.05). And the histoscore of intensity of TH in neurons preserved in dopaminergic neurons was significantly decreased in group 2, 3, 4, 5 as compared with group 1. While there was significant increase in group 3, 4, 5 as compared with group 2.
Keywords: Parkinson's disease ; salvia officinalis; patience

1. 1. Introduction

parkinson's disease (PD) is an intractable disease resulting in localized neurodegeneration of dopaminergic neurons of the substantia nigra pars compact (SNc) (1).
Usually, both selective degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the appearance of Lewy bodies, whose main component is aggregated α-synuclein, in the remaining neurons are thought to be the major pathological hallmarks of PD (2) (3).

Parkinson's disease is a recognisable clinical syndrome with a range of causes and clinical presentations. Parkinson's disease represents a fast-growing neurodegenerative condition; the rising prevalence worldwide resembles the many characteristics typically observed during a pandemic, except for an infectious cause.(4)
PD is considered the second most common neurodegenerative disorder with an estimated 6 million people affected worldwide.(5) PD prevalence has increased by74% between 1990 and 2016 and prevalence figures are expected to further increase

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by 2- to 3-fold until 2030. The disease is involving gradual loss of motor and non motor function. The motor symptoms (MS) consist of the cardinal motor features, including bradykinesia, rigidity, and tremor of the distal extremities. (6) Bradykinesia is the anchor symptom defining PD and is associated with reduced speed and amplitude of movement, the latter typically showing a decrement with repetitive motor sequences.

The risk of developing PD is twice as high in men than in women; particularly, women have a higher mortality rate and faster
progression of the disease (7).

1.2.Rotenone

is a natural insecticide and pesticide obtained from the roots of plants belonging to the genera Lonchoccarpus and Deriss . Rotenone block complex I of the mitochondrial electron transport chain, resulting in lower ATP generation, which can result in ROS such superoxide, lowering GSH and causing oxidative stress(8) and (9).Rotenone-induced PD models occurred through progressive destruction of DA-ergic neurons and the development of LBs in the SN simulating experimental aspects of idiopathic PD. For developing PD models in rats, many exposure routes like oral, subcutaneous(Sc), osmotic pumps and IP routes are used. Because rotenone is highly lipophilic, it easily crosses all biological membranes, including the BBB, and hence does not require transporters to enter cells(10).

1.3.Salvia officinalis

Salvia officinalis L., (sage) belongs to family Lamiaceae, herbaceous plants cultivated worldwide, is used as a traditional herbal medicine against various diseases, such as gastric disturbances and inflammatory processes (11). It presents many pharmacological properties, most of them associated with its largest polyphenol content . Various extracts of Salvia have been evaluated for biological effects, and their neuroprotective , antimicrobial ,anti-inflammatory , immune-modulatory, antioxidant , spasmolytic, anticancer , and cholinergic effects (12). Rosmarinic and carnosic acids, which have antioxidant properties, are present in high concentrations in the sage extract. The inhibition of lipid peroxidation by rosmarinic acid and carnosol as well as the anti- inflammatory and pro-inflammatory activities of ursolic acid

2. Materials and method

In this experiment we used one hundred male adult Albino rats whose weights range between 150-300 grams. The rats were housed in the Animal House of the College of Medicine/ University of Babylon. They were kept in 20 cages, five rats in each cage on twenty-five centigrade temperatures with fourteen hours in daylight and ten hours in darkness cycle with water and food ad libitum. The animals were randomly divided into five groups, 10 rats per each group, after two weeks of adaptation. The study was carried on at the College of Medicine/ University of Babylon, from September 2021 to March 2022.

2. 1. Plant Preparation

The dried leaves and roots of Salvia officinalis, was approved to be S. officinalis with the help of College of Agriculture/ Medicinal Plant Department/ Al-Qasim Green University according to
document No.1067 on 7/3/2022.

Infusion of sage leaves, or so-called production of sage tea, is a very popular preparation in folk medicine. According to (13), 100 mL of boiling water are poured over 5 g of leaves of Salvia officinalis L. and filtered after 30 min.

increasing the extraction temperature from 25 ◦C to 80 ◦C caused extracts yielded with higher phenolic content. These results were explained by Dent at al. who also studied sage aqueous extracts, reporting that the mass fraction of total polyphenols significantly depends on the extraction temperature (14). The highest total phenolic content and maximum antioxidant capacity of aqueous extracts of sage at temperatures were achieved at 80◦C.(15)

2.2. Rotenone Preparation

Rotenone (2.5 mg/kg BW) was given intraperitonially to the rats to induce Parkinsonism. Rotenone was initially dissolved in a 50X stock solution of dimethyl sulfoxide (DMSO)(16),125 mg rotenone dissolved in 1 mL DMSO.The stock solution was then diluted in 1960 µl of olive oil with 40 µl of the stock solution, fresh solution was prepared twice a week, the solution was vortexed to obtain a uniform mixture before administration to rat. Each rat was given 1ml/kg of prepared solution, while the control group of animals were given simply the vehicle (olive oil/DMSO) (17).

2.3. Sinemet Preparation

Sinemet(25/250 mg)tablet after crashing in water, prepared daily in a dose of 10 mg/kg for each rat in group 3 with shake before administration (18).

2.4. Study Design

The fifty rats were randomly divided into five groups, ten animals per each group as follow:

•Group I: healthy control group.

•the other fourty rats were Induced
with parkinsonism by rotenone IP 2.5mg/kg every 48hr (every other day) for 4 weeks (19) and subdivided as follow:

a) Group II: untreated PD rats.

b) Group III: 10mg/kg of Sinemet tablet every day for 4 weeks orally by a gavage.

c) Group IV: 500mg/kg of Salvia officinalis every day for 4 weeks orally by a Gavage.

d)Group V: 500mg/kg of Salvia officinalis + 10mg/kg of Sinemet tablet every day for 4 weeks orally by a gavage

•The rats were weighed in day 0, 15, and 30 of the experiment.

•Twenty-four hours after the last dose, at the day 30 behavioral tests had been performed for comparison of parkinsonism development and treatment

effectiveness. Each animal was placed in the open field for 10 mint, then on the narrow beam for three trials, then on rotarod for three trials and all behaviors were recorded by video camera.

•Then, each animal sacrificed by cervical dislocation to get mid brain samples to detect tissue levels of MDA and immunohistochemical measures

2.5. Brain Dissection

On 30th day, each rat was sacrificed and the brains were removed after dissection of skull from foramen magnum posteriorly. Cerebellum and olfactory pulps were removed and the brain gently removed from the skull and the mid and forebrain were taken and dissected out and rinsed with phosphate buffer solution and weighted.

Behavioral tests

Rotarod Apparatus

The study examined rodents' physical capacity and coordination with a digital rotarod test. Rats should balance on a rotating cylinder with a variable speed for it to work. (22). Each rat was placed on the 20 rpm rotating cylinder and studied for three minutes. Rat performance for motor coordination was graded depending on the number of spins. After each test, a 10 % ethanol solution was used to clean the instrument. (23).

Biochemical Assessments

Assessments of MDA using ELISA kit

Malondialdehyde (MDA) is an accepted marker of lipid oxidative damage. Malondialdehye was produced when highly reactive oxygen metabolites, particularly hydroxyl radicals, act on unsaturated fatty acids of phospholipids components of membranes (24)

MDA was measured by enzyme linked immunosorbent assay (ELISA).

Immunohistochemical study

The most popular method for locating and detecting specific antigens in cells and tissue is immunohistochemistry (IHC), which is a potent technique that takes advantage of the specific interaction between an antibody and antigen.

With the development of antigen retrieval techniques, which make it possible to perform IHC on formalin fixed paraffin embedded (FFPE) tissue comfortably, it has evolved from a standard tool in many domains to an essential adjunct technique in clinical diagnoses in anatomic pathology (25). Immunohistochemical score for tyrosine hydroxylase : Histoscore is based on the percentage of cells that fall into each of the four immunohistochemical categories: negative (0), weak (1+), moderate (2+), and stronge(3+) stained membranes. Each case's histoscore, which might vary from 0 to 300, was determined in the manner described below: HistoScore (H-score) is calculated as follows: HistoScore (H-score)=((1 × % weakly stained cells)+(2 × % moderately stained cells)+(3 × % strongly stained cells)) (26).

Analytical Statistics

The SPSS version 20 was used to statistically evaluate the study's findings.One-way ANOVA and the post hoc test are statistical formulas used to determine if differences are statistically significant. Statistical significance was set at 5% thus p value ≤ 0.05 was considered significant.

2. Results

The Rotarod Test

Number of Rotations

The number of rotations significantly decreased (P value <0.05) in group 2, group 3, group 4 as compared with group 1, while, the number of rotations significantly increased (P value <0.05) in group 3, group 4, group 5 as compared with group 2, furthermore the number of rotations insignificantly decreased (P value >0.05) in group 5 as compared with group 1. (Table 1 and Figure 1).

Table 1: A comparing mean differences of number of rotations between the groups

no. of rotations	G. 1	G. 2	G. 3	G. 4	G. 5
G. 1	X	9.300*	5.548*	5.718*	2.946
G. 2	-9.300*	X	-3.746*	-3.578*	-6.347*
G. 3	-5.548*	3.746*	X	0.169	-2.603
G. 4	-5.718*	3.578*	-0.165	X	-2.769
G. 5	-2.946	6.347*	2.598	2.769	X

* The mean difference is significant at the 0.05 level.

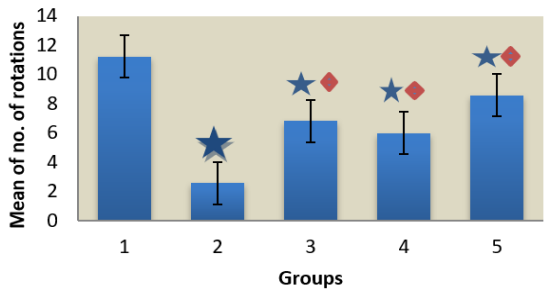

Figure 1: showing Means of rotations no. ± SEM of all groups

= significantly decreased (p value <0.05) when compared with group 1.

= significantly increased (p value <0.05) when compared with group 2.

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). No. of rat = 10 rats for each group.

Rotations Distance

Rotations distance considerably decreased (P value <0.05) in group 2, group 3, group 4 and group 5 as compared with group 1. furthermore, the rotations distance considerably increased (P value <0.05) in group 3, group 4, group 5 as compared with group 2 (Table 2 and Figure 2).

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected grotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). No. of rat = 10 rats for each group.

Table 2: Comparing the mean differences of rotations distance (cm) between the groups.

Rotation distance	Group1	Group2	Group3	Group4	Group5
Group1	X	121.91*	81.82*	93.14*	32.54*
Group2	-121.91*	X	-40.09*	-28.77*	-89.37*
Group3	-81.82*	40.09*	X	11.32*	-49.28*
Group4	-93.14*	28.77*	-11.32*	X	-60.6*
Group5	-32.54*	89.37*	49.28*	60.6*	X

* The mean difference is significant at the 0.05 level.

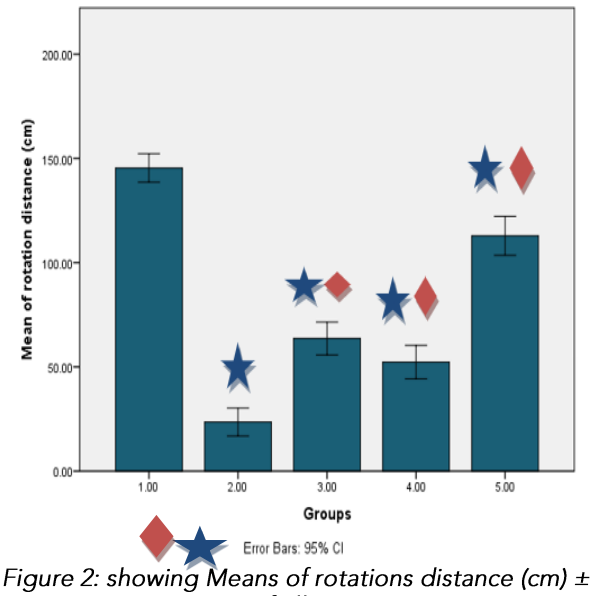

Figure 2: showing Means of rotations distance (cm) ± SEM of all groups.

= significantly decreased (p value <0.05) when compared with group 1.
= significantly increased (p value <0.05) when compared with group 2.

Time of Rotations

The time of rotations considerably decreased (P value <0.05) in group 2, group 3, group 4 and group 5 when compared with group 1. Furthermore, the time of rotations considerably increased (P value <0.05) in group 3, group 4, group 5 when compared with group 2 (Table 3 and Figure 3).

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). No. of rat = 10 rats for each group.

Table 3: Comparing the means differences of rotation time between the groups

Time of rotation	Group1	Group2	Group3	Group4	Group5
Group1	X	25.2*	14.8*	17.5*	7.8*
Group2	-25.2*	X	-10.4*	-7.7*	-17.4*
Group3	-14.8*	10.4*	X	2.7	-7.0
Group4	-17.5*	7.7*	-2.7	X	-9.7*
Group5	-7.8*	17.4*	7.0	9.7*	X

* The mean difference is significant at the 0.05 level.

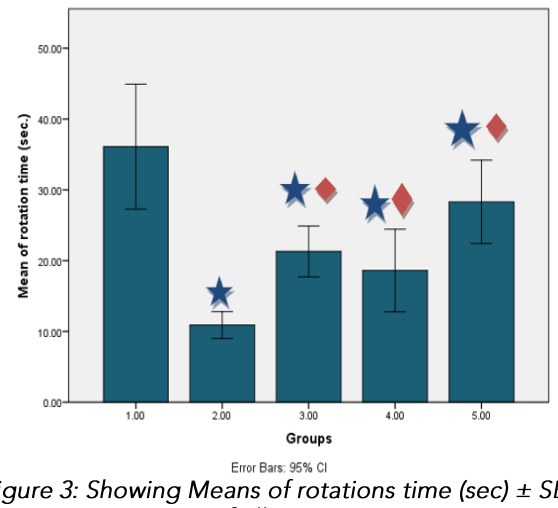

Figure 3: Showing Means of rotations time (sec) ± SEM of all groups.

= significantly decreased (P value <0.05) when compared with group 1.
= significantly increased (P value <0.05) when compared with group 2.

Biochemical study

Malondialdehyde levels

Malondialdehyde (MDA) levels considerably increased (P value <0.05) in group 2 and group 3 as compared with group 1 while considerably decreased (P value <0.05) in group 4 and group 5 as compared with group 2 and group 3, Furthermore group 4 and group 5 insignificantly decreased as compared with group 1 (Table 4 and Figure 4).

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and

injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). No. of rat = 10 rats for each group.

Table 4: Comparing the mean differences of MDA levels between different groups					
MDA	Group1	Group2	Group3	Group4	Group5
Group1	X	3.43*	-1.963*	0.945	0.339
Group2	3.432*	X	1.469	4.377*	3.771*
Group3	1.963*	-1.469	X	2.908*	2.302*
Group4	-0.945	-4.377*	-2.908*	X	-0.606
Group5	-0.339	-3.771*	-2.302*	0.606	X
* The mean difference is significant at the 0.05 level.					

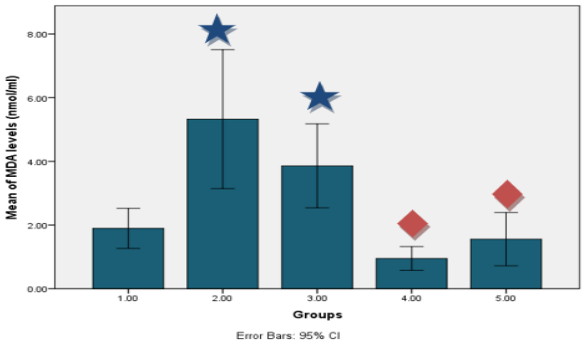

Figure 4: Showing Means of MDA levels (nmol/ml) ± SEM of all groups

= significantly increased (p value < 0.05) when compared with group 1
= significantly decreased (p value < 0.05) when compared with groups 2 and3.

Immunohistochemical study (IHC)

Number of neuron cells with positive tyrosine hydroxylase

Considerably decreased (P value <0.05) in group 2, group3, group4 and group 5when compared with group 1 while considerably increased (P value <0.05) in group 3, group 4 and group 5 when compared with group 2 (Table 5 and Figure 5).
Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and sinemet and injected rotenone). No. of rat = 10 rats for each group

Table 5: Comparing mean differences of number Of neurons with +ve stain between different groups					
No. of neurons	Group1	Group2	Group3	Group4	Group5
Group1	X	58.1*	38.7*	47.2*	32.1*
Group2	-58.1*	X	-19.4*	-10.9*	-26.0*
Group3	-38.7*	19.4*	X	8.5*	-6.6
Group4	-47.2*	10.9*	-8.5*	X	-15.1*
Group5	-32.1*	26*	6.6	15.1*	X
* The mean difference is significant at the 0.05 level.					

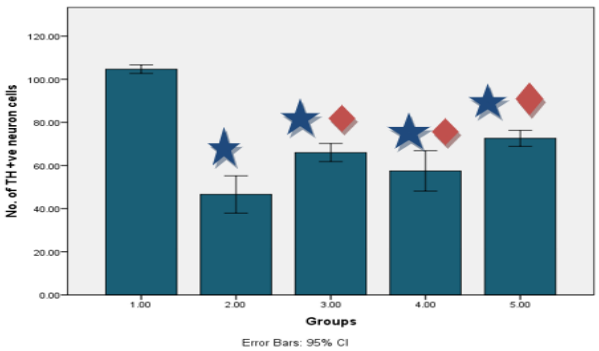

Figure 5: Showing Means of no. Of neurons with +ve stain ±SEM of all groups.

= significantly decreased (p value < 0.05) when compared with group1
= significantly increased (p value < 0.05) when compared with group2.
In order to determine the Histoscore of various groups at magnification (40x), IHC was carried out in this study on formalin-fixed, paraffin-embedded tissue samples using an SNCA poly clonal antibody and a 2-step plus poly-HRP anti-rabbit/mouse IgG detection system (with DAB solution) Based on four categories of immunohistochemistry that are reported as a percentage of cells, the HistoScore is called Intensity of Neuron with Positive Stain (BAD Stain): negative (0), weak (1+), moderate (2+), and strongly (3+) stained membranes. In each case, a histo Score with a possibility range of 0–300 was counted as follows: Histo Score (H-score) = ((1×% weakly stained cells) + (2×% moderately stained cells) + (3×% strongly stained cells))

Table 6: Histoscore (intensity of neurons with positive stain) as %	
Groups	Histoscore
G1	351.65
G2	198.84
G3	250.756
G4	235.65
G5	255.0285

Group 1 (control group, untreated and not exposed to rotenone), group 2 (untreated and injected rotenone), group 3 (treated with sinemet and injected rotenone), group 4 (treated with 500mg/kg S. Officinalis and injected rotenone), group 5 (treated with 500mg/kg S. Officinalis and injected rotenone). No. of rat = 10 rats for each group.

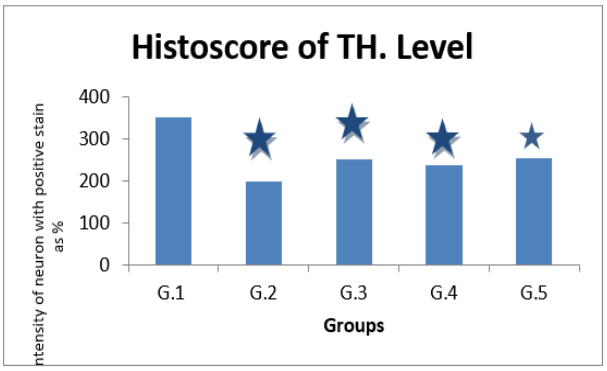

Figure 6:Showing No. Of TH +ve neurons as %

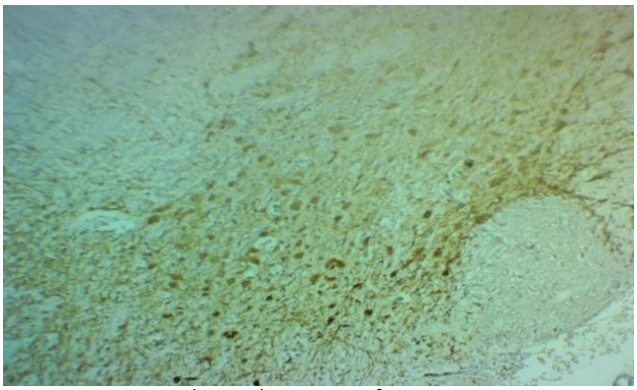

Figure 7: Histological section of SNPs in G1 a group treated with Distal water show positive stain in neurons and the fibers (HIC with BAD stain 40x)

3. Discussion

The Behavioral Tests

Rotarod Apparatus

In the rotarod apparatus, repeated rat exposure to rotenone significantly reduced the coordination of muscles (rotations number, rotations distance and time of rotations) when put in comparison with the control group which agrees with the previous studies of (27). The decrease in the DA level is linked to decrease movement in some previous studies (28).

S. Officinalis treatment preserved the coordination of muscles in the rotarod apparatus and improved rotation number, rotation distance and time of rotation as compared to group 2. This indicates that S. Officinalis has a good effect on improving the symptoms of PD in rats especially with a group of salvia officinalis and sinemet, which showed insignificant difference as compared to control group. This finding by the current study has not been arrived at by previous studies, as far as the researcher could investigate.

This improving effect of S. Officinalis is attributed to its powerful neuroprotective impact that has shown an efficient outcome as a neuroprotective agent in other neurodegenerative diseases as Alzheimer's disease because of retaining its anti-inflammatory and antioxidant agents Aqueous extracts of S. officinalis leaf have a high ability to protect the brain and liver homogenates from both Fe(II)- and SNP-induced lipid peroxidation in vitro; this high protective ability of the extract may be due to the antioxidant effect of the high vitamin C and total phenol content of the leaf. However, the main mechanism through which they bring about their protection is by their Fe(II) chelating ability, reducing power, and NO radical scavenging ability, but their OH radical scavenging ability is low. The antioxidant and protective effects of this leaf could be harnessed in the management and prevention of degenerative diseases associated with oxidative stress.(29)

In sinemet group there was a major progress of the rotarod performance on day 30 was the result of the typical treatment of L-dopa and carbidopa when compared with animals treated with rotenone due to increasing in DA level, which agrees with the study of (30).

Biochemical Parameters

Malondialdehyde

Malondialdehyde (MDA) is an accepted marker of lipid oxidative damage. Malondialdehye was produced when highly reactive oxygen metabolites, particularly hydroxyl radicals, act on unsaturated fatty acids of phospholipids components of membranes (31)

The present study has found that the rotenone group has shown highly significant increase in the MDA level as compared with the control group which agrees with the study of (32).

The development of neurodegenerative disorders is influenced by oxidative stress. Oxidative stress has been associated with the development of PD in both preclinical and clinical investigations especially elevation the concentrations of oxidative markers such as MDA. Similarly, pre-clinical investigations clearly demonstrated that oxidative stress in PD is caused by environmental factors such as neurotoxins, insecticides, pesticides, and DA itself. Pesticides, such as rotenone, have been shown to enhance ROS by blocking mitochondrial complex I activity, resulting in oxidative stress, which may be the cause of SNCA accumulation (33).

In Parkinson's disease-affected rat groups treated with Salvia officinalis extract revealed a significant reduction in MDA level there is a considerable reduction in MDA levels as compared to the rotenone with respect to lipid peroxidation and anti-oxidant concentration, indicating that the plant has anti-oxidant capacity, Aqueous extracts of S. officinalis leaf have a high ability to protect the brain from both Fe(II)- and SNP-induced lipid peroxidation in vitro; this high protective ability of the extract may be due to the anti-oxidant effect of the high vitamin C and total phenol content of the leaf. However, the main mechanism through which they bring about

their protection is by their Fe(II) chelating ability, reducing power, and NO radical scavenging ability, but their OH radical scavenging ability is low. The antioxidant and protective effects of this leaf could be harnessed in the management and prevention of degenerative diseases associated with oxidative stress.(28).(_34), These results confirmed that Salvia officinalis can significantly modulate oxidative stress parameters (35) Thus, oxidative stress inhibition could be one of the mechanisms underlying S. Officinalis's anti-Parkinson benefits.

In Sinemet group, there was also a significant increase in MDA level as compared with control group but less than rotenone group which agrees with the study of (36).

Treatment with L-dopa resulted in a rise in MDA and oxidized GSH levels, in addition to a depletion in reduced GSH. This impact could be explained by the fact that recurrent L-dopa treatment increases DA synthesis, which could lead to an excess of free radical generation, which would overwhelm the endogenous defense mechanism, causing an excess of oxidative stress. Previous investigations have shown that repeated L- dopa administration causes oxidative stress and inflammation, which supports this theory (37).

Histoimmunochemical study

The decrease in TH due to degeneration of dopaminergic neurons have been reported to play a prominent role in the reduced brain concentrations of dopamine and the manifestations of the clinical motor symptoms in patients with PD (38). The changes in TH expression have been used to show the rate of dopamine turnover or as an indirect measurement of dopaminergic activity, hence could serve as a predictor of the severity or progression of the disease (39). Consequently, TH enzyme is being viewed as an additional target for identifying new therapeutics for the disease. TH is an enzyme which converts the amino acid tyrosine into dihydroxyphenylalanine (DOPA).

This reaction is the first step in the production of dopamine It is a rate-limiting enzyme that controls the first step of dopamine biosynthesis. The expression of TH is in the right-side brain tissue of SNpc was detected by Western blotting . Lower expression of TH was observed in the right side of SNpc in the rotenone group than in the control group here was a significant reduction in expression of TH immuno-positive neurons in the substantia nigra of rats treated with rotenone. The loss of TH cells and dopaminergic neurons that serves as a local source of dopamine has been reported to be responsible for the motor deficits c a u s e d b y rotenone (40). The IHC DAB staining images revealed a significant increase in the number of TH-positive cells in the substantia nigra 30 days after sinemet exposure compared to the rotenone group which agrees with the study (41)

In the present study S. Officinalis cause increase in tyrosine hydroxylase neuron cells as it decreased the destruction of dopaminergic neurons by its mechanisms as antioxidant and anti-inflammatory . The antioxidant potential is directly related to the radical scavenging ability. High levels of radicals can cause far-reaching damage to cellular structures. Antioxidants play an important role in protecting the cells against oxidative damage (4, 5). Polyphenols(components of salvia officinalis) including flavonoids, belong to a group of natural compounds characterized by strong antioxidant properties.

the polyphenols and essential oils of Salvia officinalis also possess biological properties such as anti-bacterial , antioxidant, antitumor (42), antinociceptive, and anti-inflammatory activities and cytotoxic and cytogenetic effects (43). As results of several studies suggest that S. officinalis has a power antioxidant activity. (e phenolic compounds are isolated from the extract of Salvia officinalis such as carnosol, rosmarinic, and carnosic acids, followed by caffeic acid, rosmanol, rosmadial, genkwanin, and cirsimaritin with the most effective antioxidant activity (44). In addition, rosmarinic acid and flavonoids of S. officinalis especially quercetin and rutin have stronger antioxidant activity (45)

in group 5 there was more increment in TH + ve neurons referring to the synergistic activity of each S. officinalis and sinemet

Immunohistochemcial data showed that there is obvious difference in intensity of stain between all groups , rotenone group show low intensity of stain as it significantly increased the loss of dopaminergic neurons in the substantia nigra,(H. score= 198.84) and decreased the striatal expression of tyrosine hydroxylase. Further, rotenone administration activated microglia and astroglia, which in turn up regulated the expression of α-synuclein, pro-inflammatory, and oxidative stress factors, resulting in PD pathology.

While in sinemet group we noticed that threre is enhancement in the stain because significant increase in H. score (H.S.= 250.756) showing neuroprotective effects that prevented TH-positive neuronal loss (46)

In S. officinalis groupthe intensity of stain better than rotenone group but slightly less than group 3 and group 5, the H. score was (H.S. = 235.65) releaving the activity of S. officinalis as anti-oxidant and inti-inflammatory in preventing loss of the dopaminergic neurons.

In group 5 (H.S. = 255.0285) the stain was significantly more than groups 2,3,4 where sinemet and S.officinalis synergistically work together to preserve the dopamimergic neurons in substantia nigra and decrease the oxidative stress and preventing loss of tyrosine hydroxylase

Conclusion

1. S. officinalis improves the motor activity in the rotenone induced Parkinson disease in male rats.
2. S. officinalis, is effective in increasing the

concentrations levels of tyrosine hydroxylase in brain tissues in the rotenone induced Parkinson disease in male rats.
3. S. officinalis, is effective in decreasing the level of MDA in the tissues which indicates its effective role as an antioxidant agent in rotenone induced Parkinson disease in male rats.
4. S. officinalis potentiate the effect of sinemet in rotenone induced Parkinson disease in male rats.

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