Neuroprotective Role of Curcumin Against Benzo[a]pyrene-Induced Neurodegeneration in Zebrafish

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Abstract: Curcuma longa L. has been shown to exhibit neuroprotection in the brain. Curcuma longa L. has been used for its neuroprotective effect in humans of neurological disorders. The present study is aimed to evaluate the neuroprotective role of curcumin, a key component of Curcuma longa L., following exposure to waterborne B[a]P. Wild-type adult zebrafish (Danio rerio) were assigned as naïve, control (dimethyl sulfoxide), curcumin, B[a]P (Benzo[a]pyrene) and B[a]P+ curcumin group. B[a]P-induced altered antioxidant levels were enhanced by curcumin in the B[a]P+curcumin group. Findings showed that B[a]P induced anti-anxiety behavioral response and altered antioxidant activity in zebrafish is reduced by curcumin. The periventricular grey zone (PGZ) located on the optic tectum (TeO) in the zebrafish brain regulates anxiety-like behavior. Our histological study showed a significant increase in pyknotic neuronal counts in PGZ of TeO in adult zebrafish brain following B[a]P exposure and was improved by curcumin co-supplementation. Recent findings showed that curcumin improves glutathione production, an antioxidant necessary for maintaining redox homeostasis and shows a neuroprotective role in brain cells. The present study’s findings address the potential role of curcumin co-supplementation as a herbal therapeutic against B[a]P-induced neurotoxicity in zebrafish.

Keywords: curcumin; zebrafish; benzo[a]pyrene; oxidative stress; neurodegeneration; neuroprotection

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1. Introduction

Environmental pollutants are a major threat to living systems. Aquatic ecosystem polluted with polycyclic aromatic hydrocarbons (PAHs) produced by incomplete combustion of organic materials [1,2]. Benzo[a]pyrene is a colorless polycyclic aromatic hydrocarbon known for its carcinogenicity [3]. Exposure of B[a]P causes neurodegeneration and directly acts on the brain [4]. It causes oxidative stress and induces neurobehavioral changes in aquatic organisms [5,6]. B[a]P easily pollutes groundwater and nearby waterways, polluting the aquatic environment [7].

Recently, the neurotoxic potential of B[a]P causing behavioral alterations and oxidative stress in zebrafish has been reported [5,6]. As the aquatic environment provides a sink for environmental pollutants like B[a]P, studies need to be conducted on the fish model to explain the effect of B[a]P on oxidative stress-induced neurodegeneration and anxiety-like behavioral response in aquatic animals [6,8]. B[a]P plays a crucial role in mechanical aspects of oxidative damage to lipid membranes, nucleic acids, and proteins and change in antioxidant status.

The oxidative stressors in the fish model have been found to be similar to the mammal model because zebrafish (Danio rerio) possesses all major neuro mediators systems and
approximately 70% of human genes have at least one zebrafish orthologue, suggesting zebrafish to be an ideal animal model for oxidative stress caused by B[a]P [6, 9-13]. Zebrafish nervous system performs complex functions that are similarly modulated by neurotransmitter molecules in mammals. Zebrafish is an ideal experimental model of the aquatic habitat to study the effect of xenobiotics, neurodegeneration, and behavioral response [6, 14, 15]. Zebrafish possess all vital antioxidant enzymes [16]. It exhibits the same defense mechanism against toxic xenobiotics as mammals, including the body's responses to oxidative stress and defense against toxic and xenobiotic metabolism. Zebrafish behavior on acute exposure to B[a]P has been well studied [6,15]. Previous findings addressed the scototaxis behavior as a measure of anxiety-like character in zebrafish [6, 17-19]. Very little research work addresses the effect of B[a]P on histological parameters of zebrafish as the brain cells are vulnerable to oxidative stress leading to several neurodegenerative diseases in the animals [20]. B[a]P-induced oxidative damage on the brain has been reported by several researchers in different animal models [21]. B[a]P undergoes catabolism inside the body of the living organism into a more potent neurotoxic compound and thus resulting in increased oxidative damage by the generation of reactive oxygen species (ROS) [22,23]. Therefore, therapeutic approaches need to be developed to counteract the hazardous effect of B[a]P on the brain.

Curcuma longa L., also known as turmeric, is used as an important herb in Ayurveda and Siddha medicine to treat several neurodegenerative diseases [6,13]. Some phytochemicals from turmeric exhibit a significant neuroprotective role in various experimental animal models of neurological disorders [24]. Preclinical trials support the neuroprotective role of turmeric against anxiety disorder, oxidative stress-induced neurodegeneration [25,26]. Study shows that Curcuma longa L. can reduce symptoms of anxiety [27,28]. The mechanism of its neuroprotective role is not completely understood. However, Curcuma longa L. has been assumed to act mainly through its antioxidant and anti-inflammatory properties. It is a potent inhibitor of reactive oxygen species (ROS) [29,30]. It also regulates several neurotransmitter levels in the brain. It can normalize specific physiological processes. The antioxidant properties of curcumin are mainly achieved by regulating the brain oxidative damage markers such as glutathione (GSH) and lipid peroxidation.

Our main objective to use the zebrafish model is to address the possible outcomes of increasing contaminants such as B[a]P in aquatic habitats. Increasing pollution in the aquatic system has a direct effect on humans and aquatic life. We have taken zebrafish as our animal model as it has significant genomic similarities with humans and has similar cellular responses to oxidative damage. Therefore, a regulated dose of curcumin provides neuroprotection against B[a]P-induced oxidative stress. Thus, the present study was performed on zebrafish to examine the potential neuroprotective role of co-supplementation of curcumin against B[a]P-induced neurodegeneration.

2. Materials and Methods

The imperative chemicals and standard reagents used in the experiments were purchased from Sigma-Aldrich unless otherwise mentioned.

2.1. Experimental animals.

Adult zebrafish (short-fin, 6–8 month-old, 3-4 cm in length, ~50:50 male: female ratio and weigh about 1.0±0.78 g) were collected from ornamental fish hatchery of the Central
Institute of Freshwater Aquaculture (CIFA), Odisha, India, and were maintained in a 50-L aquarium at room temperature. Tanks were filled with filtered water, and the pH of the system water was maintained at 7.5. The salinity and hardness of water were maintained at 0.1 ppt and 100 mg/L CaCO₃, respectively [6]. They were left for acclimatization for 9 days (48 hours settling-in) and fed twice per diem with commercial fish food collected from a local market. Illumination was provided by ceiling-mounted light tubes on a 14:10 h (day: night) cycle. Hundred zebrafish were assigned into five experimental groups viz. naïve, control (vehicle-treated, i.e., dimethyl sulfoxide (DMSO)), B[a]P, curcumin, and B[a]P + curcumin and maintained in 1-L capacity rectangular aerated glass containers.

2.2.1. Dose standardization of B[a]P.

Waterborne acute toxicity test for B[a]P and dose standardization (0.4 μg mL⁻¹) has already been reported. The neurobehavioral response in adult zebrafish abruptly changed at 1.6 mg/L of B[a]P [31]. Therefore, we have chosen a concentration of 0.4 μg mL⁻¹ for B[a]P exposure to adult zebrafish for 7 days.

2.2.2. Dose standardization of curcumin.

Waterborne acute toxicity test for curcumin and dose standardization has already been reported using a dose-response curve [13]. Median lethal concentration (LC₅₀) was 173.516 μM (63.91 mg L⁻¹) [13]. Therefore, we have chosen a much lower concentration was chosen for neurobehavioral assessment, same as B[a]P concentration. Curcumin was supplemented at a concentration of 0.4 μg mL⁻¹ for waterborne administration for 7 days.

2.3. Neurobehavioral tests.

2.3.1. Light and dark box test.

Light and dark box test (LDBT) is used to quantify the scototaxic behavior. Zebrafish exhibit a marked preference for dark environments. It has already been reported that B[a]P-treated group spent more time in the light compartment as compared with naïve, control (vehicle-treated DMSO), curcumin, and B[a]P + curcumin, and the number of entries to the dark compartment was significantly increased in the B[a]P + curcumin-co-supplemented group as compared to B[a]P treated groups [6].

2.3.2. Novel tank diving test.

A novel tank diving test (NTDT) is also used to quantify anxiety-like behavior. It has already been reported that B[a]P-treated groups spent more time in the top zone of the diving tank compared with naïve, control (vehicle-treated DMSO), curcumin, and B[a]P + curcumin. Contrarily, B[a]P + curcumin co-supplemented group spent significant time in the bottom zone, and latency to enter the top zone was significantly decreased in B[a]P treated group as compared to curcumin and B[a]P + curcumin co-supplemented groups [6].

2.4. Biochemical test.

On completion of the time period of B[a]P + curcumin co-supplementation, zebrafishes were sacrificed, and the brains were removed over ice trays. Tissue homogenate was prepared in a ratio of 1g of wet tissue to 10 times ice-cold 50 mM phosphate buffer saline and processed in Tris-HCl buffer at pH 7.4 to prepare 10% tissue homogenate as the protocol suggested.
previously [32, 33]. The homogenate was used for the estimation of oxidative stress markers and antioxidant activity. 100 μL of homogenate was used to estimate thiobarbituric acid reactive substance (TBARS) [34, 35]. The remaining part of the homogenate was mixed with an equal volume of phosphoric acid (200 μL), centrifuged at 4000 xg (4°C, for 15 min) and the supernatant was incubated with 5, 5′-dithiobis (2- nitrobenzoic acid) and used for GSH assay [36-38].

2.4.1. Estimation of lipid peroxidation.

Lipid peroxidation was measured by observing the formation of thiobarbituric acid reactive substance (TBARS) as per the protocol suggested previously [34]. Briefly, to 100 μL of the sample, an aliquot of brain homogenate, a 3.8 mL thiobarbituric acid reagent, was added and heated in a water bath at 95 °C for 60 min and centrifuged at 12000 xg for 10 min. The pink chromogen formed was measured in a spectrophotometer at 532 nm. TBARS concentration was calculated from its molar extinction coefficient at 1.56 x 10⁵ M⁻¹ cm⁻¹, and the results were expressed as nanomoles of TBARS formed per milligram of protein.

2.4.2. Estimation of glutathione (GSH).

The GSH in brain tissue homogenate was measured as per the procedure suggested previously [36-38]. Briefly, 200 μL of the homogenate was mixed with an equal volume of phosphoric acid (H₃PO₄) and subsequently centrifuged at 4000 xg for 15 min at 4 °C. The supernatants were used for estimation of GSH by 30 min incubation with Ellman's reagent. Readings were taken at 412 nm using a spectrophotometer. The amount of GSH was calculated using a standard curve and expressed in micromoles per gram of tissue.

2.5. Histopathological study by hematoxylin and eosin staining (HES).

Hematoxylin and eosin staining was conducted as per the protocol suggested previously [5]. The whole-brain of zebrafish was collected and isolated at 4 °C followed by the addition of 30 % sucrose solution after the completion of the experimental period of exposure. Then serial cryosectioning of 10 μm thickness was carried out, and HES was done for histopathological studies. The sections were observed under a bright-field microscope, and images of brain sections were taken with 100x magnification (Olympus BX43, Japan made). Neurons showing staining patterns of pyknosis were counted using stereo investigator software (MBF Bioscience, USA). The obtained results were expressed as a percent of control, considering the control value as 100 %.

2.6. Statistical analysis.

All data were presented as mean ± SEM. Results were compared using one-way ANOVA analysis of variance followed by Newman-Keuls post-hoc test for comparisons between naïve, control, curcumin, and B[a]P + curcumin groups. In all the groups, the significance level was considered to be p<0.05.
3. Results and Discussion

3.1. Biochemical assay.

The exposure to benzo[a]pyrene (B[a]P) for 7 days significantly increased lipid peroxidation as compared with the naïve and control group (Figure 1A). The B[a]P + curcumin co-supplemented group showed a significant reduction in lipid peroxidation as compared with B[a]P treated group (Figure 1A). GSH levels also significantly decreased following B[a]P exposure compared with the naïve, control group, and KH groups. Supplementation of curcumin in the B[a]P + curcumin group significantly increased the GSH level when compared with B[a]P (Figure 2A).

![Figure 1](https://biointerfaceresearch.com/)

**Figure 1.** Lipid peroxidation. Graph showing a significant increase in lipid peroxidation (nmol TBARS formed mg⁻¹ of protein) (A) in the whole brain of zebrafish following B[a]P exposure for a period of 7 days. Curcumin supplementation in B[a]P-treated groups significantly decreased lipid peroxidation. Values are expressed as mean ± SEM. *p < 0.05 when compared with the naïve group; *p < 0.05 when compared with the control group; "p < 0.05 when compared with the curcumin group and "p < 0.05 when compared with the B[a]P group.

3.2. Histopathological study hematoxylin and eosin staining (HES).

Hematoxylin and eosin staining of the periventricular grey zone (PGZ) of the optic tectum (TeO) in the zebrafish brain's section showed a significant increase in pyknotic neuronal counts in B[a]P-exposed group as compared with the naïve and control groups (Figure 3A). However, curcumin co-supplementation significantly decreases the pyknotic cell count in B[a]P + curcumin groups as compared with B[a]P groups (Figure 3A). Further observations showed that waterborne B[a]P exposure significantly increases pyknotic neuronal counts in PGZ region, whereas curcumin co-supplementation leads to a reduction in pyknosis, suggesting the neuroprotective efficacy of curcumin (Figure 3B).

The present experiment was conducted on zebrafish to explain B[a]P-induced neurodegeneration, and to prevent these effects, a standardized dose of curcumin was used as a neuroprotectant. Thus, our present study was intended to address the neurotoxic effect of B[a]P, and as a therapeutic measure, we have considered co-supplementation of curcumin in an aquatic environment using zebrafish.
Figure 2. Biochemical assay of GSH level. Graphs show a significant decrease in the concentration of GSH (µM g\(^{-1}\) of protein) (A) in the whole brain of zebrafish following B[a]P exposure for 7 days. Curcumin supplementation significantly increased the antioxidant activity. Values are expressed as mean ± SEM. \(^a p < 0.05\) when compared with the naïve group; \(^b p < 0.05\) when compared with the control group; \(^c p < 0.05\) when compared with the curcumin group and \(^d p < 0.05\) when compared with the B[a]P group.

Figure 3. Neuropathological study by hematoxylin and eosin staining. Graph showing significant changes in the number of pyknotic cells in the PGZ region of TeO of zebrafish brain (A) following waterborne supplementation of B[a]P and curcumin for 7 days. Representative images of a cross-sectional view of zebrafish brain following hematoxylin and eosin staining (B). Arrowheads represent pyknotic neuronal cells in the PGZ region of TeO (B). Magnification and scale bar 100x correspond to 10 µm of different experimental groups. Values are expressed as mean ± SEM. \(^a p < 0.05\) when compared with the naïve group; \(^b p < 0.05\) when compared with the control group; \(^c p < 0.05\) when compared with curcumin group and \(^d p < 0.05\) when compared with the B[a]P group.
A dose for B[a]P for waterborne exposure was chosen (0.4 µL per mL) based on a concentration in the aquatic environment. The dose of curcumin chosen for the present study is 0.4 µL per mL of water which is much lower than the doses that have caused teratogenic effects and embryotoxicity in zebrafish embryos [13, 39-41]. B[a]P has a direct impact on the brain antioxidant defense system and antioxidant activity. Curcumin has neuroprotective role [42]. Curcumin is a powerful anti-aging, anti-anxiety, anti-cancer, anti-inflammatory, and antiviral compound [43-57]. We assumed that curcumin co-supplementation could restore B[a]P-induced oxidative stress in zebrafish.

4. Conclusions

The present study demonstrated that a standard dose of curcumin co-supplementation improves the adverse effect of B[a]P. From the above findings, it is evident that curcumin is capable of protecting the brain against B[a]P-induced oxidative stress leading to neurodegeneration by maintaining the concentrations of antioxidants. The present study elucidated the role of curcumin as a neuroprotective agent against B[a]P-induced biochemical and pathological alternations in the zebrafish brain.

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Conflicts of Interest

The authors declare no conflict of interest.

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