

## EVALUATION OF ANTIPARKINSONIAN ACTIVITY OF HYDROALCOHOLIC EXTRACT OF THE SEEDS OF *VIGNA ACONITIFOLIA* IN WISTAR ALBINO RAT

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### ABSTRACT

**Objective:** The objective of the study is to evaluate the antiparkinsonian activity of hydroalcoholic extract of the seeds of *Vigna aconitifolia* (HEVA) in Wistar albino rat.

**Methods:** In rats, catalepsy was induced using haloperidol (4 mg/kg i.p.). Treatment groups received bromocriptine (4 mg/kg) and HEVA at the dose of (100, 200, and 300 mg/kg) orally. Bar test for catalepsy, motor coordination test by rotarod, and locomotor activity by actophotometer were carried out to assess behavioral changes. Assay of dopamine and catalase was also carried out to assess biochemical parameters.

**Results:** Bromocriptine and HEVA-treated groups showed a significant difference in behavioral and biochemical parameters as compared to haloperidol control group in the experimental models.

**Conclusion:** *Vigna aconitifolia* seeds exhibited significant antiparkinsonian activity in haloperidol mouse model.

**Keywords:** *Vigna aconitifolia*, Catalepsy, Haloperidol, Wistar albino rat.

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### INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease of the central nervous system (CNS) which primarily affects the motor system. It is also called as a slowly progressive neurodegenerative disease where there is a loss of dopaminergic neurons projecting from *Substantia nigra* pars compacta toward neostriatum leading to the imbalance between dopamine and acetylcholine. In addition to this, proteins called Lewy bodies accumulate in dopaminergic neurons [1,2].

The main pathological features of PD are cell death in the brain's basal ganglia (affecting up to 70% of the dopamine secreting neurons in the *S. nigra* pars compacta by the end of life) [3] and the occurrence of Lewy bodies (gatherings of the protein alpha-synuclein) in many of the remaining neurons. This loss of neurons is accompanied by the death of astrocytes (star-shaped glial cells) and a significant increase in the number of microglia (another type of glial cell) in the *S. nigra* [4].

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. The resultant effect is the blockade of striatal dopamine transmission, which in turn causes the abnormal downstream firing within the basal ganglia circuits that are manifested as symptoms of muscle rigidity, loss of locomotor activity, and catalepsy [5]. Haloperidol use is limited as it produces a wide range of extrapyramidal movement disorders such as tardive dyskinesia (TD), akathisia, dystonia, and parkinsonism. It has been postulated that the pathophysiology underlying TD may be oxidative stress [6].

*Vigna aconitifolia* (VA) seeds are highly rich in amino acids, alkaloids, proteins, phenols, flavonoids, tannins, lectins, L-dopa, and trypsin [7]. The seeds of VA contain 0.20% of L-dopa [8].

Phytomedicines are well tolerated, with fewer side effects; in contrast, synthetic drugs can be highly effective, their usage is often hampered by severe side effects [9]. Hence, in this research, we studied the

antiparkinsonian activity of hydroalcoholic extract of the seeds of *Canavalia gladiata* (HECG) (test drug) and bromocriptine (standard drug) in albino Wistar rats.

### METHODS

#### Collection and authentication of plant

Fresh dried seeds of VA were collected from market and specimen was submitted to the Department of Botany, authenticated by Dr. Bindugopalakrishnan, Botanist at Mithibai College, Mumbai - 400 001.

#### Drying and grinding

The seeds of VA were washed, air dried for 2 days, and crushed to coarse powder. The powder obtained was passed through sieve No. 40 and used for further studies.

#### Extraction

Dried coarse powder of VA seeds was extracted with a mixture of 90% v/v ethanol (50%) and distilled water (50%) in a 250 ml Soxhlet at 60°C. The solvent obtained was evaporated to remove excess of the solvent, concentrated and then used for the study. The yield was observed to be 18% w/w.

#### Phytochemical analysis [10,11]

Preliminary phytochemical investigations were conducted employing various phytochemical tests and the phytochemical constituents were detected as elaborated by Khandelwal (2001) and Kokate *et al.*, (2001).

The hydroalcoholic extract of the seeds of VA (HEVA) was dissolved in distilled water (q.s.) and used for the presence of phytoconstituents such as alkaloids, flavonoids, glucosides, saponins, steroids, tannins, and phenolic compounds.

#### Animal

##### *Wistar albino rats*

Wistar albino rats procured from Bharat Serums and Vaccines Limited, RD Number 27, CP Talav, Wagle Industrial Estate, Thane

West, Maharashtra 400604 (Registration no. 103/99/CPCSEA dated 07/01/2019), were used for the study. They were acclimatized in the animal house of Oriental College of Pharmacy. Animals were fed standard diet and water was given *ad libitum*. 12:12 h light/dark cycle was maintained. The Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy approved the experimental protocol No. OCP/IAEC/2017-2018/07.

#### Acute toxicity studies

The acute toxicity study of VA aqueous extract was performed using up and down procedure at a dose level of 2000 mg/kg body weight orally in rats, as per OECD 423 guidelines in two different groups of three females each and observed for mortality for 24 h [12]. The dose 2000 mg/kg was found to be safe for all animals. From this, 1/10<sup>th</sup> of 200 mg dose was selected for further study.

#### Chemicals and reagents

1. HEVA (100 mg/kg, 200 mg/kg, and 300 mg/kg)
2. Standard drug: Bromocriptine (Ambaji Medical stores, Mumbai)
3. Drug to induce catalepsy: Haloperidol (injection Serenace) (RPG Life Sciences, India)

4. 0.1 M perchloric acid
5. Hydrogen peroxide
6. Levodopa
7. Distilled water.

#### Pharmacological evaluation

Rat was randomly divided into six groups (n=6), namely vehicle control (vehicle treated), haloperidol control, bromocriptine, and HEVA-treated group (low dose [100 mg/kg], intermediate dose [200 mg/kg], and high dose [300 mg/kg]). Bromocriptine and HEVA were administered orally. One hour after the drug administration, the animals were challenged with haloperidol 4 mg/kg intraperitoneal (i.p.) administration.

#### Estimation of behavioral parameters

##### Bar test [13]

A bar test was used to measure the catalepsy. In the bar test, front paw of the animals was placed on a horizontal bar located 3 cm and 9 cm above and parallel to the base alternately. The time at which the animal removes its paw from the bar was noted. Catalepsy scoring was given as follows:

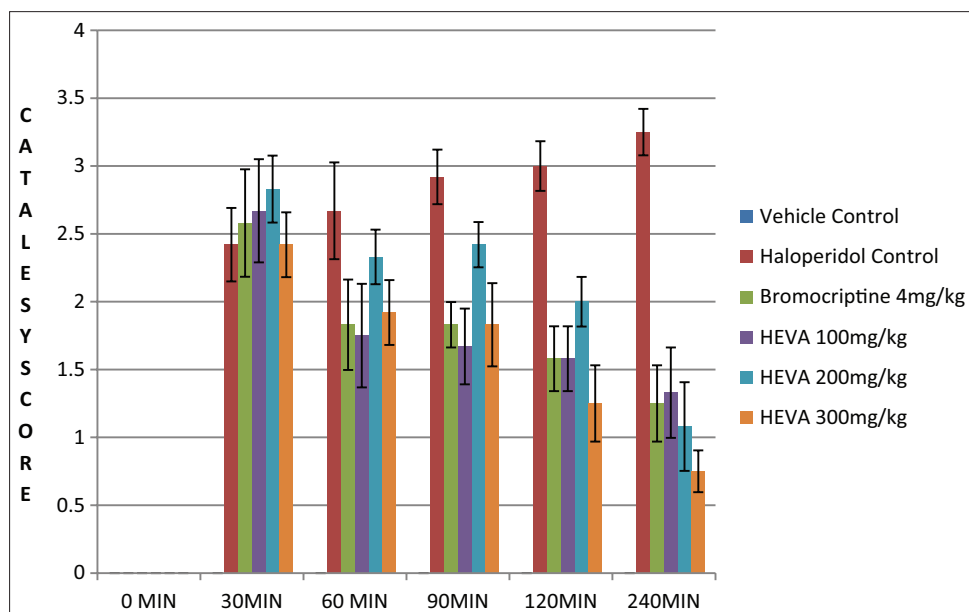


Fig. 1: Effect of bromocriptine and hydroalcoholic extract of the seeds of *Vigna aconitifolia* on catalepsy in bar test

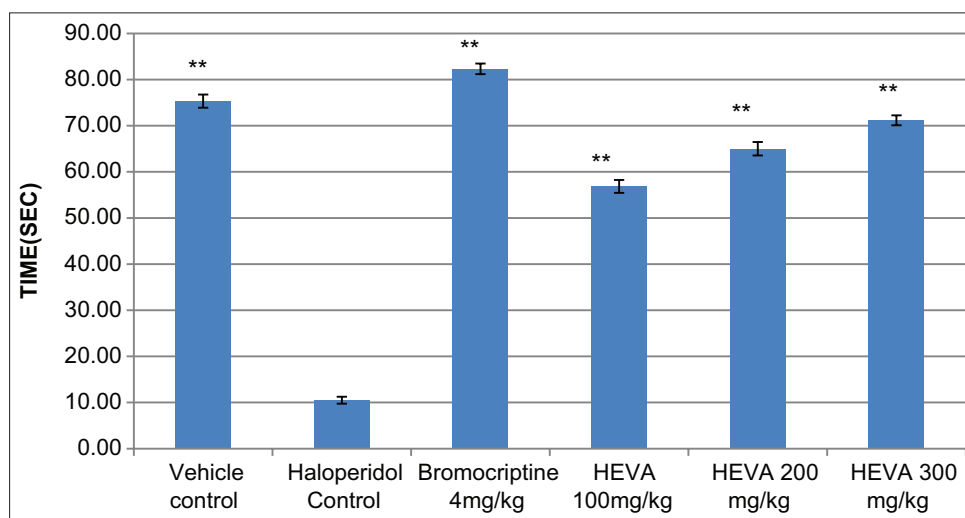


Fig. 2: Effect of bromocriptine and hydroalcoholic extract of the seeds of *Vigna aconitifolia* on motor coordination test using rotarod

Step I: The rat was taken 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 rat were placed alternately on a 3-cm-high block. If the rat failed to correct the posture within 15 s, 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 s, a score of 1 for each paw was added to the scores of Steps I and II.

#### Motor coordination test (rotarod test) [14]

A motor coordination test was conducted using a rotarod apparatus. The animals were placed on the moving rod before the treatment and the rat stayed on the rod without falling for 120 s was chosen for the study. The time at which animals take for falling from the rotating rod was noted before and after the treatment. The starting speed of rotarod was adjusted to 4 rpm with the acceleration rate to 20 rpm. The maximum speed was 40 rpm.

#### Test for locomotor activity (actophotometer) [15]

The locomotor activity was measured using actophotometer. It consists of cage which has six lights and six photocells, which are placed in the outer periphery of the bottom in such a way that single rat block only one beam at a time. Photocell is activated when the rays of light fall on photocells, the beam of light is interrupted as and when animal crosses the light beam, number of cut interruptions was recorded for 10 min.

#### Biochemical test

##### Determination of dopamine by high-performance liquid chromatography (HPLC)

###### Preparation of brain sample

Dissected striata were immediately frozen on dry ice and stored at  $-80^{\circ}\text{C}$ . Striatal tissues were sonicated in 0.1 M of perchloric acid (about 100  $\mu\text{l}$ /mg tissue). The supernatant fluids were taken for measurements of levels of dopamine by HPLC [16].

#### Assay

##### Preparation of standard solution [17]

Levodopa: Accurately weighed quantity, 50 mg of levodopa was transferred into 50 mL of volumetric flask and added 30 mL of mobile phase and sonicated for 15 min. Make up the volume with mobile phase. From the above solution, 5 ml was taken into 50 ml volumetric flask and make up the volume with mobile phase. From the above solution, 2 ml was taken into 10 ml volumetric flask and 1  $\mu\text{l}$  was injected for analysis in HPLC.

##### Preparation of sample solution

Taken accurately 5 ml of supernatant fluid and transferred into 50 ml of volumetric flask dissolved and diluted to volume with mobile phase and sonicated for 10 min. From the above solution, take 5 ml into 50 ml volumetric flask make up the volume with mobile phase. From the above solution, 2 ml was taken into 10 ml volumetric flask and 1  $\mu\text{l}$  was injected for analysis in HPLC. Concentrations of dopamine were expressed as nanogram per milligram of brain tissue. Briefly, 20  $\mu\text{l}$  supernatant fluid was isocratically eluted through a 4.6-mm C18 column as the internal standard with a mobile phase containing water and methanol (70:30), respectively. The flow rate was 1 ml/min.

##### Determination of catalase (CAT) principle [18]

###### Preparation of brain sample

After assessing the bar test, motor coordination test and locomotor activity in haloperidol-induced Parkinson rats from each group were euthanized y using a carbon dioxide chamber; brains were removed quickly and placed in ice-cold saline. The tissues were weighed and homogenized in 0.1 M phosphate buffer (pH 8). The samples of rat brain homogenates were collected in different test tubes to analyze CAT activity. The supernatant was used for CAT assay.

#### Assay

CAT activity was measured by ultraviolet (UV). 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0 ml of freshly prepared 30 mM  $\text{H}_2\text{O}_2$ . The rate of decomposition of  $\text{H}_2\text{O}_2$  was measured spectrophotometrically from changes in absorbance at 240 nm. The activity of CAT was expressed as units/mg protein. The reaction occurs immediately after the addition of  $\text{H}_2\text{O}_2$ . Solutions were mixed well, and the first absorbance (A1) was read after 15 s ( $t_1$ ) and the second absorbance (A2) after 30 s ( $t_2$ ). The absorbance was read at wavelength 240 nm.

#### Statistical analysis

Values were presented as mean $\pm$ SEM. Data were statistically evaluated by one-way analysis of variance followed by Dunnett's test for intergroup comparison using Instat software. Results were considered to be statistically significant at \* $p\leq 0.05$ . \*\*\*indicated  $p\leq 0.001$ , \*\*indicated  $p\leq 0.01$ , and \*indicates  $p\leq 0.05$  when compared with standard.

## RESULTS

#### Phytochemical analysis

The phytochemical analysis of the extract revealed that the HEVA shows the presence of carbohydrates, proteins, saponins, flavonoids, alkaloids, phenolic compounds, and tannins.

| Phytochemicals         | Observations |
|------------------------|--------------|
| Carbohydrates          | +            |
| Proteins               | +            |
| Steroids               | -            |
| Saponins               | +            |
| Flavonoids             | +            |
| Alkaloids              | +            |
| Phenolic compounds     | +            |
| Tannins                | +            |
| Present (+)/Absent (-) |              |

#### Catalepsy in rat

##### Bar test

In bar test Table 1 and Fig. 1, haloperidol control group significantly increases cataleptic score as compared to the vehicle control group. Bromocriptine 4 mg/kg and HEVA 300 mg/kg showed significant inhibition against catalepsy by decreasing cataleptic score.

#### Motor coordination test

Fall of time from rotarod was significantly decreased in haloperidol-treated group as compared to the vehicle control group and it was significantly improved with bromocriptine 4 mg/kg, HEVA 200, and 300 mg/kg (Table 2 and Fig. 2).

#### Test for locomotor activity

Spontaneous motor activity was significantly decreased in haloperidol-treated group as compared to the vehicle control group. Bromocriptine 2.5 mg/kg and HECG 300 mg/kg, significantly increased the locomotor activity as compared to haloperidol-treated animals (Table 3 and Fig. 3).

#### Determination of dopamine by HPLC

In bar test Table 4 and Fig. 4, haloperidol control group significantly decreases in dopamine level as compared to the vehicle control group. Bromocriptine 4 mg/kg and HEVA 300 mg/kg showed significant increase in dopamine level.

#### Determination of CAT by UV

In bar test Table 5 and Fig. 5, haloperidol control group significantly decreases in CAT level as compared to the vehicle control group. Bromocriptine 4 mg/kg and HEVA 100 mg/kg showed significant increase in CAT level.

Table 1: Effect of bromocriptine and HEVA on catalepsy in bar test

| Time interval in mins | Mean±SEM (cataleptic score) |                     |                       |                |                |                |
|-----------------------|-----------------------------|---------------------|-----------------------|----------------|----------------|----------------|
|                       | Vehicle control             | Haloperidol control | Bromocriptine 4 mg/kg | HEVA 100 mg/kg | HEVA 200 mg/kg | HEVA 300 mg/kg |
| 0                     | 0.00±0.00**                 | 0.00±0.00           | 0.00±0.00             | 0.00±0.00      | 0.00±0.00      | 0.00±0.00      |
| 30                    | 0.00±0.00**                 | 2.42±0.271          | 2.58±0.396            | 2.67±0.38      | 2.83±0.247     | 2.42±0.239     |
| 60                    | 0.00±0.00**                 | 2.67±0.357          | 1.83±0.333            | 1.75±0.382     | 1.92±0.201     | 1.92±0.239     |
| 90                    | 0.00±0.00**                 | 2.92±0.201          | 1.83±0.167**          | 1.67±0.279**   | 1.83±0.167**   | 1.83±0.307**   |
| 120                   | 0.00±0.00**                 | 3±0.183             | 1.58±0.239**          | 1.58±0.239**   | 1.25±0.183**   | 1.25±0.281**   |
| 240                   | 0.00±0.00**                 | 3.25±0.171          | 1.25±0.281**          | 1.33±0.333**   | 0.75±0.327**   | 0.75±0.154**   |

All values are expressed in mean±SEM (n=6). Significance: \*\*p<0.01 when compared with negative control. HEVA: Hydroalcoholic extract of the seeds of *Vigna aconitifolia*

Table 2: Effect of bromocriptine and HEVA on motor co-ordination test using rotarod

| Treatment groups      | Fall of time (S) mean±SEM |
|-----------------------|---------------------------|
| Vehicle control       | 75.33±1.43**              |
| Haloperidol control   | 10.50±0.76                |
| Bromocriptine 4 mg/kg | 82.33±1.15**              |
| HEVA 100 mg/kg        | 56.83±1.40**              |
| HEVA 200 mg/kg        | 65.00±1.46**              |
| HEVA 300 mg/kg        | 71.17±1.08**              |

All values are expressed in mean±SEM (n=6). Significance: \*\*p<0.01 when compared with negative control. HEVA: Hydroalcoholic extract of the seeds of *Vigna aconitifolia*

Table 3: Effect of bromocriptine and HEVA on locomotor activity using actophotometer

| Treatment groups      | Ambulations counts/10 min mean±SEM |
|-----------------------|------------------------------------|
| Vehicle control       | 198±2.43**                         |
| Haloperidol control   | 39.17±1.74                         |
| Bromocriptine 4 mg/kg | 156.00±2.89**                      |
| HEVA 100 mg/kg        | 83.33±1.85**                       |
| HEVA 200 mg/kg        | 92.50±3.06**                       |
| HEVA 300 mg/kg        | 100.17±1.35**                      |

All values are expressed in mean±SEM (n=6). Significance: \*\*p<0.01 when compared with negative control. HEVA: Hydroalcoholic extract of the seeds of *Vigna aconitifolia*

## DISCUSSION

### Haloperidol-induced catalepsy in rats

Catalepsy (rigidity in movements), akinesia (slowing of movement), tremors, and memory loss are some of the major symptoms of PD. Among this catalepsy is one of the major symptoms which make the life of PD patient uneasy. Bromocriptine is well-known dopamine (D2) receptor agonist and is commonly used to improve the symptoms related to rigidity. Hence, this drug was used as standard in the present study to compare the efficiency of both the models (zebrafish and mice).

Catalepsy was induced in the rat by i.p. administration of haloperidol (4 mg/kg). This cataleptic behavior induced by haloperidol and the protective effect of standard (bromocriptine) and HEVA used was evaluated using bar test, rotarod apparatus, and actophotometer.

### Bar test

This test gives the idea of the extent of catalepsy induced in an animal. In a present study, bromocriptine 4 mg/kg and HEVA 300 mg/kg reversed the effects of haloperidol in a bar test by decreasing cataleptic score significantly.

### Motor co-ordination test by rotarod

The imbalance is one of the symptoms of PD, to evaluate it, this test was carried out. The test consists of a rotating rod on which the

Table 4: Effect of bromocriptine and HEVA on dopamine level using high-performance liquid chromatography

| Treatment groups      | Dopamine (ng/mg) of tissue mean±SEM |
|-----------------------|-------------------------------------|
| Vehicle control       | 3.81±0.18**                         |
| Haloperidol control   | 1.96±0.18                           |
| Bromocriptine 4 mg/kg | 8.95±0.24**                         |
| HEVA 100 mg/kg        | 7.33±0.06**                         |
| HEVA 200 mg/kg        | 7.61±0.33**                         |
| HEVA 300 mg/kg        | 8.86±0.18**                         |

All values are expressed in mean±SEM (n=6). Significance: \*\*p<0.01 when compared with negative control. HEVA: Hydroalcoholic extract of the seeds of *Vigna aconitifolia*

Table 5: Effect of bromocriptine and HEVA on dopamine level using high-performance liquid chromatography

| Treatment groups      | Unit/mg Mean±SEM |
|-----------------------|------------------|
| Vehicle control       | 16.72±0.08**     |
| Haloperidol control   | 14.92±0.08       |
| Bromocriptine 4 mg/kg | 17.90±0.54**     |
| HEVA 100 mg/kg        | 19.65±0.12**     |
| HEVA 200 mg/kg        | 19.03±0.67**     |
| HEVA 300 mg/kg        | 17.66±0.16**     |

All values are expressed in mean±SEM (n=6). Significance: \*\*p<0.01 when compared with negative control. HEVA: Hydroalcoholic extract of the seeds of *Vigna aconitifolia*

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. Bromocriptine 4 mg/kg, HEVA 200, and 300 mg/kg prevented the motor impairment significantly, which was altered by haloperidol. It indicates that HEVA may have active constituents with CNS stimulant activity.

### Locomotor activity by actophotometer

Due to the catalepsy; movement restrictions or sometimes freezing of the movements is exhibited by PD patient. Hence, the drug which improves the locomotor activity can modify the condition of PD patient. The results indicated that haloperidol caused significant decreased locomotor counts in actophotometer. Bromocriptine 4 mg/kg and HEVA 300 mg/kg, significantly increased the locomotor activity as compared to haloperidol-treated animals. Daily treatment with HEVA significantly reversed the decrease in locomotor activity as assessed on day 14.

### Determination of dopamine by HPLC

The turnover of dopamine in nigral cells plays a major role in controlling motor function. In the present study, HEVA 300 mg/kg caused a pronounced increase in dopamine levels in midbrain regions of haloperidol-induced rats and it could a result of protection of dopaminergic neurons by these drugs. The beneficial roles of HEVA retaining dopamine levels demonstrated the protection of nigral neuron by test drugs.

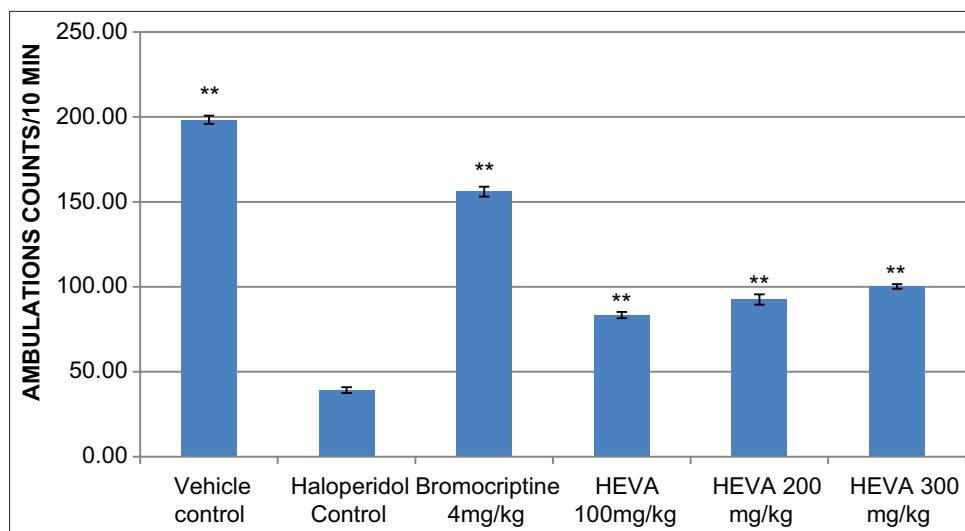


Fig. 3: Effect of bromocriptine and hydroalcoholic extract of the seeds of *Vigna aconitifolia* on locomotor activity using actophotometer

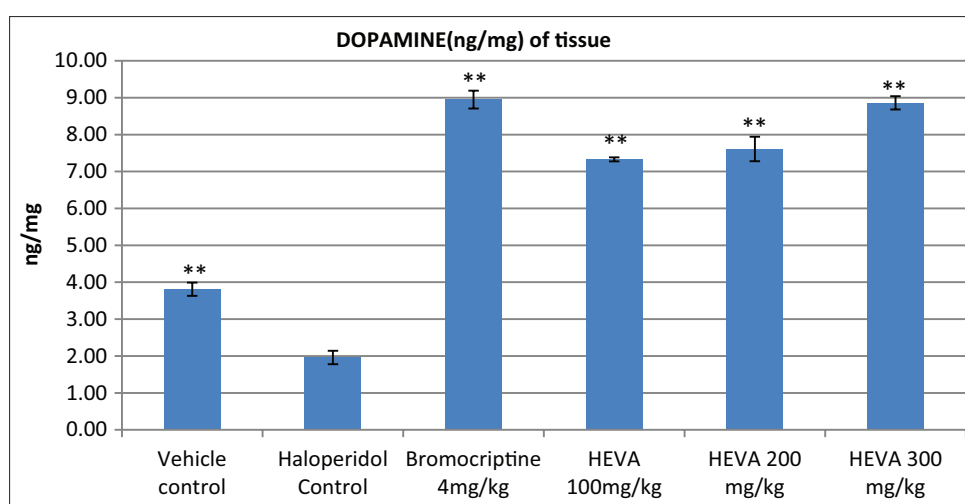


Fig. 4: Effect of bromocriptine and hydroalcoholic extract of the seeds of *Vigna aconitifolia* on dopamine level using high-performance liquid chromatography

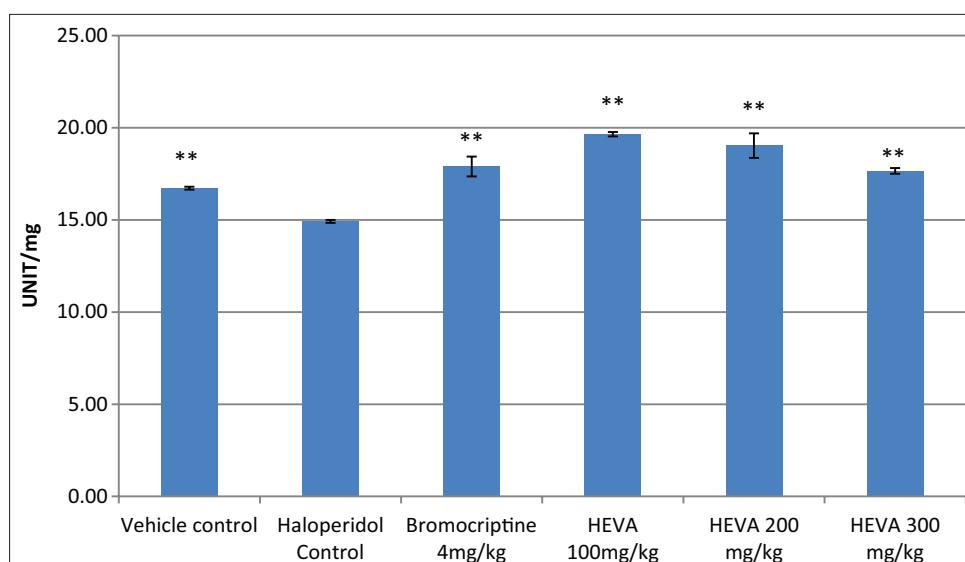


Fig. 5: Effect of bromocriptine and hydroalcoholic extract of the seeds of *Vigna aconitifolia* on catalase level using ultraviolet

**Determination of CAT by UV**

CAT is an antioxidant which helps in neutralizing the toxic effects of hydrogen peroxide. Hydrogen peroxide is converted by the CAT enzyme to form water and non-reactive oxygen species, thus preventing the accumulation of precursor to free radical biosynthesis. Oxidative stress results in decrease in CAT level. Bromocriptine 4 mg/kg and HEVA 100 mg/kg, significantly increased the CAT level as compared to haloperidol-treated animals.

**CONCLUSION**

VA exhibited significant antiparkinsonian activity in haloperidol mouse model. It appears to be the most promising plant due to its L-dopa content and potential antioxidant activity. The predictable mode of action of this plant may be due to increased synthesis of dopamine from L-dopa and decreased lipid peroxidation due to the presence of flavonoids and polyphenols. These findings provide evidence for its use as antiparkinsonian medication, including the prevention of PD and improvement of PD symptoms. Future studies are required to investigate the phytoconstituents responsible for the activity and also to establish the exact mode of action.

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**AUTHORS' CONTRIBUTIONS**

Sushmita Singh designed the experimental study and carried out the analysis. Mr. Imtiyaz Ansari contributed to preparing the manuscript and revision. Both authors have read and approved the final manuscript.

**CONFLICTS OF INTEREST**

The authors have none to declare.

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