

# Evaluation of Neuroprotective Activity of Delo nix Regia Leaves on Haloperidol Induced Parkinsonism in Experimental Animals

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**Abstract:** One of the most serious and fatal neurodegenerative disorders, Parkinson disease (PD) is characterized by low levels of dopamine. Reactive oxygen species (ROS) from both endogenous and exogenous sources have a role in the oxidative stress that leads to PD, which is a complex condition. Delo nix Regia is a prominent medicinal plant that is frequently used in Indian systems of medicine. The fruit, leaves, and bark of this plant have been utilised for its antifungal, antiemetic, larvicidal, hepatoprotective, antibacterial, antioxidant, anti-diarrheal, anti-inflammatory, wound-healing, and ant carcinogenic potential, according to Ayurvedic literature. The "antioxidant strategy" is the main topic of this study's therapeutic intervention for Parkinson's disease (PD). The investigation was completed in wistar rats, in which Parkinson's disease (PD) was induced with haloperidol 2 mg/kg, P.O. The rats were randomly divided into six groups and the test animals received the ethanolic extract of Delo nix regia (DRLE) at a dose of 100, 250 and 500 mg/kg, orally for 15 days. Various behavioural and biochemical parameters were estimated in haloperidol exposed rats. The results of this study conclusively show that Delo nix regia has anti-oxidant activity and neuroprotective activity in haloperidol experimental model of PD.

**Key Words:** Parkinson diseases, Oxidative stress, Haloperidol, Delo nix regia, Neuroprotective.

## 1. Introduction

Parkinson's disease is a neurodegenerative condition that results in movement abnormalities due to the loss of dopaminergic neurons in the substantia nigra. [1] After Alzheimer's disease, Parkinson disease is the most prevalent neurological condition. Tremor, stiffness, postural instability, bradykinesia, and akinesia are some of the clinical symptoms. [2] The condition often affects people between the ages of 55 and 64, while occasionally considerably younger people are also affected. Resting tremor, muscular stiffness, bradykinesia, and postural instability are the four cardinal, incapacitating signs of dopaminergic neuron degeneration in Parkinson's disease, despite the fact that the origins of this degeneration are poorly understood. [3] One of the main causes of Parkinsonism is oxidative stress, which results in the death of dopaminergic neurons. [4]

The pathogenetic process of oxidative stress is brought on by an increase in the production of reactive oxygen species (ROS) and a decrease in the antioxidant defense mechanisms. Treatment with haloperidol blocks the dopamine receptor, speeding up the breakdown of dopamine. As a result, a byproduct of their metabolism could be ROS. [5,6] In addition to the production of free radicals, haloperidol treatment is associated with a considerable decrease in antioxidant glutathione levels [7].

Levodopa is still the cornerstone of treatment because it is the most successful pharmacological option currently available. Levodopa's use is constrained because to the potentially debilitating effects of long-term treatment, including dyskinesias and motor irregularities. Due to worries about the negative effects of conventional treatment, natural products are being carefully evaluated and sought after as an alternative. These organic resources may offer an improvement in Parkinson's disease treatment since they contain anti-oxidant and neuroprotective effects (PD).

The development of new medicines for the treatment of Parkinson's disease can be derived from natural products that are both safe and have physiological qualities. As a result, several researchers are now concentrating on the potential anti-

## Evaluation of Neuroprotective Activity of *Delonix regia* Leaves on Haloperidol Induced Parkinsonism in Experimental Animals

Parkinson effects of plants. As a result, there is a constant need for novel agents, and natural goods are now a top focus. The active ingredients flavonoids are playing a critical part in the anti-parkinson treatment, according to a variety of research and literature. These kinds of flavonoids are abundant in the leaves of *Delonix regia*. This study uses ethanolic extracts of *Delonix regia* leaves to examine and assess anti-parkinson activity in a model of Parkinson's disease caused by haloperidol. *Delonix regia* commonly known as royal, flamboyant, poinciana, gulmohr, shima, sunkesula. The tree is native to Madagascar and has been widely planted for the last 150 years as a garden and avenue tree in both dry and moist regions of tropical India. The literature survey shows plant with beneficial bioactivities of *Delonix regia* such as antifungal [8], antibacterial [9], antioxidant [10], antiemetic [11], larvicidal [12], hepatoprotective [13], anti-diarrhoeal [14], anti-inflammatory [15], antimalarial [16], anthelmintic [17], antiarthritic [18], wound healing [19] and anticarcinogenic potential [20], along with their experimental evidence and mode of action.

Studies demonstrating *D. regia*'s anti-effect Parkinson's are scarce in the existing literature. Therefore, efforts were undertaken in the current study to look into the effects of *D. regia* on behavioural and biochemical changes brought on by haloperidol-induced oxidative stress in rats, as well as the effects of *D. regia* on the animal model of PD.

## 2. Material and Method

### Animals

Healthy male Wistar rats (120-160g) were procured from Global Bio Research Solution Pvt. Ltd. Bhor, Pune. Animals were housed in a group of 6 per cage in standard polypropylene cages (32.5x21x14) cm lined with raw husk. The animal house was maintained on 12 light/dark cycle approximately  $22\pm 2^{\circ}\text{C}$ , relative humidity 60-70%. All animals were provided with standard laboratory diet (Nutrivet Life science, Maharashtra, India) and water. All experimental procedures were carried out in accordance with the guidelines prescribed by committee for the purpose of control and supervision on experiments on animals i.e., CPCSEA (884/PO/05/ac/CPCSEA) were approved by institutional animal ethics committee (IAEC) Protocol no. (MCP/IAEC/006/2020).

### Collection and authentication of plant material

The leaves of plant *Delonix regia* were collected from the surrounding areas of Burhanpur district, Madhya Pradesh, India during the month of December and authenticated by Botanical Survey of India (BSI) - Pune. The authentication certificate number is BSI/WRC/Iden. Cer./2021/0608210007169. Soon after collection the Leaves were cleaned, dried in shade and crushed to a coarse powder, stored in an air tight plastic container, until further use.

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नं./No.BSI/WRC/Iden. Cer./2021/0608210007169 दिनांक/ Date: 09.08.2021

प्रमाणपत्र/CERTIFICATE

यह प्रमाणित किया जाता है कि/This is to certify that Mr. Nitesh Chandrakant Chaudhary, M Pharm, student from Modern College of Pharmacy, Nigdi, Pune-411044, Maharashtra के द्वारा प्रस्तुत किये गये नमूने, हमारे विशेषज्ञ एवं वैज्ञानिक द्वारा निम्नलिखित अनुसार पहचाने गये हैं।/the specimen/specimens submitted by aforesaid is/are identified by our expert's & Scientist as :

नमूना संख्या Specimen No.	वनस्पति का नाम Plant Name	वनस्पतिक कुल Family
NCCDR1	Delonix regia (Boj. ex Hook.) Raf.	Leguminosae (Fabaceae)

09.08.2021  
(डॉ.एल.शिरोडकर) D.L.Shirodkar  
वनस्पतिज्ञ/Botanist

### Drug treatment and experimental design

Thirty-six animals were grouped into six, having six in each group (n=6). Group I received normal 0.9% saline solution Group II received haloperidol (2.0 mg/kg, p.o), Group III received haloperidol (2.0 mg/kg, p.o) + L-DOPA + carbidopa (100+25 mg/kg, p.o) suspended in 1% tween 20, and Groups IV, V, VI received 100, 250 and 500 mg/kg DRLE (p.o), respectively, 30 minutes before haloperidol administration for 15 days.

After the treatment for 15 days, all six groups of animals underwent the behavioural assessment tests. Then, cervical decapitation was performed to isolate the striatum of the brains of the animals, and homogenate was prepared using ice-cold phosphate-buffered saline solution and stored.

### Process of extraction: Maceration process:

The leaves were taken and they were dried and powdered Then the powdered leaves were taken in a container and

## Evaluation of Neuroprotective Activity of *Delonix Regia* Leaves on Haloperidol Induced Parkinsonism in Experimental Animals

added 70% ethanol. And were kept for 7 days in an airtight container. After 7 days the filtration is done and the filtrate was made to evaporate in a cold evaporator. The extract which is produced is stored in an airtight container and kept in the refrigerator for further use. [21].

### Phytochemical analysis:

Phytochemical analysis involves the qualitative analysis of herbal plants. The preliminary qualitative tests have been attempted in *Delonix regia* Leaves to find out the presence or absence of certain bio active Compounds. Chemical tests were carried out on the ethanolic extract using standard procedures to identify the constituents as described by Harborne. [22].

### Behavioral Assessment:

1. **Assessment of Locomotor activity by Actophotometer:** The locomotor activity (horizontal activity) can be easily measured using an actophotometer. A count was recorded, when the beam of light falling on the photocell was cut off by movement of animal, for a period of 10 minutes. [23].
2. **Assessment of catatonia by Block method:** Catalepsy is defined as the failure to correct an externally imposed posture. A condition characterized by inactivity, decreased responsiveness to stimuli, and a tendency to maintain an immobile posture. The catalepsy test is widely used to evaluate motor effects of drugs that act on the extrapyramidal system. The method of assessment of catalepsy were determined by scoring method. [24].
3. **Assessment of muscles coordination by Rotarod activity:** Motor Co-ordination test was conducted using rota rod apparatus. Motor coordination can be tested by comparing the latency to fall on the very first trial between treatment groups. The time taken by animals to fall from the rotating rod was noted. [27].

### Biochemical assessment:

#### Preparation of brain homogenate:

All the treated animals of each protocol were anesthetized by using Chloroform on the 15th day of treatment after all behavioural and locomotors assessment. Brains were removed and rinsed with cold normal saline followed by addition of 0.1 M phosphate buffer (7.4), a 10% w/v of tissue. The isolated brain tissues were homogenized in the phosphate buffer (Ph 7.4, 0.1 M). Homogenate were centrifuge at 4°C for 10 minutes on 4000 rpm. Supernatant was removed for the further analysis. [28]

#### 1. Estimation of Catalase activity:

Catalase is determined according to the method of Aebi et.al, 1974 by the depletion rate of H<sub>2</sub>O<sub>2</sub> at 240 nm in a reaction buffer. [29]

$$\text{Catalase} = \frac{\delta \text{ O.D}}{E \times \text{Vol. of sample} \times \text{mg of protein}}$$

Where,  $\delta$  O.D. is change in absorbance per minute, E is extinction coefficient (0.071 mmol cm<sup>-1</sup>) of hydrogen peroxide.

#### 2. Estimation of Malondialdehyde level [lipid peroxidation]:

MDA (indicator of lipid peroxidation) was estimated as described by Ohkawa et al. Thiobarbituric acid was added to the brain homogenate under acidic conditions and the absorbance of colour that developed after heating was estimated spectrophotometric ally at 535 nm. [30]

Measurement of Malondialdehyde was done by using following formula

$$\text{Concentration of Malondialdehyde} = \frac{\text{Abs}_{532} \times 100 \times V_T}{(1.56 \times 10^5) \times W_T \times V_U}$$

Where Abs<sub>532</sub> is absorbance, V<sub>T</sub> = volume of total mixture, 1.56×10<sup>5</sup> is molar extinction coefficient, W<sub>T</sub> = weight of brain in grams, V<sub>U</sub> = aliquot volume 1 mL

#### 3. Estimation of reduced glutathione (GSH):

Reduced GSH was estimated by the method described by Srivastava et al. This method is based on the development of a yellow colour when 5,5'-dithio-bis-2-nitrobenzoic acid is added to compounds containing sulfhydryl groups.[31]

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

Y is absorbance taken at 412nm, BT is brain tissue homogenate, DF is dilution factor and VU is aliquot volume (1 ml)

## 4. Estimation of Monoamine Oxidase-B [MAO-B]:

The Monoamine Oxidase-B enzyme were determined by method of Charles M et al. MAO-B activity is measured in nanomoles per milligram of protein. [32]

$$X = \frac{\text{sample (abs)} - \text{blank (abs)} \times \text{conc. of standard}}{\text{Standard (abs)} - \text{Blank (abs)}}$$

## 5. Estimation of Neurotransmitter (Dopamine):

Biogenic amines i.e., dopamine was estimated by HPLC with UV detector. Mobile phase of 2% perchloric acid and methanol were used in ratio of 1:4 (100 ml). After all behavioural parameters the brain homogenate were prepared by above method and are analyzed for concentration of dopamine. [33]

DA level was expressed as ng/mg. protein.

$$\text{DA level} = \frac{\text{Abs. of sample} \times \text{Concentration of standard}}{\text{Absorbance of standard} \times \text{mg protein}}$$

## Statistical analysis

One-way analysis of variance followed by Dunnett's test was employed for the analysis of data in catalepsy test, biochemical and other behavioural parameters. the statistically significance between the groups. \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001 was considered as significant.

## 3.Results

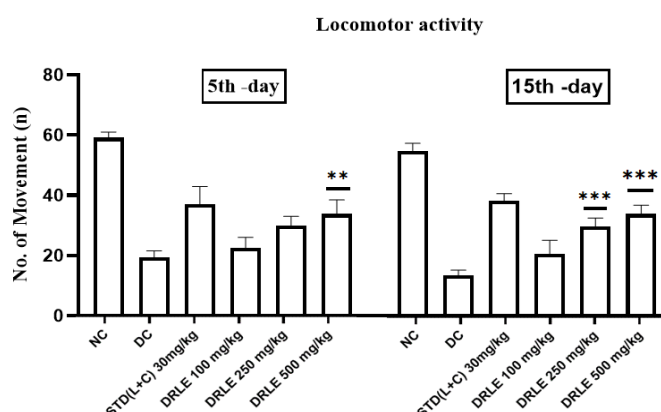
### 1. Preliminary phytochemical analysis of Ethanolic extract of Delonix regia

Sr. No	Phytochemical Constituents	Results
1.	Test for flavonoids	+
2.	Test for alkaloids	+
3.	Test for Glycosides	-
4.	Test for tannins	+
5.	Test for steroids	-

### 2. Total flavonoid content:

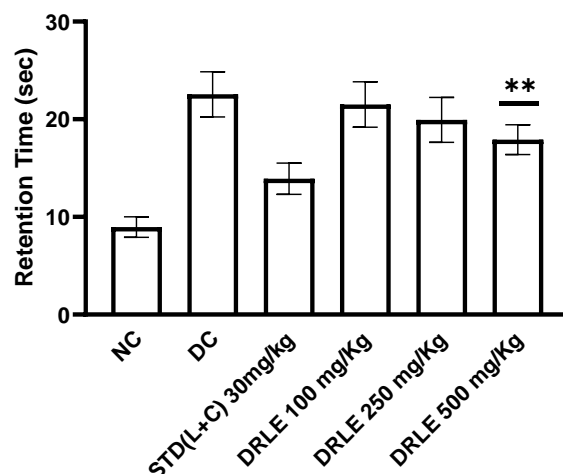
PRODUCT	Total Flavonoid Content
Ethanolic extract of Delonix regia leaves.	16.5 mg/g

### 3. Effect of DRLE on Locomotor activity in Haloperidol induced Parkinsonism model of rats.



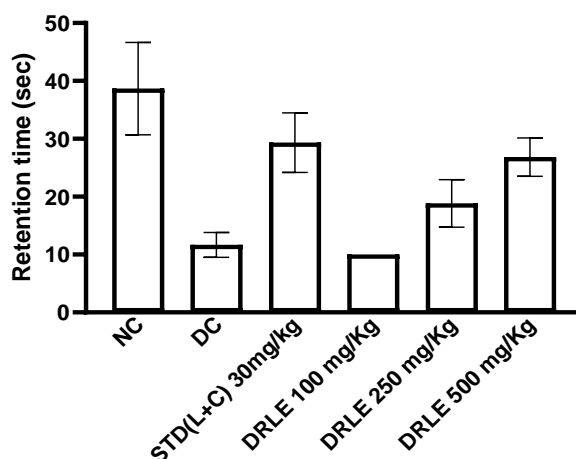
Values are expressed as mean ± SEM; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett's multiple tests for comparison. Level of significance †P < 0.05; #P < 0.01; \*P < 0.001.

#### 4. Effect of DRLE on Catatonic response in Haloperidol induced Parkinsonism model of rats.



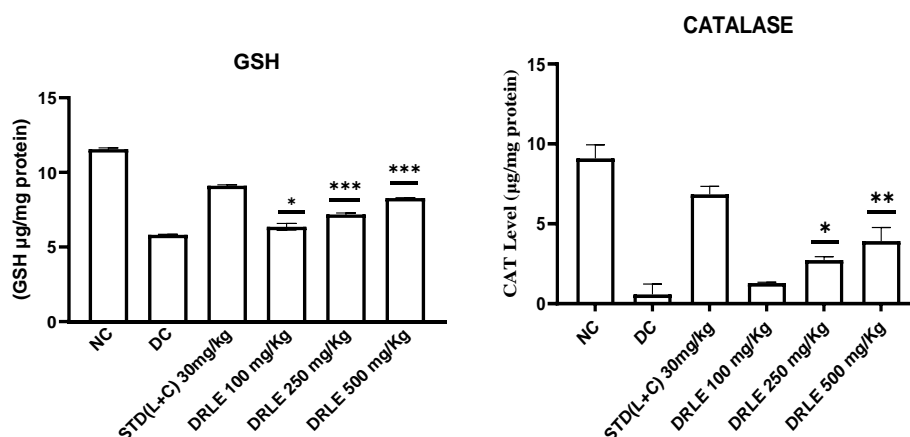
Values are expressed as mean  $\pm$  SEM; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett's multiple test for comparison. Level of significance †P < 0.05; #P < 0.01; \*P < 0.001.

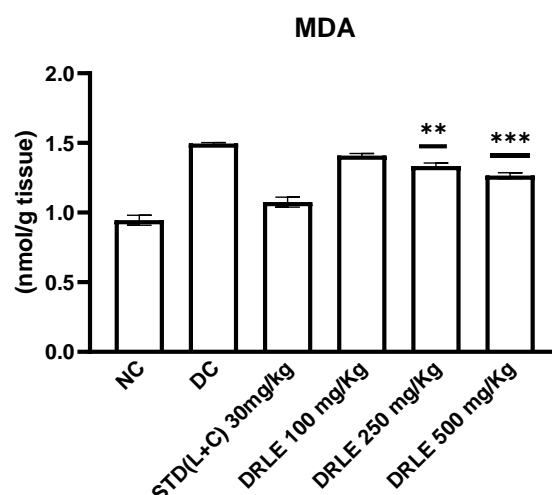
#### 5. Effect of DRLE on muscles coordination in Haloperidol induced Parkinsonism model of rats.



Values are expressed as mean  $\pm$  SEM; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett's multiple tests for comparison. Level of significance †P < 0.05; #P < 0.01; \*P < 0.001.

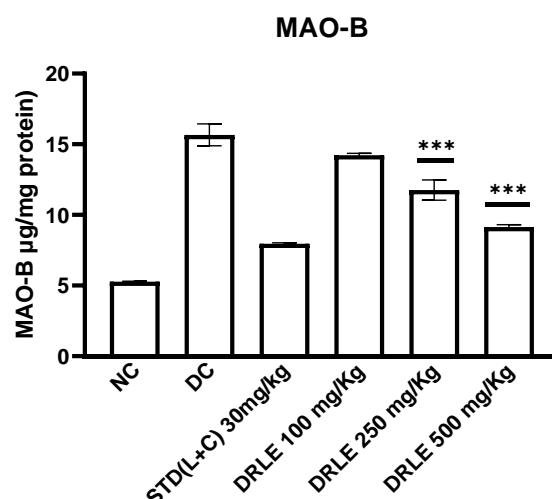
#### 6. Effect of DRLE on *IN VIVO* antioxidants of brain in Haloperidol induced Parkinsonism model of rats





Values are expressed as mean $\pm$  SEM; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett's multiple test for comparison. Level of significance †P < 0.05; #P < 0.01; \*P < 0.001.

#### 7. Effect of DRLE on monoamine oxidase enzymes of brain in Haloperidol induced Parkinsonism model of rats.



Values are expressed as mean $\pm$  SEM; n = 6; Data analyzed by One-way ANOVA test followed by Dunnett's multiple tests for comparison. Level of significance †P < 0.05; #P < 0.01; \*P < 0.001.

#### 8. Effect of DRLE on brain neurotransmitters (Dopamine) in Haloperidol induced Parkinsonism model of rats.

Groups	Dopamine (mg/g of brain tissue)
Normal Control	5.630 $\pm$ 0.380
Disease control (Haloperidol) 2 mg/kg	1.090 $\pm$ 0.140
Standard control (Levodopa+ Carbidopa) 30mg/kg	2.520 $\pm$ 0.220
D. regia 100 mg/kg	1.175 $\pm$ 0.085
D. regia 250 mg/kg	2.700 $\pm$ 0.250 <sup>#</sup>
D. regia 500 mg/kg	3.185 $\pm$ 0.065 <sup>#</sup>

#### 4. Discussion

PD is a commonly occurring neurodegenerative disorder that produces muscular rigidity, bradykinesia, tremor in resting limbs and loss of postural balance. The basic neuropathology of PD involves the selective degeneration of dopaminergic cells in specific brain regions like the striatum; when degeneration in these neurons reaches a threshold reduction of 80% dopamine, the motor symptoms of PD emerge.

Although the pathogenesis of PD is still elusive, it is believed to be multifactorial. Oxidative and nitrosative stress is one of the important contributing factors in PD pathogenesis as dopaminergic neurons being vulnerable to the generated oxidative stress. Also, oxidative stress is interlinked with other components leading to the neurodegenerative process, which includes mitochondrial dysfunction, nitric oxide toxicity and inflammation.

This study aims to investigate the Neuroprotective effect of *Delonix regia* Leaves on Haloperidol intoxicated animal models of PD by analyzing behavior patterns, brain antioxidant, brain neurotransmitters studies.

Haloperidol is a broadly used neuroleptic drug for the treatment of psychosis. It acts by antagonizing dopamine D2 and to smaller extent D1 receptors in medium spiny neurons that include indirect and direct pathways of the motor circuit, respectively.

In the present study, animals were treated with haloperidol (2 mg/kg) showed cataleptic behavior similar to the symptoms of PD. Haloperidol, an antipsychotic drug, which blocks central dopamine receptor in the striatum, produces a behavioral state in animals such as mice and rats in which they fail to correct externally imposed postures. This is referred to as catalepsy.

The haloperidol, (a nonselective D 2 dopamine antagonist) induced catalepsy is primarily due to the blockade of dopamine receptors in the striatum. The agent, increasing dopamine transmission inhibits neuroleptic induced catalepsy.

Haloperidol (HP) is converted to potentially toxic (HHP+) metabolites which may play a role in the extrapyramidal side effects which are observed in the patients who are treated with haloperidol (Garrod et al., 1993).

The DRLE (500 mg/kg, p.o.) showed a significant reduction in the duration of catalepsy demonstrating Antiparkinson activity. The inhibition of catalepsy indicates the ability of the drug to potentiate dopaminergic transmission in the striatum.

Anxiety and hypnosedation are principally mediated in the CNS by the GABA<sub>A</sub> receptor complex, which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, anxiety, depression, Parkinson syndrome and Alzheimer's disease.

Diverse drugs like Haloperidol that are used in various psychological and neurological disorders might modify the GABA system at the level of the synthesis of GABA, induce anxiolysis or hypnosis in animals by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors (Charney et al., 2001). In some studies, it was seen that mice/rats with dopamine depletion showed poor motor skill learning as dopamine degeneration impairs spatial memory tasks. Locomotor activity indicates attentiveness and the decline indicates sedative action. These inhibitions of GABA and depletion of Dopaminergic Transmission leads to depression and anxiety like effects which were evaluated by Actophotometer and Rotarod.

The results indicated that the haloperidol caused significant decreased locomotor counts in Actophotometer. Levodopa + carbidopa 30 mg/kg and DRLE [500 mg/kg] has shown the increased locomotor activity as compared to haloperidol-treated animals. Daily treatment with DRLE significantly reversed the decrease in locomotor activity as assessed on day 15<sup>th</sup>.

Similarly, the motor coordination test in is one of the evaluation parameters in Parkinson model which were evaluated by rotarod.

The test consists of a rotating rod on which the animal balances. Haloperidol-treated rat, subjected to the rotarod test, exhibited a significant loss of muscular coordination, it could be due to loss of muscular strength.

Levodopa and carbidopa 30 mg/kg and DRLE 500 mg/kg prevented the motor impairment significantly, which was altered by haloperidol. It indicates that DRLE which consist of active constituents like flavonoids, sterols, triterpenes, have a stimulant effect on CNS.

It is also well established that the administration of haloperidol leads to an increase in the oxidative stress in the brain tissue (Sagara et al., 1998).

The reduced levels of endogenous antioxidant molecules such as glutathione (GSH), antioxidant enzymes such as Catalase (CAT), and lipid peroxidation product malondialdehyde (MDA) in the brain could contribute to neuronal death. Indeed, post-mortem 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. These findings introduced the requirement of using antioxidants as a therapeutic intervention in PD in addition to other protective agents.

In the ongoing study Haloperidol treated animals will show increased levels of MDA and decreased levels of GSH and CAT, when compared with normal control animals which supports the earlier finding that haloperidol alters the levels of GSH, CAT and MDA.

Treatment with *Delonix regia* levels has reversed the altered oxidative stress markers due to its antioxidant properties in rat brain.

CAT is an antioxidant which helps in neutralizing the toxic effects of hydrogen peroxide. Hydrogen peroxide is converted by the CAT enzyme to form water and non-reactive oxygen species, thus preventing the accumulation of precursor to free radical biosynthesis. Oxidative stress results in decrease in CAT level. Levodopa + carbidopa 30 and DRLE, has increases the CAT level as compared to haloperidol-treated animals.

Malondialdehyde (MDA) is an aldehyde which is a marker of lipid per oxidation. Increased MDA level in brain is an indicator of mitochondrial dysfunction due to oxidative stress. Due to increased oxidative stress NADPH oxidase cause



## Evaluation of Neuroprotective Activity of *Delonix Regia* Leaves on Haloperidol Induced Parkinsonism in Experimental Animals

oxidative destruction in brain. DRLE treatment will show dose dependent decrease in MDA level as compare to in haloperidol induced Parkinson disease.

GSH is important antioxidant which protects from free oxygen radicals by converting  $\text{H}_2\text{O}_2$  into  $\text{O}_2$ , and  $\text{H}_2\text{O}$ . Decrease in glutathione level led to mitochondrial damage. GSH is a cofactor for antioxidant and detoxifying enzymes. Thus, the antioxidant property of DREL has reverse the level of GSH level in dose dependent manner in treatment group.

MOA-B enzyme contributed to dopamine neurodegenerative process and oxidative stress so it appeared that peripheral concentration of enzyme was low. Monoamine oxidase B level has occurred at low level in diseases control group when compared to Treatment group which means that enzyme will be recovered to normal level in treatment group. The dopaminergic neuron function was assessed by measuring striatal DA levels by HPLC. The haloperidol treated group showed significantly decreased striatal DA levels, whereas DRLE 500 mg/kg and 250mg/kg and levodopa caused a significant increase in striatal DA levels.

The above behavioural and biochemical results suggest that *D. regia* has the ability to improve symptoms of Parkinsonism, in part, by the restoring the level of dopamine, and by the regulation of the antioxidant system. Thus, antioxidant and neuroprotective activities may be responsible for antiparkinson's effect. Hence, *D. regia* may be useful as a neuroprotective agent in the treatment of PD. The above observed beneficial effects of *D. regia* may be attributed to diverse chemical components namely flavonoids, glycosides, saponins, and tannins.

## 5. Conclusion

From the present study, it can be considered that the Ethanolic extract of *Delonix regia* leaves exhibited significant anti-parkinsonism activity in Haloperidol induced Parkinson model in rats. The behavioural and biochemical results suggest that *D. regia* has the ability to improve symptoms of Parkinsonism, in part, by the restoring the level of dopamine, and by the regulation of the antioxidant system. Thus, antioxidant and neuroprotective activities may be responsible for antiparkinson's effect. Hence, *D. regia* may be useful as a neuroprotective agent in the treatment of PD. All the Parameters of extract treated group animals have shown better results when compared with Haloperidol induced group and the standard L-dopa treated group. These findings provide a preliminary evidence for its potential as anti-parkinsonian medication, including Parkinson's disease prevention and improvement of symptoms. The present study showed that ethanolic extract of *Delonix regia* leaves possesses anti-Parkinson activity. The extract of *Delonix regia* leaves contains mixture of tannins, flavonoids, alkaloids and other phytoconstituents which will be responsible for different pharmacological activities as a result, more research on the ethanolic extract of *Delonix regia* leaves is urgently needed in order to find a safer, more effective anti-Parkinson alternative.

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**Conflicts of interest:** None

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## Evaluation of Neuroprotective Activity of Delonix Regia Leaves on Haloperidol Induced Parkinsonism in Experimental Animals

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### Supplementary Data:

#### 1. Effect of DRLE on Locomotor activity in Haloperidol induced Parkinsonism model of rats.

Groups	No. of Movements	
	5 <sup>TH</sup> DAY	15 <sup>TH</sup> DAY
Normal Control	45.33±4.37	42.33± 3.59
Disease control (Haloperidol) 2 mg/kg	20.00±2.22	18.16± .02
Standard control (Levodopa+ Carbidopa) 30mg/kg	37.83±6.063	39.66±4.29
D. regia 100 mg/kg	23.33±2.60	20.24±2.404
D. regia 250 mg/kg	25.50±2.18	23.16±1.95*
D. regia 500 mg/kg	41.33±3.748 <sup>#</sup>	38.83±3.98*

#### 2. Effect of DRLE on Catatonic response in Haloperidol induced Parkinsonism model of rats.

Time interval in min	Groups					
	Normal Control	Disease control (Haloperidol) 2 mg/kg	STD (L+ C) 30mg/kg	D. regia 100 mg/kg	D. regia 250 mg/kg	D. regia 500 mg/kg

## Evaluation of Neuroprotective Activity of Delonix Regia Leaves on Haloperidol Induced Parkinsonism in Experimental Animals

<b>30 min</b>	7.600±1.122	19.600±1.503	11.200±1.158	18.600±1.208	16.600±0.927	15.400±0.927 <sup>†</sup>
<b>60 min</b>	8.800±0.735	21.200±1.655	13.800±0.970	20.200±1.625	19.400±1.631	17.600±1.691 <sup>†</sup>
<b>90 min</b>	8.400±0.510	22.600±1.327	14.600±1.208	21.800±1.158	20.400±1.166	18.600±1.122 <sup>†</sup>
<b>120 min</b>	9.800±0.583	23.200±0.583	14.800±0.374	22.200±1.393	20.200±1.744	18.800±1.068 <sup>†</sup>
<b>180 min</b>	10.200±0.583	25.600±1.030	15.200±0.374	24.800±1.158	23.000±1.049	19.200±0.800 <sup>*</sup>

### 3. Effect of DRLE on muscles coordination in Haloperidol induced Parkinsonism model of rats.

Groups	Retention time (in sec)
Normal Control	38.66±3.26
Disease control (Haloperidol) 2 mg/kg	11.66 ±0.88
Standard control (Levodopa+ Carbidopa) 30mg/kg	29.33±2.09
<i>D. regia</i> 100 mg/kg	10.00±00
<i>D. regia</i> 250 mg/kg	18.83±1.68 <sup>†</sup>
<i>D. regia</i> 500 mg/kg	26.83±1.35 <sup>*</sup>

### 4. Effect of DRLE on IN VIVO antioxidants of brain in Haloperidol induced Parkinsonism model of rats.

Groups	Cat value in (µg/mg protein)	MDA value in (µg/mg protein)	GSH value in (µg/mg protein)
Normal Control	9.10±0.66	0.954±0.025	11.56±0.060
Disease control (Haloperidol) 2 mg/kg	0.58±0.46	1.495±0.005	5.825±0.025
Standard control (Levodopa+ Carbidopa) 30mg/kg	6.85±0.35	1.075±0.025	9.100±0.050
<i>D. regia</i> 100 mg/kg	1.29±0.04	1.410±0.04	6.360±0.160 <sup>†</sup>
<i>D. regia</i> 250 mg/kg	2.73±0.14 <sup>†</sup>	1.335±0.015 <sup>#</sup>	7.175±0.075 <sup>*</sup>
<i>D. regia</i> 500 mg/kg	3.91±0.60 <sup>#</sup>	1.265±0.015 <sup>*</sup>	8.275±0.025 <sup>*</sup>

### 5. Effect of DRLE on monoamine oxidase enzymes of brain in Haloperidol induced Parkinsonism model of rats.

Groups	MAO-B value in (µ/mg protein)
Normal Control	5.275±0.025
Disease control (Haloperidol) 2 mg/kg	15.665±0.54
Standard control (Levodopa+ Carbidopa) 30mg/kg	7.955±0.055
<i>D. regia</i> 100 mg/kg	14.220±0.100
<i>D. regia</i> 250 mg/kg	11.755±0.505 <sup>*</sup>
<i>D. regia</i> 500 mg/kg	9.135±0.125 <sup>*</sup>