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ORIGINAL ARTICLE

# Evaluation of antioxidant and antiparkinsonian activities of *Brassica oleracea* in haloperidol-induced tardive dyskinesia

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**Background:** *Brassica oleracea* is a leafy green vegetable commonly called as cabbage. It belongs to the family Brassicaceae. Studies reveal that it is rich in flavonoids and has high antioxidant property. **Aims:** The present study was aimed at evaluating antiparkinsonian activity of *B. oleracea* in haloperidol-induced tardive dyskinesia. **Settings and Design:** The present study was designed for 14 days administration of the plant extract then behavioral and biochemical parameters were estimated in the rats. **Materials and Methods:** *B. oleracea* was collected, dried in the shade, powdered, and subjected to the extraction by cold maceration using water and methanol mixture of $H_2O/CH_3OH$ as a solvent. The extract was subjected to preliminary phytochemical screening. Haloperidol model was selected for screening antiparkinsonian activity, and it was administered at a dose of 1 mg/kg i.p., for 14 days. Behavioral and biochemical parameters were estimated in all the groups. **Statistical Analysis Used:** Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni's compare all columns test using the computer-based fitting program. **Results:** In groups treated with hydroalcoholic extract of *B. oleracea* (250 and 500 mg/kg p.o.,) decreased the elevated levels of lipid peroxidation in the haloperidol-treated animals and elevated the cellular defense mechanisms such as glutathione, further suggesting the role of free radicals in the pathophysiology of the haloperidol-induced extrapyramidal syndrome. **Conclusion:** This study reveals the use of *B. oleracea* in parkinsonian disease.

**Key words:** Behavioral parameters, biochemical parameters, *Brassica oleracea*, haloperidol, parkinsonian disease

## INTRODUCTION

Neuroleptics are extensively used in the treatment of schizophrenia and other affective disorders. Unfortunately, their use often associated with distressing side effects involving Parkinsonism and tardive dyskinesia.[1] Neuroleptic-induced catalepsy has long been used as a model for the extrapyramidal side effects (EPS), such as parkinsonian-like bradykinesia associated with antipsychotic use in humans. Evidences indicate that haloperidol induces catalepsy in animals, and this behavior response has long been used as a model for EPS effects.[2] Besides, dopamine receptor blockade and catecholamine depletion, other neurochemical hypotheses have been proposed for the development of catalepsy such as striatonigral GABAergic, cholinergic, glutamate, and serotonergic depletion, etc.[3,4] Several phytochemicals have been shown to protect neurons against injury and disease by stimulating the production of antioxidant enzymes, neurotrophic factors, protein chaperones, and other proteins that help to withstand oxidative stress.[5] *Brassica oleracea* belongs to the family Brassicaceae is a leafy green vegetable commonly called as cabbage. According to the literature, this plant contains different phytoconstituents like phenolic compounds such as quercetin 3-O-sophoroside-7-O-glucoside, 3-p-coumaroylquinic acid, kaempferol 3-O-sophoroside-7-O-glucoside, kaempferol 3-O-(caffeoyl)-sophoroside-7-O glucoside, sinapoyl glucoside glucoside acid, kaempferol 3-O-(sinapoyl)-sophoroside 7-O glucoside.[6,7] Organic acids such as aconitic acid, citric acid, ascorbic acid, malic acid, quinic acid, shikimic acid, and fumaric acids have been identified and quantified

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from *B. oleracea*.[8] Several studies reveal that *B. oleracea* is rich in various flavonoids and has high antioxidant property.[9-12] Recently, this plant showed acceptable results such as analgesic, anti-inflammatory,[13] anticarcinogenic,[14] antiulcer,[15] and antidiabetic activities.[16] In the present study, an attempt was made to evaluate antioxidant parameters and antiparkinsonian activity of *B. oleracea* in haloperidol-induced tardive dyskinesia.

## MATERIALS AND METHODS

### Collection and Authentication of Plant Material

Fresh cabbage is collected from local cultivating areas in and around of Anantapuramu District of Andhra Pradesh, India, and it is identified and authenticated by Dr. J. Ravindra Reddy, Professor and Vice Principal, Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Anantapuramu and the voucher specimen (01/13) was preserved in the department of pharmacology, RIPER, Anantapuramu for further reference. The collected plant material was chopped into small pieces and dried at room temperature. The shade-dried cabbage pieces were made into powder mechanically.

### Extraction Procedure

Finely grounded powder was mixed and agitated with a hydroalcoholic solution in the ratio of 1:4 (water:methanol) and subjected to maceration with intermittent shaking for 4 days. The obtained extract was collected by filtration using a muslin cloth. Then the filtrate was subjected to solvent evaporation to obtain semisolid extract which was weighed and stored in an airtight container.[17,18]

### Phytochemical Screening

In the present study, phytochemical screening was carried out using standard procedures.[18,19]

## ANIMALS

Wistar albino rats (150–180 g) were used. They were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to experimentation. The rats were acclimatized to laboratory environment 1 h before the experiments. All experiments were conducted during the light period (08.00–16.00 h).[20]

### Ethical Approval

All the protocols were approved by Institutional Animal Ethical Committee and conducted according to Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) registered no. 878/ac/05/CPCSEA/002/2013 at Postgraduate Department of Pharmacology, RIPER, Anantapuramu.

### Acute Toxicity Studies

Acute toxicity study was carried out using Swiss albino mice (150–180 g) and plant extract was administered orally to different groups of mice at doses of 100 mg, 500 mg, 1000 mg, 2000 mg, and 4000 mg/kg body weight, respectively. Animals were observed for 48 h to study their general behavior, signs of discomfort, and nervous manifestations.[20]

### Pharmacological Evaluation

In this research, Wistar albino rats were randomly divided into four groups consisting of six animals in each group. The first group is normal which received distilled water 10 ml/kg body weight p.o., the second group is negative control which received haloperidol 1 mg/kg body weight i.p., and the third and fourth groups were test groups which received 250 and 500 mg/kg body weight of hydroalcoholic extract of *Brassica oleracea* (HABO). All the groups were treated with above for 14 days. The tabulated treatment schedule is given in Table 1.

## ESTIMATION OF BEHAVIOURAL PARAMETERS

### Locomotor Activity

#### *Actophotometer*

This test measures the exploration and the voluntary locomotion within an enclosed area. The objective value for the spontaneous motor activity was obtained using a photoactometer (INCO Ltd., India). The animal was placed individually into a 30 cm × 30 cm black metal chamber with a screen floor and a light-tight lid. Six beams of red light were focused 2 cm above the floor into photocells on the opposite side. Each beam interruption was registered as an event on the external counter. The light beam breaks were counted for 5 min.[21]

#### *Open Field Test*

The open field apparatus consists of a big square area 76 × 76 with walls 42 cm high. The floor was divided into 25 equal squares. To determine activity, an animal was placed at the corner of a square of the open field and immediately after the placement the number of squares crossed was scored for 5 min.[21]

### Motor Coordination

#### *Rotarod Test*

The rotarod apparatus consists of a motor rod with a drum of 7.0 cm diameter. It was adjusted to a speed of

**Table 1: Treatment schedule of different groups**

| Group | Haloperidol-induced tardive dyskinesia |
|---|---|
| Normal | Distilled water 10 ml/kg p.o./14 days |
| Negative control | Haloperidol (1 mg/kg, i.p.,)/14 days |
| Low dose (250 mg/kg) | Haloperidol (1 mg/kg, i.p.,)/14 days+HABO (250 mg/kg, p.o.,)/14 days |
| High dose (500 mg/kg) | Haloperidol (1 mg/kg, i.p.,)/14 days+HABO (500 mg/kg, p.o.,)/14 days |

HABO – Hydroalcoholic extract of *Brassica oleracea*

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12 revolutions/min during the test session. The latency to fall in a test session of 180 s was taken as a measure of motor coordination.[21,22]

*Despair Swim Test*

Each animal was introduced into a pool (45 cm long; 22 cm wide diameter, and 20 cm high) filled with 10 cm deep water. The animals were allowed to make rotations. The number of rotations made per 3 min was recorded.[22,23]

### Cataleptic Behavior

*Bar Test*

Catalepsy, defined as a reduced ability to initiate movement and a failure to correct abnormal posture, was measured by means of the bar test. To test of catalepsy, animals were positioned so that their hindquarters were on the bench, and their forelimbs rested on a 1 cm diameter horizontal bar, 6–9 cm above the bench. The length of time that animal maintained this position was recorded by stopwatch to a maximum of 180 s (mean of three consecutive trials; interval: 1 min). Animals would determine judge to be cataleptic if they maintained this position for 30 s or more.[22,23]

## ESTIMATION OF BIOCHEMICAL PARAMETERS

Oxidative parameters in brain tissue homogenate for the estimation of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and lipid peroxidation (LPO) were estimated as per the reported protocols.[24]

### Tissue Parameters

*Dopamine Assay*

Preparation of tissue extract

Weighed quantity of tissue was homogenized in 3 ml HCl-Butanol in a cool environment. The sample was centrifuged for 10 min at 2000 rpm then 0.8 ml of supernatant phase was removed and added to an Eppendorf reagent tube containing 2 ml of heptanes and 0.25 ml of 0.1M HCl. After 10 min, the tube was shaken and centrifuged under same conditions to separate two phases. Upper organic phase was discarded, and the aqueous phase was used for dopamine assay (DA).

### Assay Procedure

To 0.02 ml of the HCl phase, 0.005 ml, 0.4 ml HCl, and 0.01 ml EDTA sodium acetate buffer (pH 6.9) were added, followed by 0.01 ml iodine solution for oxidation. The reaction was stopped after 2 min by the addition of 0.1 ml sodium thiosulfate in 5M sodium hydroxide. 10M acetic acid was added 1.5 min later. The solution was then heated to 100°C for 6 min. When the samples again reached to room temperature, excitation and emission spectra were read (330–375 nm) in a spectrofluorimeter. Then the tissue values (fluorescence of tissue extract – fluorescence of tissue blank) compared with an internal reagent standard (fluorescence of internal standard – fluorescence of internal reagent blank). Tissue blanks for the assay were prepared by adding the reagents of the oxidation step in reverse order (sodium thiosulfate before iodine). Internal reagent standards were obtained by adding 0.005 ml of double-distilled water and 0.1 ml HCl Butanol to 20 ng of dopamine standard. It is expressed in pg/mg protein.[25]

### Statistical Analysis

All the data were expressed as mean ± standard error of the mean. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni's compare all columns test using the computer-based fitting program Prism Graph Pad version 5.0 (Graph Pad softaware, Inc., CA , USA.) Statistical significance was set accordingly.

## RESULTS AND DISCUSSION

### Phytochemical Screening

Phytochemical screening of the HABO showed the presence of carbohydrates, proteins, flavonoids, and saponins.

### Haloperidol-induced Tardive Dyskinesia

*Effect of Hydroalcoholic Extract of Brassica Oleracea on Behavioral Parameters-locomotor Activity*

Actophotometer

Animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a significant ($P < 0.01$) decrease on 4$^{th}$ day, ($P < 0.0001$) decrease on 8$^{th}$ day, ($P < 0.0001$) decrease on 14$^{th}$ day in the locomotor activity when compared to normal group. Animals treated with low dose of HABO (250 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a significant ($P < 0.05$) increase on 4$^{th}$ day, nonsignificant increase on 8$^{th}$ day, ($P < 0.01$) increase on 14$^{th}$ day in the locomotor activity when compared to negative control group. Animals treated with high dose of HABO (500 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a significant ($P < 0.01$) increase on 4$^{th}$ day, ($P < 0.001$) increase on 8$^{th}$ day, ($P < 0.0001$) increase on 14$^{th}$ day in the locomotor activity when compared to negative control group [Table 2].

**Table 2: Actophotometer (number of counts/5 min)**

| Group | 4$^{th}$ day | 8$^{th}$ day | 14$^{th}$ day |
|---|---|---|---|
| Normal | 217.6±10.1 | 238.6±19.2 | 238.8±20.2 |
| Negative control | 118.3±14.9$^{\#\#}$ | 82.8±23.8$^{\#\#\#\#}$ | 14±2.5$^{\#\#\#\#}$ |
| Low dose | 182±23.5* | 116±5.0$^{ns}$ | 83.6±11.8** |
| High dose | 222±14.3** | 204.8±13.3*** | 191.1±13.0**** |

Values are expressed as Mean ± SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. $^{\#\#\#\#}$indicates $P<0.0001$ when compared to normal, $^{\#\#}$indicates $P<0.01$ when compared to normal, **** indicates $P<0.0001$ when compared to negative control, *** indicates $P<0.001$ when compared to negative control, ** indicate $P<0.01$ when compared to negative control, * indicate $P<0.05$ when compared to negative control and ns- Nonsignificant

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### Open Field Test

Animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a nonsignificant decrease on 4th day, significant ($P < 0.0001$) decrease on 8th day, ($P < 0.0001$) decrease on 14th day in the number of squares traveled when compared to normal group. Animals treated with low dose of HABO (250 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a nonsignificant increase on 4th day, significant ($P < 0.0001$) increase on 8th day, ($P < 0.0001$) increase on 14th day in the number of squares traveled when compared to negative control group. Animals treated with high dose of HABO (500 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a nonsignificant increase on 4th day, significant ($P < 0.0001$) increase on 8th day, ($P < 0.0001$) increase on 14th day in the number of squares travelled when compared to the control group [Table 3].

### Motor Coordination

#### *Rotarod Test*

Animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a nonsignificant decrease on 4th day, significant ($P < 0.01$) decrease on 8th day ($P < 0.01$) decrease on 14th day in the latency of fall when compared to normal group. Animals treated with low dose of HABO (250 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a nonsignificant increase on 4th day, increase on 8th day, significant ($P < 0.05$) and on 14th day in the latency of fall when compared to negative control group. Animals treated with high dose of HABO (500 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a nonsignificant increase on 4th day, significant ($P < 0.05$) increase on 8th day, ($P < 0.05$) increase on 14th day in the latency of fall when compared to negative control group [Table 4].

#### *Despair Swim Test*

The animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a nonsignificant decrease on 4th day, nonsignificant decrease on 8th day, significant ($P < 0.01$) decrease on 14th day in the number of rotations when compared to normal group. The animals treated with low dose of HABO (250 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a nonsignificant increase on 4th day, nonsignificant increase on 8th day, nonsignificant increase on 14th day in the number of rotations when compared to negative control group. The animals were treated with high dose of HABO (500 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a nonsignificant increase on 4th day, nonsignificant increase on 8th day, significant ($P < 0.05$) increase on 14th day in the number of rotations when compared to negative control group [Table 5].

### Effect of Hydroalcoholic Extract of *Brassica oleracea* on Cataleptic Activity

#### *Bar Test*

The animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a significant ($P < 0.001$) increase on 4th day, ($P < 0.001$) increase on 8th day, ($P < 0.0001$) increase on 14th day in the cataleptic behavior when compared to normal group. Animals treated with low dose of HABO (250 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a significant ($P < 0.05$) decrease on 4th day, ($P < 0.05$) decrease on 8th day, ($P < 0.0001$) increase on 14th day in the cataleptic behavior when compared to the negative control group. The animals treated with high dose of HABO (500 mg/kg) along with haloperidol (1 mg/kg, s.c.,) for 14 days showed a significant ($P < 0.01$) decrease on 4th day, ($P < 0.01$) decrease on 8th day, ($P < 0.001$) increase on 14th day in the cataleptic behavior when compared to the negative control group [Table 6].

**Table 3: Open field test (number of squares traversed/5 min)**

| Group | 4th day | 8th day | 14th day |
|---|---|---|---|
| Normal | 61±5.9 | 63±6 | 85.5±2.7 |
| Negative control | 55.5±3.8$^{ns}$ | 23.3±2.3$^{####}$ | 3.8±1.4$^{####}$ |
| Low dose | 74.5±5.3$^{ns}$ | 62.8±5.6**** | 52.6±3.9**** |
| High dose | 83±7.5* | 93.3±2.8**** | 83.5±4.6**** |

Values are expressed as mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. *$P$<0.05 when compared to negative control; ****$P$<0.0001 when compared to negative control; $^{####}P$<0.0001 when compared to normal; $^{ns}$Nonsignificant; SEM – Standard error of the mean

**Table 4: Rotarod (number of seconds/3 min)**

| Group | 4th day | 8th day | 14th day |
|---|---|---|---|
| Normal | 55±2.7 | 61.5±11.0 | 68±8.8 |
| Negative control | 41±3.0$^{#}$ | 23±4.4$^{##}$ | 4.8±1.5$^{##}$ |
| Low dose | 52±10.2$^{ns}$ | 39.6±7.4$^{ns}$ | 33±5.7* |
| High dose | 59.6±3.3* | 53.6±6.2* | 46.8±7.5* |

Values are expressed as Mean ± SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. $^{##}$Indicates $P$<0.01 when compared to normal, $^{#}$indicates $P$<0.05 when compared to normal, *indicate $P$<0.05 when compared to negative control, ns indicates non significant

**Table 5: Despair swim test (number of rotations/3 min)**

| Group | 4th day | 8th day | 14th day |
|---|---|---|---|
| Normal | 7.1±0.7 | 8.5±0.7 | 9.8±1.9 |
| Negative control | 4.1±0.5$^{#}$ | 4.8±0.6$^{###}$ | 1.6±0.5$^{##}$ |
| Low dose | 4.5±0.8$^{ns}$ | 4.8±1.6$^{ns}$ | 5.3±1.4$^{ns}$ |
| High dose | 5.3±1.4$^{ns}$ | 7.1±0.3* | 8.3±1.5* |

Values are expressed as mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. *$P$<0.05 when compared to negative control; $^{#}P$<0.05 when compared to normal; $^{##}P$<0.01 when compared to normal; $^{###}P$<0.001 when compared to normal; $^{ns}$Nonsignificant; SEM – Standard error of the mean

**Table 6: Bar test (number of seconds/3 min)**

| Group | 4th day | 8th day | 14th day |
|---|---|---|---|
| Normal | 3.5±1 | 5.6±1.6 | 7.1±1.7 |
| Negative control | 11.1±1.7$^{###}$ | 56.6±14.1$^{###}$ | 118.8±21.7$^{####}$ |
| Low dose | 10±4.4* | 21.3±8.5* | 31.2±9.9**** |
| High dose | 8.3±1.7** | 8.6±1.4** | 13.3±2.6*** |

Values are expressed as mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. *$P$<0.05 when compared to negative control; **$P$<0.01 when compared to negative control; ***$P$<0.001 when compared to negative control; ****$P$<0.0001 when compared to negative control; $^{###}P$<0.001 when compared to normal; $^{####}P$<0.0001 when compared to normal; SEM – Standard error of the mean

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### Effect of hydroalcoholic Extract of *Brassica oleracea* on Biochemical Parameters

*Oxidative Parameters*

#### Effect of hydroalcoholic extract of *Brassica oleracea* on superoxide dismutase

The animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a nonsignificant decrease in the SOD when compared to the normal group. Animals treated with low dose (250 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a nonsignificant increase in the SOD when compared to the negative control group. Animals treated with high dose (500 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a nonsignificant increase in the SOD when compared to negative control group [Table 7].

#### Effect of hydroalcoholic extract of *Brassica oleracea* on catalase

The animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a significant ($P < 0.001$) decrease in the catalase when compared to the normal group. The animals treated with low dose (250 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.001$) increase in the catalase when compared to the negative control group. The animals treated with high dose (500 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.001$) increase in the catalase when compared to negative control group [Table 7].

#### Effect of hydroalcoholic extract of *Brassica oleracea* on glutathione

The animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a significant ($P < 0.001$) decrease in the GSH when compared to the normal group. Animals treated with low dose (250 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.01$) increase in the GSH when compared to negative control group. The animals treated with high dose (500 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.001$) increase in the GSH when compared to the negative control group [Table 7].

#### Effect of hydroalcoholic extract of *Brassica oleracea* on lipid peroxidation

The animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a significant ($P < 0.0001$) increase in the LPO when compared to the normal group. The animals treated with low dose (250 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.0001$) decrease in the LPO when compared to negative control group. Animals treated with high dose (500 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.0001$) decrease in the LPO when compared to negative control group [Table 7].

### Effect of hydroalcoholic extract of *Brassica oleracea* on Tissue Parameters

*Effect of hydroalcoholic extract of Brassica oleracea on dopamine*

The animals treated with haloperidol (1 mg/kg, s.c.,) alone for 14 days showed a significant ($P < 0.001$) decrease in the dopamine when compared to the normal group. Animals treated with low dose (250 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.001$) increase in the dopamine when compared to the negative control group. The animals treated with high dose (500 mg/kg) of HABO along with haloperidol (1 mg/kg, s.c.,) caused a significant ($P < 0.001$) increase in the dopamine when compared to negative control group [Table 8].

## DISCUSSION

In the present study, the animals which were treated for 14 days with haloperidol showed severe cataleptic responses along with decreased locomotor and motor coordination. Further, the animals (haloperidol-treated) showed decreased levels of glutathione and catalase and increased levels of LPO products and superoxide dismutase as compared to the control animals. The exact mechanism by which haloperidol increases free radical production was not clear. The enzymatic degradation by MAOs was associated with the production of hydrogen peroxide, which was readily converted to the hydroxyl radical in the presence of iron.[21] Thus, it could initiate a destructive LPO cascade, but an increased dopamine (DA) turnover, leading to hydrogen peroxide production which might not be exclusively involved in the degeneration of oxidative stress.[23] The auto-oxidation of DA which resulted in the production of superoxide radicals might have contributed to the unbalanced production of the free radicals. However, other mechanisms may also be involved. Haloperidol was reported to suppress the activity of certain detoxifying enzymes, thus leaving the cell unprotected,

**Table 7: Effect of HABO on oxidative parameters in brain tissue homogenate**

| Group | SOD (U/mg protein) | CAT (µmol $H_2O_2$ decomposed/mg protein) | GSH (nmol GSH/mg protein) | LPO (nmol MDA/mg protein) |
|---|---|---|---|---|
| Normal | 2.2±0.05 | 164.5±14.1 | 32.9±3.3 | 8.7±0.28 |
| Negative control | 2.2±0.06$^{ns}$ | 57.2±6.8$^{###}$ | 7.2±1$^{###}$ | 27.8±0.19$^{####}$ |
| Low dose | 2±0.01$^{ns}$ | 130.2±4.6*** | 29.9±3.9** | 12.6±0.36**** |
| High dose | 2.2±0.12$^{ns}$ | 176.1±7.2*** | 38.04±1.3*** | 4.8±0.20**** |

HABO – Hydroalcoholic extract of *Brassica oleracea*; SOD – Superoxide dismutase; CAT – Catalase; GSH – Glutathione; LPO – Lipid peroxidation Values are expressed as Mean ± SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. ####indicates $P<0.0001$ when compared to normal, ###indicates $P<0.001$ when compared to normal, ****indicates $P<0.0001$ when compared to negative control, *** indicates $P<0.001$ when compared to negative control, **indicate $P<0.01$ when compared to negative control, ns indicates non significant

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**Table 8: Effect of HABO on DA levels in brain tissue homogenate**

| Group | DA (pg/mg tissue) |
|---|---|
| Normal | 613±23 |
| Negative control | 221.5±19.5### |
| Low dose | 485.5±12.5*** |
| High dose | 612±11.5*** |

Values are expressed as mean±SEM, one-way ANOVA followed by Dennett's multiple comparisons test. ***$P$<0.001 when compared to negative control; ###$P$<0.001 when compared to normal; HABO – hydroalcoholic extract of *Brassica oleracea*; DA – Dopamine assay; SEM – Standard error of the mean

especially if the basal enzyme activity was low or if the free radical scavenging mechanisms were less effective.[23] 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. Another possible mechanism could be the effect of neuroleptics on the mitochondrial respiration. The metabolites of haloperidol inhibit complex-I of the electron transport chain. The capability of the anti-psychotic drugs to clinically induce the extrapyramidal syndrome seems to correlate well with their inhibitory effect on the complex-I inhibition.[23] Whatever could have been be the mechanism of the unbalanced production of the reactive oxygen species and the oxidative stress by haloperidol, HABO was found to be effective in decreasing the oxidative stress in the haloperidol-treated animals. The anti-oxidative properties of HABO reduced the duration of the catalepsy and increased the locomotor activity along with motor coordination. The group treated with HABO 500 mg/kg showed normal locomotor activity and motor coordination without any cataleptic behavior when compared with the haloperidol-treated group. The group treated with HABO 250 mg/kg showed some cataleptic behavior when compared to HABO 500 mg/kg treated group. Treatment with HABO (250 and 500 mg/kg) decreased the elevated levels of LPO in the haloperidol-treated animals and elevated the cellular defense mechanisms such as glutathione, further suggesting the role of free radicals in the pathophysiology of the haloperidol-induced extrapyramidal syndrome.

## CONCLUSION

In this research, the animals which were treated for 14 days with haloperidol showed severe cataleptic responses along with decreased locomotor and motor coordination. Further, the animals (haloperidol-treated) showed decreased levels of glutathione and catalase and increased levels of LPO products and superoxide dismutase as compared to the control animals. The anti-oxidative properties of HABO reduced the duration of the catalepsy and increased the locomotor activity along with motor coordination. The group treated with HABO 500 mg/kg showed normal locomotor activity and motor coordination without any cataleptic behavior when compared with haloperidol-treated group. The group treated with HABO 250 mg/kg showed some cataleptic behavior when compared to HABO 500 mg/kg treated group. Treatment with HABO (250 and 500 mg/kg) decreased the elevated levels of LPO in the haloperidol-treated animals and elevated the cellular defense mechanisms such as glutathione, further suggesting the role of free radicals in the pathophysiology of the haloperidol induced extrapyramidal syndrome. The anti-oxidant activity of HABO could be possibly due to the direct scavenging of the superoxide radicals by the flavonoids which are known to be present in the hydroalcoholic extract of *B. oleracea*.[26,27] From the present results, it can be concluded that HABO may prove to be a beneficial adjuvant in the treatment of drug-induced EPS effects and related disorders.

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### Conflicts of Interest

There are no conflicts of interest.

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