



## Evaluation of Neuroprotective Activity of Bauhinia Variegata on Haloperidol Induced Catalepsy in Rats

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### ABSTRACT

An acetone soluble leaf extract from *Bauhinia variegata* was investigated for its neuroprotective effects in the haloperidol induced catalepsy rat model of the disease by measuring behavioural and biochemical parameters in the model. In rat model catalepsy was induced by administration of haloperidol (1 mg/kg, p.o) in wistar rats of either sex. A significant reduction in the catalepsy was observed in the drug treated groups when compared to the disease induced group. The biochemical parameters were Lipid peroxidation, Glutathione (GSH), Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were assessed in brain. Administration of haloperidol resulted in increased lipid peroxidation and decreased levels of antioxidant enzymes i.e GSH, GSH-Px and SOD. The study showed that the plant extract significantly decreased lipid peroxidation levels and increased antioxidant enzyme levels.

**KEYWORDS:** *Bauhinia variegata*, neuroprotective, antioxidant, antioxidant enzymes

### 1. INTRODUCTION

The term neurodegeneration is a combination of two words - "neuro," referring to nerve cells and "degeneration," referring to progressive damage. Thus, in the strict sense of the word, neurodegeneration corresponds to any pathological condition primarily affecting neurons. In practice, neurodegenerative diseases represent a large group of neurological disorders with heterogeneous clinical and pathological expressions affecting specific subsets of neurons<sup>[1]</sup>. The process of neurodegeneration begins at the cellular level in which two main processes, oxidative stress and excitotoxicity, act

relentlessly to inflict the majority of cell damage and death. Although many factors can play a direct role in the initiation of neurodegeneration, the two forces which interact at the cellular level are free radicals formed by the reactive oxygen species and reactive nitrogen species, and secondly, excitotoxins, such as glutamate. Excitotoxins are neurotransmitters which can cause cell death when their actions are prolonged<sup>[2]</sup>.

Parkinson's Disease is a rare occurrence, affecting approximately 1 in 300 people. As per current statistics, about 7-10 million people in the world are living with Parkinson's disease<sup>[3]</sup>. Parkinson's

disease (PD) is one of the major neurodegenerative disorders of old age, and was originally described by James Parkinson in 1817. It is characterized by a trio of prime symptoms—muscle rigidity, tremor and bradykinesia—but can also involve postural deficits and impaired gait in marginal patients. The major symptoms of PD result from the profound and selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). The pathological hallmarks of PD are round proteinaceous inclusions termed Lewy bodies (LBs)<sup>[4]</sup>. PD is primarily a sporadic disorder and its specific etiology is incompletely understood, but important new insights have recently been provided through studying the genetics, epidemiology, and neuropathology of PD, in addition to the development of new experimental models.

*Bauhinia variegata* Linn., commonly known as 'Kachnar', is a small sized deciduous tree with dark brown and more or less smooth bark, up to 8m tall; propagated by seed. The roots and bark are astringent, acrid, cooling, constipating, depurative, anthelmintic, vulnerary, anti-inflammatory and styptic. They are useful in treating diarrhoea, dysentery, skin diseases, leprosy, intestinal worms, tumours, inflammations, scrofula, proctoplosis, haemorrhoids, haemoptysis, cough, menorrhagia and diabetes. Root decoction is used in dyspepsia and flatulence and act as an antidote to snake poison<sup>[5]</sup>.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

The leaves of *Bauhinia variegata* were collected from Lila Nursery, Santacruz, Mumbai. *Bauhinia variegata* leaves were authenticated by Dr H.M.Pandit, Khalsa College, Matunga Mumbai. *Bauhinia variegata* is also commonly known as kachnara.

### 2.2. Extraction of *Bauhinia variegata*

Air dried leaves of *Bauhinia variegata* were powdered. The powdered leaves were macerated with Acetone and water in a closed flask. This

mixture was shaken frequently during the first 6 hours. Thereafter it was allowed to stand for 18 hours. The solution was then filtered and the filtrate obtained was air dried to obtain the required extract. Test suspension was prepared by suspending 15mg of acetone soluble extract in 15 ml of distilled water in the presence of 0.5% Sodium Carboxymethyl Cellulose.

### 2.3 Experimental animals

Wistar rats weighing 180-220 gm body weight of either sex, were procured from Bharat Serum and Vaccines Thane. The animals were maintained in a well-ventilated room with a 12-hour light/dark cycle in standard polypropylene cages under controlled temperature ( $26 \pm 1^\circ\text{C}$ ) and humidity (30%–40%). They were fed with a standard pellet diet. Experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) of C.P.C.S.E.A.

### 2.5. Acute toxicity studies of *Bauhinia variegata*

Rats selected by a random sampling technique were used in the study. Acute oral toxicity was performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines. Three male Wistar rats weighing between 180–220 g were used for each dose. The dose level of 2000 mg/kg/body weight was selected. The drug was administered orally to rats, which were fasted overnight with water ad libitum before the administration of the drug. The body weight of the rat was noted before and after treatment. The animals were observed for toxic symptoms, such behavioral changes, locomotion, convulsions, and mortality for 14 days.

### 2.6. Haloperidol induced catalepsy<sup>[6]</sup>

Haloperidol is a neuroleptic which causes extrapyramidal side effects like catalepsy, akinesia (immobility), bradykinesia (slow movements), muscular rigidity and tremor. Chronic treatment with haloperidol increases free radical production and oxidative stress and causes decrease in the

activity of antioxidant defense enzymes, superoxide dismutase (SOD) and catalase. The dose of ASE i.e 800mg/kg was prepared by suspending ASE in the required volume of 0.5% sodium carboxy methyl cellulose (CMC) solution in water. Dose of Selegiline 15mg/kg was prepared by suspending it in saline. Vehicle containing 0.5 % sodium carboxy methyl cellulose (CMC) in water was used as control. All the solutions were freshly prepared everyday prior to the administration.

Animals were divided into the four groups, viz control, disease control, test and standard groups, each consisting of six rats. The animals in the respective groups were administered ASE suspended in 0.5 % sodium CMC orally, and the standard drug group received Selegiline p.o, daily one hour prior to the administration of haloperidol for 21 days.

Induction of catalepsy was done by the administration of haloperidol (1 mg/kg) in filtered distilled water p.o once daily for 21 days.

#### [A] Behavioral Parameters:

##### 2.6.1. Rotarod Apparatus:

The rotarod test is used to assess motor coordination and balance in rodents. It is measured the time (latency) it takes the rat to fall off the rod rotating at under continuous acceleration i.e 4 rpm. Before the beginning of the study, the animals were trained at the rotarod apparatus for 5 days. On the day of testing, rats were kept in their home cages and allowed to acclimatize to the testing room for at least 15 min (acclimation phase). After induction of catalepsy with haloperidol, the animals were placed on the rod in the apparatus. The rod was rotated under continuous acceleration of 4rpm. The time (latency) when the rat falls off the rod was recorded. The readings were recorded on the 5<sup>th</sup>, 9<sup>th</sup>, 13<sup>th</sup>, 17<sup>th</sup> and 21<sup>st</sup> days.

##### 2.6.2. Locomotor activity using actophotometer

Assessment of locomotor activity was done using an actophotometer. Before subjecting the animal

to cognitive task they were individually placed in actophotometer and the total activity count was registered for 10 min. The locomotor activity was expressed in terms of total counts/10 min per animal. In the present study the locomotor activity was assessed on the 5<sup>th</sup>, 9<sup>th</sup>, 13<sup>th</sup>, 17<sup>th</sup> and 21<sup>st</sup> days.

##### 2.6.3. Muscular Rigidity by Block Test

This scoring method followed is in three steps.

Step 1: The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back a score of 0.5 was assigned.

Step II: The front paws of the rats were placed alternately on a 3-cm high block. If the rat failed to correct the posture within 15 seconds, a score of 0.5 for each paw was added to the score of step 1.

Step III: The front paws of the rat were placed alternately on a 9-cm high block, if the rat failed to correct the posture within 15 seconds a score of 1 for each paw was added to the scores of steps I and II.

Thus, the highest score for any animal was 3.5 (cutoff score) and that reflects total catalepsy

#### [B] Biochemical parameters:

Evaluation of Antioxidant Enzymes as follows:

On 21<sup>st</sup> day after behavioral assessments, animals were sacrificed by cervical dislocation and brains were removed. The cerebellum was discarded and the remaining brain tissue was weighed and preserved at -20°C in deep freezer till further analysis. The known weight of brain tissue was homogenized for the estimation of antioxidant enzymes as follows.

##### (a) Lipid Peroxidation (LPO)

Lipid peroxidation was estimated colorimetrically in brain tissue by quantifying TBARS according to the method of Niehaus and Samuelson. In brief; for the estimation of TBARS the supernatant of the tissue homogenate was treated with tertiary butanol-trichloroacetic acid-hydrochloric acid, (TBA-TCA-HCl) reagent and mixed thoroughly. The mixture was kept in boiling water bath for 15

minutes. After cooling, the tubes were centrifuged for 10 minutes and the supernatant taken for measurement. The developed color was read at 535 nm using a UV spectrophotometer against a reagent blank and expressed as mM per 100g tissue.

#### (b) Glutathione (GSH)

The amount of GSH in mice brain was measured according to the method of Sedlak and Lindsay (1968). Briefly, brain tissue was deproteinized with an equal volume of 10% trichloroacetic acid and was allowed to stand at 40C for 2 h. The contents were centrifuged for 15 min. The supernatant was added to Tris buffer (pH 8.9) containing ethylene diamine tetraacetic acid (EDTA) (pH 8.9) followed by the addition of 0.01 M 5,5-dithiobis(2-nitrobenzoic acid) (DTNB). Finally, the mixture was diluted with distilled water, to make the total mixture to 3 ml and absorbance was read in a spectrophotometer at 412 nm and results are expressed as lg GSH/g tissue

#### (c) Glutathione Peroxidase (GSH-Px)

Rotruck and his coworkers measured the activity of glutathione peroxidase. The reaction mixture contained 0.4 M tris HCl buffer (pH =7.0),0.2ml standard glutathione (GSH) and 0.2mM H<sub>2</sub>O<sub>2</sub>.The contents were incubated at 37°C for 10 minutes. The supernatant was assayed for glutathione content by using Ellman's reagent.

## 4.2 Haloperidol induced Catalepsy in rats:

### 4.2.1 Muscular rigidity using Rotarod

**Table: 1:** Muscular rigidity using Rotarod apparatus

Day	Vehicle Control	Haloperidol (Disease Control)	ASE (800mg/kg)	Standard Selegiline (15mg/kg)
5 <sup>th</sup>	79.80 ± 1.135	24.77 ± 1.072*	51.21 ± 0.7105**	55.12 ± 0.5126**
9 <sup>th</sup>	79.59 ± 2.587	20.14 ± 1.086*	53.04 ± 1.008**	58.48 ± 1.023**
13 <sup>th</sup>	81.62 ± 1.197	17.98 ± 0.7854*	53.43 ± 1.100**	56.69 ± 1.096**
17 <sup>th</sup>	80.88 ± 1.723	17.26 ± 1.065*	53.79 ± 1.152**	58.12 ± 0.7589**
21 <sup>st</sup>	82.28 ± 1.734	17.44 ± 1.115*	52.74 ± 1.050**	58.43 ± 1.180**

Values are expressed as Mean ± SEM for 6 rats in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple

GPx activity was expressed as mg of GSH utilized /minute/mg tissue.

#### (d) Super Oxide Scavenging Activity (SOD)

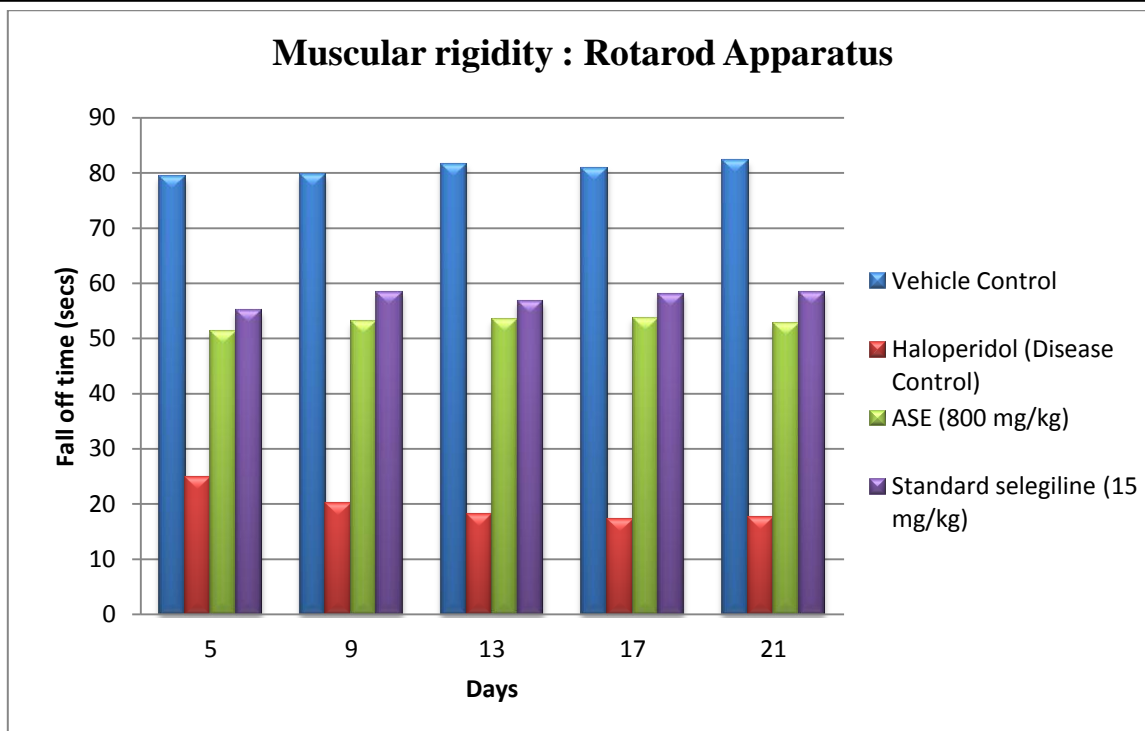
The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome. To the supernatant, carbonate buffer and EDTA were added. The reaction was initiated by addition of 0.5 mL of epinephrine and the auto-oxidation of adrenaline to adrenochrome at pH 10.2 was measured by following changes in optical density at 480 nm. The changes in optical density every minute were measured at 480 nm against a reagent blank. The results are expressed as units of SOD activity . One unit of SOD activity induced approximately 50% inhibition of adrenaline. The results were expressed as nmol SOD U per mg wet tissue.

## 3. STATICAL ANALYSIS

All values were reported as mean + S.E.M. Results were significantly analyzed using one way ANOVA by Dunnett test p<0.05 was considered to be significant.

4.1. Acute toxicity studies: Acute toxicity studies was carried out to evaluate toxicity and to determine the minimum lethal dose of the drug extract .ASE did not show any toxic or deleterious effects up to 2000 mg/ kg oral dose. As the rats were administered up to maximal possible dose, the LD50 value of ASE could not be determined.

comparison tests. \*: p<0.05 when compared with Vehicle control group, \*\*: p<0.05 when compared with Disease control group.



**Graph 1:** Muscular rigidity by Rotarod apparatus

Vehicle control group showed significant difference in muscular rigidity as compared to disease control group. Standard group (Selegiline 15mg/kg + Haloperidol 1 mg/kg) and Test group ASE (800mg/kg) showed a significant difference when compared to Disease control group (Haloperidol 1mg/kg). From above result it is seen

that Test drug ASE 800 mg/kg decreases muscular rigidity in Parkinson disease induced rats as compared to Disease control Haloperidol group. Thus test drug treated group has lowered muscular rigidity compared to that of disease controlled group.

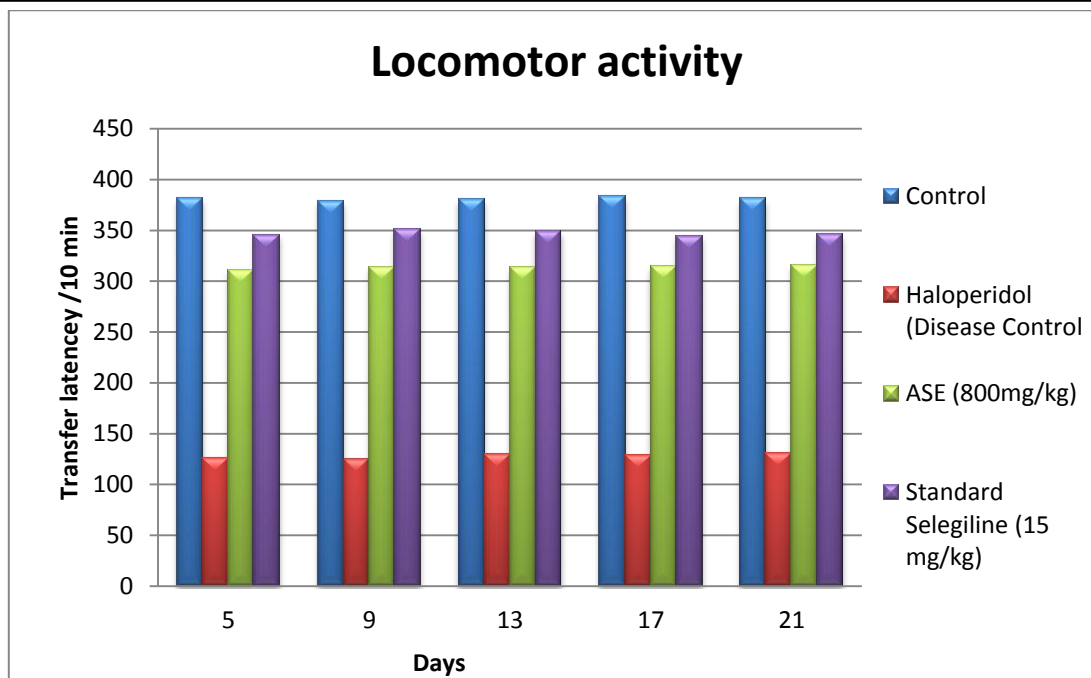
**4.2.3 Locomotor Activity using Actophotometer**

**Table 2:** Locomotor activity using Actophotometer

Day	Vehicle Control	Haloperidol (Disease Control)	ASE (800mg/kg)	Standard Selegiline (15mg/kg)
5 <sup>th</sup>	381.8 ± 6.237	126.3 ± 2.472*	311.0 ± 2.840**	345.3 ± 2.996**
9 <sup>th</sup>	378.3 ± 6.323	125.2 ± 3.240*	314.2 ± 2.600**	350.7 ± 2.963**
13 <sup>th</sup>	379.7 ± 6.015	130.0 ± 4.115*	313.5 ± 3.181**	348.7 ± 2.603**
17 <sup>th</sup>	383.5 ± 4.794	128.7 ± 4.835*	314.7 ± 2.813**	344.5 ± 3.191**
21 <sup>st</sup>	380.8 ± 4.285	130.5 ± 2.172*	316.0 ± 2.033**	345.8 ± 3.114**

Values are expressed as Mean ± SEM for 6 rats in each group. Significance was determined by One-way ANOVA followed by Dunnett’s multiple

comparison tests. \*: p<0.05 when compared with Vehicle control group, \*\*: p<0.05 when compared with Disease control group.



**Graph 2:** Locomotor activity using Actophotometer

The locomotion of the animals belonging to the Haloperidol treated group was decreased significantly when compared to the vehicle treated group. Standard group (Selegiline 15mg/kg + Haloperidol 1 mg/kg) and Test group ASE (800mg/kg) showed a significant difference when compared to Disease control group (Haloperidol

1mg/kg). From above result it is seen that Test drug ASE 800 mg/kg increases locomotion in Parkinson disease induced rats as compared to Disease control Haloperidol group. Thus test drug treated group has increased locomotor activity when compared to that of disease controlled group.

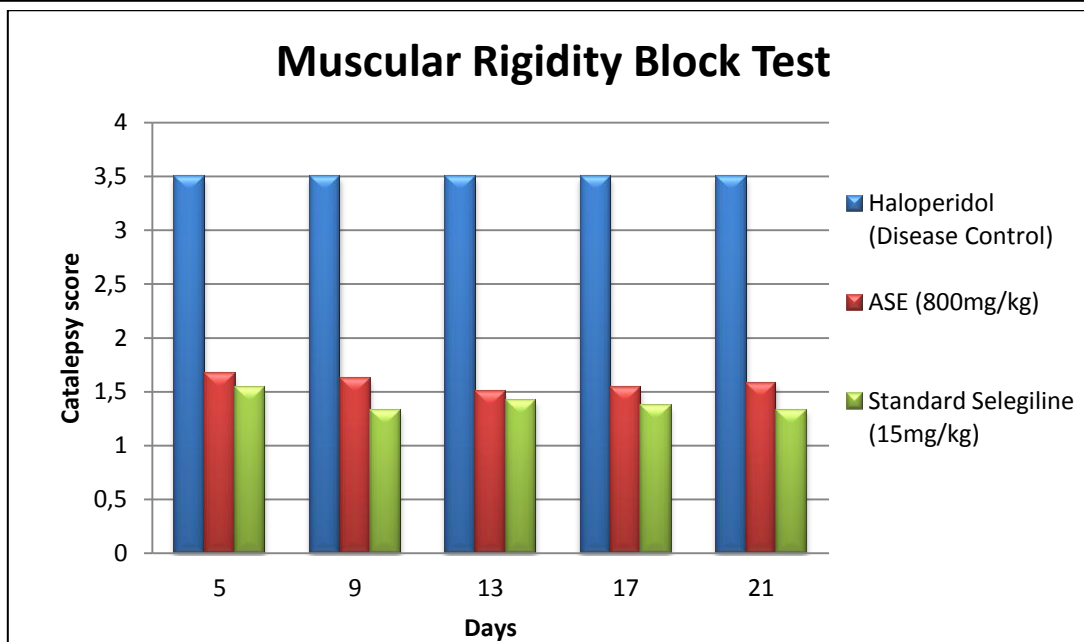
**4.2.3 Muscle rigidity using Block Test**

**Table 3 :** Muscle rigidity using Block Test

Day	Vehicle Control	Haloperidol (Disease Control)	ASE (800mg/kg)	Standard Selegiline (15mg/kg)
5 <sup>th</sup>	0.0 ± 0.0	3.5 ± 0.0*	1.667 ± 0.0527**	1.542 ± 0.1005**
9 <sup>th</sup>	0.0 ± 0.0	3.5 ± 0.0*	1.625 ± 0.0559**	1.333 ± 0.0527**
13 <sup>th</sup>	0.0 ± 0.0	3.5 ± 0.0*	1.500 ± 0.0912**	1.417 ± 0.0833**
17 <sup>th</sup>	0.0 ± 0.0	3.5 ± 0.0*	1.542 ± 0.0768**	1.375 ± 0.0559**
21 <sup>st</sup>	0.0 ± 0.0	3.5 ± 0.0*	1.583 ± 0.0527**	1.333 ± 0.0527**

Values are expressed as Mean ± SEM for 6 rats in each group. Significance was determined by One-way ANOVA followed by Dunnett's multiple

comparison tests. \*: p<0.05 when compared with Vehicle control group, \*\*: p<0.05 when compared with Disease control group.



**Graph 3 :** Catalepsy score of Muscular rigidity using Block test

Catalepsy score of 3.5 shows maximum catalepsy. The vehicle control group showed no catalepsy. The ASE (800 mg/kg) group showed significant decrease in the catalepsy score when compared with the disease control group. Also the standard Selegiline (15 mg/kg) showed significant decrease in the catalepsy score when compared with the

disease control group. From above result it is seen that Test drug ASE 800 mg/kg decreases catalepsy in Parkinson disease induced rats as compared to Disease control Haloperidol group. Thus test drug decreased catalepsy when compared to that of disease controlled group.

**4.2.4 Biochemical parameters (Antioxidant Enzymes)**

**Table 4 :** Biochemical parameters

Groups	Lipid Peroxidation (LPO) (mM/mg tissue)	Glutathione (GSH) (uM/mg tissue)	Glutathione peroxidase GSH-Px (nmol/min/ml)	Superoxide dismutase (SOD) (U/g tissue)
Vehicle Control	5.214 ± 0.0593	0.08915 ± 0.0021	4.170 ± 0.03425	0.6650 ± 0.0033
Haloperidol (Disease Control)	9.095 ± 0.0903*	0.03127 ± 0.0002*	2.087 ± 0.048*	0.2689 ± 0.0031*
ASE (800mg/kg)	6.696 ± 0.0494**	0.0679 ± 0.0006**	3.37 ± 0.0587**	0.5847 ± 0.0023**
Standard Selegiline (15mg/kg)	5.864 ± 0.0258**	0.07412 ± 0.0002**	4.630 ± 0.0262**	0.6424 ± 0.0018**

Values are expressed as Mean ± SEM for 6 rats in each group. Significance was determined by One-way ANOVA followed by Dunnett’s multiple comparison tests. \*: p<0.05 when compared with

Vehicle control group, \*\*: p<0.05 when compared with Disease control group.

GSH-Px and SOD were found to be significantly increased in Standard treatment and ASE

100mg/kg and ASE 500 mg/kg treatment group as compared to Haloperidol treatment group. These enzymes were found to be significantly decreased in Haloperidol group as compared to Control group. Also the levels of the lipid peroxidation (LPO) and GSH were found to significantly decrease in Standard and ASE treated groups when compared to the disease controlled group. This shows that Test drug has increased antioxidant enzyme levels in Haloperidol treated rats. Thus ASE shows anti parkinsonian effect by possessing antioxidant activity.

## 5. DISCUSSION

Haloperidol is a typical neuroleptic drug that exhibits EPS by blocking dopamine D2 receptors which reduces dopaminergic transmission. In the present study involving rats, in haloperidol treatment group decrease in the levels of dopamine than vehicle control was observed. This decrease in the level of dopamine is responsible for catalepsy and other motor defects. ASE treated group restored the dopamine levels dose dependently in haloperidol treated groups. Dopamine deficiency in the brain is the major biochemical deficit in PD. Chronic administration of haloperidol for a period of 21 days in rats resulted in decrease in Rotarod (time for grip on rod at 4 rpm) task, also there are decreased counts in Actophotometer activity cage and an increased in muscular rigidity determined by block test, in Haloperidol treated group as compared to ASE (800mg/kg) treated group. ASE dose decreased the transfer latency time and increased Rotarod (time for grip on rod at 4 rpm) task. Neurodegeneration and decrease in the Antioxidant enzyme levels after chronic haloperidol administration can cause this cognitive impairment. The levels of the antioxidant enzyme (GSH-Px and SOD) were greatly reduced in case to the disease controlled animals. Also the level of lipid peroxidation and GSH was increased to a large extent. The animals treated with the ASE extract showed significant increase in the antioxidant enzymes and a further

decrease in the Lipid peroxidation and GSH levels was observed.

## 6. CONCLUSION

The present study thus, provides sufficient evidence that ASE; an antioxidant of natural origin medicinal plant can be used as an effective anti-PD drug due to its neuroprotective activity. ASE can be employed as an effective anti-PD drug as it shows improvement in dopamine neurotransmission and also prevents neurodegeneration. Further studies are required on molecular mechanism and molecular pathways for the potential use of ASE as a neuroprotective in Parkinson's disease.

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