

Evaluation of In-Vivo Antioxidant of *Ethanollic* 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 antioxidant was evaluated by MDA, serum catalase, superoxide dismutase and reduced glutathione reactive substance assay method. Male albino rats were treated at a dose of 200 and 500mg/kg I.P and behavior was observed on these models.

Keywords: Antioxidant, MDA, Serum catalase, superoxide dismutase, reduced glutathione, *Jasminum sambac*

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicines (Gupta S *et al.*, 2022). Antioxidants act as a defense mechanism that protect against deleterious effects of oxidative reaction produced by reactive oxygen species (ROS) in a biological system. Antioxidant are substances that are capable of counter acting the damaging effects of the physiological process of oxidation in animal tissues. Oxidation stress occurs when the production of harmful molecules called free radicals is beyond the protective capability of the antioxidant defenses (Gupta S *et al.*, 2022). Reactive oxygen species not only are produced naturally in cell following stress or respiration but also have been reported to be produced by radiation, bacterial and viral toxin, smoking, alcohol, and

psychological or emotional stress (L. Chen *et al.*, 2008). Overproduction of ROS and/or inadequate antioxidants has been implicated in the pathogenesis and complications of some disease conditions like diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative disease, and aging process. Antioxidants have been reported to prevent oxidative damage caused by ROS by reacting with free radicals, chelating, and catalytic metals and by acting as oxygen scavengers (F. Zoccarato *et al.*, 2005). The enzymatic antioxidants include catalase, superoxide dismutase, and glutathione which catalyse neutralization of many types of free radicals, while the nonenzymatic antioxidants include Vitamin C, selenium, vitamin E, carotenoids, and polyphenols. There is growing evidence that antioxidants play a pivotal role in the prevention of heart disease, cancer, DNA degeneration, pulmonary disease, and

neurological disorder (Tariquo S *et al.*,1985). Plants, herbs, and spice, rich in phenolic compounds like flavonoids, have been demonstrated to have anti-inflammatory, antiallergetic, antiviral, antiaging, and anticarcinogenic activities which can be attributed to their antioxidant properties (T. B. Sherer *et al.*,2002).

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 the antioxidant potential and 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°C 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 *Ethanollic* 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.

Biochemical studies

Determination of the In -Vivo Antioxidant Effect of *Jasminum sambac*

Thirty male Wistar strain rats were randomly divided into five groups of six animals each. Group 1 served as the control and received 2 mL of distilled water. Group 2 received Vehicle treated, lesioned with 6 hydroxy dopamine. Group 3 received 250 mg/kg of the *Jasminum sambac*. Group 4 received 500 mg/kg of the *Jasminum sambac* and group 5 received 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). The animals were dosed daily for 21 days and were observed daily for changes and other signs of toxicity and death throughout the period of study. Twenty-four hours after the last treatment, blood obtained through direct cardiac puncture was used to assay for *in vivo* antioxidant activity of JS.

Analytical Methods

Serum Preparation- The blood used for serum preparation was collected via direct heart puncture with 21 G needle attached to 5 mL syringe, following mild chloroform anaesthesia of the rats. The serum was prepared using standard method as described by Yesufu *et al.* Briefly, the method used is as follows. Blood was allowed to clot for 30 minutes and then centrifuged at 2500 rpm for 15 minutes and serum was harvested.

Malondialdehyde (MDA) Level.

The method was used for the estimation of lipid peroxidation. Briefly, 0.2ml homogenate was pipetted in Eppendorf tube and incubated at 37±1°C in a metabolic water bath shaker for 60 min at 120 strokes up and down; another 0.2ml was pipetted in an Eppendorf tube and placed at 0°C incubation. After 1h of incubation, 0.4ml of 5% TCA and 0.4 ml of 0.67% TBA was added in both samples (i.e., 0°C and 37°C). The reaction mixture from the vial was transferred to the tube and centrifuged at 3500×g for 15min. The supernatant was transferred to another tube and placed in a boiling water bath for 10min. Thereafter, the test tubes were cooled and the absorbance of the color was read at 535nm. The rate of lipid peroxidation

expressed as nmol of thiobarbituric acid reactive substance formed/min/mg protein (H. Ohkawa *et al.*, 1989).

Results were expressed as nanomoles per mg of protein. The concentration of MDA was calculated using the formula

$$\text{Conc. of MDA} = \frac{\text{Abs}_{532} \times 100 \times VT}{(1.56 \times 10^5) \times WT \times VU}$$

where Abs₅₃₂ is absorbance, *VT* is total volume of mixture (4 mL), 1.56×10^5 is molar extinction coefficient, *WT* is weight of dissected brain (1 g), and *VU* is aliquot volume (1 mL).

Determination of Superoxide Dismutase (SOD)

Procedure

To 1 ml of tissue homogenates 0.25 ml of ethanol and 1.25 ml of chloroform were added, kept in a mechanical shaker for 15 min and centrifuged. To 0.5 ml of the supernatant, 2.0 ml of 0.1 M Tris-HCl buffer pH 8.2; 1.5 ml of distilled water and 0.5 ml of pyrogallol were added. Change in optical density at 0, 1 and 3 min was read at 420 nm in a UV-VIS Spectrophotometry. Control tubes containing 0.5 ml of distilled water were also treated in a similar manner against a buffer blank. The enzyme activity is expressed as Unit/mg protein. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogallol auto-oxidation (W. F. Beyer *et al.*, 1987).

Assay of Catalase (CAT)

Procedure

To 0.1 ml of tissue homogenates 1.0 ml of buffer and 0.5 ml of hydrogen peroxide were added and the time was noted (H. Aebi, *et al.* 1984). The reaction with hydrogen peroxide in the range of 4 to 20 μ moles were taken and treated similarly. The tubes were heated in a boiling water bath for 10 min. The green colour developed was read at 570 nm using a UV-VIS Spectrophotometry.

Catalase activity is expressed as μ moles of H₂O₂ consumed/min/mg protein.

$$\text{CAT Activity} = \frac{\delta O.D.}{E \times \text{Vol. of sample (ml)} \times \text{mg of protein}}$$

where $\delta O.D.$ is change in absorbance/minute; *E* is extinction

coefficient of hydrogen peroxide (0.071mmol cm⁻¹).

Estimation of reduced glutathione

Procedure

One ml of tissue homogenate was precipitated with 1.0 ml of 10% TCA. The precipitate was removed by centrifugation. To an aliquot of the supernatant, 1.0 ml of DTNB reagent was added and the final volume was made up to 5.0 ml with phosphate solution (S. K. Srivastava *et al.*, 1968). The colour developed was read at 420 nm using a UV-VIS Spectrophotometry. The values were expressed as nM of reduced glutathione per mg of protein:

$$\text{GSH level} = \frac{Y - 0.00314}{0.0314} \times \frac{DF}{BT \times VU}$$

where *Y* is Abs₄₁₂ of tissue homogenate, *DF* is dilution factor, *BT* is brain tissue homogenate (1 mL), and *VU* is aliquot volume (1 mL).

Statistical Analysis

Statistical Analysis. All the values were expressed as mean \pm 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

The In Vivo Antioxidant Effects of JS in Rats- The In Vivo Antioxidant Effects of *JS* in Rats. The results of the in vivo antioxidant effect of *JS* on rats are presented in Table. The extract produced a dose dependent decrease in the MDA levels in the serum. The serum MDA level of the group treated with 500 mg/kg of *JS* was significantly ($P < 0.05$) lower when compared to other treatment groups and the negative control group. The extract also produced a dose dependent increase in the

serum level of catalase activity. The serum catalase activity of the group treated with 500 mg/kg of *JS* was significantly ($P < 0.05$) higher when compared to other treatment groups and the negative control group. Furthermore, the extract produced dose dependent increase in the

serum level of superoxide dismutase activity. The serum superoxide dismutase activity of the groups treated with *JS* were significantly ($P < 0.05$) higher when compared with the negative control group.

Table 1: Preliminary Phytochemical studies of Extracts *Jasminum sambac*

Constituents	Ethanollic Extract (<i>JS</i>)
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.

Biochemical studies

The Effects of *Jasminum sambac* on 6-OHDA Induced Parkinson's Disease in MDA, CAT, SOD, and GSH Level. Administration of 6-OHDA resulted in significant changes in biochemical parameters when compared to the vehicle control animals. The inoculation of 6-

OHDA induced oxidative stress, as indicated by increased MDA level, and decreased CAT, SOD and GSH levels when compared to vehicle control animals in brain levels. The treatment with *Ethanollic* extract of *Jasminum sambac* (500mg/kg, p.o.) showed significant ($p < 0.001$) decrease in MDA level compared to 6-OHDA rats. Similarly, daily administration *J. sambac* (500mg/kg) attenuated the increase in SOD and CAT activity with 6-OHDA treated group. Pre-treatment with *JS* (500mg/kg) significantly ($p < 0.001$) increased GSH levels in the brain as compared to 6-OHDA treated animals, thus preventing the reduction in GSH induced by 6-OHDA (Table 2).

Table 2: Effect of JS on the levels of lipid peroxidation (MDA), catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) in the brain of 6-OHDA treated animals

Group	MDA (nM/mg of protein)	CAT (μ moles of H ₂ O ₂ used /min/mg protein)	SOD (units/mg protein)	GSH (nM/mg of protein)
Vehicle Control	1.411±0.08215	7.921±0.98	6.932±1.01	23.504±1.43
6-OHDA Control	2.415±0.1502 ^{a#}	1.412±0.40 ^{a#}	1.411±2.24 ^{a#}	5.701±0.76 ^{a#}
Meclobemide	2.648±0.02541 ^{b**}	6.312±0.62 ^{b***}	7.615±0.54 ^{b***}	23.415±1.63 ^{b***}
JS (250)	1.475±0.2143 [*]	2.991±0.78 [*]	3.581±0.28 ^b	15.717.26±1.57 [*]
JS (500)	1.837±0.2402 ^{***}	6.12±0.62 ^{***}	6.01±0.48 ^{***}	17.37±1.65 ^{***}

Values are expressed as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ as compared to 6-OHDA treated control group (Group II) [Groups III to VI were compared with Group II], # $p < 0.001$ as compared to vehicle treated group (Group I) [Group II was compared with Group I].

Discussion

Oxidative stress generated as a result of mitochondrial dysfunction particularly mitochondrial complex-I impairment plays an important role in the pathogenesis of PD. The oxidative stress was measured through determination of levels of malondialdehyde, catalase, superoxide dismutase, and reduced glutathione in the brain tissue.

6-OHDA generates an increase in the production of hydrogen peroxide and free radicals. (G. Cohen *et al.* 1974 and D. G. Graham *et al.*, 1978). These reactive oxygen species are generated through the nonenzymatic breakdown of 6-OHDA or direct inhibition of complex- I and complex-IV of the mitochondrial electron transport chain (Y. Y. Glinka *et al.*, 1995 and Y. Glinka, M. Gassen, *et al.*, 1997). The resulting ROS production from 6-OHDA breakdown leads to lipid peroxidation, protein denaturation, and increases in glutathione, which are found in PD patients (P. Jenner *et al.*, 1998).

Lipid peroxidation, a sensitive marker of oxidative stress, was estimated by measuring the levels of thiobarbituric acid. Lipid peroxidation occurs due to attack by radicals on double bond of unsaturated fatty acid and arachidonic acid which generate lipid peroxy radicals and that initiate chain reaction of further attacks on other unsaturated fatty acid. As we know, lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, impaired structural integrity (B. Halliwell *et al.*, 1985), decreased fluidity, and inactivation of number of membranes bound enzymes. Increased levels of the lipid peroxidation product have been found in the substantia nigra of PD patient. In the present investigation, similar results were observed in

the brain homogenate of 6-OHDA treated control animals.

Catalase is an antioxidant which helps in neutralizing the toxic effects of hydrogen peroxide. Hydrogen peroxide is converted by the catalase enzyme to form water and nonreactive oxygen species, thus preventing the accumulation of precursor to free radical biosynthesis. Oxidative stress results in decrease in catalase level. 6-OHDA inoculation in rats induced oxidative stress, as indicated by a decrease in the catalase levels.

Superoxide dismutase (known as SOD) is an enzyme which acts as a catalyst in the process of dismutation of superoxide into nonreactive oxygen species and hydrogen peroxide. It is therefore a critical antioxidant defence which is present in nearly all cells which are exposed to oxygen (L. S. Monk *et al.*, 1989 and C. Bowleret *et al.*, 1992). Superoxide dismutase helps in neutralizing the toxic effects of free radicals (J. G. Scandalios *et al.*, 1990 and E. B. Gralla *et al.*, 1992). 6-OHDA treated control group showed a decrease in the level of SOD in the brain of animals, thus, indicative of production of oxidative stress.

GSH, potent enzymes, are an important factor in aetiology of PD (P. Jenner *et al.*, 1998). The depletion of reduced glutathione in the substantia nigra in Parkinson's disease could be the result of neuronal loss. As a matter of fact, the positive correlation has been found to exist between the extent of neuronal loss and depletion of glutathione (P. Riederer *et al.*, 1989). A decrease in the availability of reduced glutathione would impair the capacity of neurons to detoxify hydrogen peroxide and increase the risk of free radical formation and lipid peroxidation. Reduction in GSH levels was evident in 6-OHDA treated control animals. Thus, the 6-OHDA group showed a significant increase in the levels of thiobarbituric acid (which is an indication of extent of lipid peroxidation) and decrease in the levels of SOD and GSH in the brain as compared to the vehicle treated control animals. All these indicate an increase in the oxidative stress in the brain of animals treated with 6-OHDA. Pre-treatment with higher dose of *Ethanol* extract of *J. sambac* (500mg/kg)

resulted in a decrease in MDA level and increase in the levels of SOD, catalase, and GSH, indicating its antioxidant effect in the brain of 6-OHDA treated animals.

Conclusion

In view of the above facts, we are concluding that *Ethanol* extract of *Jasminum sambac* plant showed to be an antioxidant and showed 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.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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