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Evaluation of Antiparkinson's Activity of *Ethanolic* Extract of *Jasminum Sambac* Flower

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Abstract:

Oxidative stress is the major role involved in the etiology of depression. Reduced oxidative stress correlates with the antidepressant treatment and brings the moderate clinical recovery of depression. Natural antioxidants that are present in herbs and spices are responsible for inhibiting and preventing the deleterious consequences of oxidative stress. Different concentrations of *Jasminum sambac* were used to evaluate the antioxidant effect. The in-vivo antiparkinson activity of *Ethanolic* extract of *Jasminum sambac* flower were evaluated by using Locomotor and Rotarod method. Male albino rats were treated at a dose of 200 and 500mg/kg I.P and behavior was observed on these models.

Keywords: *Jasminum sambac*, Locomotor, Rotarod.

Introduction

In 1817, an English physician named James Parkinson wrote a renowned monograph titled "An essay on the shaking palsy" (Parkinson, 1817) in which he officially characterized Parkinson's disease (PD) for the first time in modern times. "involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace: the senses and intellects being uninjured" are some of the symptoms listed in this report, which initially referred to the disease as shaking palsy (paralysis agitans). In terms of prevalence, Parkinson's disease (also known as idiopathic PD or primary parkinsonism) is second only to Alzheimer's disease (AD) among age-related neurodegenerative diseases (Fahn andPrzedborski, 2000;Tanner and Aston, 2000;Mayeux, 2003;Fahn and Sulzer, 2004). In addition, PD accounts for over 80% of all instances of chronic progressive Parkinsonism. Parkinsonism is a neurological condition characterized by bradykinesia, stiffness, and tremor; it is also known as Parkinson's syndrome, atypical Parkinson's, and secondary Parkinson's*Jasminum sambac* is a sub erect shrub with young shoots of ovate or elliptic glabrous simple leaves, entire margin, and acute apex with opposite arrangement, grown as an ornamental shrub in gardens and cultivated throughout the tropical and subtropical parts of India. The plant has numerous applications in traditional medicine but there is a lack of data on the standards of flower of the plant. Leaves, roots, and flowers are used as lactifuge. The plant also exhibited antilactation effect, antibacterial, antiviral, antiproliferative, anti acne and anti-inflammatory effect. The present study is an attempt to investigate antiparkinson's

activity of *Jasminum sambac (*AB Upaganlawar *et al., 2009).*

Materials and Methods

Procurement and Authentication of the Plant

The flowers of *Jasminum sambac* plant were collected from National botanical Research Institute, Lucknow, India in March 2021 and authenticated by Dr. Sunita Garg, Former Chief Scientist, NISCAIR, Delhi (Ref. No.- NISCAIR/RHMD/ Consult/2020/3767-70).

Preparation of extracts of *Jasminum sambac*

Flowers of the plant were collected and dried under shade at room temperature. The plant material was then chopped and ground to fine powder using a mechanical blender. 20gm of powder of flowers of *Jasminum sambac* was taken into conical flask. The phytoconstituents were extracted by adding 100ml of ethanol to the powder. The flask was incubated in orbital shaker for 48 hrs. The extract was filtered through five layer of muslin cloth. The process was repeated twice. The collected extract was pooled and concentrated by evaporation. The extract was preserved and stored at 40^0C in airtight bottles for further study (Gupta S *et al*.,2022)

Animals

Healthy male albino rats of Wistar strain weighing 250-300g were used for the present study. The animals were procured from CDRI, Lucknow, UP. The animals were housed in a large spacious cage, bedded with husk, and were given food and water. The animal house was ventilated with a 12hr light/dark cycle, throughout the experimental period. The feed contains 5% fat, 21% protein, 55% nitrogen free, 4% fiber (wt/wt) with adequate vitamin and mineral content. Experimental animals were handled according to the University and institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests (Animal Welfare Division), Government of India.

Chemicals

6- Hydroxy dopamine (6-OHDA) was purchased from Sigma. GSH, glutathione oxidized (GSSG), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate reduced (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), 5-5′-dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), Thio barbituric acid, ethylene diamine tetra acetic acid (EDTA) was purchased from Sisco Research Laboratories (SRL). 3, 4-dihydroxy phenyl acetic acid (DOPAC) were purchased from Sigma Aldrich. Other chemicals used were of analytical grade.

Preliminary phytochemical analysis

To determine which phytoconstituents were present in each extract, a preliminary phytochemical study was performed.

Toxicity studies

The mice were divided into 5 groups of 10 animals each. The mice were fasted for 6 h and had access to only water *ad libitum* before experimental study. Group I received only vehicle (distilled water). Groups II, III, IV, and V received different doses of *Ethanolic* extract of *Jasminum sambac* (JS), that is, 1000, 2000, 3000, 4000 and 5000mg/kg, respectively. All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality.

All the extracts were subjected to an acute toxicity test in accordance with OECD 423 guidelines (V. Ravichandran *et al.,* 2007).

6-hydroxydopamine induced Parkinsonism

The rats were anesthetized with an intraperitoneal injection of 50 mg/kg of sodium pentobarbital and were fixed in a stereotaxic apparatus (R. Deumens *et al*.,2002 and S. Wang *et al.,* 2005). A stainless-steel needle (0.28 mm o.d) was

inserted unilaterally into the substantia nigra with the following coordinates: anterior/posterior: −4.8 mm; medial/lateral: −2.2 mm; ventral/dorsal: −7.2 mm–3.5 mm from bregma, and injection of 6-OHDA (12 µg of 6-OHDA moclobemide 2 µL 0.1% ascorbic acid-saline) was then made over 5 min and the needle was left in place for a further 5 min. Then the skull was secured with stainless metallic screws and the wound area was covered by dental cement. Each rat was housed individually following the surgical procedure. Sham operated animals were also treated in the same manner, but they received equivalent volumes of normal saline instead of 6-OHDA.

Experimental design:

Animals were divided into 5 group of 6 rats in each group ($n = 6$)

Group I: Vehicle treated; control group received 2µl of vehicle (0.1% ascorbic acid-saline)

Group II: Vehicle treated, lesioned with 6 hydroxy dopamine on 22nd day (L).

Group III: Rats pretreated with methanol extract of *Jasminum sambac (MEJS) (250mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid-saline) injected into right striatum.

Group IV: Rats pretreated with methanol extract *Jasminum sambac (MEJS) (500mg/kg,bw)* orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid-saline) injected into right striatum.

Group V: Rats pretreated with Moclobemide orally for 21 days; on 22nd day single dose of 6-hydroxydopamine (12 µg of 6-OHDA/2µl in 0.1% ascorbic acid-saline) injected into right striatum.

Behavioral Assessment

All the behavioral studies were performed at room temperature in a calm room without any outside interference. All the experiments were performed between 10.00 am and 6.00 pm.

Locomotor activity

On day 36, all animals were tested for locomotor activity. This animal activity monitor consists of a chamber ($50\times50\times35cm^3$) a video camera fixed over the chamber by an adjacent rod and its locomotor activity was monitored by activating the camera (D. S. Reddy *et al*., 1998). The activity chamber was furnished with black paper to provide a good contrast on the screen. Each animal was assessed for locomotor activity for three sessions of 5min each. After each animal, the activity chamber was swabbed with 10% alcohol to avoid any interferencedue to animal odors.

Rota rod (muscular coordination) Activity

Rota rod (Instruments and Chemicals, Ambala, New Delhi) was used to evaluate the muscular coordination on the 40th day. It consists of a rotating rod (75mm diameter), which is divided into four parallel compartments, permitting testing of 4 rats at a time. The apparatus automatically records the time in 0.1sec when the rats fall of the rotating shaft. The speed was setat 10rpm, and cut-off time was 180sec. The drug-naïve animals were trained on the rod, so that they could stay on it at least for the length of the cut-off time (S. Raja Sankar *et al*., 2009).

Histopathological Studies:

Histology of striatum was studied using haematoxylin and eosin (H and E) staining. Portions of striatum were fixed in 10% formalin. The washed tissues were dehydrated in the descending grades of isopropanol and finally cleared in xylene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 µm thickness, stained with haematoxylin and eosin (H&E) (100X). The sections were then viewed under light microscope (Nikon microscope ECLIPSE E400, model 115, Japan) for histopathological observation.

Statistical Analysis

Statistical Analysis. All the values were expressed as mean ± SEM. Statistical evaluation of the data was done by one-way ANOVA (between control and drug treatments) followed by Dunnett's t-test for multiple comparisons and two-way ANOVA followed by Bonferroni's multiple comparison test, with the level of significance chosen at $p < 0.001$ using Graph-Pad Prism 5 (San Diego, CA) software.

Results

Table1: Preliminary Phytochemical studies of Extracts *Jasminum sambac*

Constituents	***Ethanolic Extract (JS)***
Carbohydrate	+
Glycosides	_
Oil and fats	_
Proteins	+
Saponins	_
Phenolic comp. and tannins	+
Phytosterols	+
Alkaloids	+
Gums and mucilage	+
Flavonoids	+

Acute Toxicity-

The *J.sambac* was found to be safe at all the doses used and there was no mortality found up to the dose of 5000mg/kg of *J. sambac* when administered orally. Therefore, we have taken 500mg/kg as the therapeutic dose and made variations by taking 250mg/kg as lower dose and 500mg/kg as higher dose.

The Effects of JS on 6-OHDA Induced Parkinson's Disease in the Locomotor Activity. Total locomotor activity of rats in 6-OHDA treated group was significantly ($p < 0.001$) reduced as compared to vehicle treated group. Oral administration of JS of different doses (250 and 500mg/kg) showed significant ($p < 0.001$) increase in the locomotor activity from day 20 to 55 as compared to 6-OHDA treated control animals. Administration of *J. sambac* (100mg/kg) did not show significant activity. Levodopa (6mg/kg) significantly ($p < 0.001$) increased locomotor activity.

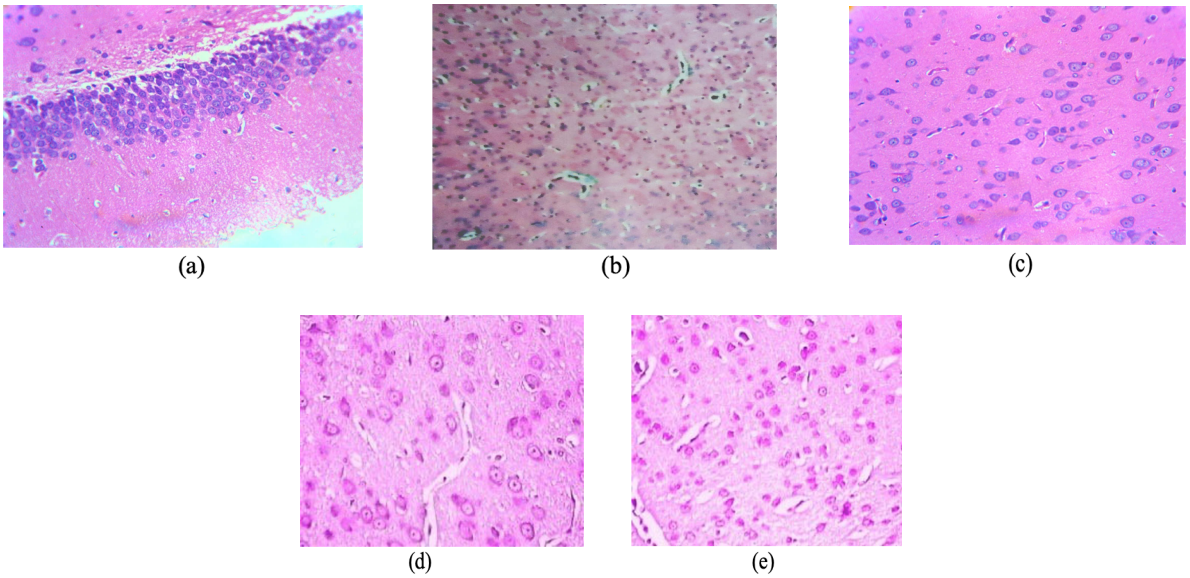
The Effects of JS on 6-OHDA Induced Parkinson's Disease in the Rotarod Performance. Treatment with 6-OHDA significantly decreased the fall of time when compared to the vehicle control animals. Chronic oral administration of *J. sambac* (250 and 500mg/kg) significantly ($p < 0.001$) increased the fall of time when compared to 6-OHDA group from day 15 to day 55. Moclobemide (5mg/kg) significantly ($p < 0.001$) increased the fall of time as compared to 6-OHDA group. Administration of *JS* (100mg/kg) did not show significant activity.

Effect of JS on Histopathological Changes in the Brain of Normal and 6-OHDA Treated Animals.

The histopathological study showed that neurotoxins, that is, 6-OHDA, caused marked hypertrophic changes, increased intracellular space, infiltration of neutrophils, decreased density of cells, alterations of architecture, hemorrhage, and neuronal damage and even cell death. Furthermore, many neurons were shrunken, pyknotic, and darkly stained with small nuclei (Figure 4(b)) compared with

normal vehicle treated rats (Figure 4(a)). There is significant reversal of neuronal

damage or neuronal alterations observed in Meclobemide (5mg/kg) treated rats (Figure 4(c) and JS treated rats at doses of 250 (Figure 4(e)) and 500mg/kg (Figure 4(f)). Treatment with JS (250mg/kg) did not show significant recovery of neuronal damage (Figure 4(d)).



Effect of *JS* on histopathological changes in the brain of normal and 6-OHDA treated animals (H&E staining; original magnification, 10x). (a) Normal control showing normal brain architecture. (b) Rats treated with 6-OHDA showing degeneration of neurons. (c) Rats treated with 6-OHDA and Moclobemide (5mg/kg) showing minimal changes in neuronal cell integrity and architecture. (d) Rats treated with 6-OHDA and JS (250mg/kg) showing mild decrease in neurons and cellular hypertrophy and (e) *JS* (500mg/kg) treated rats showing minimal changes in neuronal cell populations.

Discussion

Parkinson's disease is a chronic neurodegenerative disorder characterized by loss of dopamine neurons of the SNpc. The pathogenesis of PD includes oxidative stress, protein accumulation like a-synuclein, mitochondrial dysfunction, apoptosis, and neuronal excitotoxicity. Among all, oxidative stress is a crucial pathological mechanism for PD. SNpc is more vulnerable to reactive oxygen species as it contains more amount of dopamine. In the present study, we evaluated the effect of *Ethanolic* extract of *J. sambac* in neurotoxins (6- OHDA) induced Parkinson disease in experimental animals. The efficacy of *J. sambac* in 6-OHDAinduced PD has not been well established. In the present study, 6-OHDA administration to rats caused a significant decrease in locomotor activity and muscle activity. Lack of motor coordination and maintenance of normal limb posture has been reported in PD condition. The evaluated data suggested damage to the dopaminergic neurons and progression of Parkinson's disease like behavioral abnormalities in rats exposed to 6- OHDA. Pre-treatment of rats with *J.sambac* at the doses of 250 and 500mg/kg exhibited significant increase in locomotor activity and increase in muscle activity and thus could be proved with possible action on CNS (F. Blandini et al., 2012).

Histopathological findings showed that *Ethanolic* extract of *Jasminum sambac* treated animals had decreased infiltration of neutrophils, reduced intracellular space, increased density of cells, and regained normal architecture and moderate necrosis in striatum region of brain.

Conclusion

In view of the above facts, we are concluding that *Ethanolic* extract of *Jasminum sambac* plant showed to be an a promising effect in animals with Parkinson's disease. And we appreciate further detailed molecular studies with this drug in anti-Parkinson's pharmacology and toxicology and characterization of active constituents responsible for neurodegenerative effect.

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