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Research Article

Investigation of banana peel powder in scopolamine induced neurobehavioral changes in zebrafish model

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ABSTRACT

The present research work was intended to carry out the neuroprotective efficacy of banana peel powder (BPP) in scopolamine induced amnesia in zebrafish. The effect of BPP was assessed by Novel tank test, Y-Maze test and Color-biased appetite conditioning T-Maze test**.** In novel tank test, the different concentrations of BPP (12.5, 25 and 50 mg/L) showed dose dependent increase in the number of entries to top, time spent in top, latency to enter top and total distance travelled in tank whereas decrease in number of entries to bottom and time spent in bottom as compared to scopolamine control group. In Y-Maze test, BPP at various strengths exhibited dose dependent significant increase in number entries to novel arm, time spent in novel arm and total distance travelled in the maze as compared to scopolamine control group. The different concentrations of BPP showed significant decrease in acetylcholinesterase (AChE) and MDA content in brain homogenate of zebrafish as compared to scopolamine control group. In T-Maze test BPP at 12.5, 25 and 50 mg/L exhibited dose dependent significant increase in number of entries to green arm and time spent in green arm whereas significant decrease in number of entries to red arm and time spent in red arm as compared to scopolamine control group in dose dependent manner. The results obtained in this study conclude that, BPP could effectively improve memory impairments in a scopolamine induced zebrafish model of amnesia by enhancing the function of the behavioral response and antioxidant enzymes in the amnesic zebrafish model.

Keywords:
Neuroprotective, Novel Tank Test, AChE, MDA, Zebrafish

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, affecting the cerebral cortex and hippocampus, leading to memory impairment. AD pathological hallmarks include extracellular accumulation of amyloid-β, aggregation of the microtubule protein tau in neurofibrillary tangles in neurons, as well as the reduction in levels of acetylcholine[1-2]. The various descriptive hypotheses regarding the cause of AD, the cholinergic hypothesis was the first proposed to explain AD based on the findings that a loss of cholinergic activity is commonly observed in the brains of AD patients[1]. In addition, this theory implied utilizing acetyl cholinesterase inhibitors (AChEIs), which reversed memory deficits in AD patients. AChEIs could diminish memory impairment in AD patients by inhibiting the degradation of acetylcholine[3]. Currently, for the treatment of mild to moderate AD, three AChEIs are used i.e. Donepezil, Rivastigmine and Galantamine[4]. Moreover, there are different side effects related to existing treatment. Herbal based compounds could be a great source of anti AD agents[5].

Zebrafish exhibit complex cognition comparable to that seen in mammals[6-7] and there are behavioral tasks protocols based on rodent protocols such as active or passive avoidance test[8], Y-maze test[9] and colour-biased appetite conditioning T-Maze test[10] for zebrafish. In zebrafish, scopolamine, a muscarinic acetylcholine receptor blocker has been characterized to induce amnestic effects and is used in combination with nootropic and cognitive-

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enhancing drugs to study memory processes[11].

Banana is the leading fruit grown in more than 122 countries. Normally has a short shelf life and start deteriorating just after plucking. The most widely used part of banana is the flesh of the fruit, the outer skin is used for animal feed and organic fertilizer. Unripe banana are cooked as vegetable, chips, snacks, powder etc., whereas, mature dessert banana is eaten raw. Banana peel has been utilized for various industrial applications including biofuel production, bio-sorbents, pulp and paper, cosmetics, energy related activities, organic fertilizer, environmental cleanup and biotechnology related processes. The banana peel is rich in phytochemical compounds than its pulp. The antifungal, antibiotic properties of banana peel can put to be good use. The peel is used for treating several skin problems including allergies and skin irritations[12].

Banana peel is used as natural wrapping paper with additive essential oils[13], antibacterial[14], antioxidant status, cytokine responses[15]. Banana peel contains cellulose (60-65%), hemicellulose (6-8%) and lignin (5-10%). The bioactive agents in banana peels include glycosides, alkaloids, saponins, tannins, flavonoids and volatile oil[16]. Upon extensive literature survey, no reports were found on neuroprotective property of banana peel powder in zebrafish model against scopolamine induced neurobehavioral changes. Hence, we intended to evaluate the neuroprotective property of banana peel powder in scopolamine induced neurobehavioral changes in zebrafish model.

2. METHODOLOGY

2.1. Procurement of banana peel powder

The banana peel powder was purchased e-store, Amazon. The BPP powder was packed and marketed by Phoenix Medicaments Pvt. Ltd, Ahmedabad-380006 bearing batch number PHX444.

2.2. Use of zebrafish[17]

Zebrafish (*Danio rerio*) (1-1.2 g) 3-4 month old were obtained from an authorized commercial supplier, Vijayapur and were randomly divided into groups of 15/10 L tank with a constant 14:10 h of the light/dark and fed twice daily with commercial flake food. All fish used in this experiment were quarantine for one week before the conduct of study.

2.3. Administration of drug[18]

In the present study, the drugs were administered by immersion of drug into the tank.

2.4. Acute oral toxicity study[19-20]

Acute oral toxicity of BPP was tested in the zebrafish (*Danio rerio*) as per the OECD guidelines. The different concentrations (12.5, 25, 50, 100, 200 and 400 mg/L) of BPP were selected for oral toxicity in zebrafish. 7 healthy fishes were selected to each concentration and were transferred to beakers containing different concentration of BPP solution. The fish were observed after 24, 48, 72, and 96 hours. The mortalities were recorded for every 24 hours and fishes were considered dead, if there was no visible movement even upon mechanical stimulation producing no reaction[21]. LC50 was determined based on the concentration of the BPP which killed 50% of fishes.

2.5. Evaluation of scopolamine induced amnesia in zebrafish

2.5.1. Novel tank test (NTT)[21-22]

Principle

NTT is a specific test used for assessing memory improvement in zebrafish. This method is used screen the behavioral parameters and cognitive impairments. The novel tank test measures locomotor and anxiety in zebrafish, and is highly sensitive both to anxiolytic and anxiogenic drugs. In this trapezoidal tank 1.5 L measured 15.2 cm (height), 27.9 cm (top), 22.5 cm (Bottom), and 7.1 cm (width) and divides into two equal horizontal portions by a line draw on the external surface of tank walls.

Method

Zebrafish of either sex, weighing 1-1.2 g were selected and divided into six groups of seven each.

- Group I: Normal control, received vehicle.
- Group II: Scopolamine control, received scopolamine (100 µM/L).
- Group III: Standard, received scopolamine (100 µM/L)+imipramine (20 mg/L).
- Group IV: Received scopolamine (100 µM/L)+BPP 12.5 mg/L.
- Group V: Received scopolamine (100 µM/L)+BPP 25 mg/L.
- Group VI: Received scopolamine (100 µM/L)+BPP 50 mg/L.

The Vehicle, different doses of BPP and standard drug were administered individually by immersion to zebrafish through transferring into 500 ml beaker for 1 hour, once daily for 8 days.

Scopolamine treatment was given individually by transferring into a 500 ml beaker, 30 min before the administration of standard and different doses of test extract.

On the 8th day, after 30 min of drug administration, behavioral parameters were recorded for 5 minutes using a mobile camera and analyzed manually.

The behavioral parameters observed were

- Number of entries to the top
- Number of entries to the bottom
- Time spent in top (s)
- Time spent in bottom(s)
- Total distance travel in tank (m)
- Latency to enter the top (s)

2.5.2. Y-Maze test[23-24]

Principle

Y-Maze spontaneous alternation is a behavioral test for measuring the willingness of zebrafish to explore new environments. Zebrafish typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited. Spatial memory and the response to novelty in zebrafish were assessed using the Y-Maze task. The position in the Y-Maze task wasconsidered as an index of memory. The zebrafish Y-Maze uses the natural tendency of the fish toexplore novelty to assess spatial preference. The response to novelty in zebrafish was assessedusing the Y-Maze test. The position in the Y-Maze test was considered an index of memory.

Method

Zebrafish of either sex, weighing 1-1.2 g were selected and divide into six groups of seven each.

- Group I: Control, received vehicle.
- Group II: Scopolamine control, received scopolamine.
- Group III: Standard, received scopolamine (100 µM/L)+donepezil (10 mg/L).
- Group IV: Received scopolamine (100 µM/L)+BPP 12.5 mg/L.
- Group V: Received scopolamine (100 µM/L)+BPP 25 mg/L.
- Group VI: Received scopolamine (100 µM/L)+BPP 50 mg/L.

The vehicle, different doses of BPP and standard drug was administered individually by immersion to zebrafish through transferring into 500 ml beaker for 1 hour, once daily for 9 days.

Scopolamine treatment was given individually by transferring into a 500 ml beaker, 30 min before administration of standard drug and different doses of BPP (12.5, 25 and 50 mg/L).

On the 8th day, after 30 min of standard and BPP administration, the behavioral parameters were recorded for 5 minutes using a mobile camera and analyzed manually.

The behavioral parameters observed were

- Time spent in novel arm (s)
- Number of entries in novel arm
- Total distance travelled (m)

2.5.2.1. Biochemical parameters assay[25]

All zebrafish were euthanized by immersion in ice water of 2-4°C until loss of opercular motions, and their whole brains were isolated for a biochemical parameters assay. The brains were gently homogenized in ice 0.1 M potassium phosphate buffer (pH 7.4), 1.15% KCl with homogenizer. The resulted homogenate was centrifuged at 2,000 rpm for 15 min. The supernatant was used for the estimation of acetylcholinesterase (AChE) and malondialdehyde (MDA) level.

2.5.2.2. Determination of acetylcholinesterase (AChE)[26]

The method of AChE activity estimation is popularly known as Ellman's method named after George Ellman who developed this method in 1961. The choline esterase activity is measured by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released because of the cleavage of ATC by AChE is allowed to react with the -SH reagent 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitro benzoic acid, a yellow coloured anion with an absorption maxima at 412 nm. The extinction coefficient of the thionitro benzoic acid is 1.36×104/molar/centimeter. The concentration of thionitro benzoic acid detected using a UV spectrophotometer is thentaken as a direct estimate of the AChE activity.

2.5.2.3. Determination of malondialdehyde (MDA)[27]

The content of malondialdehyde (MDA), which is an indicator of lipid peroxidation, was measured via the usage of the approach previously described by Ohkawa et al. 200 µL ofsupernatant was taken and mixed in 0.1 M HCl, 1 mL of 50% trichloroacetic acid and 1 mL of 26 mM thiobarbituric acid. Samples were incubated at 95°C for 20 min after mixing. Samples were then centrifuged for 10 min at 2,000 rpm, and the supernatants were read at 532 nm. The findings were presented as nmol/mg protein.

2.6. Colour-biased appetite conditioning T-Maze test[28]

Principle

T-Maze is an instrument that has been utilized to evaluate spatial learning and memory in rodents and fish. T-Maze has also been applied to evaluate the effect of chemical pollution or the pharmacological effect of drugs in the behavior of animal models. In zebrafish, the T-Maze test is based on food reward or stimulus with the

conspecifics. However, the long training period, which usually lasts from eight to ten days, becomes one of the challenges to perform spatial learning andmemory test in zebrafish. Thus, modifications in the T-Maze method are necessary to increase its effectiveness and efficiency.

Method

Zebrafish of either sex, weighing 1-1.2 g were selected and divided into six groups of seven each.

- Group I: Control, received only vehicle.
- Group II: Scopolamine control, received scopolamine 100 µM/L.
- Group III: Standard, received scopolamine (100 µM/L)+rivastigmine 0.5 µg/L.
- Group IV: Received scopolamine (100 µM/L)+BPP 12.5 mg/L.
- Group V: Received scopolamine (100 µM/L)+BPP 25 mg/L.
- Group VI: Received scopolamine (100 µM/L)+BPP 50 mg/L.

Each group were administered with vehicle, scopolamine, standard and different doses of BPP for a period of 7 days.

Then the zebrafish were trained for 3 days from 8th to 10th day.

On 11th day, the zebrafish were exposed for behavioral testing of time spent by zebrafish in red and green arm and also total number of entries into the green and red arm.

The experimental design of the study was as follows

Day 1 to Day 7=7 days of pre-treatment

Day 8-Day 10=3 days of training.

Day 11=1 day for testing

Training session

In the training session, zebrafish was placed in the terminal of long arm. 1 minute after that, the sliding door was open to allow the fish to swim towards the short arms. Once the fish entered any of the short arms, another sliding door at the junction was closed. The fish was authorized to swim in the short arms and was observed for 4 minutes.

Statistical analysis

The results were expressed in mean±SEM. The data obtained from the study subjected to One way ANOVA followed by Tukey's Kremer Multiple Comparison Test by using Prism Pad 5 software.

3. RESULTS

3.1. Acute toxicity study

The acute oral toxicity of the banana peel powder was carried out on zebrafish. In our study the BPP did not show mortality in 12.5 and 25 mg/L but showed death in 50, 100, 200 and 400 mg/L dose. (Medial lethal concentration (LC 50) is considered as the most accepted basis to determine the acute toxicity). The LC 50 value with 100% confidence intervals of different concentrations of BPP were 12.5, 25, 50 mg/L for 24, 48, 72 and 96 hours.

Table 1. Effect of BPP on oral toxicity in Zebrafish.

Sl. No.	Concentration (mg)	% Mortality	LC 50
01.	12.5	0	140 mg/L
02.	25	0	
03.	50	29	
04.	100	57	
05.	200	100	
06.	400	100	

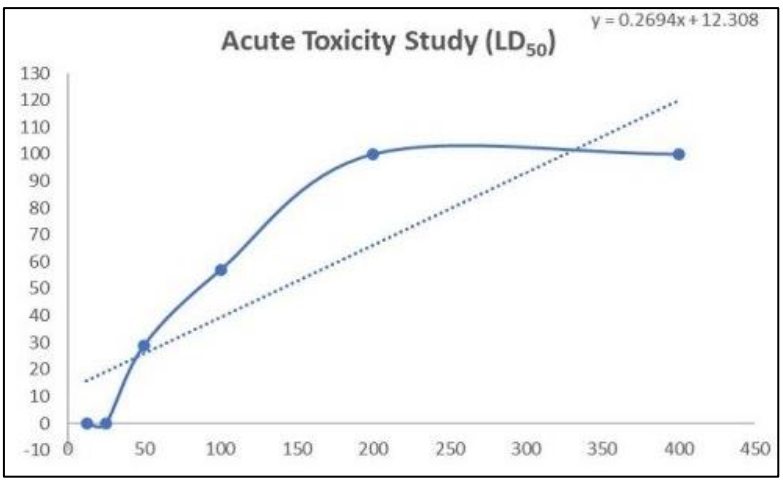

Figure 1. The effect of BPP on oral toxicity in Zebrafish.

The BPP was found to be 100% lethal on 1st day of experiment within 24 hours at the dose of 200 and 400 mg/L since it killed all the seven fishes in the experiment. Whereas the mortality rate of the zebrafish found to be 14.28, 28.57 and 71.42% in acute toxicity study of BPP at doses of 12.5 mg/L, 25 mg/L and 50 mg/L respectively. The LC50 value of the banana peel powder was found to be 140 mg/L which was calculated on concentration dependent mortality during 24, 48, 72, and 96 hours of exposure to BPP. Based on the LC50 value the different doses such as 12.5, 25 and 50 mg/L were selected for the study. The results are presented in Table 1 and Figure 1.

3.2. Effect of BPP in novel tank test (NTT)

In NTT model, the histograms in the Figure 2 indicated that, the various concentrations of BPP showed dose dependent increase in the number of entries to the top, time spent in the top, latency to enter the top and total distance travelled in the tank. Whereas decrease in number of entries to bottom and time spent in bottom as compared to scopolamine control group.

3.3. Effect of BPP on spatial memory and novelty response in Y-Maze

In Y-Maze test, the results depicted in Figure 3 showed that BPP at different concentrations exhibited dose dependent significant increase in number entries to novel arm, time spent in novel arm and total distance travelled in the maze as compared to scopolamine control group.

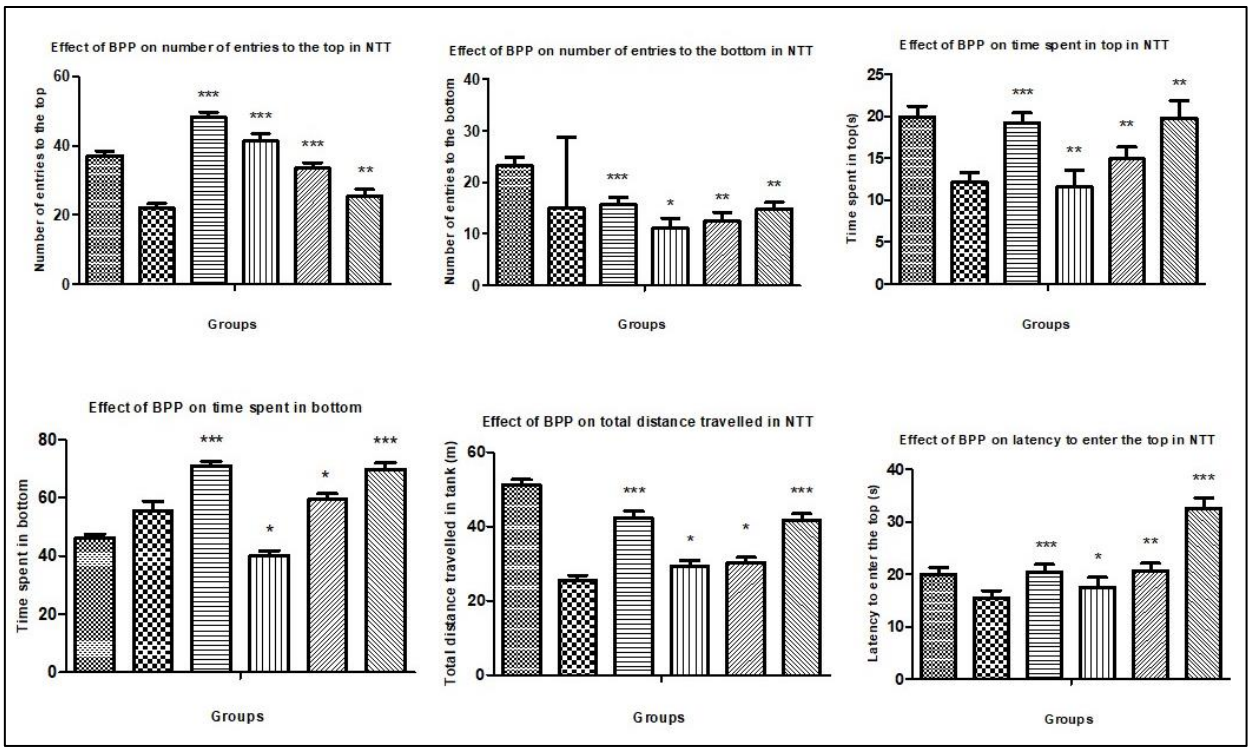

Figure 2. Histograms showing the effect of BPP in Novel Tank Test (NTT).
Values are expressed as mean±SEM, n=7, $^{@}p<0.001$ as compared to normal control group, and $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ as compared to scopolamine control group.

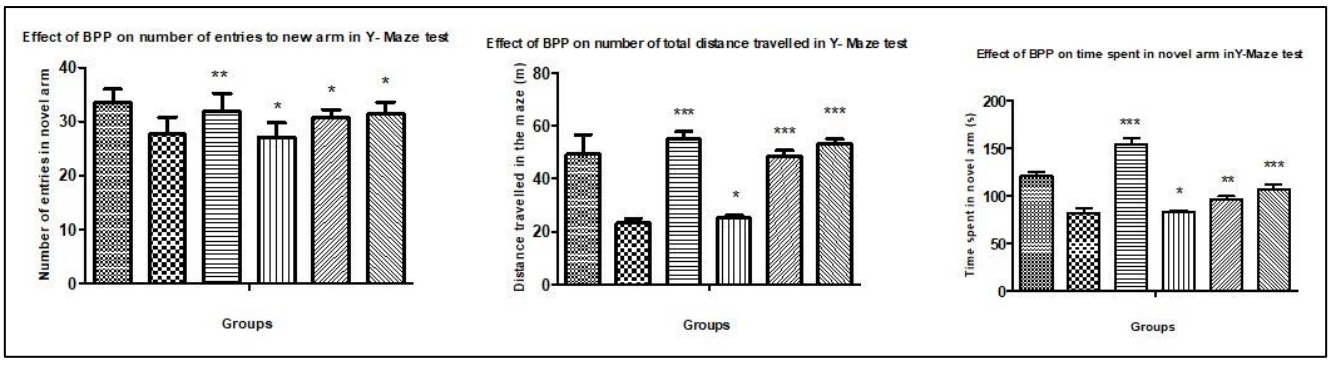

Figure 3. Effect of BPP on spatial memory and novelty response in Y-Maze.
Values are expressed as mean±SEM, n=7, $^{@}p<0.001$ as compared to normal control group, and $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ as comparedto scopolamine control group.

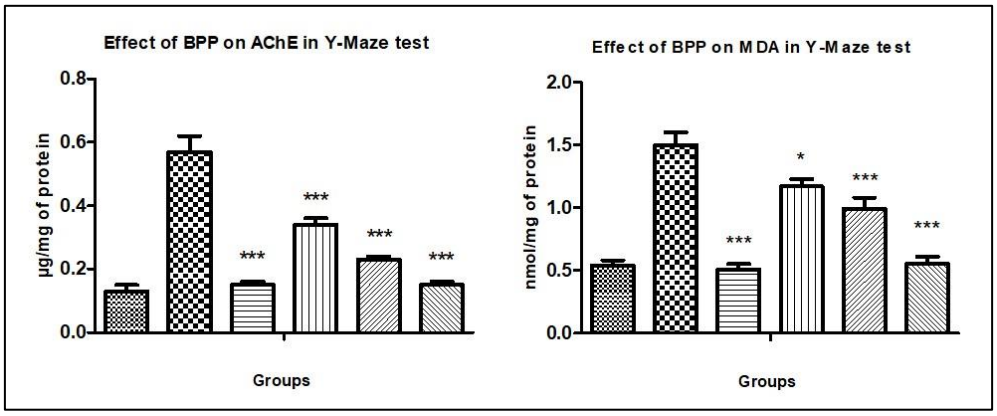

Figure 4. Histogram showing the Effect of BPP on biochemical parameters in Y- maze.
Values are expressed as mean±SEM, n=7, $^{@}p<0.001$ as compared to normal control group, and $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ as comparedto scopolamine control group.

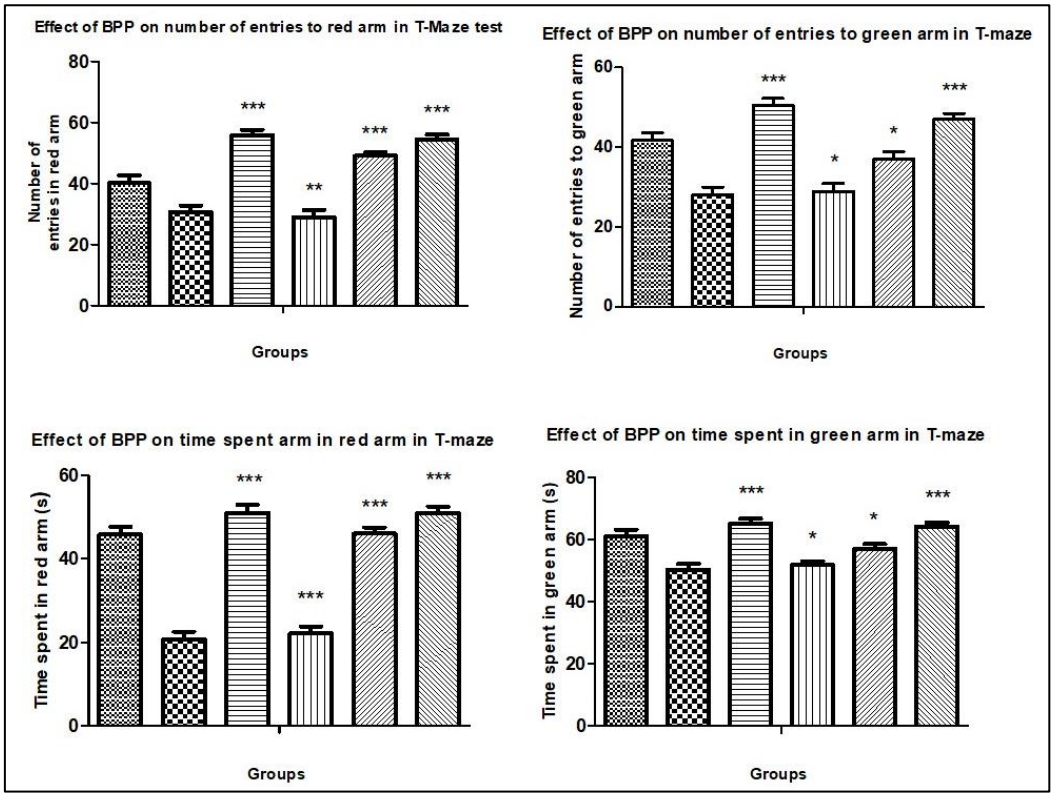

Figure 5. Histogram showing the effect of BPP on spatial memory in T-Maze.
Values are expressed as mean±SEM, n=7, $^{@}p<0.001$ as compared to normal control group, and $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ as comparedto scopolamine control group.

3.4. Effect of BPP on AChE and MDA levels in zebrafish brain homogenate

Results included in the Figure 4 indicated that, various concentrations of BPP showed significant decrease in AChE and MDA content in brain homogenate of zebrafish as compared to scopolamine control group.

3.5. Effect of BPP on spatial memory and novelty response in colour-biased appetite conditioning T-Maze test

The graphical representation of results at various concentrations of BPP exhibited dose dependent significant increase in number of entries to green arm and total time spent in green arm in the maze as compared to scopolamine control group. The results are depicted in Figure 5.

4. DISCUSSION

Treatment of scopolamine at 100 µM/L in group II zebrafish demonstrated anxiety which was evident by a significant decrease in time spent in the top zone of the tank, entrees to top zone, latency to enter the top and total distance travelled as compared to group I zebrafish served

as normal control in NTT. In our study the pretreatment of BPP in zebrafish significantly reversed (increase in the number of entries to the top, time spent in the top, latency to enter the top and total distance travelled in NTT) the scopolamine mediated effects in a dose depended fashion.

In Y-Maze model, the zebrafish immersed with scopolamine at a dose of 100 μM exhibited significant decrease in number entries to novel arm, time spent in novel arm and total distance travelled in the maze as compared to normal control group. In contrast, scopolamine treated fish subjected to pretreatment with BPP demonstrated a significant increase in number entries to novel arm, time spent in novel arm and total distance travelled in the Y-Maze. The effect of BPP at higher dose in novel tank test found to be comparable to the results of the standard drug Donepezil.

Acetyl-cholinesterase is an enzyme known to play a significant role in hydrolysis of Acetyl choline, a crucial cholinergic neurotransmitter[29]. Scopolamine administered zebrafish exhibited a significant increase in the AChE activity when compared to the normal control group. BPP treated zebrafish showed a significant decrease in the AChE activity when compared to Scopolamine alone treated zebrafish and this could be correlated to the improvement of memory parameters, as evidenced in the behavioral approaches.

Scopolamine induced anxiety and amnesia are closely related to increased oxidative stress in the zebrafish brain. Scopolamine administered zebrafish clearly showed increased level of lipid peroxidation (MDA) when compared to the normal control group.

Alternatively, BPP treatment inhibited in a dose-dependent manner, scopolamine-induced oxidative stress in zebrafish by enhancing the antioxidant enzyme activity and suppressingthe lipid peroxidation levels when compared to scopolamine treated animals.

In colour maze model, the zebrafish immersed with scopolamine at a dose of 100 μM exhibited significant decrease in number entries to novel arm, time spent in novel arm and total distance travelled in the maze as compared to normal control group. In contrast, scopolamine treated fish subjected to pretreatment with BPP demonstrated a significant increase in number entries to novel arm, time spent in novel arm and total distance travelled in the Y-Maze. The effect of BPP at higher dose in novel tank test found to be comparable to the results of the standard drug Donepezil.

Many studies have shown that citrus peels exhibit neuroprotective effects[30]. The condensed tannins have been investigated for their effects on cognitive functions in different animal models and improved spatial memory impairments, lipid peroxidation and oxidative stress[31].

Previous studies suggest that administration of tannins as dietary supplement have beneficial effects on blood flow to the brain, hence improves learning and memory. Results of present study also reveal favorable effect on memory therefore it may be suggested that tannins abundantly present in *Musa acuminata* may be responsible for the memory boosting effect of banana peel powder.

5. CONCLUSION

The results of our study conclude that the BPP could effectively improve memory impairments in a scopolamine induced zebrafish model of amnesia by enhancing thefunction of the behavioral response and antioxidant enzymes in the amnesic zebrafish model.

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Conflict of interest
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Ethics approval
Experiments were performed after obtaining Institutional Animal Ethics Committee approval. (BLDEACOP/IAEC/2021/01).

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Author contribution
SG: Involved in carrying out the research work, collection of research articles and typing. SH: Took the lead in writing the manuscript, provided critical feedback on the research and helped in writing the manuscript. VPP: Involved in carrying out the research work, collection of research articles and contributed to sample preparation. SA: Developed the theoretical formalism, performed the numerical simulations, conceived and planned the experiments. LH: Designed the model and the computational framework, analysed the data and helped in writing the manuscript.
NHM: Developed the theory and performed the computations, supervised the findings of work, contributed to the design and implementation of the research, to analyze the results and helps in writing of the manuscript.

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