

Pharmacological Evaluation of Anti-Parkinson Activity of 'Ananas comosus' Fruits Ethanolic Extract in Mice

Ankit Sharma*, Ajeet Pal Singh, Amar Pal Singh

Department of Pharmacology, St. Soldier Institute of Pharmacy, Lidhran Campus, Behind NIT (R.E.C.), Jalandhar –Amritsar by pass, NH-1, Jalandhar -144011, Punjab, India..

ABSTRACT

Objective: The purpose of the study was to assess the neuroprotective potential of ethanolic extract from the pineapple fruit (*Ananas comosus*) against MPTP-induced Parkinsonism in mice.

Method: Five groups of male Swiss mice weighing between 30 and 35 grammes were randomly assigned: control, disease (MPTP 20 mg/kg), standard (Syndopa 10 mg/kg + MPTP), low dose extract (200 mg/kg + MPTP), and high dose extract (400 mg/kg + MPTP). For seven days, MPTP was injected intraperitoneally to produce symptoms similar to Parkinson's disease. On day seven, behavioural tests were performed, such as the Rota rod, Hanging/Grip, Forced Swim, Elevated Plus Maze, Catalepsy, Open Field, and Actophotometer tests. Water intake, feed, and body weight were also noted.

Results: MPTP caused anxiety-like behaviour and markedly hampered motor coordination and movement. Performance in every behavioural test was significantly enhanced by treatment with *Ananas comosus* extract. Superior outcomes were demonstrated by the high-dose extract, which was on par with the standard Syndopa group. Better feed and water intake, decreased anxiety and catalepsy, and improved motor coordination were among the improvements.

Conclusion: Ethanolic extract from *Ananas comosus* significantly protects mice from MPTP-induced Parkinsonism. Its potential as a supplemental treatment for neurodegenerative diseases like Parkinson's disease.

Keywords: Parkinson's disease, neuroprotection, behavioral testing, motor coordination, *Ananas comosus*, MPTP model, and herbal therapy.

INTRODUCTION

Parkinson's disease (PD) is a neurological disorder that makes it hard to move, think, sleep, feel pain, and deal with other health problems. PD happens when neurones, which are nerve cells in the brain, break down or die. The substantia nigra, a part of the brain near the base, is where neurones start to die off. This causes the typical symptoms of PD. In the early stages of the disease, you may notice that your face doesn't move as much, your arms don't swing as much when you walk, and your speech changes, like becoming softer or slurred. As the disease gets worse, the symptoms of PD get worse too. Some of the first signs of PD are tremors, small handwriting, loss of smell, trouble sleeping, trouble moving, constipation, a low voice, a masked face, dizziness, stooping or hunching. A 2015 study called the Global Burden of Disease Study estimated that around 6.2

million people worldwide had PD. However, this number could be much higher because many cases go undiagnosed. Since PD becomes more common as people get older and life expectancy increases, the number of people with Parkinson's is expected to rise significantly in the coming years. The Global Burden of Disease Study from 2015 says that by the year 2040, there could be almost 13 million people with Parkinson's. The risk of getting PD goes up with age, usually starting around 60 years old. It is more common in males or people who were assigned male at birth (DMAB) than in females or people who were assigned female at birth (DFAB)²¹. The global impact of PD has more than doubled in 26 years, from an estimated 2.5 million people (with a 95% uncertainty interval of 2.0–3.0 million) in 1990 to about 6.1 million people (with a range of 5.0–7.3 million) in 2016²². PD is not very common in other

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parts of the country, but with India's population of over 1 billion, the estimated number of people with PD in India is 7 million [1-3].

Traditional herbal medicines have been an important part of traditional medical practices in places like China and India for a long time. Now, they are becoming more popular around the world for their ability to treat a wide range of illnesses. People value them for their many uses and, in some cases, for how well they work with few side effects [4]. Many researchers have looked into the possible use of different natural products and herbs to treat PD in the last few years. Some herbs have been shown to work better and more reliably than standard synthetic drugs [5]. Some medicinal plants that can help with PD are *Mucuna pruriens*, *Withania somnifera*, *Bacopa monnieri*, *Ginkgo biloba*, *Camellia sinensis*, and *Curcuma longa*. Studies have shown that these plants can protect neurones from PD, and their main goal is to improve the production of ROS [6]. The scientific name for pineapple is *Ananas comosus*. It belongs to the Bromeliaceae family, which has three subfamilies: Tillandsioideae, Bromelioideae, and Pitcarnioideae. The *Ananas comosus* plant is part of the Bromelioideae subfamily. The genus *Ananas* is part of the Bromeliaceae family because of its unique characteristics [7]. The pineapple is a perennial herbaceous plant that usually grows to be 1 to 2 meters tall and wide and looks like a spinning top. The stem, leaves, peduncle, multiple fruit, crown, shoots, and roots are its main morphological parts. The multiple fruit forms when several fruitlets join together on a single stalk, with several flowers spiralling around the stalk. Each flower makes a fleshy fruitlet that joins with those of nearby flowers to make a single fleshy fruit [8]. Pineapple is a good source of sugars, organic acids, essential minerals, vitamins, and fibre, which makes it good for people to eat. Its fruits also have healthy antioxidants like ascorbic acid, flavonoids, and carotenoids that help keep you healthy. The chemical makeup of pineapple changes depending on the type. It can have sugars, organic acids, minerals, fibre, aromatic compounds, vitamins, amino acids, flavonoids, and carotenoids. It is interesting because it is one of the few fruits that has a lot of B-complex vitamins. Tyrosine and tryptophan are the main amino acids in pineapple. Other amino acids include asparagine, proline, aspartic acid, serine, glutamic

acid, α -alanine, aminobutyric acid, valine, and isoleucine. Bromelain, a proteolytic enzyme that helps digestion by breaking down proteins, is also found in pineapple. Bromelain is known to reduce inflammation, stop blood clots, and possibly fight cancer [9]. *Ananas comosus* has a number of pharmacological effects, such as abortive effects, anticholinesterase effects, anticancer potential, antidepressant activity, antioxidant properties, cardioprotective effects, hepatoprotective qualities, anticoagulant activity, and anthelmintic properties [10].

MATERIAL & METHOD

Chemicals and equipment requirement: MPTP was purchased from Scientific Sales Corporation. All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers.

Plant Material

The extract of *Ananas comosus* fruit was procured from Shivay Herbals and Healthcare and Certificate of Analysis was also got for the same saying it complies with all the required specifications.

Standard drug: Syndopa tablet 110mg/kg is used as a standard drug.

Test component: Extract of Fruits of *Ananas comosus* (Pineapple) were used as the test component.

Procurement of animals

Healthy Swiss Mice, weighing between- 30 to 35 g procured from Lovely Professional University, Phagwara and kept in Animal House Facility, St. Soldier institute of Pharmacy, Jalandhar, Punjab, India. The animals will keep in polypropylene cages and maintained under standardized conditions. Standard laboratory conditions: 12 hours light and dark cycle, maintain temperature: 22°C Relative humidity: 60%. Standard pellet diet and water ad libitum is given to animals [9].

Experimental protocol

Induction of Disease: MPTP model is used for the study. MPTP will be dissolved in saline (1mg



MPTP/1ml saline) and will be kept frozen until the time of use. After, this MPTP will be given to animals for 7 Consecutive days to create the symptoms of PD [10].

Experimental Design

Male Swiss mice weighing between- 30 to 35 g will be used for the study. The animals will be kept in polypropylene cages and maintained under standardized conditions. Standard laboratory conditions: 12 hours light and dark cycle, maintain temperature: 22°C Relative humidity: 60%. Standard pellet diet and water ad libitum is given to animals¹¹⁴. Animals were divided into the 5 groups each group contains 6 rats.

Group I was designated as the 'Control group' and received regular food and drinking water for seven days. Control group also received 0.9% w/v NaCl (10ml/kg) once daily orally.

Group II was the 'Disease group' and was treated with MPTP at 20 mg/kg i.p. once daily for seven consecutive days.

Group III was the 'Standard group' and was treated with Syndopa (Levodopa + Carbidopa) at 10 mg/kg orally once daily before receiving MPTP and then received MPTP 20 mg/kg i.p. once daily for 7 consecutive days (from the 1st to 7th day)[11]

Groups IV and V were categorized as the 'Treatment Groups.'

Group IV was treated with a low dose (200 mg/kg) of *Ananas comosus* ethanolic extract orally once daily before receiving MPTP 20 mg/kg i.p. once daily for seven consecutive days (from the 1st to 7th day)

Group V was treated with a high dose (400 mg/kg) of *Ananas comosus* ethanolic extract orally once daily before receiving MPTP 20 mg/kg i.p. once daily for seven consecutive days (from the 1st to 7th day) [12].

30 mice will be used for the evaluation of the Antiparkinson activity of herbal preparation. The study will be conducted for approx. 7 days. The animals

will allow to acclimatizing for one week before the experiments. Animals will be divided into 5 groups each group containing 6 mice. MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine) model will be used for study. MPTP will be given to animals for 7 Consecutive days to create the symptoms of PD. Behavioral parameters will be conducted at the end of study.

In Vivo Study

Pharmacological activity of *Ananas comosus* on MPTP induced PD in mice.

Why MPTP is best model for induction of Parkinson disease?

The use of MPTP in PD is very useful due to its ability to closely replicate PD symptoms observed in humans and it's cost-effective, manageable and ethically less complex than other toxin-induced models. It also unveils non-motor symptoms associated with PD and this model helps in exploring deep brain stimulation therapy, as demonstrated by Wallace et al. According to Filograna et al., the MPTP-mouse model significantly advances clinical PD research [13].

Why Mice are used for the study?

As per the literature survey. Mice are preferred over rats because rats are very resistant to MPTP, although they are sensitive to MPP⁺ when injected directly into the SNpc.

How many days are required for the study?

Approx. 7 Days are required for the study of Antiparkinson activity in animals¹¹⁵.

What are the objectives of this In Vivo Study?

In these in vivo studies, we will assess the antioxidant, anti-inflammatory, and anticholinesterase properties of the herbal preparation. Additionally, we will evaluate its impact on PD. The effects of the test preparation will be evaluated through the examination of various behavioural parameters, including locomotor activity, catalepsy, rota rod performance in mice.



Table 1: Grouping of animals & Dosing

Groups	Treatment	Dose (mg/kg)	Animal required	Days
I	Vehicle only	0.9% w/v NaCl	6	7
II	MPTP	20mg/kg i.p	6	7
III	Levodopa + Carbidopa (10 mg/kg) + MPTP	10mg/kg p.o.+20mg/kg i.p.	6	7
IV	Ethanolic extract of Fruit of Ananas comosus Low dose (200 mg/kg b.wt) + MPTP	200mg/kg p.o.+20mg/kg i.p.	6	7
V	Ethanolic extract of Fruit of Ananas comosus High dose (400 mg/kg b.wt) + MPTP	400mg/kg p.o.+20mg/kg i.p.	6	7

Dosing:

1. MPTP-20 mg/kg
2. ACE (Low Dose) – 200 mg/kg
3. ACE (High Dose) – 400 mg/kg
4. L-dopa – 10 mg/kg

Total No. of Animals Required:

No. of the animal in each group (n) = 06

No. of groups (N) = 05

Total no. of animals required = 30

Behaviour Test performed in-Vivo Studies in detail:

Body Weight Analysis-Body weight analysis was conducted for each group of animals at the start and at the end of the study, and changes in body weight were recorded for each group.

Feed Intake Analysis-Feed intake analysis was performed on each group of animals at both the beginning and the end of the study, and the changes in feed intake were documented for all groups.

Water Intake Analysis-Water intake was measured for each group of animals at the beginning and end of the study, and any changes in water intake were recorded accordingly.

Hanging/Grip Test-The Bar Test, as outlined by Deacon (2013), evaluates motor coordination in mice by assessing their ability to remain on a horizontal bar. The apparatus consists of brass bars, each 38 cm long

and positioned 49 cm above the bench surface, supported by wooden columns secured to a heavy wooden base. Three bar diameters—0.5 mm, 2 mm, and 4 mm—are provided, with larger diameters increasing the difficulty due to reduced grip efficiency. The procedure followed Deacon's (2013) protocol with modifications to assess both forelimb and hindlimb coordination. To conduct the test, the mouse was lifted by its tail and placed on the bench in front of the apparatus. The mouse was slid backward approximately 20 cm, aligned perpendicular to the bar, quickly lifted, and positioned so its forepaws could grasp the midpoint of the bar. Once the mouse gripped the bar, its tail was gently released, and the timer was started. If the mouse failed to grasp the bar properly, the trial was repeated after a brief rest, with a maximum of three attempts permitted. Special care was taken to release the tail gently to avoid disrupting the mouse's grip. The best performance among the attempts was recorded. The scoring method was consistent for all bar diameters (0.5 mm, 2 mm, and 4 mm), and the final score was noted [14].

Rotarod Test- The setup included a sturdy base and a metal rod that wasn't slippery, measuring 3cm across and 30 cm long. Before getting the MPTP injection,

the mice were trained by placing them on a rotating drum moving at 20 revolutions per minute (rpm). They ran on the drum until they could stay on it for



more than 200 seconds without falling off. During the test, the mice were placed on the rotating rod also moving at 20 rpm, and their time staying on the rod without falling off was measured for up to 5 minutes.

Forced Swim Test- The forced swim test was used to assess behavior similar to depression. Mice were put into a cylinder filled with water at 24°C for 5 minutes. The test was recorded using a video camera, and the time it took for them to stop moving and their time spent immobile were measured. An increase in immobility time and a decrease in the time it took for them to become immobile were considered signs of a depressive-like state.

Elevated Plus Maze- The elevated plus maze had two open arms and two closed arms extending from a central platform, elevated above the floor. Each animal was placed individually in the center of the maze, facing one of the open arms, and allowed to explore for 5 minutes. After each session, the maze was cleaned with 10% ethanol. We recorded the test using a video camera and analyzed behavior. The recorded behavioral parameters included the number of entries into the open and closed arms.

Catalepsy Bar Test -To measure catalepsy score, we used the bar test. In this test, the animal was positioned with both front paws on a horizontal bar, which was 3 cm above and parallel to the base, while in a half rearing position. We then measured the time the animal spent with at least one forepaw on the bar. Once the animal removed its forepaw, we recorded the time.

Open Field Test- To evaluate locomotor behavior, we used the open field test. The rat was placed in the center of a square arena measuring and allowed to explore for 5 minutes. We recorded the total distance traveled, time spent resting, number of rearing's, and ambulatory episodes. The arena was cleaned with 10% ethanol between each animal.

Actophotometer Test- We used an actophotometer to measure locomotor activity. The animals were first given 2 minutes to get used to the chamber, and then their movement was monitored for the next 5 minutes. We calculated the total locomotor activity by averaging the number of photo beam counts per animal over the 5-minute period.

RESULTS

Changes on Body Weight: - The study aimed to assess changes in body weight across different groups of mice during the experimental period. Groups IV and V exhibited a significant increase in body weight compared to Groups I and II. Group III also showed a moderate gain in body weight. In contrast, Group II displayed a reduction in body weight over the course of the study. Among all groups, the weight gain in Groups IV and V was most prominent, whereas Group II showed a clear decline, indicating varying physiological responses among the groups.

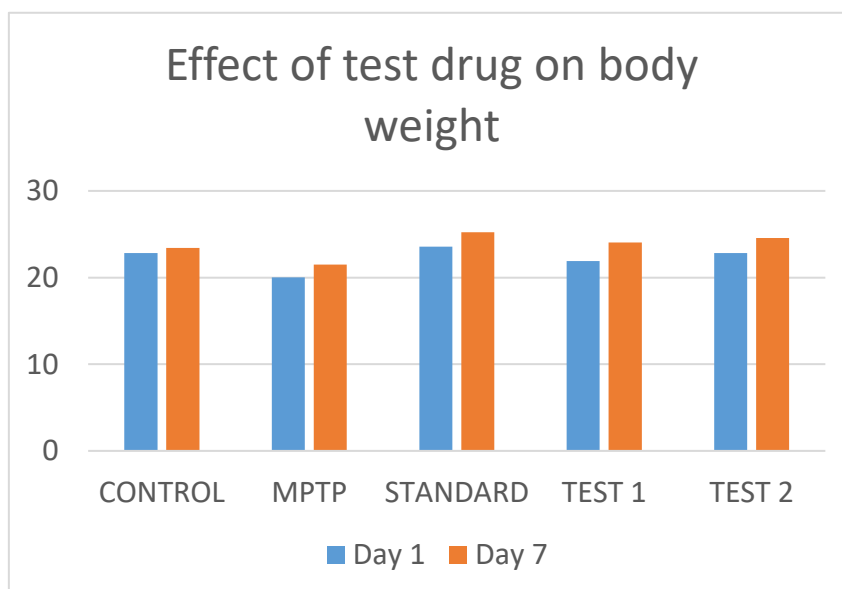


Figure 1: Effect of *Ananas comosus* on Body Weight

Changes on Feed intake: - The study aimed to assess changes in feed intake across different groups of mice during the experimental period. Groups IV and V exhibited a significant increase in feed intake compared to Groups I and II. Group III also showed a moderate increase in feed consumption. In contrast,

Group II displayed a reduction in feed intake over the course of the study. Among all groups, the increase in feed intake was most prominent in Groups IV and V, whereas Group II showed a noticeable decline, indicating varying dietary responses among the groups.

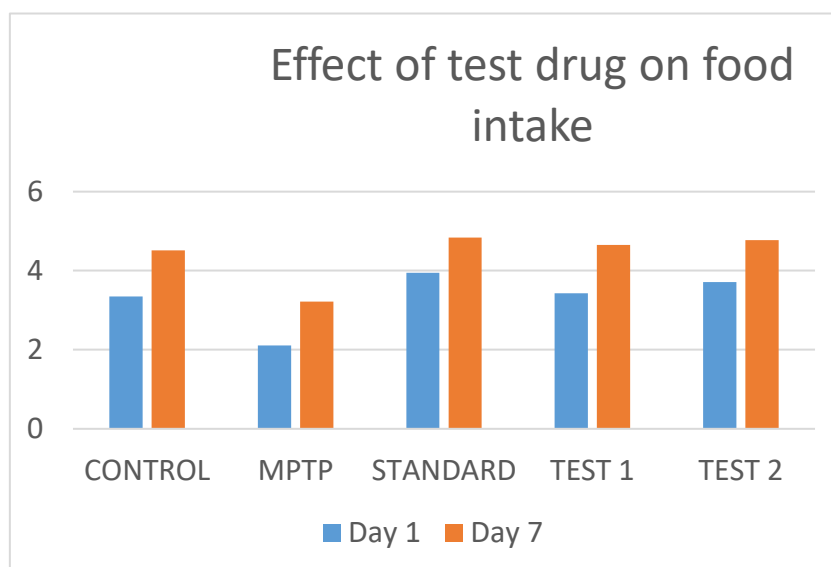


Figure 2: Effect of Ananas comosus on Feed intake

Changes on Water intake:- The study aimed to assess changes in water intake across different groups of mice during the experimental period. Group II showed an increase in water intake compared to the other groups. Groups IV and V also exhibited a

significant increase in water consumption compared to Group I. Group III demonstrated a higher increase in water intake than Groups IV and V. Overall, Groups II and III showed the highest water intake, indicating a stronger hydration response in these groups.

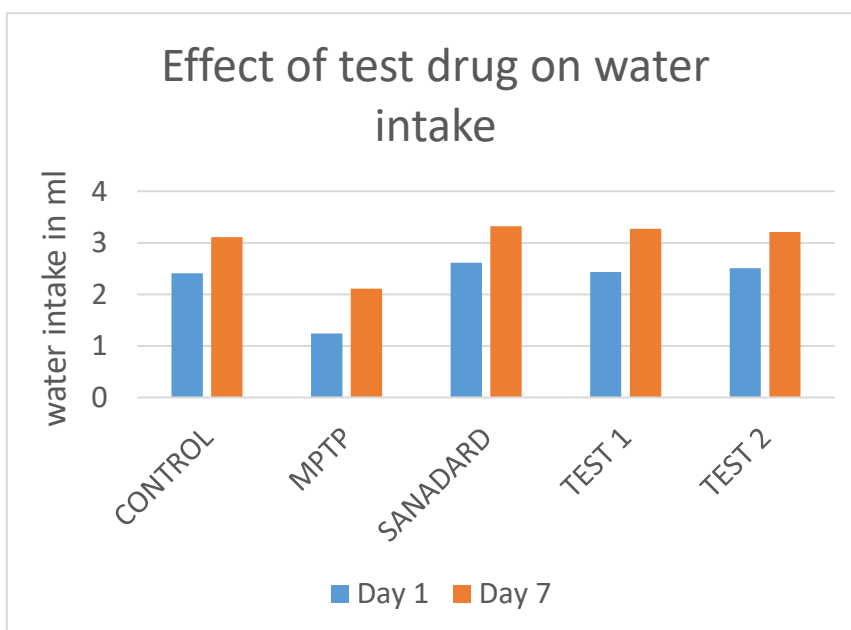


Figure 3: Effect of Ananas comosus on Water intake



Hanging/Grip Test: -The test was conducted to evaluate motor coordination by measuring how long mice could balance on a horizontal bar with varying diameters—0.5 mm, 2 mm, and 4 mm—where larger diameters posed greater difficulty due to reduced grip efficiency. Group I demonstrated the highest performance, maintaining balance on the bar for the longest duration, while Group II showed the lowest performance, staying on the bar for the shortest time.

Groups III, IV, and V performed better than Group II but did not match the performance of Group I. Among these, Group V remained on the bar longer than Group IV, while Group III outperformed both but still fell short of Group I. Notably, the performance of Group V closely approached that of Group III, indicating relatively strong motor coordination.

(A) Bar Test 0.5mm:

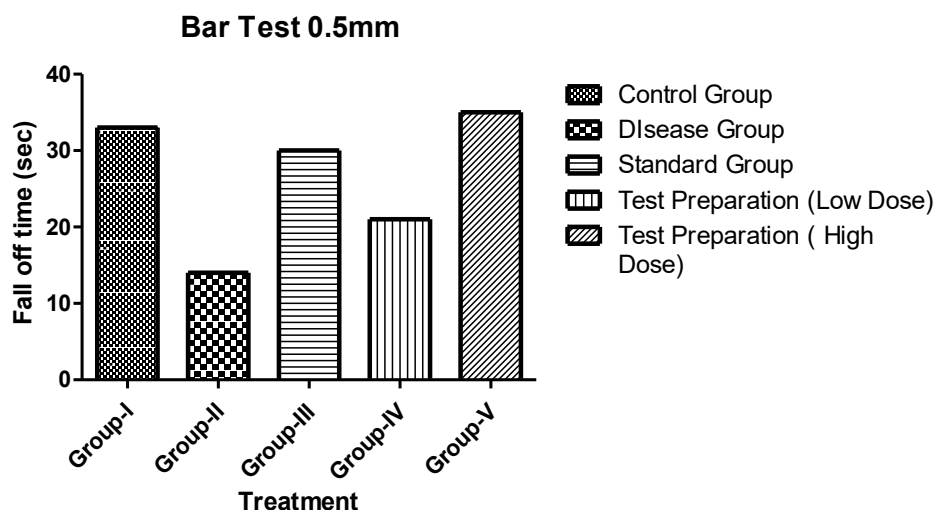


Figure 4: Effect of Ananas comosus on Bar Test 0.5mm

(B) Bar Test 2mm:

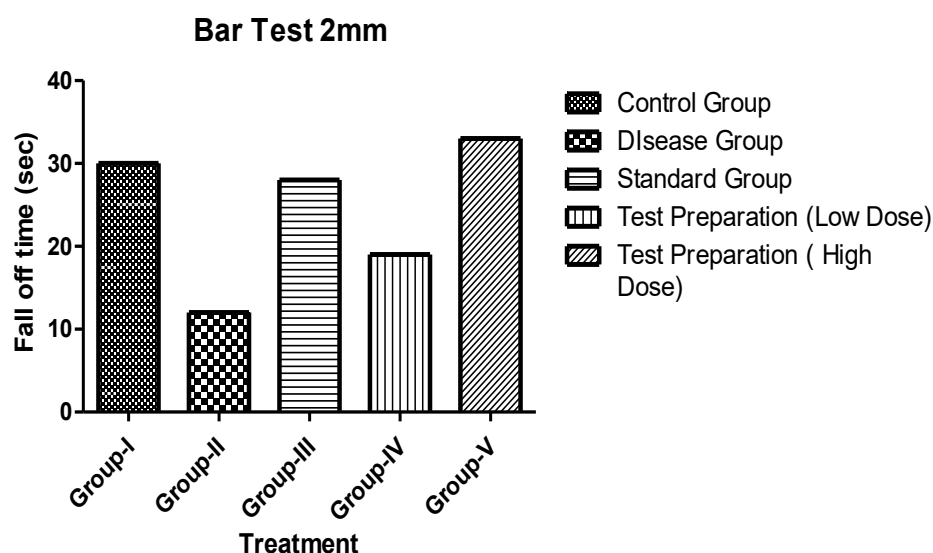


Figure 5: Effect of Ananas comosus on Bar Test 2mm

(C) Bar Test 4mm:

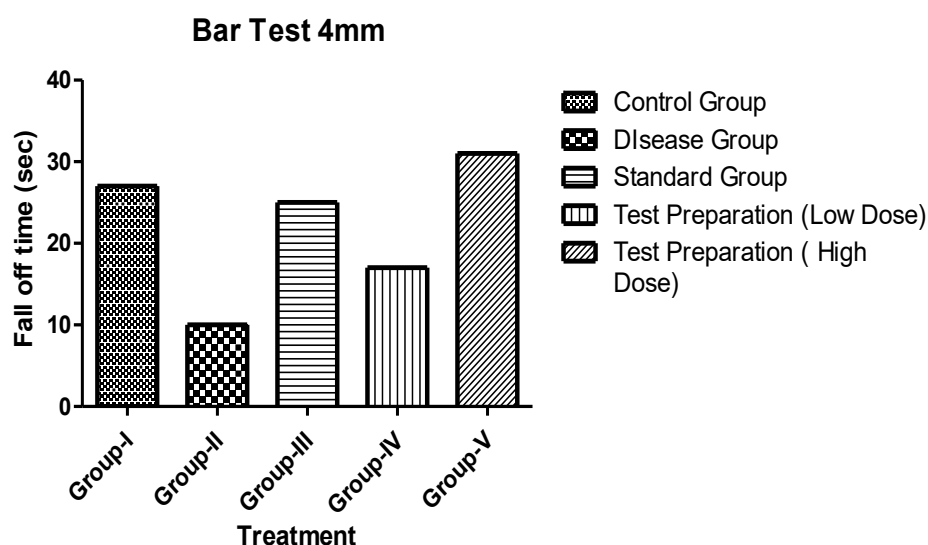


Figure 6: Effect of Ananas comosus on Bar Test 4mm

Rota Rod Test: -In the rotarod test, the length of time spent on the rotating rod was measured in Groups I–V to assess motor coordination, balance, physical condition, and motor planning. Group I demonstrated the highest performance, staying on the rod for the longest duration, while Group II showed the lowest

performance, spending the least amount of time on the rod. Groups III, IV, and V remained on the rod for longer than Group II but for a shorter time than Group I. Among these, Group V stayed on the rod longer than Group IV, with Group III outperforming both but still falling short of Group I. Notably, the performance of Group V closely approached that of Group III.

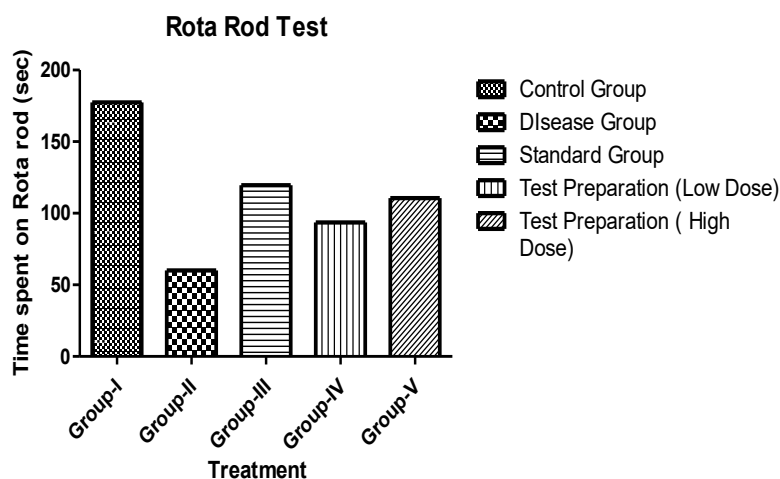


Figure 7: Effect of Ananas comosus on Rota Rod

Forced Swim Test: -The time taken by mice to stop moving and their time spent immobile were measured in Groups I–V during the forced swim test. The time spent by the animals as immobile in water represents depression-like behavior. Group II exhibited the

maximum immobility period, while Group I showed the minimum immobility time, indicating the least depression-like behavior. Groups III, IV, and V displayed longer immobility times compared to Group I but shorter than Group II, suggesting intermediate levels of depression-like behavior.



Among these, Group III demonstrated the shortest immobility period, followed by Group V, with Group IV showing the longest immobility time. Notably, the

immobility time of Group V was close to that of Group III.

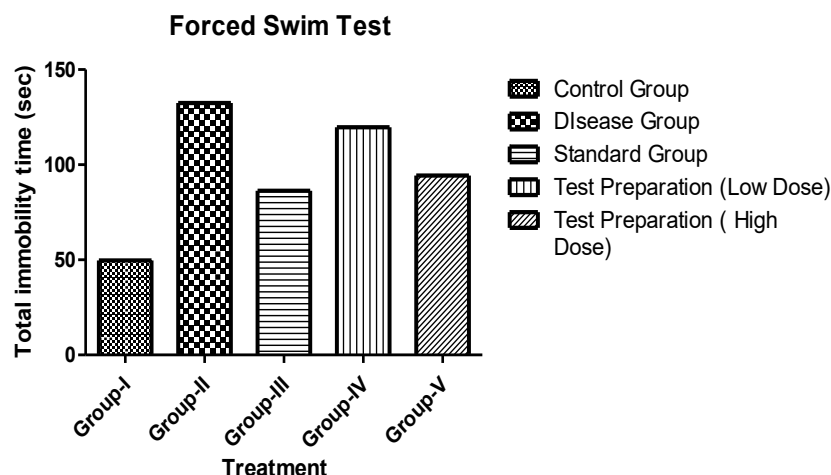


Figure 8: Effect of *Ananas comosus* on Forced Swim Test

Elevated Plus Maze: -In the elevated plus maze test, the number of entries and the time spent in open and closed arms were measured in Groups I–V. Group I spent the maximum time in the closed arm, while Group II spent the minimum time in the closed arm, indicating the highest and lowest anxiety-like behaviour, respectively. Groups III, IV, and V spent less time in the closed arm than Group I but more than Group II. Among these, Group IV spent the most time in the closed arm, followed by Group V and then

Group III. Group III spent the maximum time in the open arm, suggesting the lowest anxiety-like behaviour, whereas Group II spent the minimum time in the open arm. Group I spent more time in the open arm than Group II but less than the other groups. Group V spent more time in the open arm than Group IV but less than Group III. The number of entries was highest for Group III and lowest for Group II. Group I had more entries than Group II but fewer than the other groups. Group V had more entries than Group IV but fewer than Group III.

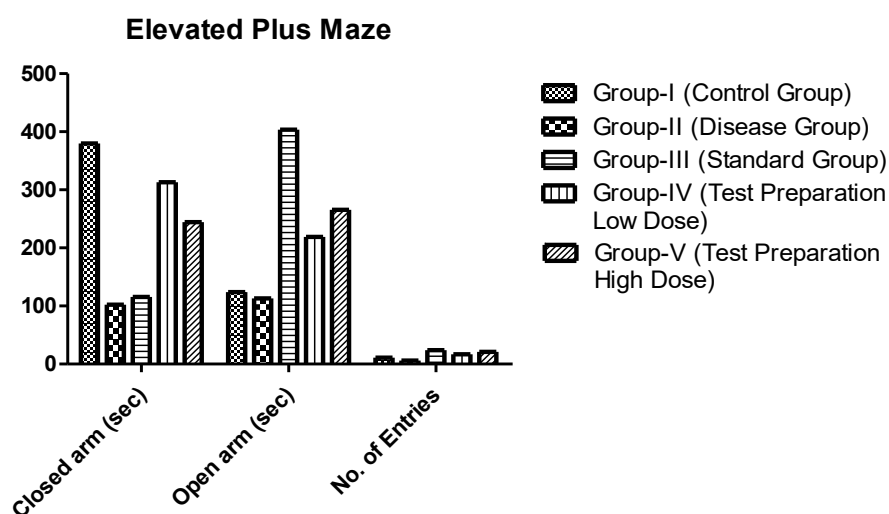


Figure 9: Effect of *Ananas comosus* on Elevated Plus Maze

Catalepsy Test: -In this test, motor coordination was measured using the catalepsy score by positioning the animals with their front paws on a horizontal bar and measuring the time spent with at least one forepaw on the bar. Once the animal removed its forepaw, the time was recorded. Group II exhibited the maximum time on the bar, indicating the highest catalepsy score and the lowest motor coordination. Group IV demonstrated the minimum time, reflecting the best motor coordination. Group I spent less time on the bar than Group II but more than the other groups. Group III stayed on the bar longer than Groups IV and V, while Group V remained on the bar for more time than Group IV and showed results close to Group III. A

lower catalepsy score corresponds to better motor coordination.

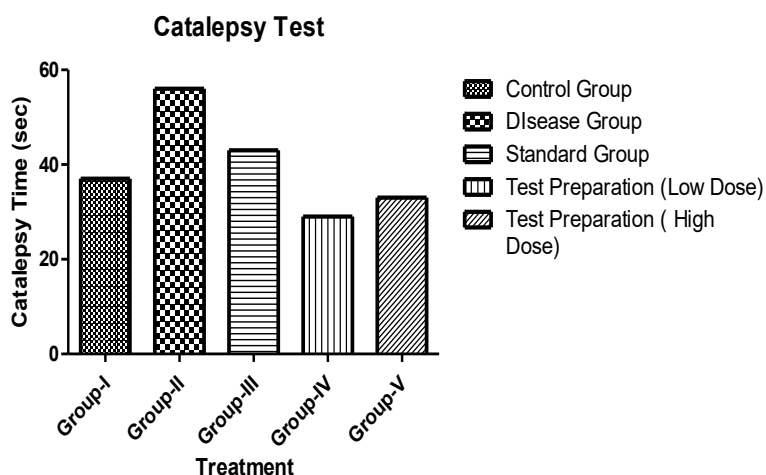


Figure 10: Effect of Ananas comosus on Catalepsy Test

Open Field Test: -The open field test was conducted to evaluate locomotor behavior by recording the time mice spent at the centre; time spent grooming, and the number of line crossings. Group III spent the maximum time at the centre, while Group II spent the least. Group I spent more time at the centre than Group II, and Groups III, IV, and V spent more time at the centre compared to Groups I and II, with Group III spending the most time, followed by Group V and then Group IV. In terms of grooming behavior, Group

I spent the most time grooming, while Group II spent the least. Groups III, IV, and V groomed longer than Group II but less than Group I, with Group IV grooming more than Group V and Group III spending the least time grooming. For the number of line crossings, Group I crossed the most lines and Group II the fewest. Groups III, IV, and V crossed more lines than Group II but fewer than Group I, with Group IV crossing more than Group V and Group III crossing the least among them.

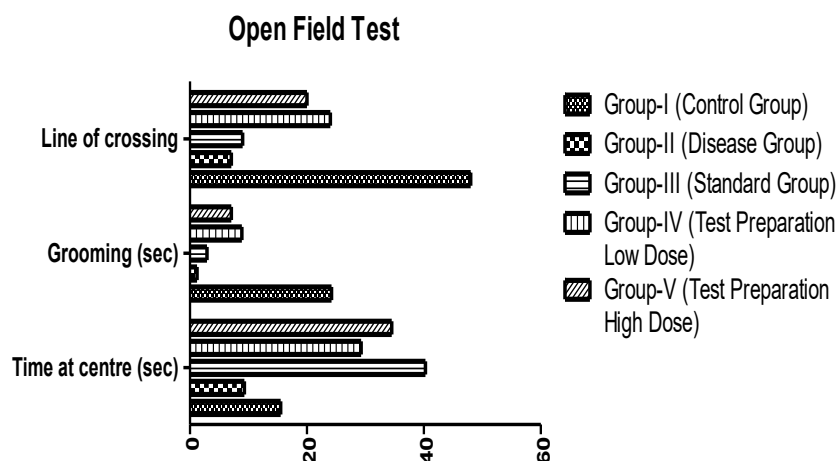


Figure 11: Effect of *Ananas comosus* on Open Field Test

Actophotometer Test: - In this test, locomotor activity was measured in Groups I–V. Group I demonstrated the highest locomotor activity, spending the maximum time in movement, while Group II exhibited the lowest activity, spending the

least time in movement. Groups III, IV, and V displayed more activity compared to Group II but less than Group I. Among these, Group III showed the highest activity, followed by Group V, with Group IV exhibiting the least activity. Notably, the locomotor activity of Group V was close to that of Group III.

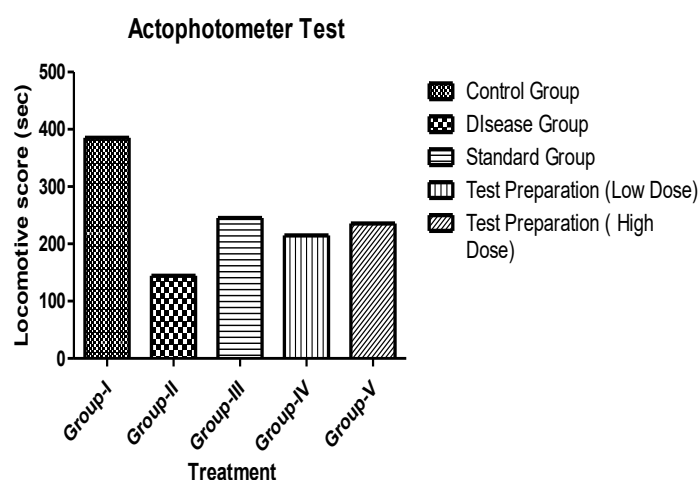


Figure 12: Effect of *Ananas comosus* on Actophotometer Test

CONCLUSION

The study showed that the ethanolic extract of the pineapple fruit, *Ananas comosus*, significantly protects mice from MPTP-induced Parkinsonism. Both low (200 mg/kg) and high (400 mg/kg) dosages of the extract successfully increased overall locomotor activity, decreased anxiety-like behaviour, and improved motor coordination. Numerous behavioural tests, such as the Rota rod, Grip strength,

Forced Swim, Elevated Plus Maze, Open Field, and Catalepsy tests, demonstrated these effects. Specifically, the high-dose group demonstrated improvements on par with the group receiving levodopa and carbidopa as part of standard treatment. The results all point to the potential of *Ananas comosus* extract as a supplemental treatment approach for neurodegenerative diseases like Parkinson's disease, most likely as a result of its neuroprotective qualities.



DISCLOSURE STATEMENT

There are no conflicts of interest.

AUTHORS CONTRIBUTIONS

All authors have contributed equally

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