Research Article

Therapeutic potential of S*esbania grandiflora* Linn. Seeds in alleviating behavioural alterations in 6-Hydroxy Dopamine lesioned rats

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ABSTRACT:

In the present study, the therapeutic potential of pet ether and ethanolic fractions of *Sesbania grandiflora* Linn. seeds in alleviating behavioural alterations induced by intracerebroventricular administration of 6-Hydroxy Dopamine (6-OHDA) in rats. Male *Wistar* albino rats were allocated to 5 groups and treated as follows: Gp-I rats treated with 0.9% saline throughout the study, served as vehicle control. The inducer, "6-OHDA (8 µg/2 µl/rat, *i.c.v*)" was administered to all rats (except Gp-I) to induce Parkinson's disease (PD). After seven days of PD induction, Gp-II, III, IV and V animals were given 0.9% saline, L-DOPA (6 mg/kg, p.o.), Pet ether fraction (PEF) (100 mg/kg, p.o.) and Ethanolic fraction (EF) (100 mg/kg, p.o.), correspondingly. Treatment with fractions of *S. grandiflora* had signicantly shown enhanced spatial learning and memory processes evidenced by decrease in Mean Escape latency time in Morris-Water Maze test and increase in Recognition index in Novel Object recognition Test (NOR) compared to the Disease control group. This study established the potential of fractions of *S. grandiflora* in alleviating behavioural alterations in 6-OHDA lesioned rats, suggesting its use as therapeutic entity for treating PD.

KEY WORDS: Parkinson's disease; 6- Hydroxy dopamine*; Sesbania grandiflora;* Morris-Water Maze test; Novel Object recognition Test

INTRODUCTION:

Parkinson's disease (PD) is a neurological ailment, originally documented in 1817 by James Parkinson. This is a progressive motor disorder, mainly caused due to disruption of dopaminergic pathway. India is the treasure house for indigenous plants, which have, inexplicable constituents with proven efficacy on mental and physical disorders.[1-4]

Parkinsonism induced by 6-hydroxydopamine (6-OHDA) injected in left corpus striatum is a recognized model of motor deficits in rats by inducing oxidative stress and Neuroinflammation leading to Neurodegeneration.[5]

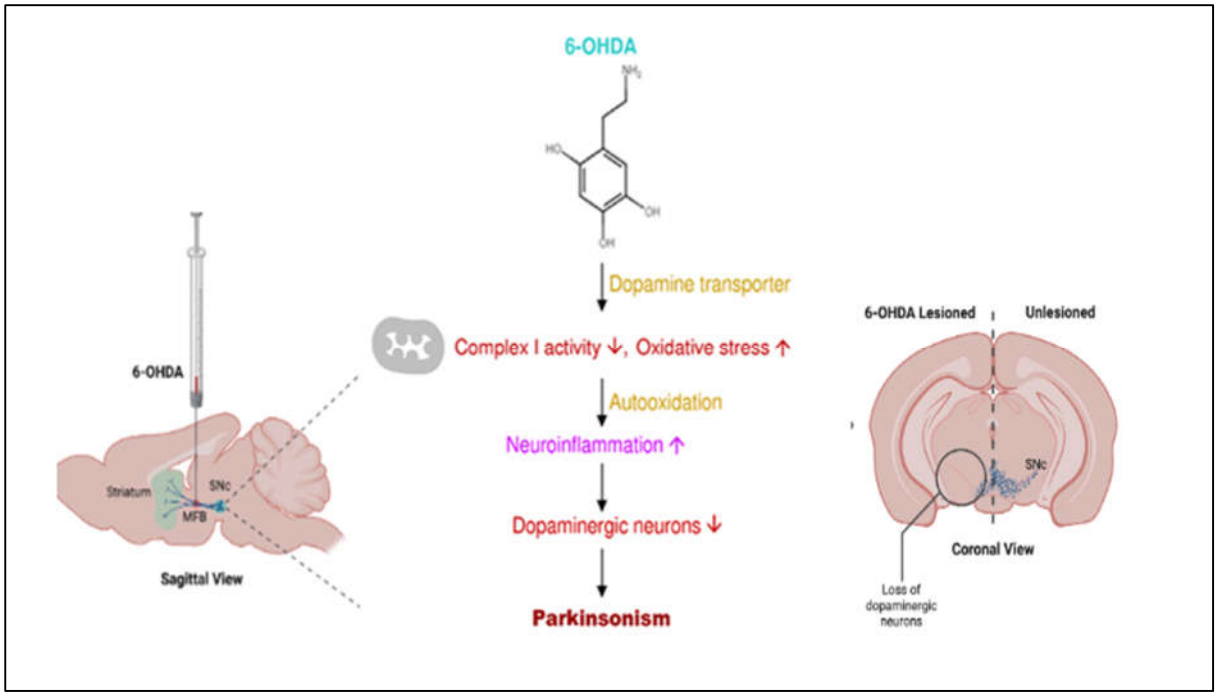

Fig 1: Diagrammatic representation of mechanism of induction of PD by 6-OHDA administration. SNc-Substantia Nigra pars Compacta; MFB- Medial forebrain bundle

Sesbania grandiflora (Fabaceae) is a small, soft-wooded, fast-growing tree which has high nutritional value. The seeds and leaves of *Sesbania grandiflora* comprises protein, minerals, Vitamin A, Folate, Thiamine, Niacin and Vitamin C. The seeds consist of phytoconstituents like leucocyanidin, cyanidin, Saponins and Sesbanimide which exert antioxidant potential.[6,7] Research studies suggested various pharmacological activities of *S. grandiflora* including anti-cancer, anti-diabetic, wound healing, hepatoprotective, anti-ulcer, anxiolytic, anticonvulsant, immunomodulatory, anti-microbial, anti inflammatory, ani-arthritis, anti-diarrhoeal, analgesic, anti pyretic, antioxidant, anti-urolithiatic[8], anti-worm[9], hypolipidemic and cardioprotective.[10]

The aim of present study is to evaluate the therapeutic potential of of pet ether and ethanolic fractions of S*esbania grandiflora* Linn. Seeds in alleviating behavioural alterations in 6-hydroxydopamine lesioned rats.

MATERIALS AND METHODS

Methods for Collecting and Identifying Plant

Sesbania grandiflora seeds were gathered, and the plant parts were authenticated by Dr. Vijaya Bhaskar Reddy, Department of Botany, Osmania University, Hyderabad.

Extraction process

Sesbania grandiflora seeds were shade-dried and pulverized. Preparation of extract involved soaking 1Kg of seed powder in ethanol and water (80: 20) for triple maceration. The extracts were filtered and concentrated by distilling off the solvent under reduced pressure. The extract was further suspended in distilled water and was successively partitioned with solvents of increasing polarity (Petroleum ether, Ethyl acetate and Ethanol). Extracts were filtered following each fractionation and the resulting products were evaporated at a reduced pressure and the extract yields were computed. Pet ether fraction (PEF) and Ethanolic fractions (EF) were selected for investigating the anti-Parkinson activity.

Experimental Animals

Thirty male *Wistar* albino rats (150-200g body weight) were procured from Sainath agencies, Hyderabad. All the animals were caged in clean polypropylene containers; the regular pellet diet was provided with free water access. The study received approval from the CPCSEA (Approval No. CPCSEA/ IAEC/JLS/011/11/19/020).

Experimental Design

The experimental protocol was scheduled for 14 days. The day on which, the inducer was administered to rats was marked as “Day 0”. Then the rats were left as such up to Day 7, for developing PD symptoms as assessed by behavioural parameters like Catalepsy score, Mean Fall-off time and Mean escape latency time. Then treatment with Standard and Pet ether fraction (PEF) and Ethanolic fraction (EF) of *S. grandiflora* was continued up to Day 14 and then behavioural and biochemical assessments were performed. Six rats each in 5 groups were allocated to experiment as follows: Gp-I rats treated with 0.9% saline throughout the study, served as vehicle control. The inducer, “6-OHDA (8 µg/2 µl/rat, *i.c.v*)”was administered to all rats (except Gp-I) to induce Parkinson’s disease (PD)[11]. After seven days of PD induction, Gp-

II, III, IV and V animals were given 0.9% saline, L-DOPA (6 mg/kg, p.o.), Pet ether fraction (PEF) (100 mg/kg, p.o.) and Ethanolic fraction (EF) (100 mg/kg, p.o.), correspondingly.

Evaluation of Anti Parkinson Activity

Behavioural Assessments

Morris Water Maze (MWM) Test

This assay is performed to measure the spatial learning behaviour of animals. The system contain round pool filled with water, divided into four quadrants with an escape platform 2 cm below the water level. The rats were trained to memorize the platform. Then, water was made hazy to conceal the escape platform. The animals were initially put facing the tank wall and given two minutes to discover and ascend to the hidden/escape platform. The "Escape latency time" was the amount of duration by each rat to investigate and ascend the concealed platform[12].

Novel Object Recognition Test

The procedure consists of three different phases: a habituation phase, an acquisition phase, and a retention phase. Initial day (habituation Phase) rats were individually subjected to an adaptation session of 10 min, during which they were introduced in the unfilled area to become familiar with the apparatus. On the 2nd day (acquisition phase), animals were subjected to a 10-min session, during which floor-fixed two objects (A and B) were placed in a symmetric situation in the central line of the area. Rats were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of rats. On the 3rd day (retention phase), rats were allowed to explore the open field in the presence of two objects: the recognizable object A and a novel object C in different shapes but in similar color and size (A and C).[13] Recognition index (for retention session), calculated for each rat as follows:

Recognition index (RI) = Time exploring novel object (T_1) / [Time exploring novel object (T_1) + Time exploring familiar object (T_2)] × 100

Statistical Analysis

The data was put through a statistical analysis by two way ANOVA accompanied with Bonferroni's multiple comparision test using Graph Pad Prism (version 9.2.0). Mean ±SEM is the statistical measure used to summarize the data. P*<0.0001 was deemed to be statistically significant.

RESULTS

Evaluation of Antiparkinsonian Activity

Assessment of Behavioural parameters

Morris Water Maze Test

A momentous increase in the "Mean escape latency time" was observed in all 6-OHDA administered rats before treatment in comparison with vehicle control animals. (#p<0.0001). However, PEF and EF fraction-treated groups exhibited significant reduction in "Mean escape latency time"(P^{****}<0.0001). ***[Table 1].*** A possible explanation for this is the neuroprotective potential of PEF and EF of *S. grandiflora* against neurodegeneration thus alleviating cognitive impairment.

Table 1: Effect on 'Mean escape latency time" before and after the treatment in 6-OHDA treated rats

Group	Treatment	Mean escape latency time in seconds(s)	
		End of Induction period	End of treatment period
I	0.9% Saline	37.16±1.88	29.33±2.12
II	6- OHDA	76.00±4.17#	111.17±2.65#
III	6-OHDA+L-DOPA	74.16±3.70#	30.17±1.88****
IV	6-OHDA+PEF (100 mg/kg)	79.69±1.39#	48.52±1.47****
V	6-OHDA+ EF (100 mg/kg)	81.33±2.32#	39.83±1.53****

*n=6, Mean ± SEM is used to convey values and the data was analysed statistically using a two-way ANOVA and Bonferroni's test. ****p<0.0001 when before treatment values are compared with after treatment; #p<0.0001, relative to vehicle control.*

Novel Object recognition test

A momentous decrease in the "Recognition Index" was observed in all 6-OHDA administered rats before treatment in comparison with vehicle control animals. (#p<0.0001). However, PEF and EF fraction-treated groups exhibited significant increase in "Recognition Index"(P^{****}<0.0001). ***[Table 2].*** A possible explanation for this is the neuroprotective potential of PEF and EF of *S. grandiflora* against neurodegeneration thus alleviating cognitive impairment.

Table 2: Effect on 'Recognition Index" before and after the treatment in 6-OHDA treated rats

Group	Treatment	Recognition index	
		End of Induction period	**End of treatment period**
I	0.9% Saline	54.38±1.05	53.98±1.05
II	6- OHDA	39.12±1.06$^{\#}$	31.16±1.32$^{\#}$
III	6-OHDA+L-DOPA	34.26±1.23$^{\#}$	59.45±1.02****
IV	6-OHDA+PEF (100 mg/kg)	40.23±1.66$^{\#}$	56.027±1.04****
V	6-OHDA+ EF (100 mg/kg)	39.34±1.22$^{\#}$	59.18±1.21****

*n=6, Mean ± SEM is used to convey values and the data was analysed statistically using a two-way ANOVA and Bonferroni's test. ****p<0.0001 when before treatment values are compared with after treatment; #p<0.0001, relative to vehicle control.*

DISCUSSION

PD is a deteriorating motor ailment, affecting individuals in their later years. The etiology of PD comprises ageing, genetic predisposition, oxidative damage and idiopathic. The pathophysiology of PD includes atrophy of pars compacta substantia nigral nerve cells and the nigrostriatal (dopaminergic) tract. Disruption in this nigrostriatal pathway leads to deficiency of Dopamine in the striatum, thus interfering with the muscle tone and voluntary movements**.** Several studies suggested the beneficial effect of plant constituents exhibiting AO activity which paved the way for evaluation of naturally occurring plants for treatment of Parkinson's disease. This also provides a cost-effective method to fight against PD. Also the constituents which can promote the dopaminergic transmission and regulates the extent of dopamine metabolism proved to be effective in the treating the complications of PD.

Parkinsonism induced by 6-hydroxydopamine (6-OHDA) injected in left corpus striatum is a recognized model of motor deficits in rats. 6-OHDA is a lethal toxin that mainly damages peripheral and central nervous systems. Nevertheless, the neurotoxin 6-OHDA cannot pass the blood-brain barrier; the central nervous system toxicity obtains only by direct administration into the brain. The toxic effects of 6-OHDA happen through a twofold mechanism involving toxins aggregating into catecholaminergic neurons and then modifying cell steadiness and neuronal destruction. Dopamine or norepinephrine membrane transport protein uptake 6-OHDA because it is similar to the structure of endogenous Catecholamine. The toxicity of 6-

OHDA is related to its ability to the production of free radicals and oxidative stress, which is similar to the hydrogen peroxide effect. The inhibition of brain mitochondrial complexes (I and IV) and oxidation to form semiquinone radicals (which participate in reactive oxygen species production) are the main biochemical properties of 6-OHDA. This toxicity results in oxidative stress leading to neuroinflammation and neurodegeneration.

NOR is being explored as an excellent model to assess cognitive decline in PD and related disorders like Alzheimer's and schizophrenia. Since this is a non-invasive model and had minimal stress on animals it's especially useful in preclinical studies to evaluate drug efficacy and neuroprotective strategies. The Key point is cognitive deficits in PD often appear before motor symptoms in many patients and hence it would be important to be able of dealing early with non-motor indicators in order to use prospective neuroprotectors to prevent the progression of the disease.

PEF and EF fraction-treated groups exhibited significant reduction in "Mean escape latency time and significant increase in "Recognition Index". A possible explanation for this is the neuroprotective potential of PEF and EF of *S. grandiflora* against neurodegeneration thus alleviating cognitive impairment.

CONCLUSION:

In conclusion, this study proved the therapeutic potential of *Sesbania grandiflora* established by alleviated PD symptoms in PEF and EF-treated rats compared to disease control as evidenced by decrease in Mean Escape Latency time and increase in Recognition Index, Additional studies are suggested to classify the active phytochemicals and illustrate the exact mechanism of action.

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