

**Original Article**

**PHARMACOLOGICAL EVALUATION OF ANTI-PARKINSON ACTIVITY OF *DELPHINIUM DENUDATUM* (JADWAR) FLOWERS ETHANOLIC EXTRACT IN MICE**

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**ABSTRACT**

**Objective:** This study aimed to evaluate the neuroprotective effects of ethanolic extracts of *Delphinium denudatum* (Jadwar) flowers in an MPTP-induced mouse model of Parkinson's disease (PD).

**Methods:** Parkinsonism was induced in Swiss albino mice using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Mice were divided into five groups: control, negative control (MPTP), standard drug (Levodopa+Carbidopa), and two test groups receiving low (200 mg/kg) and high (400 mg/kg) doses of the ethanolic extract. The extract was administered orally for 7 d, followed by MPTP injections for another 7 d. Behavioral assessments were conducted using rota rod test, hanging test, forced swim test, open field test, actophotometer, elevated plus maze, and catalepsy bar test.

**Results:** The high-dose group (400 mg/kg) demonstrated significant improvement in motor coordination, grip strength, reduced immobility time, enhanced locomotor activity, and decreased cataleptic behavior compared to the MPTP group. These results were comparable to those observed in the standard treatment group, indicating a potential neuroprotective effect of the plant extract.

**Conclusion:** *Delphinium denudatum* ethanolic flower extract showed promising anti-parkinsonian activity by mitigating MPTP-induced motor and behavioral deficits in mice. This suggests its potential as a complementary therapeutic strategy for managing Parkinson's disease, warranting further clinical exploration.

**Keywords:** Parkinson's disease (PD), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), *Delphiniumdenudatum* (Jadwar) flowers ethanolic extract, Rota rod test, Hanging test, Swimming test, Open field test, Actophotometer test, Elevated plus maze test, Catalepsy bar test, Neuroprotective activity

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**INTRODUCTION**

Parkinson's disease (PD), with a prevalence of 1% to 2% in the population aged over 60 years, is believed to be a multicentric second most common neurodegenerative disease in world surpassed in frequency only by Alzheimer's disease [1], characterized by a slowly expanding degeneration of neurons particularly in the mesencephalon [2]. PD is associated with the oxidative stress and mitochondrial dysfunction and characterized clinically by combination of motor symptoms (rest tremor, bradykinesia, rigidity, postural instability, stooped posture and freezing of gait [FoG]) and non-motor symptoms (including psychiatric and cognitive disorders) [3]. In Parkinson's disease (PD), also visual signs and symptoms as part of sensory dysfunction are a common complaint that leads to a significant decrease in the vision related quality of life [4] as the Cognitive impairment and depression are more common in patients with Parkinson disease (PD) whereas dementia commonly occurring in advanced stage of disease. Pathologically, PD is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the deposition of synuclein and cytoplasmic protein aggregates (Lewy bodies) inside degenerated neurons [5]. The Progressive loss of nigrostriatal dopamine (DA) neurons is the neuropathological hallmark of Parkinson's disease (PD) [6]. PD is not considered a hereditary condition; however, genetic risk factors should be considered as an etiology, along with environmental factors. The available treatment options for early Parkinson's disease have changed little in the past decade and include carbidopa/levodopa, dopamine agonists, and monoamine oxidase type B (MAO-B) inhibitors to reduce or delay the appearance of motor complications and along with this current therapy produce adverse events like "on-off phenomenon" [7] *Delphinium denudatum* (Jadwar) belongs to Family-Ranunculaceae, an annual or perennial erect and hardy ornamental herb grows in the western Himalayas from Kashmir to

Uttarakhand at an altitude of 2400-3600 m especially on grassy slopes. The plant density in Himalayan region was most in Sirmaur followed by Solan and Shimla with only a few plants found in Bilaspur district. The roots are used in various medical formulations in Unani and Ayurveda to reduce the withdrawal symptoms in people on de-addiction therapy. Jadwar is Arabic word Persian Zadwar, which means the antidote. In Persian it is also called Mah-parwin (Moon and Pleiades), as it blossoms in the beginning of summer when the Pleiades rise. Indian Jadwar named as Narbisi/Nirbisi due to its antidotal properties. Nir means to oppose or to remove and Bisi means Bis or Vish (Poison). Some of its vernacular names are Antila, Balootularz, Jadwar, Mahpervin, Zadwar, Nirbisi, Nirbishi, Vishalakarani, Nilobikh, Nirvisha, Mahferin, Apavisha, Avisha. It is one of the most important drugs used in the indigenous system of medicine in India, especially in Unani Medicine [8, 9]. Folk medicines are the tribal people's attitudes and response towards the natures, they are the result of the nature interrelation towards the natural vegetation or the flora of the adjoin area, primitive man live on the mercy of the natures and they use the nature for the several purposes, well the folk knowledge transmit from the one generation to the another by them means of the talks and the other means. Traditional knowledge is the nature excavation for the different purposes. *Delphinium denudatum* (Jadwar) is a potent drug of Central Nervous System, an annual herb belongs to family Ranunculaceae occurring in the western Himalayas from Kashmir to Uttarakhand. The word Jadwar is Arabic form of Persian Zadwar means the great purifier or antidote. It is also called Nirbisi due to its antidotal properties [10, 11].

**MATERIALS AND METHODS**

**Plant material**

The flower extract of *Delphinium denudatum* was collected from Shivay herbal healthcare along with COA. Flower parts of *Delphinium*

*denudatum* were shade dried and powdered. Ethanolic Extract was prepared by soxhlet apparatus. Yield: 10 g/kg. Ethanolic extract of *Delphinium denudatum* was administered in different doses (200 and 400 mg/kg, p. o) to mice.

#### Animal protocol

All the experiments were carried out using Adult male Swiss Albino Mice weighing 20–25 g, approximately 2 mo old, procured from the Disease Free Small Animal House, Lovely Professional University, Phagwara. The animals had free access to food and water, and they were housed in a natural (12h each) light-dark cycle. Food given to mice consisted of wheat in the form of dahlia boiled in water. The animals were acclimatized for at least 5 d to the laboratory conditions before behavioral experiments. The experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC) and the care of animals was taken as per guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

#### Drugs and chemicals

1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine hydrochloride (MPTP-HCL) was purchased from Scientific Sale Corporation, HSP. All other chemicals used in the present study were of analytical quality. All drug solutions were freshly prepared before use.

#### MPTP-induced Parkinson's disease model in mice

The 30 mice will be used for the evaluation of the Antiparkinson activity of ethanolic extract. The study will be conducted for 7 d. The animals will allow to acclimatizing for one week before the experiments. Animals will divide into 5 groups each group containing 6 mice. MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine) model will use for study. MPTP will be given to animals for 7 Consecutive days to create the symptoms of Parkinson's disease. Behaviour parameters will be conducted regularly.

#### Experimental protocol

Swiss mice weighing between 30-35 g will be used for the study. The animals will keep in polypropylene cages and maintained under standardized conditions. Standard laboratory conditions: 12 h' light and dark cycle, maintain temperature: 25±2 °C Relative humidity:

60±5%. Standard pellet diet and water ad libitum is given to animals.

Animals were divided into the 5 groups each group contain 6 mice.

Group I Taken as 'Control group' and receives regular food and drinking water for 7 d.

Group II Treated with MPTP for 7 d.

Group III Treated with L-Dopa+Carbidopa for 7 d.

Group IV Treated with *Delphinium denudatum* (Jadwar) Flowers extract 200 mg/kg and then received MPTP for 7 consecutive days.

Group IV is Treated with *Delphinium denudatum* (Jadwar) Flowers extract 400 mg/kg and then received MPTP for 7 consecutive days.

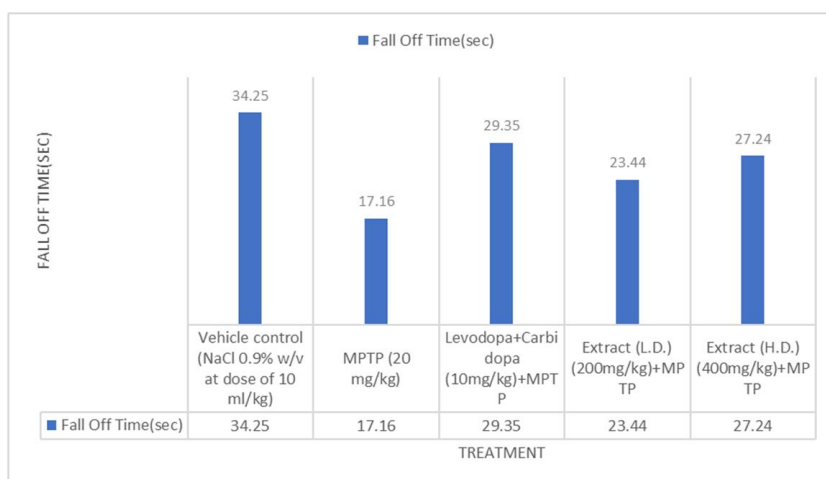
#### Extraction of *Delphinium denudatum* (Jadwar) flowers

Jadwar samples were taken from a plantation, and the flowers were separated from the flesh, sliced thinly, and dried in an oven at 40-50 °C. The dried flowers were crushed, sieved through a 30-mesh sieve, and 100 gs of the material that passed through was mixed with 300 ml of 70% ethanol. This mixture was stirred for 2 h, left to macerate for 24 h, filtered, and then macerated two more times. The resulting filtrate was concentrated using a rotary vacuum evaporator.

#### Experimental parameters

##### Behaviour parameters

- Rotarod Test
- Hanging/Grip Test
- Forced Swim Test
- Open field Test
- Actophotometer Test
- Elevated plus maze Test
- Catalepsy bar Test/wooden bar test



**Fig. 1:** Effect of ethanolic extracts of *Delphinium denudatum* on fall time in seconds in 0.5 mm Bar Test. All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup> represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup> represents significant difference (P<0.05) compared to MPTP control. Results were analysed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEHcontrol; b =P<0.05 vs. MPTP control

#### STATISTICAL ANALYSIS

The results were expressed as mean±standard deviation (S. D.). Statistical analysis was performed for all the result by using one-way ANOVA followed by Tukey's test as post hoc analysis. A value of P<0.05 was considered to be statistically significant.

#### RESULTS

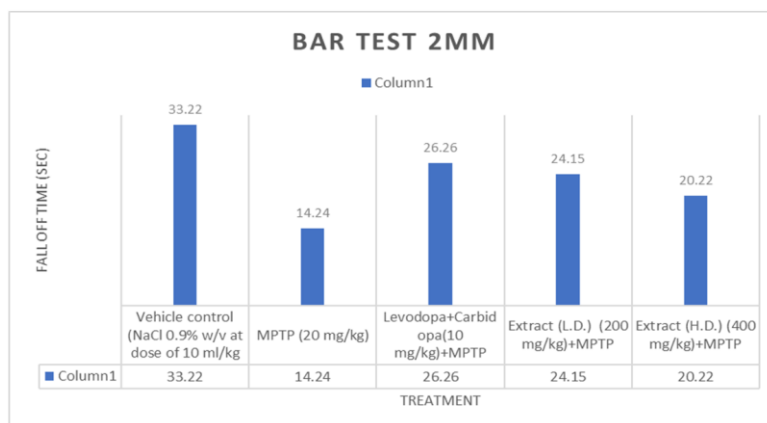
The results are discussed as

##### Bar test (0.5 mm)

Effect of *Ethanolic extract of Delphinium denudatum* (Jadwar) on bar test (0.5 mm)

##### Bar test (2 mm)

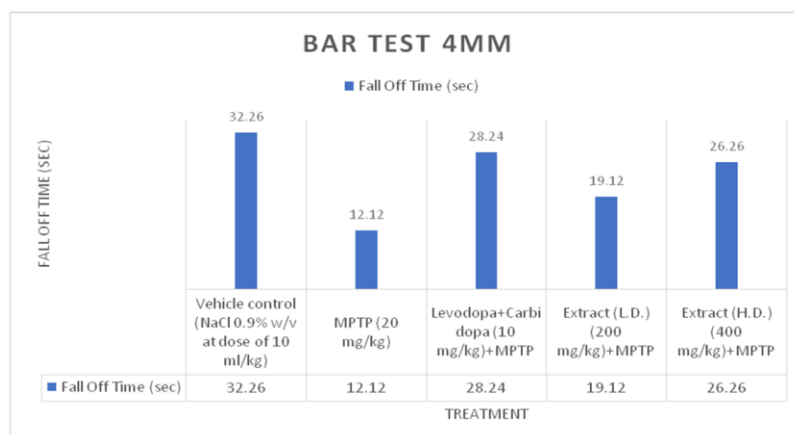
Effect of *Ethanolic extract of Delphinium denudatum* (Jadwar) on bar test (2 mm)



**Fig. 2:** Effect of ethanolic extracts of *Delphinium denudatum* on fall time in seconds in 2 mm Bar Test. All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup> represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup> represents significant difference (P<0.05) compared to MPTP control. Results were analysed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

#### Bar test (4 mm)

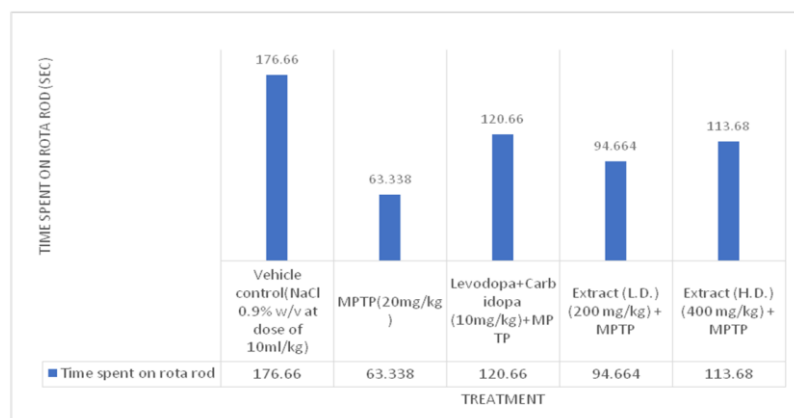
Effect of *Ethanolic extract of Delphinium denudatum (Jadwar)* on bar test (4 mm)



**Fig. 3:** Effect of ethanolic extracts of *Delphinium denudatum* on fall time in seconds in 4 mm Bar Test. All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup> represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup> represents significant difference (P<0.05) compared to MPTP control. Results were analysed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

#### Rota rod test

Effect of *Ethanolic extract of Delphinium denudatum (Jadwar)* on rota rod test



**Fig. 4:** Effect of ethanolic extracts of *Delphinium denudatum* on fall time in seconds in Accelerated Rotarod Test. All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup> represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup> represents significant difference (P<0.05) compared to MPTP control. Results were analyzed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

Table 1: Details of experimental protocols: for 7 d

Group name	Number of animals required
Naïve animal received a standard pellet diet and water <i>ad libitum</i> daily. Control group, normal saline 0.9% NaCl w/v (10 ml/kg, or 1 ml of/100 g body weight orally) for 7 consecutive days	1 X 6=6
Group treated with MPTP 20 mg/kg for 7 consecutive days	1 X 6=6
Group treated with Syndopa(Levodopa (100 mg)+Carbidopa (10 mg)/kg) Followed by MPTP 20 mg/kg for 7 consecutive days	1 X 6=6
Ethanol extract of the Flower of <i>Delphinium denudatum</i> (Jadwar)Low dose (200 mg/kg b. wt) Followed by MPTP 20 mg/kg for 7 consecutive days	1 X 6=6
Ethanol extract of the Flower of <i>Delphinium denudatum</i> (Jadwar)Low dose (400 mg/kg b. wt) Followed by MPTP 20 mg/kg for 7 consecutive days	1 X 6=6
Total number of animals required: n=6; N=5; = 30 mice	

Note: All the parameters will perform with suitable time interval to prevent unwanted stress in animals.

### Forced swim test

Effect of *Ethanol extract of Delphinium denudatum (Jadwar)* on forced swim test

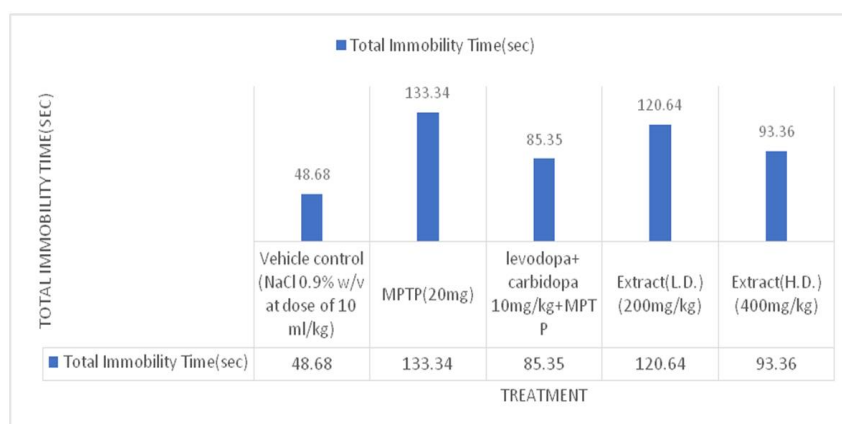


Fig. 5: Effect of ethanolic extracts of *Delphinium denudatum* on immobility time in seconds in Forced Swim Test. All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup> represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup> represents significant difference (P<0.05) compared to MPTP control. Results were analysed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

### Accelerated rotarod test

Effects of ethanolic extract of *delphinium denudatum (Jadwar)* on accelerated rota rod test

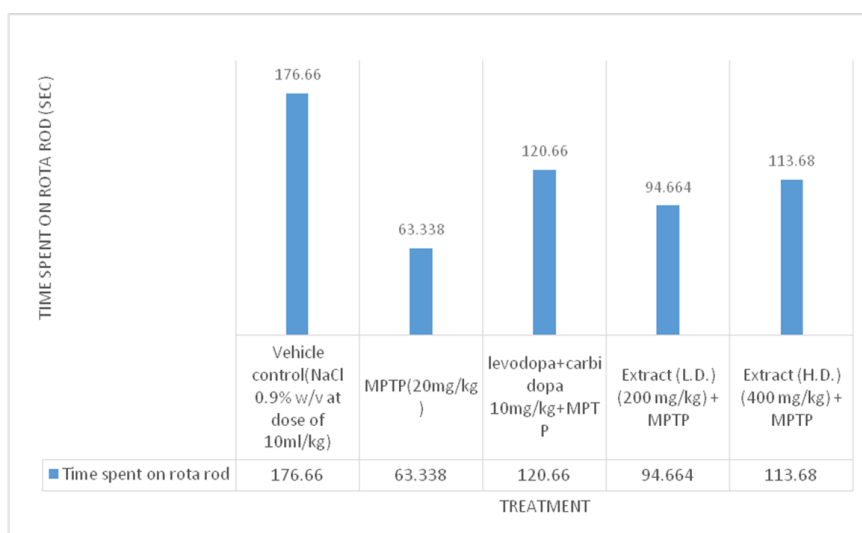
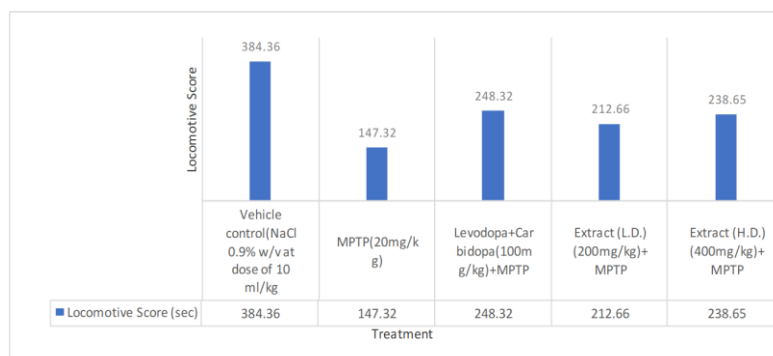


Fig. 6: Effect of ethanolic extracts of *Delphinium denudatum* on fall time in seconds in Accelerated Rotarod Test. All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup> represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup> represents significant difference (P<0.05) compared to MPTP control. Results were analysed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

**Actophotometer test**

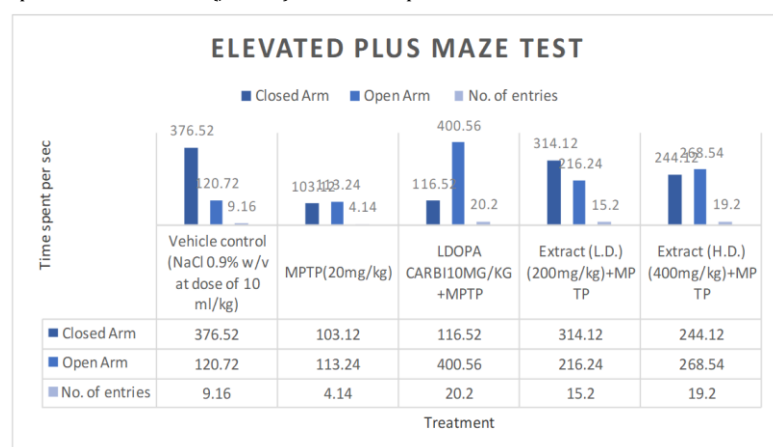
Effects of ethanolic extract of delphinium denudatum (Jadwar) on actophotometer test



**Fig. 7: Effect of ethanolic extracts of *Delphinium denudatum* on locomotor activity in actophotometer test.** All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup>represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup>represents significant difference (P<0.05) compared to MPTP control. Results were analysed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

**Elevated plus maze test**

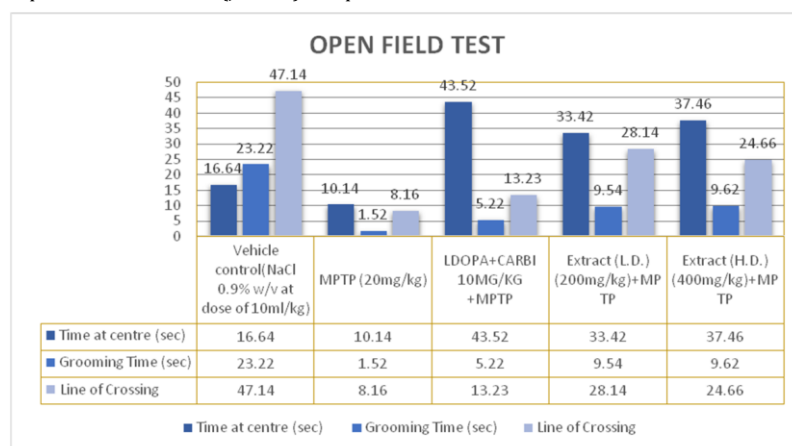
Effects of ethanolic extract of delphinium denudatum (Jadwar) on elevated plus maze test



**Fig. 8: Effect of ethanolic extracts of *Delphinium denudatum* on locomotor activity in Elevated Plus Maze test.** All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup>represents significant difference (P<0.05) compared to vehicle control and <sup>b</sup>represents significant difference (P<0.05) compared to MPTP control. Results were analysed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

**Open field test**

Effects of ethanolic extract of delphinium denudatum (Jadwar) on open field test



**Fig. 9: Effect of ethanolic extracts of *Delphinium denudatum* on locomotor activity in the Open Field test.** All values are represented as mean±standard error of the mean; n = 6, <sup>a</sup>represents significant difference (P<0.05) compared to vehicle control, and <sup>b</sup>represents significant difference (P<0.05) compared to MPTP control. Results were analyzed using analysis of variance Tukey's post-hoc test. a =P<0.05 vs. VEH-VEH control; b =P<0.05 vs. MPTP control

## CONCLUSION

The studies showed that the anti-Parkinsonian actions of natural medicinal plants and active components. We identified a wide range of anti-Parkinsonian effects of different herbal extracts and natural compounds. Several PD neurotoxin models allow for the formulation of anti-Parkinsonian drugs. Even herbal treatment can be used for anti-Parkinsonian drugs. However, researchers need to utilize different types of plant extracts and active components to see their actions and efficacy in PD models. Additionally, there is insufficient discussion about the active components and the action of herbal extracts.

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## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All authors have contributed equally

## CONFLICT OF INTERESTS

Declared none

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