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# NEUROPHARMACOLOGICAL EVALUATION OF *BRASSICA NIGRA* SEED EXTRACT IN PARKINSON'S DISEASE

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# ABSTRACT

The present study was carried out to evaluate Anti Parkinson's Activity of Hydro-alcoholic Extract of *Brassica nigra* seeds (HABN) in Chlorpromazine (CPZ) induced experimental animal model and Dexamethasone (DEX) test animal model. In Chlorpromazine (CPZ) induced experimental animal model, the effects of Hydro-alcoholic extracts of *Brassica nigra* (400 mg/kg, p.o) was studied using in - vivo parameter like bradykinesia and catalepsy in male Wistar rats. Along with it effect of HABN on Neurochemical parameters (TBARS, GSH) were also assessed. Bradykinesia was measured using actophotometer and Catalepsy was measured using block method. For neurochemical estimations all groups were given CPZ dosing for 21 days to induce Parkinson's Disease (PD). The cataleptic scores was significantly (P<0.05) found to be reduced, with the *Brassica nigra* (400 mg/kg,

p.o.). Trihexyphenidyl and *Brassica nigra* increase the depleted level of Reduced Glutathione (GSH) (P<0.05) and decrease the elevated levels of TBARS (P<0.05) preferably at higher dose (400 mg/kg) as compared to group II receiving Chlorpromazine. Our results suggest the Anti Parkinson's activity of *Brassica nigra* due to its behavioural and Neurochemical responses. In Dexamethasone (DEX) test animal model, the effects of Hydro-alcoholic extracts of Brassica nigra (400 mg/kg, p.o.) was studied using in – vivo parameter like bradykinesia. Male Albino Swiss mice received DEX at a single dose (8 mg/kg). After a single dose (in the chronic experiment) of DEX, dopamine agonist was given in the following regimen: bromocriptine (10 mg/kg) 180 min before the measurement of locomotor activity. The obtained results show that DEX may decrease the locomotor activity in mice.

**KEYWORDS:** Bradykinesia, Catalepsy, Chlorpromazine (CPZ), Dexamethasone (DEX), Hydro-alcoholic extract of *Brassica nigra* (HABN), Trihexyphenidyl, Bromocriptine, Parkinson's disease (PD).

# **INTRODUCTION**

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease caused when a small group of brain cells that control body movements die. This disease was first described by James Parkinson in 1817. It is characterized clinically by bradykinesia, resting tremor, rigidity and postural instability. Pathological features of PD include loss of dopamine neurons in substantia nigra and the presence of Lewy bodies in surviving dopamine neurons. The disease occurs in about 1% of the people over the age of 65 years. The peak onset of the disease is in the sixth decade of the life. The available treatments are levodopa, carbidopa, apomorphine, Amantadine, orphenadrine, benzhexol, benztropine, selegeline, pergola and many more. These drugs effectively reverses the symptoms of Parkinson and improves the level of dopamine. The available drug treatments for PD possess various side effects like nausea and vomiting, depression, respiratory disturbances, hallucinations, mania, convulsions and anxiety, arrhythmia, mydriasis, orange discoloration of saliva and urine, dyskinesia on long term use, postural hypotension, peripheral vasospasm, ankle edema, nervousness, insomnia, constipation, dry mouth, sore throat, transient dizziness, diarrhea and abdominal pain, sleepiness, increased hunger.

*Brassica nigra* (B. nigra) seeds have an immense medicinal value and are known to have numerous medicinal properties, mainly in the Ayurveda systems of medicine. It belongs to family Brassicaceae. The phytochemical analysis of the different extracts revealed that it contains flavonoids, carbohydrates, glycosides, proteins and alkaloids. The values for the phenolic contents ranged between 260 and 516 mg GAE/100g fw. Traditionally, they are known to cure heaviness of head, headache, rhinitis, hemicrania (single side headache), infectious diseases of head, epilepsy, insomnia and fainting. The studies suggests that, they also possess good Antimicrobial activity, Larvicidal activity, Antibacterial activity, Antibyperglycemic effect, Antiepileptic activity, Antioxidant activity. It contains Gallic acid (an antioxidant) having potential to protect brain from neurodegenration as free radicals are most important cause of neuronal cell death. Experimental studies have shown central nervous actions like Anticonvulsant and antioxidant by using pentylenetetrazole - induced kindling in mice.

The treatment of the dopaminergic MN9D cell line with dexamethasone transactivated two major autosomal dominant familial PD genes, LRRK2 and  $\alpha$ -synuclein which when mutated, are known to cause PD in an autosomal dominant manner. *Fernandez et al.* have indicated that corticosterone administered chronically may depress locomotor activity and exploratory behavior of rats. The results of the above studies suggest that glucocorticoids may reduce the activity of dopamine agonists in the mesolimbic system. Studies showing the presence of glucocorticoids binding sites in central nervous system indicate that these hormones may affect the central neurotransmission. High glucocorticoids can reduce dopamine uptake by dopaminergic synaptosomes. The results imply that increased glucocorticoid levels during stress or disease, can directly modulate the neuronal activity of specific dopaminergic systems in the brain. There are suggestions that the decline of the response to dopamine agonists may be the result of glucocorticoid influence on other than dopaminergic, neurotransmitter system like cholinergic, adrenergic or GABAnergic.

#### **MATERIAL AND METHOD**

#### **Plant material**

Brassica nigra seeds were procured from herbal store at Dombivli, Thane. The plant was identified and authenticated by Mr. Harshad M. Pandit, Ph.D.(Botany), Guru Nanak Khalsa College, Matunga, Mumbai.

# **Experimental animals**

Wistar rats weighing 150 - 200 grams body weight of male sex and Albino swiss mice weighing 18 - 25 grams body weight of male sex, were procured from Bharath serums and vaccines Limited Plot no. A-371-372 Road NO .27, Wagle Industrial Estate, Thane-400604, Maharashtra, Registration no. 103/99/CPCSEA. The animals were maintained in hygienic conditions in our animal house in groups of 6 in clean plastic cages containg husk bedding. The animals were fed standard pellet food and water ad libitum. The animal house conditions maintained were : temperature  $(22\pm1)^0$  C, relative humidity (65±10) % and 10 hr (light) and 14 hr (dark) cycle. The animals were allowed to acclimatize to our animal house is registered with Govt. of India, having registration No. (879/AC/05/CPCSEA) and conforms to the Indian National Science Academy guidelines for the use and care of experimental animal research. All experimental protocols involving animal studies were placed before the

Institutional Animal Ethics Committee. The committee granted approval after carefully reviewing the research project.

# Acute toxicity study

Wistar rats weighing 150 - 200 g and Albino Swiss mice 18 - 20 g (six male animals per strain) were used in the study. Acute oral toxicity was performed as per the OECD - 423 guidelines. The animals were fasted overnight, provided with water after which HABN was administered orally at a dose level of 2000 mg/kg, p.o. intubation. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 14 days.

#### **Experimental design**

In – vitro studies

# I. DPPH Assay

# Principle

The DPPH system is stable radical generating procedure. The free radical scavenging activity of the extract was evaluated based on the ability to scavenge the synthetic DPPH. DPPH shows a strong absorption band at 517 nm in uv-visible spectrum. As the unpaired electron becomes paired in the presence of scavenger, the absorption vanishes and resulting discoloration stoichiometrically coincides with respect to the number of electron taken up. The bleaching of DPPH absorption in representative of the capacity of the test extracts to scavenge free radicals independently. The absorbance was monitored spectrophotometrically at 517 nm exactly 30 min after addition of HABN extract.

#### Procedure

1. 50 mg of HABN extract and std. Quercetin were separately dissolved in the Methanol and final volume was makeup to 50 ml. which was used as the stock solution with the conc.  $1000 \ \mu g/ml$ .

Solution in µg/ml.	HABN extract / Std. Quercetin	Methanol
20	0.1	4.9
40	0.2	4.8
60	0.3	4.7
80	0.4	4.6
100	0.5	4.5

2. 5 ml of HABN extract and Std. Quercetin solution were made as follows,

- 3. Solution of DPPH (0.1 mM) in methanol was prepared by dissolving 1.9 mg of DPPH in methanol and volume made upto 100 ml with methanol.
- 4. The solution was kept in dark for 30 min. to complete the reaction.
- 5. 1 ml of each concentration of HABN extract and Std. Quercetin was taken in different volumetric flasks.
- 6. To these add 3 ml of methanolic solution of DPPH and incubate it at  $37^{\circ}$ C for 30 min.
- 7. The absorbance was measured, against methanol as blank at 517 nm.

# Methodology

The percentage scavenging activity was determined by comparing the result of HABN extract with those of standard antioxidant Quercetin. The result was expressed as IC50 value that is the concentration of extract required for 50% inhibition of DPPH radicals.

# Evaluation

The percentage of DPPH radical scavenging activity was calculated according to the following equation

% Inhibition = 
$$A_0 - A_1 \ge 100$$
  
 $A_0$ 

Where,

A<sub>0</sub> - Absorbance of the Control (blank)

A<sub>1</sub>. Absorbance in the presence of HABN extract and Std. Quercetin.

# **II.** Metal chelation assay

# Principle

Metal chelation of seed extract was tested for their anti-oxidant activity. It is apparent that the antioxidative activity of medicinal plants plays an important role in the healing of the various diseases. The mixture of ferrozine and ferric chloride shows a strong absorbance at 562 nm in colorimetry. The ferrous ions are known to stimulate the free radical reaction decomposing the lipid peroxides to chain propagating alkoxyl radicals and also reacting with hydrogen peroxide to produce hydroxyl radicals and other highly reactive species.

# Procedure

 1 ml of HABN extract and Standard EDTA (0-5 mg/ml) was diluted with 3.75 ml of distilled water seperately.

- 2. The above solutions were mixed with  $\text{FeCl}_2$  (2 mm, 0.1 ml) and 4,4<sup>1</sup>-[3-(2-pyridinyl)-1,2,4-triazine-5,6-dryl] bisbenzene sulphonic acid (ferrozine) (5 mm, 0.2 ml).
- 3. After 10 min the absorbance at 562 nm was determined.

# Methodology

The percentage metal chelating activity was determined by comparing the result of HABN extract with those of standard EDTA. The result was expressed as IC50 value that is the concentration of extract required for 50% inhibition of free Ferrous ions.

# Evaluation

The percentage of DPPH radical scavenging activity was calculated according to the following equation :

% Inhibition = 
$$\frac{A_0 - A_1 \times 100}{A_0}$$

Where,

A<sub>0</sub> - Absorbance of the Control (blank).

A<sub>1</sub> – Absorbance in the presence of HABN extract and Std. Quercetin.

#### In – vivo studies

## I. Chlorpromazine induced catalepsy in Wistar rats

# Procedure

Adult male Wistar rats (150 – 200gm) were divided into four groups each containing six animals. Group I received the vehicle distilled water and served as the control, group II, III, IV received chlorpromazine (3 mg/kg,i.p.). Group II receiving chlorpromazine only served as the negative control without any drug treatment. Group III received trihexyphenidyl hydrochloride (1.5 mg/kg, i.p.) and served as standard group and Group IV received hydro-alcoholic extract of Brassica nigra at dose (400 mg/kg, p.o.), respectively for 21 days. Chlorpromazine was given 30 minutes prior to standard and test drug. Catalepsy was induced by the intraperitoneal administration of chlorpromazine at a dose of 3 mg/kg body weight in distilled water. All the behavioural studies were performed at room temperature in a calm room without any external interference. After the 21 days, animals were sacrificed by cervical dislocation and the whole brain was immediately dissected out and washed in ice - cold saline to remove all traces of blood. The brains were weighed and a 10% tissue homogenate was prepared in 0.1 M Potasium Phosphate pH 8 for the activities of Thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH).

### **Behavioral studies**

# Measurement of locomotor activity using actophotometer

One of the cardinal features of PD is bradykinesia which refers to slowness in movement, which will result in a decrease in locomotor activity. Assessment of locomotor activity was done using an actophotometer. An actophotometer consists of infrared beam, which will get recorded and displayed digitally. This principle is used to count total locomotor activity of an animal. 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>, 21<sup>st</sup> day.

# Measurement of catalepsy by block method

This scoring method was followed 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 I.

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 (cut off score) and that reflects total catalepsy. In the present study the catalepsy was assessed on the 5<sup>th</sup>, 9<sup>th</sup>, 13<sup>th</sup>, 17<sup>th</sup>, 21<sup>st</sup> day.

# **Neurochemical studies**

# **Dissection and homogenization**

Chronic chlorpromazine treated animals on day 22nd after behavioural quantification were sacrificed by cervical dislocation. The brains were removed, forebrain was dissected out and cerebellum was discarded. Brains were put on ice and the cortex, striatum and subcortical regions were separated and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 8).

# > LIPID PEROXIDATION ESTIMATION (TBARS ASSAY)

- 1. Supernatant treated with 10 % TCA and was kept in ice bath for 15 min.
- 2. Then centrifuged solution for 5 min at 2000 rpm.
- 3. 2 ml of supernatant was taken out and mixed with freshly prepared 2 ml of 0.67 % TBA solution, the mixture was kept in boiling water bath for 10 min.

- 4. After cooling, tubes centrifuged for 10 min and supernatant taken for measurement.
- 5. The color developed was read at 532 nm against reagent blank.
- The concentration of TBARS in the supernatant was expressed in nM/mg of tissue. Formula for calculation of MDA concentration in tissue (brain) homogenate (Mm/g = 1000 nM/mg).

Conc. Of MDA =  $Abs_{532} \times 100 \times V_T$ 

 $(1.56 \text{ x } 10^5) \text{ x } W_T \text{ x } V_U$ 

Where,

 $Abs_{532} = Absorbance$  of tissue homogenate.

 $V_T$  = Total volume of mixture.

 $W_T$  = Weight of dissected brain.

 $V_U$  = Aliquote volume.

1.56 x  $10^5$  = Extinction coefficient.

# **GLUTATHIONE ESTIMATION (GSH) :**

- 1. 1ml of tissue homogenate was precipitated with 1 ml of 10% TCA.
- 2. The precipitate was removed by centrifugation.
- 3. To an aliquot of the supernatant was added 4 ml of phosphate solution and 0.5 ml of DTNB reagent.
- 4. The color developed was read at 412 nm.
- 5. The concentration of GSH in the supernatant was expressed in nM/mg of tissue.

Formula for calculation of glutathione in tissue (brain) homogenate :  $(\mu M/g = nM/mg)$ .

X =	Y - 0.00314	Х	Dilution factor
	0.0314	ŀ	Brain tissue homogenate x aliquote volume

#### Where,

 $Y = Abs_{412}$  of tissue homogenate.

# II. Dexamethasone test in Albino Swiss mice

# Procedure

DEX was given at a single dose (8 mg/kg). Locomotor activity was registered 3.5 h after a single dose (in the chronic experiment) of DEX, for 1 h. Three and a half hours after a single dose (in the chronic experiment) of DEX, dopamine agonists were given in the following regimen: BRO (10 mg/kg) 180 min before the measurement of locomotor activity.

# **Behavioral studies**

# > Measurement of locomotor activity using actophotometer

# > LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER

One of the cardinal features of PD is bradykinesia which refers to slowness in movement, which will result in a decrease in locomotor activity. Assessment of locomotor activity was done using an actophotometer. An actophotometer consists of infrared beam, which will get recorded and displayed digitally. This principle is used to count total locomotor activity of an animal. The locomotor activity was expressed in terms of total counts/10 min per animal.

# STATISTICAL ANALYSIS

The result of Antiparkinson's activity for in vivo model are expressed as Mean  $\pm$  SEM. Results were significantly analyzed using one-way analysis of variance (ANOVA) by Dunnett's multiple comparison test. The criterion for significance was set at a level of \*p < 0.05.

# RESULTS

# IN VITRO MODEL FOR ANTIOXIDANT ACTIVITY

# I. DPPH free radical scavenging activity of HABN extract

Concentration (µg/ml)	% Inhibition of DPPH radicals	IC <sub>50</sub> value
20	47.21	
40	53.71	26.54 µg/ml
60	67.81	
80	74.14	
100	81.25	

Graphical representation of effect of HABN extract in DPPH free radical scavenging activity



From above graphical representation it can be seen that HABN extract inhibits DPPH free radical scavenging in concentration dependent manner up to 100  $\mu$ g/ml and thus inhibit oxidative mechanism that lead to degenerative diseases. The IC<sub>50</sub> value of HABN extract was 26.54  $\mu$ g/ml (calculated from slope). The correlation coefficient (R<sup>2</sup>) was calculated from the graph and was found to be 0.9801.

Concentration (µg/ml)	% Inhibition of DPPH radicals	IC <sub>50</sub> value
20	51.34	
40	60.47	18.32 µg/ml
60	72.15	
80	85.20	
100	92.54	

# DPPH free radical scavenging activity of Quercetin

Graphical representation of effect of Quercetin in DPPH free radical scavenging activity



Quercetin showed promising free radical scavenging effect of DPPH in concentration dependent manner up to 100  $\mu$ g/ml. The extract was compared with Quercetin which was used as standard antioxidant having IC<sub>50</sub> value 18.32  $\mu$ g/ml and the correlation coefficient (R<sup>2</sup>) was calculated from the graph and was found to be 0.9934. HABN extract can be used as an antioxidant in degenerative diseases as per in vitro study as it shows higher IC<sub>50</sub> value when compared with Quercetin.

Concentration mg/ml	% Inhibition of HABN	% Inhibition of EDTA
0	0	0
0.05	37.74	59.74
0.1	48.03	64.88
0.5	59.30	71.40
1	68.10	79.05
2.5	74.44	89.40
5	83.30	95.00

II. Metal chelating activity using HABN extract and standard EDTA :

Graphical representation of effect of HABN extract and EDTA in DPPH metal chelating activity - % Inhibition Vs Concentration



From above graphical representation it can be seen that HABN extract binds free ferrous ions in concentration dependent manner up to 5 mg/ml and thus inhibit oxidative mechanism that lead to degenerative diseases.

EDTA showed promising metal chelating activity in concentration dependent manner up to 5 mg/ml. The extract was compared with EDTA which was used as standard metal chelator. HABN extract can be used as an antioxidant in degenerative diseases as per in vitro study as when compared with EDTA.

The HABN extract showed good metal chelating activity against the free ferrous ions by binding with it and thereby showing reduction in absorbance in concentration dependent manner.

# Acute toxicity

The HABN did not produce any toxic symptom or mortality at a dose level of 2000 mg/kg body weight orally in rats and mice, and hence the drug was considered safe for further pharmacological screening.

# **IN – VIVO MODEL**

#### I. Chlorpromazine induced catalepsy in Wistar rats

# Effect of chronic administration of HABN on CPZ induced bradykinesia

The locomor activity of the present study are given in Table 1 and Figure 1 assessed by actophotometer. Chlorpromazine induced catalepsy significantly (P<0.05) reduced the locomotor activity at a dose of 3 mg/kg (intraperitoneal administration). Significant reversal in chlorpromazine - induced catalepsy was observed, with the administration of hydro-alcoholic extract of Brassica nigra and trihexyphenidyl hydrochloride. In fig. 1, shows the graph of Acitivity count/10 min. vs Treatment groups. Treatment groups ; 1,2,3,4,5 represents  $5^{th}$ , $9^{th}$ , $13^{th}$ , $17^{th}$ , $21^{st}$  day of study.

Sr. no.	Vehicle Control (D.W.)	Chlorpromazine (Disease control) Chlorpromazi (Disease contro Trihexyphenic Hcl (standard		Chlorpromazine (Disease control) + HABN extract (Test)
Day 5	$250.2\pm0.477$	$57\pm0.7303$	$192 \pm 0.7303*$	$181 \pm 0.365*$
Day 9	$249.7\pm0.557$	$56\pm0.365$	$194 \pm 0.365*$	$181 \pm 0.365*$
Day 13	$248.3 \pm 0.557$	$54.67\pm0.557$	$196 \pm 0.365*$	$183 \pm 0.7303*$
Day 17	$250.3 \pm 0.76$	$51.67 \pm 0.557$	$197 \pm 0.7303*$	$185 \pm 0.365*$
Day 21	$248.7 \pm 0.76$	$48.33 \pm 0.587$	$197.8 \pm 0.477 *$	$186.7 \pm 0.558*$

Table 1: It shows	Effect of Brassica	nigra on lo	comotor activity	in rats

Values were expressed as Mean  $\pm$  SEM for 6 rats in each group. Significance was determined by One – way ANOVA followed by Dunnett's multiple comparison test. The \*P value < 0.05 or less was considered statistically significant.



Fig.1: It shows Effect of Brassica nigra on locomotor activity in rats.

# Effect of chronic administration of HABN on CPZ induced catalepsy

The cataleptic scores of the present study are given in Table 2 and Figure 2 assessed by block method. Chlorpromazine induced catalepsy significantly (P<0.05) at a dose of 3 mg/kg (intraperitoneal administration). Significant reversal in chlorpromazine - induced catalepsy was observed, with the administration of hydro-alcoholic extract of Brassica nigra and trihexyphenidyl hydrochloride. The maximal decrease (P<0.05) in catalepsy was observed in the groups receiving hydro - alcoholic extract of Brassica nigra at a dose of 400 mg/kg. In fig. 2, shows the graph of Catalepsy count vs Treatment groups. Treatment groups ; 1,2,3,4,5 represents 5<sup>th</sup>,9<sup>th</sup>,13<sup>th</sup>,17<sup>th</sup>,21<sup>st</sup> day of study.

Sr. no.	Vehicle Control (D.W.)	Chlorpromazine (Disease control)	ChlorpromazineChlorpromazine(Disease control)(Disease control) +(Disease control)TrihexyphenidylHcl (standard)	
Day 5	0	$1.667 \pm 0.1054$	$1.000 \pm 0.0$ *	$1.333 \pm 0.1054*$
Day 9	0	$1.750 \pm 0.1118$	$0.833 \pm 0.1054*$	$1.250 \pm 0.1118*$
Day 13	0	$1.750 \pm 0.1054$	$0.750 \pm 0.1118*$	$1.167 \pm 0.1054*$
Day 17	0	$1.833 \pm 0.1054$	$0.666 \pm 0.1054*$	$1.000 \pm 0.0*$
Day 21	0	$1.917 \pm 0.0833$	$0.500 \pm 0.0*$	$0.833 \pm 0.1054*$

Values were expressed as Mean  $\pm$  SEM for 6 rats in each group. Significance was determined by One – way ANOVA followed by Dunnett's multiple comparison test. The \*P value < 0.05 or less was considered statistically significant.



Fig.2: It shows Effect of Brassica nigra on catalepsy in rats.

# Effect of chronic HABN on the brain MDA or TBARS level in chronic CPZ treated rats

Chronic CPZ treatment to rats for 21 days induced lipid peroxidation as indicated by a significant (P<0.05) rise in brain MDA levels compared with the vehicle treated rats. Chronic administration of trihexyphenidyl (1.5 mg/kg i.p.) and HABN (400 mg/kg) to CPZ treated animals significantly (P<0.05) and (P<0.05) respectively reversed the extent of lipid peroxidation compared with CPZ only treated rats (Table 3) (Figure 3).

# Effect of chronic HABN on the brain glutathione (GSH) levels in chronic CPZ treated rats

Statistical analysis of brain GSH levels showed a significant difference (P<0.001) between the vehicle treated and CPZ treated rats. However, chronic administration of EENS (200 and 400 mg/kg) showed a significant increase (P<0.5) and (P<0.001) respectively in the level of GSH compared with CPZ treated rats (Table 3) (Figure 4).

**Table 3: Lipid Peroxidation and Glutathione Estimation** 

Parameters / Groups	Lipid Peroxidation (TBARS assay)	Glutathine (GSH)			
Vehicle Control (D.W.)	$0.9667 \pm 0.009545$	$2.243 \pm 0.02231$			
Chlorpromazine (Disease control)	$1.400 \pm 0.003651$	$1.502 \pm 0.007032$			
Trihexyphenidyl Hcl (Standard)	1.117 ± 0.006146*	$2.080 \pm 0.01265*$			
HABN extract (Test)	$1.117 \pm 0.008028 *$	$1.928 \pm 0.01352*$			

Values were expressed as Mean  $\pm$  SEM for 6 rats in each group. Significance was determined by One – way ANOVA followed by Dunnett's multiple comparison test. The \*P value < 0.05 or less was considered statistically significant



Fig. 3 : Graphical representation of lipid peroxidation estimation



nM/mg of tissue vs treatment groups

Fig. 4 : Graphical representation of Glutathione estimation

# II. Dexamethasone test in Albino Swiss mice

# Effect of chronic administration of HABN on DEX induced bradykinesia

The locomor activity of the present study are given in Table 4 and Figure 5 assessed by actophotometer. Dexamethasone induced (P<0.05) reduced locomotor activity at a dose of 8 mg/kg (subcutaneous administration). Reversal in Dexamethsone - induced reduced locomotor activity was observed, with the administration of hydro-alcoholic extract of Brassica nigra and trihexyphenidyl hydrochloride.

T.I.I. 4	T4		T100	C.	<b>D</b> •	• • • •		1		•	• • •
I anie 4	: IT	snows	влест	OT 1	Brassica	nigra	on	locomotor	activity	/ <b>in</b>	mice
I upic I	• ••	0110 110	Lincer	••	Diabbica			locomotor	activity		mee

Sr. no.	Vehicle Control (D.W.)	Dexamethasone (Disease control)	Dexamethasone (Disease control) + Bromocriptine (standard)	Dexamethasone (Disease control) + HABN extract (Test)
Day 1	$251.5\pm0.99$	$168.3\pm0.88$	215.5 ± 0.76 *	201 ± 1.25*

	•	4
	win	r net
<b>VV VV VV</b>	• • • • •	<b>I</b> .IICt

Values were expressed as Mean  $\pm$  SEM for 6 mice in each group. Significance was determined by One – way ANOVA followed by Dunnett's multiple comparison test. The \*P value < 0.05 or less was considered statistically significant



Fig. 5: Graphical representation of Locomotor Activity using Actophotometer

# DISCUSSION

In the present study we evaluated the antiparkinson's effect of *Brassica nigra*, in rodents using chlorpromazine induced catalepsy. Chlorpromazine induced catalepsy is a widely accepted animal model of Parkinson's disease. Some authors have demonstrated that chlorpromazine provides a pharmacological model of Parkinsonism by interfering with the storage of catecholamines in intracellular granules, resulting in monoamine depletion (norepinephrine, 5-hydroxytryptamine and dopamine) in nerve terminals. Antipsychotic effects and extrapyramidal symptoms are also produced due to dopamine depletion. In the present study, Chlorpromazine (3 mg/kg, i.p.) induced significant catalepsy in rats as evidenced by a significant increase in the time spent on the block as compared to the vehicle treated control rats. Treatment with *Brassica nigra*, a neuroprotectant, dose dependently reduced the catalepsy in chlorpromazine - treated rats. The protective effect of *Brassica nigra* at the dose 400 mg/kg against chlorpromazine induced catalepsy suggested that this plant has influence on the dopaminergic receptor mediated neurotransmission.

Animal data has demonstrated elevated oxidative stress markers with 21 - days administration of chlorpromazine. With chronic dosing in rats for 21 - days, chlorpromazine is associated with the greatest level of oxidative stress and increased lipid peroxidation. There was a significant increase in catalepsy, decrease in movements following chlorpromazine administration to rats. The current data thus suggested damage to motor control system (DA-

ergic neurons) and development of Parkinson's disease like behavioral symptoms in chlorpromazine treated rats. The oxidative stress was measured through determination of levels of TBARS (or MDA), reduced glutathione.

The extent of lipid peroxidation was estimated by measuring the levels of thiobarbituric acid, a product of lipid peroxidation. 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, decreased fluidity and inactivation of number of membrane bound enzymes. There is substantial evidence of oxidative damage in the brains of PD patients. Increased levels of the lipid peroxidation product, thiobarbituric acid have been found in the substantia nigra of PD patients. Similar results were observed in the brain homogenates of chlorpromazine - treated negative control animals.

A defect in one or more of the naturally occurring antioxidant defences particularly GSH is an important factor in etiology of PD. A reduction in GSH levels may impair H2O2 clearance and promote hydroxyl radical formation leading to the generation of pro-oxidant milieu.

Chlorpromazine significantly induce catalepsy as compared to control group. Chlorpromazine group also showed a significant increase in the levels of thiobarbituric acid (which is an indication of extent of lipid peroxidation). Whereas, a decrease in reduced glutathione and was observed in the brain as compared to the vehicle treated control animals, indicate an increase in the oxidative stress in the brain of animals treated with chlorpromazine. Brassica nigra significantly reduce cataleptic scores showing its anti cataleptic activity. Pretreatment with Brassica nigra also resulted in a decrease in TBARS, increase in glutathione level. This suggested that Brassica nigra acts by increasing activity of antioxidant or decreasing oxidation and thus may contribute to increased availability of GSH to act against increased oxidative stress and decreased level of TBARS showing decrease in free radical induced oxidation. Since, oxidative stress produced in brain due to chlorpromazine toxicity seems to be important in producing motor defects, therefore use of antioxidants could prove beneficial. The present study which thus explored the potential of Brassica nigra in neurodegenerative disorders showed a promising effect in animals with Parkinson's disease or symptoms. Gallic acid, is already claimed to be an antioxidant. Although fractions rich in Gallic acid were found to be most potent in terms of antioxidant capacity.

BRO administered at a single dose of 10 mg/kg significantly increased locomotor activity (by 67%) in dexamethasone induced bradykinesia in mice. The findings of the present study show that DEX decreases spontaneous locomotor activity. The chronic Dexamethasone administration in mice leds to decreased locomotor activity as compared to HABN extract test group. Dexamethasone is known to affect the central neurotransmission like dopaminergic, cholinergic, adrenergic and GABAnergic. This may be the reason, for decreased locomotor activity in chronic dexamethsone administered group.

# CONCLUSION

*Brassica nigra* was found to possess a potential activity against Parkinson's disease in chlorpromazine induced animal PD models and dexamethasone test animal model. So, we conclude that Brassica nigra may possess anti – parkinson's activity, mainly due to its antioxidant activity, based on chemical constituent like polyphenolic tannin – Gallic acid.

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