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Evaluation of Neuroprotective Activity of Delonix 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 characterised 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. Delonix 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 anticarcinogenic 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. **2 The rats were randomly divided into six groups and the test animals received the ethanolic extract of Delonix 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 Delonix regia has anti-oxidant activity and neuroprotective activity in haloperidol experimental model of PD.

**Keywords:-** Parkinson diseases, Oxidative stress, Haloperidol, Delonix regia, Neuroprotective.

## 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. [2] 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] [8] One of the main causes of Parkinsonism is oxidative stress, which results in the death of dopaminergic neurons. [4]

The pathogenetic process [16] 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. [8] As a result, a byproduct of their metabolism could be ROS. [5,6] [11] In addition to the production of free radicals, haloperidol treatment is associated with a considerable decrease in antioxidant glutathione levels [7]. Levodopa [26] is still the cornerstone of treatment because it is the most successful pharmacological option currently available. [2] 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 [12] in Parkinson's disease treatment since they contain anti-oxidant and neuroprotective effects (PD).

[20] 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. [8] As a result, several researchers are now concentrating on the potential anti-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. <sup>1</sup> 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 <sup>9</sup> 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.

## Material and Method

### Animals

Healthy <sup>7</sup> 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±2°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

<sup>1</sup> 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.

#### 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. <sup>4</sup> 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.

#### <sup>2</sup> 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 added 70% ethanol. And were kept for 7 days in an airtight container. <sup>4</sup> 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]

#### <sup>2</sup> 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. <sup>15</sup> Chemical tests were carried out on the ethanolic extract using standard procedures to identify the constituents as described by Harborne.[22]

#### Behavioural Assessment:

1. Assessment of Locomotor activity by Actophotometer: <sup>18</sup> 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. <sup>13</sup> 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. [8] 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 [4] 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]

□ O.D

Catalase =

$$E \div \text{Vol. of sample} \div \text{mg of protein}$$

Where, □ 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. [2] Thiobarbituric acid was added to the brain homogenate under acidic conditions and the absorbance of colour that developed after heating was estimated spectrophotometrically at 535 nm.[30]

Measurement of Malondialdehyde was done by using following formula

$$\text{Abs}_{532} \times 100 \times \text{VT}$$

Concentration of Malondialdehyde =

$$(1.56 \times 105) \times \text{WT} \times \text{VU}$$

Where Abs<sub>532</sub> is absorbance, VT = volume of total mixture, 1.56×105 is molar extinction coefficient, WT = weight of brain in grams, VU = 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]

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

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.

$$\text{Abs. of sample} \times \text{Concentration of standard}$$



DA level =

Absorbance of standard x mg protein

#### 4 Statistical analysis

One-way analysis of variance followed by Dunnett's test was employed for the analysis of data in catalepsy test, biochemical and 2 other behavioural parameters. the statistically significance between the groups. \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001 was considered as significant.

#### 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. **1** Total flavonoid content:

PRODUCT

Total Flavonoid Content

Ethanollic extract of Delonix regia leaves.

16.5 mg/g

**4** 3. Effect of DRLE on Locomotor activity 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.

**12** 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. **2** 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) <sup>12</sup> in Haloperidol induced Parkinsonism model of rats.

Groups

<sup>4</sup> 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±0.220

D. regia 100 mg/kg

1.175±0.085

D. regia 250 mg/kg

2.700±0.250#

D. regia 500 mg/kg

3.185±0.065#

## 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.

All Though 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 analysing behaviour 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 behaviour similar to the symptoms of PD. Haloperidol, an antipsychotic drug, which blocks central dopamine

receptor in the striatum, produces a behavioural 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.

6 Anxiety and hypnosedation are principally mediated in the CNS by the GABAA receptor complex, which is also involved in other physiological functions related to behaviour 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 inhibition of GABA and depletion of Dopaminergic Transmission leads to depression and anxiety like effects which were evaluated by Actophotometer and Rotarod.

3 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 15th. Similarly, the motor coordination test 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.

3 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 4 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), 7 antioxidant enzymes such as Catalase (CAT), and lipid peroxidation product malondialdehyde (MDA) in the brain could contribute to neuronal death. Indeed, post-mortem 11 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. 5 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 7 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 5 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 3 + 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. 5 Increased MDA level in brain is an indicator of mitochondrial dysfunction due to oxidative stress. Due to increased oxidative stress NADPH oxidase cause oxidative destruction in brain. DRLE treatment will show dose

dependent [4] decrease in MDA level as compare to in haloperidol induced Parkinson disease.

GSH is important antioxidant which protects from free oxygen radicals by converting H<sub>2</sub>O<sub>2</sub> into O<sub>2</sub>, and H<sub>2</sub>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 [5] the level of GSH level in dose dependent manner in treatment group.

MOA-B enzyme contributed to dopamine neurodegenerative process [9] 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 [4] 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 [5] 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 [3] 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.

Conclusion: From [4] 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 [3] 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 [12] 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. <sup>24</sup> The present study showed that ethanolic extract of *Delonix regia* leaves possesses anti-Parkinson activity. The <sup>1</sup> 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 <sup>11</sup> needed in order to find a safer, more effective anti-Parkinson alternative.

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Conflicts of interest: None

#### References:

- [1] Johnson, M. E., Bobrovskaya, L., 2015. An update on the rotenone models of Parkinson's disease: their ability to reproduce the features of clinical disease and model gene environment interactions. *Neurotoxicology* 46, 101-116
- [2] <sup>17</sup> Erro R, Stamelou M. The motor syndrome of Parkinson's disease. *Int Rev Neurobiol.* 2017; 132:25–32.
- [3] Husnul Khotimah, Sutiman B. Sumitro, M. Aris Widodo, Zebrafish <sup>14</sup> Parkinson's Model: Rotenone decrease motility, Dopamine, and increase  $\alpha$ -synuclein Aggregation and Apoptosis of Zebrafish Brain, *International Journal of Pharm Tech Research*, 2015:614-621
- [4] Prasad KN, Cole WC, Kumar B. Multiple antioxidants in <sup>9</sup> the prevention and treatment of Parkinson's disease. *J Am Coll Nutr.* 1999;18(5):413-23.
- [5] Perera J, Tan JH, Jeevathayaparan S, Chakravarthi S, Haleagrahara N. Neuroprotective effects <sup>21</sup> of alpha lipoic acid on haloperidol-induced oxidative stress in the rat brain. *Cell Biosci* 2011;1:2e6.
- [6] Mosley RL, Benner EJ, Kadiu I, Thomas M, Boska MD, Khader Hasan, et al. Neuroinflammation, <sup>7</sup> oxidative stress and the pathogenesis of Parkinson's disease. *Clin Neurosci Res* 2006;6(5):261e81.
- [7] Shivakumar BR, Ravindranath V. Oxidative stress and thiol modification induced by chronic administration of haloperidol. *J Pharmacol Exp Therapeut* 1993;265:1137e41.



- [8] 1 Shabir G, Anwar F, Sultana B, Khalid ZM, Afzal M, Khan QM, et al. Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf.] *Molecules* 2011;1:7302-19.
- [9] Sama K, Raja AXV, Yadav RH. Antibacterial and pharmacognostical evaluation of *Delonix regia* root bark. *Int J Pharm Life Sci* 2012;3:1628-30.
- [10] Rani PMJ, Kannan PSM, Kumaravel S. Screening of antioxidant activity, total phenolics and gas chromatograph and mass spectrometer (GC-MS) study of *Delonix regia*. *Afr J Biochem Res* 2011;5:341-7.
- [11] Salman A, Toseef S, Mohtasheemul HM, Iqbal A. Antiemetic activity of leaves extracts of five leguminous plants. *Int J Res Ayurveda Pharm* 2012;3:251-3.
- [12] Deepa B, Remadevi OK. Larvicidal activity of the flowers of *Delonix regia* (Bojer ex Hook.) Rafin. (Fabales: Fabaceae) against the teak defoliator, *Hyblaea puera*. *Cramer Curr Biotica* 2011;5:237-40
- [13] Ahmed J, Nirmal S, Dhasade V, Patil A, Kadam S, Pal A, et al. Hepatoprotective activity of methanol extract of aerial parts of *Delonix regia*. *Phytopharmacology* 2011;1:118-22.
- [14] Shiramane RS, Biradar KV, Chivde BV, Shambhulingayya HM, Goud V. In-vivo antidiarrhoeal activity of ethanolic extract of *Delonix regia* flowers in experimental induced diarrhea in wistar albino rats. *Int J Res Pharm Chem* 2011;1:442-7
- [15] Shewale VD, Deshmukh TA, Patil LS, Patil VR. Anti-inflammatory activity of *Delonix regia* (Boj. Ex. Hook). *Adv Pharmacol Sci* doi 2012;10:1155/2012/789713.
- [16] Fatmawaty, Fadilah, Astuti H. Antimalarial activity of *Delonix regia* on mice with *Plasmodium berghei*. *J Nat Prod* 2013;6:61-6
- [17] Ahirrao RA, Patel MR, Hamid S, Patil JK. In-vitro anthelmintic property of Gulmohar flowers against *Pheritima posthuma*. *Pharmacologyonline* 2011;1:728-32.
- [18] Chitra V, Ilango K, Rajanandh MG, Soni D. Evaluation of *Delonix regia* Linn. Flowers for antiarthritic and antioxidant activity in female wistar rats. *Ann Biol Res* 2010;1:142-7.
- [19] Khan MA, Saxena A, Fatima FT, Sharma G, Goud V, Husain A. Study of wound healing activity of *Delonix regia* flowers in experimental animal models. *Am J PharmTech Res* 2012;2:380-90

- [20] Azab SS, Abdel-Daim M, Eldahshan OA. Phytochemical, cytotoxic, hepatoprotective and antioxidant properties of *Delonix regia* leaves extract. *Med Chem Res* 2013;22:4269-77
- [21] Rani MJJ, Chandramohan G, Kumaravel S. Evaluation of antimicrobial activity of some garden plant leaves against *Lactobacillus* sp, *Streptococcus mitis*, *Candida albicans* and *Aspergillus niger*. *Afr J Basic Appl Sci* 2012; 4:139-42.
- [22] Harborne JB. <sup>22</sup> *Phytochemical Methods A guide to modern techniques of plant analysis*. 3rd edition. Chapman & Hall; 1998. 40-96 p
- [23] Kulkarni S K. To study the phenothiazine induced catatonia in rats; *Hand book of experimental pharmacology* Third edition; 2003: 140.
- [24] Anil kumar, Kulkarni SK. Effect of BR-16A(Mentat), a poly herbal formulation on drug <sup>4</sup> induced catalepsy in mice. *Indian J Exper Biol*. 2006;44:45–48.
- [25] Samanta M.K et al, Development <sup>23</sup> of Mono ingredient Herbal neuroleptic tablet for better psychiatric therapy, *Indian Journal of Pharmaceutical Sciences*, 2005, 67(1): 51-56.
- [26] Sakina M.R et al; A Neuropsychopharmacological profile of "cinkara", a polyherbal preparation, *Ind. Physio. Pharmac.*, Volume 33, Number 1,1989.
- [27] Rabelo, I.D. Oliveira, A.G. Guimaraes, J.S.S Quintans, A.P.N. Prata , D.P. Gelain, <sup>19</sup> Antinociceptive, anti-inflammatory and antioxidant activities of aqueous extract from *Remirea maritima* (Cyperaceae), *Journal of ethnopharmacology*, 145 (2013): 11-17
- [28] Maham Sanawar, Uzma Saleem, Fareeha Anwar, Samra Nazir, Muhammad Furqan Akhtar, Bashir Ahmad & Tariq Ismail (2020): Investigation of Anti Parkinson Activity of Dicyclomine, *International <sup>5</sup> Journal of Neuroscience*, DOI: 10.1080/00207454.2020.1815732
- [29] H. Aebi, "Catalase in vitro," in *Methods in Enzymology*, vol. 105, pp. 121–126, Academic Press, New York, NY, USA, 1984.
- [30] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Analytical Biochemistry*, vol. 95, no. 2, pp. 351–358, 1979.
- [31] S. K. Srivastava and E. Beutler, "Accurate measurement of oxidized glutathione content of human, rabbit, and rat red blood cells and tissues," *Analytical Biochemistry*, vol. 25, pp. 70– 76, 1968
- [32] Charles M, McEwen J, Tabor <sup>9</sup> H et al (eds). *Methods of Enzymologists*. 1977. Academic press.

New York.

[33] Rahman H, Eswaraiah M. 29 Simple spectroscopic methods for estimating brain

neurotransmitters, antioxidant enzymes of laboratory animals like mice: A review. Pharmatutor Art. 2008;1244.

Supplementary Data:

1. Effect of DRLE on 4 Locomotor activity in Haloperidol induced Parkinsonism model of rats.

Groups

No. of Movements

5TH DAY

15TH 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#

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

30 min

7.600±1.122

19.600±1.503

11.200±1.158

18.600±1.208

16.600±0.927

15.400±0.927†

60 min

8.800±0.735

21.200±1.655

13.800±0.970

20.200±1.625

19.400±1.631

17.600±1.691†

90 min

8.400±0.510

22.600±1.327

14.600±1.208

21.800±1.158

20.400±1.166

18.600±1.122†

120 min

9.800±0.583

23.200±0.583

14.800±0.374

22.200±1.393

20.200±1.744

18.800±1.068†

180 min

10.200±0.583

25.600±1.030

15.200±0.374

24.800±1.158

23.000±1.049

19.200±0.800\*

### 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

D. regia 100 mg/kg

10.00±00

D. regia 250 mg/kg

18.83±1.68†

D. regia 500 mg/kg

26.83±1.35\*

### 4. Effect of DRLE on IN VIVO antioxidants of brain <sup>12</sup> 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

D. regia 100 mg/kg

1.29±0.04

1.410±0.04

6.360±0.160†

D. regia 250 mg/kg

2.73±0.14†

1.335±0.015#

7.175±0.075\*

D. regia 500 mg/kg

3.91±0.60#

1.265±0.015\*

8.275±0.025\*

5. Effect of DRLE on monoamine oxidase enzymes of brain **12 in Haloperidol induced** Parkinsonism model of rats.

Groups

MAO-B value in

(µ/mg protein)

Normal Control

5.275±0.025

Disease control 4 (Haloperidol) 2 mg/kg

15.665±0.54

Standard control (Levodopa+ Carbidopa) 30mg/kg

7.955±0.055

D. regia 100 mg/kg

14.220±0.100

D. regia 250 mg/kg

11.755±0.505\*

D. regia 500 mg/kg

9.135±0.125\*



## Sources

1	<a href="https://www.academia.edu/114007146/PHYTOCHEMICALS_AND_PHAR...">https://www.academia.edu/114007146/PHYTOCHEMICALS_AND_PHAR...</a> INTERNET 5%
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