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Original Article

Evaluation of Neuroprotective Potential of Water-Dispersible Turmeric Extract and Sustained-Release Ashwagandha Root Extract against B[A]P Induced Neurotoxicity in Zebrafish

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ABSTRACT

Background: Numerous advantages of using herbs in the management of neurodegenerative disorders have been documented. Water-Dispersible Turmeric Extract Containing 60% Natural Curcuminoids (WDTE60N) and Sustained-Release Ashwagandha Root Extract (AshwaSR) are the herbal formulations prepared from the extracts of turmeric rhizome and Ashwagandha root, respectively. Benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon, has recently emerged as a neurotoxic compound that impacts zebrafish behaviour and triggers oxidative stress. This neurotoxic effect illustrates the link between oxidative stress and neurodegeneration, as well as the development of anxiety-like behaviours in zebrafish. This study aimed to investigate the neuroprotective potential of WDTE60N and AshwaSR against B[a]P-induced neurotoxicity in zebrafish. **Materials and Methods:** In geometric sequence, 5 different concentrations of each formulation were prepared and administered to zebrafish for 7 days via bath immersion technique. Before the administration of active concentration, fish were exposed to B[a]P for seven days. Neurobehavioral patterns were assessed by using novel tank diving test and light and dark box test. **Results:** Exposure to B[a]P for seven days reduced the locomotor activity of zebrafish and increased their preference for the lighter part of the glass tank. A notable increase in locomotor activity and higher preference for diving towards lower zone of tank was observed in the zebrafish treated with WDTE60N or AshwaSR. The histopathological findings showed that supplementation with these herbal formulations resulted in reduction in congestion of the corpus cerebella region of adult zebrafish brain. **Conclusion:** Collectively, WDTE60N and AshwaSR demonstrated neuroprotective potential against B[a]P-induced neurobehavioral alterations in the zebrafish.

Keywords: Ashwagandha, B[a]P, Neurodegeneration, Neurobehavioral, Turmeric, Zebrafish.

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INTRODUCTION

Benzo[a]pyrene-B[a]P is a colourless polycyclic aromatic hydrocarbon known for its carcinogenicity and environmental pollutant.[1] Exposure to B[a]P causes neurodegeneration in the brain region.[2] It causes oxidative stress and induces neurobehavioral changes.[3,4] Neurodegenerative effects refer to the progressive deterioration of neuron structure and function in the nervous system, consequently leading to the loss of cognitive and motor impairment. Neurodegeneration is a major cause of Alzheimer's, Parkinson's, Huntington's, multiple sclerosis, amyotrophic lateral sclerosis, pontocerebellar hypoplasia, dementia and other related brain disorders.

Zebrafish (*Danio rerio*) is rapidly emerging as a popular non-mammalian vertebrate model in the scientific research. The oxidative stressors in zebrafish closely resemble those in humans, mainly due to their major neuro-mediator system. Approximately 70% of human gene have at least one zebrafish orthologue, making zebrafish an ideal animal model for studying B[a]P-induced oxidative stress.[4-6] Their high physiological and genetic homology to humans makes zebrafish a powerful model for the study of neurological diseases and identification of potential therapeutic targets.[1] There are various brain structures (such as telencephalon, diencephalon, mesencephalon and rhombencephalon) in zebrafish that are homologous to those found in humans.[4,7] Additionally, the hippocampus, amygdala,

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striatum, and basal ganglia are among the cerebral nuclei in the zebrafish brain that exhibit the highest degree of resemblance to those found in mammals.[7] Zebrafish have been shown to possess several key neurotransmitter systems that are present in the human brain, including the glutamatergic, GABAergic, dopaminergic, serotonergic, and cholinergic systems. While the D2 and D4 dopaminergic receptors have 85-95% homology, the D1 and D3 dopaminergic receptors show full amino acid homology in the binding site between humans and zebrafish.[8] These resemblances enhance the utility of zebrafish as a model for studying neural processes and disorders that are relevant to human biology.

The neurotoxic effects of B[a]P in zebrafish highlights the oxidative stress-induced neurodegeneration and anxiety-like behaviours in aquatic animals. Herbal ingredients such as *Curcuma longa* (Turmeric) and *Withania somnifera* (Ashwagandha) have been shown to exhibit neuroprotective effects against the B[a]P-induced neurodegeneration.[4,9-11] This study evaluated neuroprotective effects of two novel plant extracts, Water-Dispersible Turmeric Extract Containing 60% Natural Curcuminoids (WDTE60N) and Sustained-Release (SR) Ashwagandha root extract (AshwaSR), on B[a]P-induced neuronal degeneration in zebrafish model. The effects were compared with the third group which was administered with levodopa as standard. The WDTE60N and AshwaSR are the herbal formulations prepared from the extracts of turmeric rhizome and ashwagandha root, respectively.

MATERIALS AND METHODS

Test products

Test Product 1: Water-Dispersible Turmeric Extract Containing 60% Natural Curcuminoids (WDTE60N).

Test Product 2: Ashwagandha root extract Sustained-Release granules (AshwaSR).

Positive control: Levodopa.

Toxicant: Benzo[a]pyrene (B[a]P).

Chemicals and instruments

Other chemicals used in this study include Dimethyl Sulphoxide (DMSO AR), antifungal solution (methylene blue AR), and formalin 10%. All the above-mentioned chemicals and standard reagent used in the experiment were purchased from Sigma-Aldrich except Test product 1 and 2 which were obtained from Nutriventia Limited.

Instruments used in this study include thermostat, aerator pump, digital thermometer, B.O.D. incubator, digital microscope, siphon tube, aeration tube, filter, and square fishing net.

Preparation of toxicant

B[a]P-induced acute toxicity is reported in zebrafish at concentrations of 16 µg/mL. For the current study, 0.8 µg/mL was selected for induction of neurodegeneration. For inducing neurodegeneration in zebrafish, 4 mg of B[a]P was dissolved in 2 mL DMSO and then added to 5 L of system water.

Preparation of standard (Positive Control)

Levodopa was chosen as positive control. Significant neuroprotection of levodopa is reported at a concentration of 25 µg/mL. Hence, 15 mg of levodopa was added to 5 L of water to prepare positive control.

Preparation of test product concentration

- **Test product 1:** WDTE60N

Five different concentrations of 16 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL were prepared in geometric proportion using water as a solvent system.

- **Test product 2:** AshwaSR

Five different concentrations of 16 µg/mL, 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL were prepared in geometric proportion. Since AshwaSR is not soluble in water, it was solubilized in 2 mL of DMSO followed by water.

Preparation of dechlorinated water

De-chlorination was performed by storing the water in a wide mouth open container and allowed to stand for 48 hr in light sunlight. The UV rays of sunlight will penetrate the chlorine molecule in water and cleave the bonds, ultimately the chlorine getting released in the atmosphere.

In vivo neuroprotective study

Experimental animals

The study protocol was approved by the animal ethics committee of H. K. College of Pharmacy (1612/PO/Re/S/2012/CCSEA) with protocol number HKCP/IAEC/232404. Adult zebrafish (short-fin, 6-8-month-old, 3-4 cm in length, ~50:50 male:female ratio, and weight about 1.0±0.78 g) were collected from the local fish market of Mumbai, Maharashtra, India, and were acclimatized at a CCSEA-approved animal house facility for a period of 2 weeks in four glass tanks (20 fish per tank) of dimension 10×7×9 inches with 5 L of system water. These tanks were well aerated with oxygen and temperature was maintained at 26.5±2°C using RS-30 thermostat. Feeding was done twice a day with commercial fish food [Micro Bits] collected from a local market, and changing of system water was done after every 4 days by syphoning out its 60% water with a syphon tube and replacing it with fresh system water. Zebrafish were assigned into the different experimental groups.

Control group received no treatment. The toxicant group received only B[a]P 0.8 µg/mL for 7 days followed by no treatment served as a negative control. Likewise, B[a]P 0.8 µg/mL -treated adult zebrafish exposed to 1,2, 4, 8, 16 µg/mL of AshwaSR 60N for 7 days were named as group P1, P2, P4, P8, 1nd P16. Groups that were B[a]P-treated adult zebrafish exposed to 1,2, 4, 8, 16 µg/mL of WDTE60N 60N for 7 days were named as T1, T2, T4, T8 and T 16. Standard group received B[a]P 0.8 µg/mL for 7 days followed by 25 µg/mL levodopa for 7 days.

After seven days of toxicant induction with B[a]P, upper view was recorded for 5 min which was uploaded in Toxtrac (A software helpful in neurobehavioral studies for determining the speed of insects based on several factors).

Mass induction of toxicant

Three glass tanks of dimension 10×7×9 inches with 5 L of system water and 20 fish per tank were well aerated with oxygen and temperature was maintained at 26.5±2°C using RS-30 thermostat. Toxicant of concentration 0.8 µg/mL was used for mass induction. A total of 4 mg of toxicant was dissolved using 20 mL of DMSO in a 100 mL beaker and then it is introduced to each tank for 7 days.

Investigational Products (IP) treatment at various concentration

Ten glass tanks of dimension 6×6×6 inches with 600 mL of system water and 6 fish per tank were well aerated with oxygen, and temperature was maintained at 26.5±2°C using RS-30 thermostat. Five glass tanks were labelled for 5 concentrations of WDTE60N and other five glass tanks were labelled for five concentrations of AshwaSR. After preparation of five concentrations of each IP in geometric sequence, its induction started in respective labelled glass tanks for 7 consecutive days, and simultaneously Toxtrac was run for each glass tank per day. The fish were exposed to the beakers containing various concentrations of the drugs for 5 min (exposure via gills) and then replaced in fresh system water. The Toxtrac was run after every exposure to assess the changes in the neurobehavioral pattern.

Neurobehavioral tests

Light and dark box test

A light and dark box test was used to observe the scototaxis behaviour. Generally, zebrafish exhibit a marked preference for darker environments because they are prone to be caught by predators in their natural habitat. In this test, 50% of rectangular glass tank with dimensions of 10×7×9 inches was covered with a black paper, which ultimately resulted in the production of two regions, namely, the light region and dark region. Tank was filled with system water, and fish of the control group, standard group, toxicant group, WDTE60N and AshwaSR treated groups were allowed to swim one by one in the glass tanks. Their preference towards lighter or darker regions of glass tanks was noted.

Novel tank diving test

A novel tank diving test was used to quantify anxiety-like behaviour. In this test, a rectangular glass tank with dimensions of 10×7×9 inches was divided into two zones, namely, the upper zone and lower zone of the glass tank. Upper zone was available with more light rays as compared to the lower zone of the glass tank. Tank was filled with system water and fish of control group, toxicant group, WDTE60N- and AshwaSR-treated groups were allowed to swim one by one in different glass tanks. For further elaboration, videos of the control group, standard (levodopa-treated) group, toxicant (B(a)P-treated) group, WDTE60N, and AshwaSR-treated groups were captured via camera (for accuracy, camera was positioned in a way that it could capture a topical view of the glass tank). Five videos having a duration of 2 min were recorded and uploaded on Toxtrac software for assessment of the following parameters.

Parameters considered in novel tank diving test for evaluation of zebrafish behaviour by employment of Toxtrac software

- **Average speed (mm/sec):** It is the average swimming speed of the organism for the duration of the assessment.[12,13]
- **Exploration Rate (%):** It is the ratio of the area explored by the organism to the total area.
- **Freezing Time (sec):** It is the duration (in seconds) for which the organism remains frozen in place.
- **Total Distance travelled (mm):** It is the total swimming distance of the organisms.
- **Mobility Rate (%):** It is the ratio of the time spent with speed above a certain value to the total time.

Histopathological Assessment

Brain specimens were collected from different groups and fixed in 10% of neutral buffered formalin. The head was removed from the body to allow for easier formalin penetration and then formalin fixed specimen was decalcified using 0.35 M EDTA disodium dihydrate (pH 7.8) solution for 10 days. Decalcification solutions were replaced with new solutions every day. Decalcified specimens were embedded in Paraffin and processed as per standard procedures. Embedded specimens were sectioned at 5 mm thickness using semi-automated rotary microtome and were stained with haematoxylin and eosin (H&E) stain. These slides were observed under digital microscope and images of required regions were captured.[14]

RESULTS

Light and dark box test

The fish in the control group were observed to spend more time in the lighter compartment of the aquarium glass tank; whereas the fish treated with B[a]P exhibited erratic behaviour towards the

lighter compartment of the tank. Upon treatment with various concentrations of WDTE60N and AshwaSR, their entries to the darker compartment were observed to be increased as compared to the B[a]P-treated group alone (Figures 1A and 1B).

Novel tank diving test

Parameters considered for evaluation of zebrafish behaviour are mentioned below:

Average speed: According to the data, the fish administered with AshwaSR have shown higher recovery to the neurodegeneration as apparent from average speed for the duration. The average speed for the ashwagandha group has shown similar recovery to positive control levodopa (Figures 2A and 2B).

Exploration rate (%): The groups of fish receiving the highest concentration of the study products have shown the highest recovery in the exploration rate. This indicates that these groups of fish experienced the least neurodegeneration and the highest tendency to examine surroundings (Figures 2C and 2D).

Freezing time: Freezing time is the time spent by the animal in a motionless state. Freezing time was progressively reduced in the zebrafish treated with increased concentrations of the study products. This indicated recovery from neurodegeneration in these fish (Figures 2E and 2F).

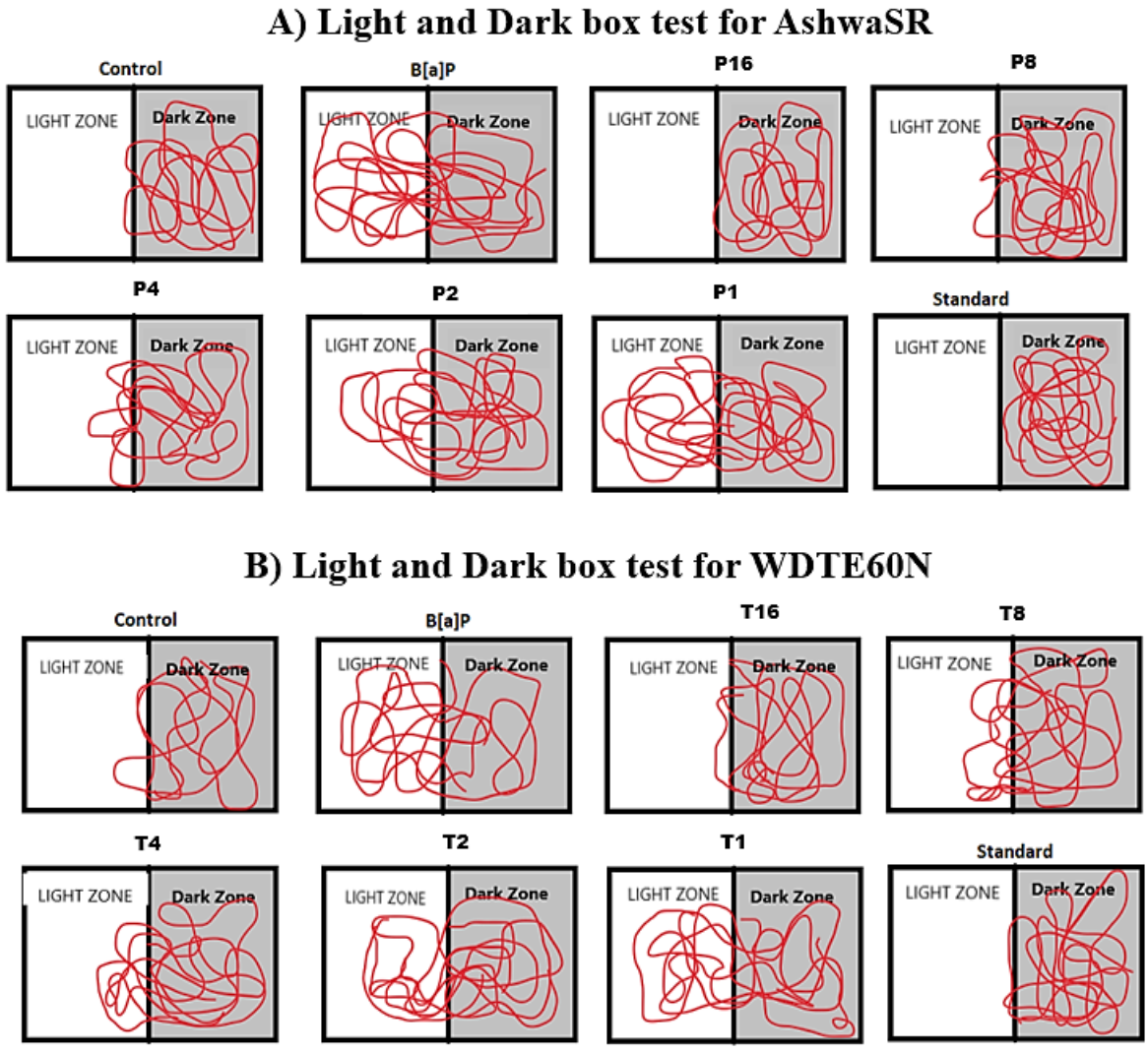

Figure 1: Light and Dark box test for AshwaSR and WDTE60N. AshwaSR, Ashwagandha root extract sustained-release granules; WDTE60N, Water-dispersible turmeric extract containing 60% natural curcuminoids. P1, B[a]P-treated adult zebrafish exposed to 1 µg/mL of AshwaSR 60N for 7 days; P2, B[a]P-treated adult zebrafish exposed to 2 µg/mL of AshwaSR for 7 days; P4, B[a]P-treated adult zebrafish exposed to 4 µg/mL of AshwaSR for 7 days; P8, B[a]P-treated adult zebrafish exposed to 8 µg/mL of AshwaSR for 7 days; P16, B[a]P-treated adult zebrafish exposed to 16 µg/mL of AshwaSR for 7 days; T1, B[a]P-treated adult zebrafish exposed to 1 µg/mL of WDTE60N 60N for 7 days; T2, B[a]P-treated adult zebrafish exposed to 2 µg/mL of WDTE60N for 7 days; T4, B[a]P-treated adult zebrafish exposed to 4 µg/mL of WDTE60N for 7 days; T8, B[a]P-treated adult zebrafish exposed to 8 µg/mL of WDTE60N for 7 days; T16, B[a]P-treated adult zebrafish exposed to 16 µg/mL of WDTE60N for 7 days.

Total Distance travelled: The increase in total distance travelled suggests that the fish have recovered from the neurodegeneration induced by B[a]P. The zebrafish treated with WDTE60N or AshwaSR showed noteworthy improvement in their movement, which is similar to the positive control (Figures 2G and 2H).

Mobility rate: Mobility rate is the ratio of the time spent with speed above a certain value to the total duration of the evaluation. Mobility rate observed in in the zebrafish treated with test products indicated the overall movement improvement and therefore, the recovery from neurodegeneration (Figures 3A and 3B).

Histopathological study

Microscopically, the Corpus Cerebella (CCe) of control group brains exhibited normal histological architecture with intact neurons. Whereas CCe region of toxicant group was found to be congested because of neuronal degeneration. After exposure of levodopa to B[a]P-treated zebrafish, congestion was reduced in CCe region of adult zebrafish brain (Figure 4A).

After treatment with various concentrations of WDTE60N and AshwaSR, congestion was reduced in CCe region of adult zebrafish brain (Figure 4B and 4C). Particularly, after exposure of WDTE60N and AshwaSR, congestion of the CCe region shifted from heavy to mild congestion.

DISCUSSION

This study was conducted in zebrafish to elucidate B[a]P-induced neurodegeneration and to evaluate the neuroprotective activity of two plant extracts, WDTE60N and AshwaSR, at various doses in an aquatic environment. In the present study, exposure to B[a]P for seven days notably reduced the locomotor activity of zebrafish, as evidenced by the novel tank diving test, and increased their preference for the lighter part of the glass tank, according to the light and dark box test. These behavioural changes indicate progressive neuron degeneration in zebrafish compared to both the control and standard groups. Notably, zebrafish co-supplemented with B[a]P and either WDTE60N or

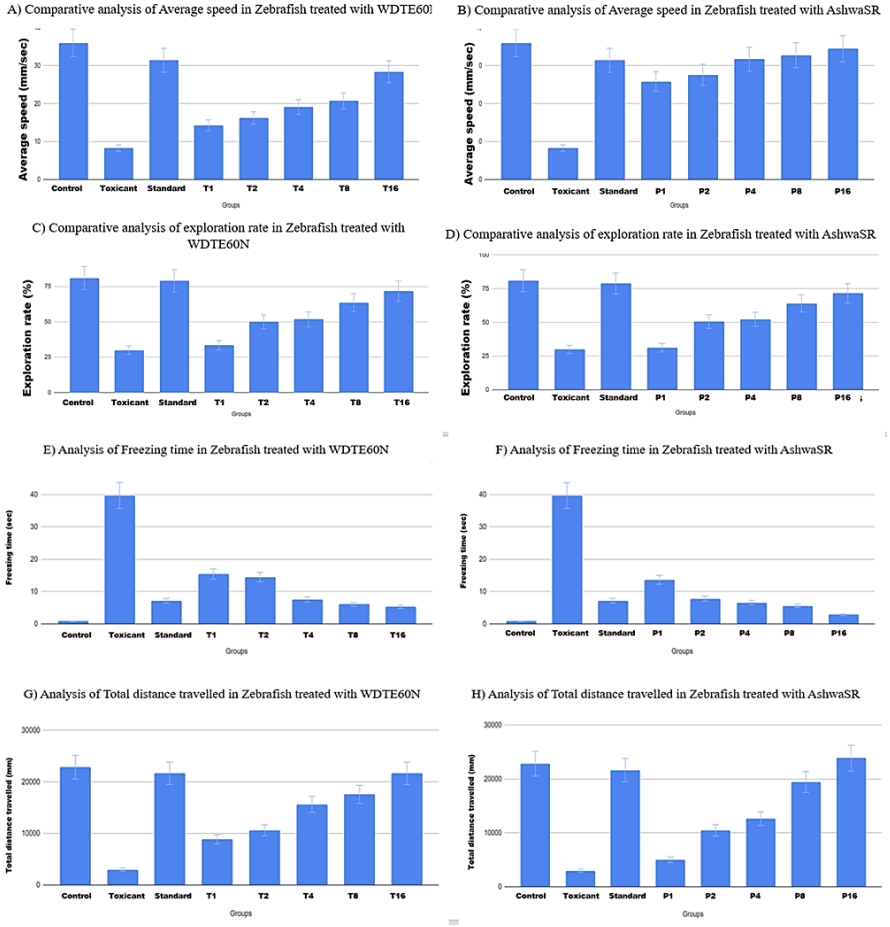

Figure 2: Average speed, exploration rate, freezing time and total distance travelled in zebrafish treated with WDTE60N and AshwaSR. AshwaSR, Ashwagandha root extract sustained-release granules; WDTE60N, Water-dispersible turmeric extract containing 60% natural curcuminoids. P1, B[a]P-treated adult zebrafish exposed to 1 µg/mL of AshwaSR 60N for 7 days; P2, B[a]P-treated adult zebrafish exposed to 2 µg/mL of AshwaSR for 7 days; P4, B[a]P-treated adult zebrafish exposed to 4 µg/mL of AshwaSR for 7 days; P8, B[a]P-treated adult zebrafish exposed to 8 µg/mL of AshwaSR for 7 days; P16, B[a]P-treated adult zebrafish exposed to 16 µg/mL of AshwaSR for 7 days; T1, B[a]P-treated adult zebrafish exposed to 1 µg/mL of WDTE60N 60N for 7 days; T2, B[a]P-treated adult zebrafish exposed to 2 µg/mL of WDTE60N for 7 days; T4, B[a]P-treated adult zebrafish exposed to 4 µg/mL of WDTE60N for 7 days; T8, B[a]P-treated adult zebrafish exposed to 8 µg/mL of WDTE60N for 7 days; T16, B[a]P-treated adult zebrafish exposed to 16 µg/mL of WDTE60N for 7 days.

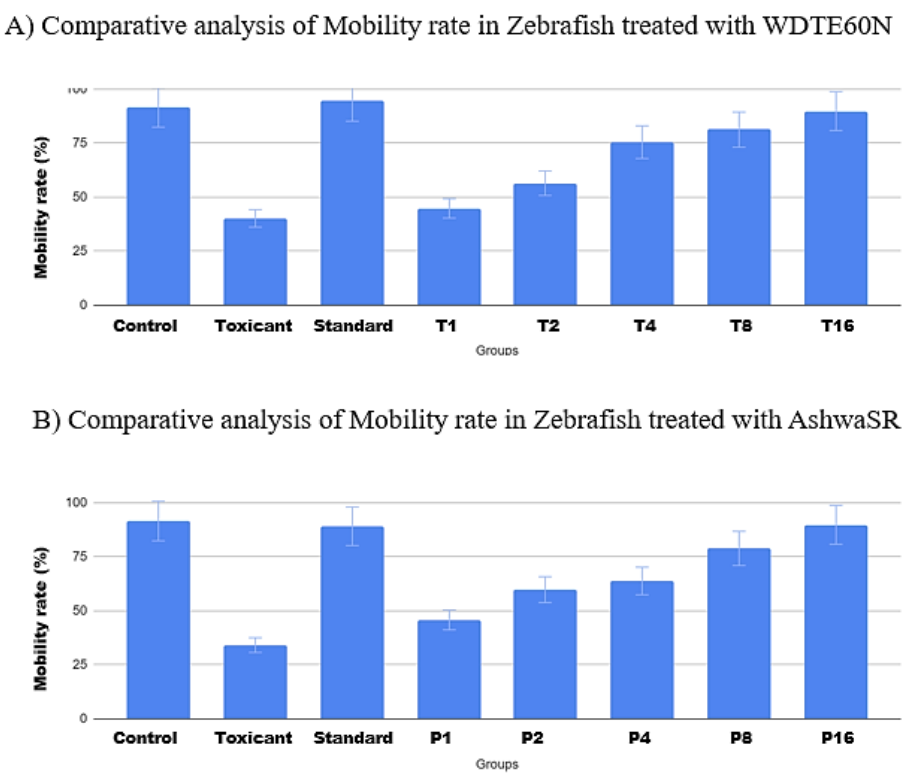

Figure 3: Mobility rate in zebrafish treated with AshwaSR and WDTE60N. AshwaSR, Ashwagandha root extract sustained-release granules; WDTE60N, Water-dispersible turmeric extract containing 60% natural curcuminoids. P1, B[a]P-treated adult zebrafish exposed to 1 µg/mL of AshwaSR 60N for 7 days; P2, B[a]P-treated adult zebrafish exposed to 2 µg/mL of AshwaSR for 7 days; P4, B[a]P-treated adult zebrafish exposed to 4 µg/mL of AshwaSR for 7 days; P8, B[a]P-treated adult zebrafish exposed to 8 µg/mL of AshwaSR for 7 days; P16, B[a]P-treated adult zebrafish exposed to 16 µg/mL of AshwaSR for 7 days; T1, B[a]P-treated adult zebrafish exposed to 1 µg/mL of WDTE60N 60N for 7 days; T2, B[a]P-treated adult zebrafish exposed to 2 µg/mL of WDTE60N for 7 days; T4, B[a]P-treated adult zebrafish exposed to 4 µg/mL of WDTE60N for 7 days; T8, B[a]P-treated adult zebrafish exposed to 8 µg/mL of WDTE60N for 7 days; T16, B[a]P-treated adult zebrafish exposed to 16 µg/mL of WDTE60N for 7 days.

AshwaSR exhibited neurobehavioral improvement. These fish showed increased locomotor activity and a higher preference for the darker part of the glass tank relative to the B[a]P-treated group. Additionally, mitigation of the neuron degeneration was observed following the administration of WDTE60N and AshwaSR. Furthermore, along the subsequent days of the experiment, the WDTE60N- or AshwaSR- treated groups showed remarkable recovery in parameters like locomotor activity and sensory exploration of the tank. In the histopathological study, we investigated the structural changes in the brains of adult zebrafish following exposure to a toxicant, control conditions, and WDTE60N and AshwaSR supplementation, respectively. Post treatment with WDTE60N or AshwaSR, a reduction in congestion of the CCe region of the adult zebrafish brain was observed. These findings support the neuroprotective activity of WDTE60N and AshwaSR against B[a]P-induced neurotoxicity in the brain of zebrafish.

The primary mechanism of B[a]P toxicity in aquatic organisms is believed to involve oxidative stress and the generation of Reactive Oxygen Species (ROS), which ultimately result in lipid peroxidation and neuronal damage.[10,15] Several preclinical studies have demonstrated neuroprotective role of curcumin and ashwagandha against B[a]P-induced neurodegeneration; however, studies involving zebrafish as an animal model are limited.[4,9,11,16]

The WDTE60N is a low-dose, water-dispersible extract ingredient containing 60% natural curcuminoids, designed using patented technology to offer enhanced absorption of curcuminoids at a single 250 mg daily dose. Formulated with nature-sourced excipients, WDTE60N provides a higher concentration of curcuminoids compared to conventional turmeric extracts, which typically contain synthetic excipients and only 6%-20% curcuminoids. The enhanced absorption of curcuminoids in a single low dose of WDTE60N compared to the multiple daily dose of standard 95% turmeric extract has been well established in the two human pharmacokinetic studies.[17,18] The sustained-release profile of AshwaSR, a unique ashwagandha root extract formulation, was confirmed in a pharmacokinetic study. The study demonstrated superior bioavailability of active constituents-withanolides, and a longer elimination half-life following a single daily

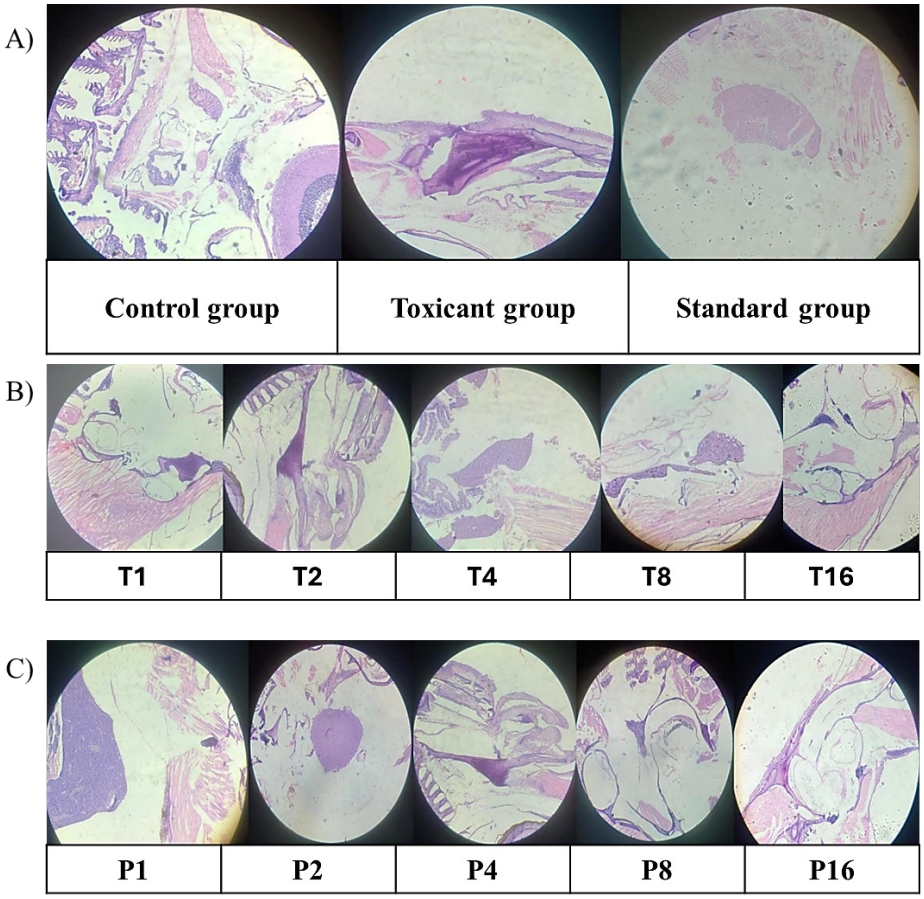

Figure 4: Microscopic view of the corpus cerebella (CCe) of adult zebrafish brain (H&E staining). T1, CCe of adult zebrafish exposed to 1 µg/mL of WDTE60N 60N for 7 days; T2, CCe of adult zebrafish exposed to 2 µg/mL of WDTE60N for 7 days; T4, CCe of adult zebrafish exposed to 4 µg/mL of WDTE60N for 7 days; T8, CCe of adult zebrafish exposed to 8 µg/mL of WDTE60N for 7 days; T16, CCe of adult zebrafish exposed to 16 µg/mL of WDTE60N for 7 days. P1, CCe of adult zebrafish exposed to 1 µg/mL of AshwaSR for 7 days; P2, CCe of adult zebrafish exposed to 2 µg/mL of AshwaSR for 7 days; P4, CCe of adult zebrafish exposed to 4 µg/mL of AshwaSR for 7 days; P8, CCe of adult zebrafish exposed to 8 µg/mL of AshwaSR for 7 days; P16, CCe of adult zebrafish exposed to 16 µg/mL of AshwaSR for 7 days.

dose of AshwaSR, compared to marketed immediate-release Ashwagandha root extract formulation.[19] Therefore, both plant extracts evaluated in this study were novel and this study adds valuable evidence to the available literature involving zebrafish as an animal model and demonstrates neuroprotective potential of these novel nutraceutical supplements, WDTE60N and AshwaSR, against B[a]P-induced neurodegeneration. In light and dark box test, the zebrafish exposed to B[a]P plus *Withania somnifera* leaf extract demonstrated notably higher preference to the darker zone compared to B[a]P group indicating role of ashwagandha leaf extract in ameliorating the behavioural alteration (anxiety-like behaviour) that was induced by B[a]P exposure.[10] Similarly, the authors also reported that ashwagandha supplementation with B[a]P improved the B[a]P-induced neurobehavioral alterations through novel dive tank test and histopathological observations further demonstrated neuroprotective potential of ashwagandha leaf extract.[10] It is important to note that in our study we observed neuroprotective effect of the formulation of root extract of Ashwagandha. A study demonstrated the potential role of curcumin supplement in reducing B[a]P induced anxiety behavioural response and altered antioxidant activity in zebrafish.[9] These two above-discussed studies along with our study corroborates neuroprotective function of turmeric extract and ashwagandha extract against B[a]P-induced neurotoxicity in zebrafish.

CONCLUSION

This study demonstrated that standard doses of WDTE60N and AshwaSR ameliorate the adverse effects of B[a]P. It indicates that these novel herbal formulations offer protection to the brain against B[a]P-induced oxidative stress and subsequent neurodegeneration by maintaining antioxidant concentrations. The study elucidates the neuroprotective roles of curcumin and ashwagandha against B[a]P-induced biochemical and pathological alterations in the zebrafish brain, with AshwaSR being more effective than WDTE60N in counteracting B[a]P-induced neurodegeneration, according to the findings of the current zebrafish model study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This study was funded by Nutriventia Limited.

ABBREVIATIONS

B[a]P: Benzo[a]pyrene; **WDTE60N:** Water-Dispersible Turmeric Extract Containing 60% Natural Curcuminoids; **AshwaSR:** Sustained-Release Ashwagandha Root Extract; **ROS:** Reactive Oxygen Species; **DMSO:** Dimethyl Sulphoxide; **CCe:** Corpus Cerebella.

AUTHOR CONTRIBUTION

All authors have contributed to the work reported; approved the final version to be published; and agree to be accountable for all aspects of the work.

ETHICAL APPROVAL

The study protocol was approved by the animal ethics committee of H. K. College of Pharmacy (1612/PO/Re/S/2012/CCSEA) with protocol number HKCP/IAEC/232404.

SUMMARY

In this study, zebrafish were used to assess the neuroprotective potential of WDTE60N (a bioavailable turmeric extract) and AshwaSR (a sustained-release ashwagandha extract) against neurotoxicity induced by benzo[a]pyrene (B[a]P). The fish were divided into various groups and treated with B[a]P to induce cognitive and motor deficits, followed by administration of WDTE60N, AshwaSR, 7 days. Behavioral assessments, including light and dark preference were conducted to evaluate learning and memory. Results showed that B[a]P caused significant neurobehavioral impairments. Treatment with WDTE60N and AshwaSR, markedly improved behavioral performance. Histopathological examination confirmed the improvement in congestion of the Corpus Cerebella (CCe) when compared to the toxicant group.

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