BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2022. Vol. **19**(3), p. 589-600

Quercetin Prevents Bisphenol S Induced Behavioral Changes and oxidative stress in the Zebrafish by Modulating Brain Antioxidant Defense Mechanism

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http://dx.doi.org/10.13005/bbra/3012

(Received: 16 June 2022; accepted: 11 September 2022)

The man-made xenoestrogen bisphenol S has been well-established and associated with developing neoplasm, dementia, neurotoxicity, anxiety, and other adverse effects in people and other organisms. The mechanisms of BPS-induced toxicity have been studied; however, it is unclear if there is any prospect for improvement by natural means. After being exposed to BPS through water, zebrafish (Danio rerio) were employed in this investigation to determine whether quercetin co-supplementation could lessen the compound's destructive potential. Laboratory tests were done to see if quercetin's antioxidant properties may shield the zebrafish brain from oxidative stress and altered behavioral responses brought on by BPS. The available evidence shows that quercetin is beneficial in reducing the abnormal behavioral response brought on by BPS. Quercetin (QU) may have therapeutic potential for reducing oxidative stress caused by BPS, according to biochemical research conducted in the zebrafish brain. In addition, quercetin guards the zebrafish brain against toxicity brought on by BPS. These preliminary findings imply that quercetin, which reduces the generation of reactive oxygen species, would be an effective treatment for BPS-induced toxicity in zebrafish.

Keywords: Bisphenol S; Oxidative Stress; Quercetin (QU); Toxicity; Zebrafish.

Synthetic polymers are used more frequently in producing high-quality plastic and micro-plastic materials due to the rising demand for consumer goods (Abdalla et al., 2013). The uncontrolled release of these compounds into the environment puts human health at risk by increasing the likelihood that serious health issues will develop in the future. Bisphenol S (BPS), a synthetic chemical with a polymeric nature and bisphenol's (BP) counterpart, has been used extensively in manufacturing plastics since its invention in the 1950s. Bisphenol S (BPS) is the chemical utilized most commonly in producing adhesive and polycarbonate resins (Ansari et al., 2009 Barboza et al., 2020). The BPS is more likely found in groundwater and surface rivers from sewage effluent discharge and waste seepage. The BPS contamination has also been detected in human urine and dust, proving the substance pervasiveness in the environment.It is, an endocrine-disrupting

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chemical (EDC), is a potent anthropogenic xenoestrogen due to its estrogenic properties. It might be an endocrine disruptor because of its estrogenic characteristics (Ahn et al., 2015., Bencan et al., 2009). Due to its lipophilic nature, BPS can pass across the blood-brain barrier, the placenta, and even mother's milk (Ben-Jonathan et al., 2016, Calafat et al., 2005, Canesi et al., 2015). Given the contrasting viewpoint, several studies have exposed a connection between BPS exposure and several contrary properties, such as depression, poor cognition, neoplasm, swelling, difficulties with reproduction, and heightened pressure brought on by oxidation. Another concern is that elevated oxidative stress has been connected to several health problems, including cancer, inflammation, aging, and cardiovascular disease. Both "within the living" and "within the glass" models using organs such as the liver, colon, pancreas and testes have been carried out on the effects of BPA and oxidative stress in many research (Ahn et al., 2015).

However, it is still unknown how this stress appears pathologically in the brain up until this point (Bindhumol et al., 2003). The BPS may tempt oxidative stress by increasing the formation of reactive oxygen species (ROS), according to several earlier studies (Cassar et al., 2020, Crain et al., 2007, Das et al., 2020, Denny Joseph et al., 2015, Dong et al., 2014, Egan et al., 2009 and Eid et al., 2015). Therefore, additional research is required into the potential contribution of elevated BPS exposure to the formation of the brain stress pattern upon oxidation (Corrales et al., 2015, Costa et al., 2016). Because BPS is released into bodies of water nearby where people live, there is a significant risk that the public could have serious health issues. Zebrafish *(Danio rerio)* is now regarded as the perfect animal model for a variety of pre-symptomatic studies since it displays a distinctive chain of behavior and responds to dissimilar pharmacological intrusions and stress situations, together with those caused by treatment (Chin-Chan et al., 2015, El-Horany et al., 2016, Ishisaka et al., 2011 and Kajta et al., 2013). Quercetin is a polyphenolic flavonoid that may have anticancer effects. The QU, widely distributed in plant food sources and a significant bioflavonoid in human food, may have tumor suppressor properties by altering the signal transduction pathways mediated by either estimated glomerular filtration rate (EGFR) or estrogen receptors. Even though the process of action is not fully understood, the ensuing effects have been observed with this substance in vitro: decreased expression of mutant P_{53} protein and P_{21}-ras oncogene, activation of cell cycle arrest in the G1 phase, and suppression of heat shock protein synthesis. This chemical also establishes synergy and the capacity to reverse the multidrug resistance phenotype when joined with chemotherapeutic drugs *in vitro*.The QU also possesses anti-inflammatory and anti-allergy properties that prevent the production of pro-inflammatory mediators by inhibiting the lipoxygenase and cyclooxygenase pathways. The ability of quercetin to stop BPS-induced changes in oxidative and behavioral patterns is not well understood. In the present work, zebrafish were employed as an animal model to investigate the detrimental effects of BPS on the brain's antioxidant defense system and the mitigating effect of quercetin on BPS-tempted changes in the zebrafish brain.

METHODOLOGY

Reagents and chemicals

Sigma-Aldrich, Ottochem, India provided all the chemicals and reagents utilized in the extant experimentations.

Experimental animals

Zebrafish aged 5-7 months were purchased from a neighbourhood fish shop in Kolathur, Chennai, and were maintained in a 50-L tank at a persistent temperature of 25 °C. The laboratory was kept on a 12–12 h light-dark cycle for zebrafish preservation.

Bisphenol S (BPS) toxicity testing and dose standardization

The BPS solution was created by liquifying it in 100 percent EtOH and dynamically dissolving the solution to calculate the LC_{50} of BPS. The final EtOH concentration for the BPS dose-response study and acute toxicity testing was made to be 0.003 percent (v/v) in all experimental groups. While the projected lethal value for BPS was 28.28 M, the dose-response study showed that BPS caused 100% death at a concentration of 38.04 M. The results of this experiment showed that a behavioral paradigm shift took place at a dose of 20.52 M after 96 hours, proving the drug's

danger. Therefore, in this investigation, a BPS dosage of 20.52 M was effective for examining zebrafish's nervous system and brain variations. We looked into the consequences of aquatic contact with BPS at a concentration much higher than the environmentally relevant dose because of the rising amount of BPS in the environment.

Acute Tolerance and Dose Tests for Quercetin Standardization

To calculate the LC_{50} and evaluate the preventative dosage of quercetin for its defensive properties against BPS-induced toxicity, a test was conducted to determine the toxicity of quercetin. The quercetin LC_{50} was discovered to be 55.83 M after a dose-dependent analysis. At a level of 12.82 M, the behavioral research showed a sharp swing in its pattern. Because of this, the current study used quercetin at a dosage of 2.96 M to encase its protective properties in zebrafish. Five experimental approaches were used to categorize the zebrafish: naive, control, BPS, quercetin, and BPS+ quercetin. In a 15-liter experimental aquarium, ten mature zebrafish were divided into each group. *Danio rerio* from the relevant, tested groups were treated with BPS and quercetin for 21 days under the experimental paradigm.

Behavioral Evaluation

The light and dark tests are used to evaluate scototaxis behaviour (LDT)

The light/dark preference test reveals that zebrafish strongly prefer darker settings (LDT). LDT was made in the present study after the obligatory 21 days of the experimental setting had passed. After a minute of acclimating in the light zone, the adult zebrafish were relocated separately and individually with a screen door between each zone to the dark chamber of the apparatus. The behavioral changes were noticed once the separating gate was removed, which was recorded on a 5-minute video recorder (Magno et al., 2015, Sera et al., 1999).

Evaluation of exploratory behavior through the use of a Novel Tank Test (NTT)

The typically suggested approach for examining tentative zebrafish behavior is NTT. Zebrafish strongly prefer spending most of their time at the bottom of the experimental tank. In this study, the zebrafish's investigative behavior was evaluated (Bencan et al., 2009, Egan et al., 2009).

Biochemical investigation

Zebrafish were slaughtered, and their brains were detached and kept at 4°C after the studies were over and their behavior had been evaluated. The biochemical examination was carried out three times on the zebrafish brains for each research (Mohanty et al., 2016). The brain samples were thoroughly blended in a glass homogenizer using an ice-cold RIPA buffer at 4°C, followed by a 25-minute incubation period and a 20-minute centrifugation period at 12,000 RPM. The supernatant needed to be collected, divided and kept at -20°C until needed.

Estimation of carbonylated protein

How many carbonyls are left after oxidation depends on protein carbonylation (Mohanty et al., 2016). After removing the 10 percent homogenate, the supernatant was centrifuged at 12,000 rpm for 20 min. The final product was created by combining 0.5 mL of 20 percent trichloroacetic acid and centrifuging it at 11,000 g for 10 minutes at 4°C. To get rid of unreactive chemicals before drying, the pellet was cleaned three times with 1 mL of ethanol-ethyl acetate (1:1). The amount of carbonyls in the protein pellet was quantified at 366 nm using a spectrophotometer. The samples were incubated with 2 M HCl to create a blank test. The aliphatic hydrazone molar extinction coefficient was used to estimate the carbonyl concentration, and the findings were represented as nmole/mg carbonyl.

Peroxidation of Lipids

One distinguishing feature of the peroxidation of lipids in the production of thymidine thiobarbituric acid reactive substances TBARS (Mohanty et al., 2017). 3.8 ml of the anti-TBARS was added to 100 ml of the brain supernatant, which was then centrifuged at 10,000 g for 10 minutes to eradicate any leftover reagent. This mixture was then incubated at 95 °C for 60 minutes to complete the process. A pink chromogen that had just been produced was calculated at 532 nm by a UV spectrophotometer at this point. Moles of TBARS produced per milligram of lipid were used to express the results.

Assay of catalase

Using the method previously described, the catalase enzyme's activity was ascertained. H_2O_2 is broken down by catalase, and the amount

of H_2O_2 broken down was determined using a spectrophotometer at a wavelength of 240 nm for up to 2 minutes. One milligram of protein (mg protein) was used to prompt the catalase activity measured in milligrams. One nano katal (nkatal) is equivalent to one mole of H_2O_2 utilized per second in the reaction mixture.

Determination of GSH level

Low levels of cytosolic oxidative stress in the tissue can be found using tissue glutathione (GSH). This investigation used the previously established method to measure the GSH level in zebrafish brain tissue homogenate. About 200 mL of brain supernatant was combined with the phosphoric acid solution, and the mixture was centrifuged at 4000 g for 15 minutes at 4 °C. The production of supernatant after a 30-minute incubation at room temperature with 5, 5-dithiobis-2-nitrobenzoic acid to calculate GSH. Following this, the amount of GSH present was determined by spectrophotometric measurement at 412 nm and quantified as micromoles per kilogram of the group of cells.

Determination of Glutathione Reductase

The method for measuring glutathione reductase (GR) activity in the zebrafish brain used in this investigation was previously described (Sarkar et al., 2014). A spectrophotometric measurement at 340 nm was made to gauge the degree of variation in reduced form GSSG to GSH, and the degree of change in GSH was estimated. The molar extinction coefficient of NADPH is used to gauge glutathione reductase activity. This value is reported as nmoles NADPH oxidized/min/mg protein.

Determination of Glutathione S-transferase (GST)

The GST activity was measured using a formerly described methodology (Pabst et al., 1974). It was essential to watch the interaction between glutathione GSH and GST to estimate the amount of this biological catalyst (GST) found in brain tissue. At an absorbance of 340 nm, the substrate CDNB (1-chloro-2,4-dinitrobenzene) was estimated. The molar extinction coefficient, a measure of GST activity, was calculated from the GSH-CDNB conjugate. The outcome was expressed as the number of CDNB conjugate nanomoles produced per minute per milligram of protein in the sample (nmole CDNB conjugate).

Determination of SOD activity

The approach developed has been slightly modified by our methods for determining total SOD activity (Beauchamp C 1971). A 100 mL flask was used to hold the reaction mixture, including an aliquot of 100 mL of brain tissue and 2.9 mL of 50 mM Na-phosphate buffer, 2 mM riboflavin, 10 mM EDTA 75 mM Nitro Blue tetrazolium (NBT) , and 13 mM methionine. To analyze its absorbance at 560 nm, further incubation was carried out at 30°C for 10 minutes. In this work, the quantity of sample protein needed to block the NBT completely was given as one unit of SOD enzyme activity.

Statistical analysis

All of the data were signified by the mean and standard error of the mean. One-way analysis of variance was used to compare the results of the various groups, and the BPS and BPS+ quercetin groups and the naive and control quercetin groups were equated using the DMRT test. All groups regarded a *p*-value of 0.05 or less as statistically substantial.

RESULTS

The scototaxis behavior of BPA-induced groups was enhanced by quercetin co-supplementation, which was connected to a decrease in BPS exposure.

After being exposed to BPS under aquatic conditions, zebrafish's scototaxis behavior was considerably changed, as evidenced by the fish spending more time in the lit circumstance than naive or control fish (Fig. 2a & b). Additionally, after BPS exposure, LDT showed a significantly longer latency to enter the black zone than the naive and control groups (Fig. 2c). When compared to the BPS group of zebrafish, quercetin dramatically decreased the scototaxis behavior abnormalities in the BPS+ quercetin group.

After receiving BPS supplements, the antioxidant quercetin aids zebrafish in regaining their bottom-dwelling and exploratory behaviors. Compared to groups 1 and 2, transition to the top zone and time spent in the bottom zone increased in the groups prone to BPS (Fig. 3a & b). Additionally, the BPS-treated group had considerably decreased latency to top zone entry compared to groups 1 and 2 (Fig. 3c). The amount of time spent in the top zone, the frequency of transitions made to

the full area, and the dormancy to reach the top site decreased in the BPS-exposed group when quercetin was added. The results of the present investigation imply that quercetin may protect against behavioural alterations brought on by BPS.

The symptoms of BPS-induced oxidative stress have been demonstrated to be improved by co-supplementing with quercetin.

BPS administration for 21 days significantly raised LPX and protein carbonylation levels compared to other groups (Fig. 4a & b). After exposure to BPS, catalase activity in zebrafish brains was significantly lowered (Fig. 4c). The current study's main findings show that, compared to the control and naive groups, the zebrafish brain's enhanced ROS production caused protein and lipid components to break down. Quercetin supplementation decreased protein carbonylation levels, lipid peroxidation, and CAT activity in zebrafish brains exposed to BPS.

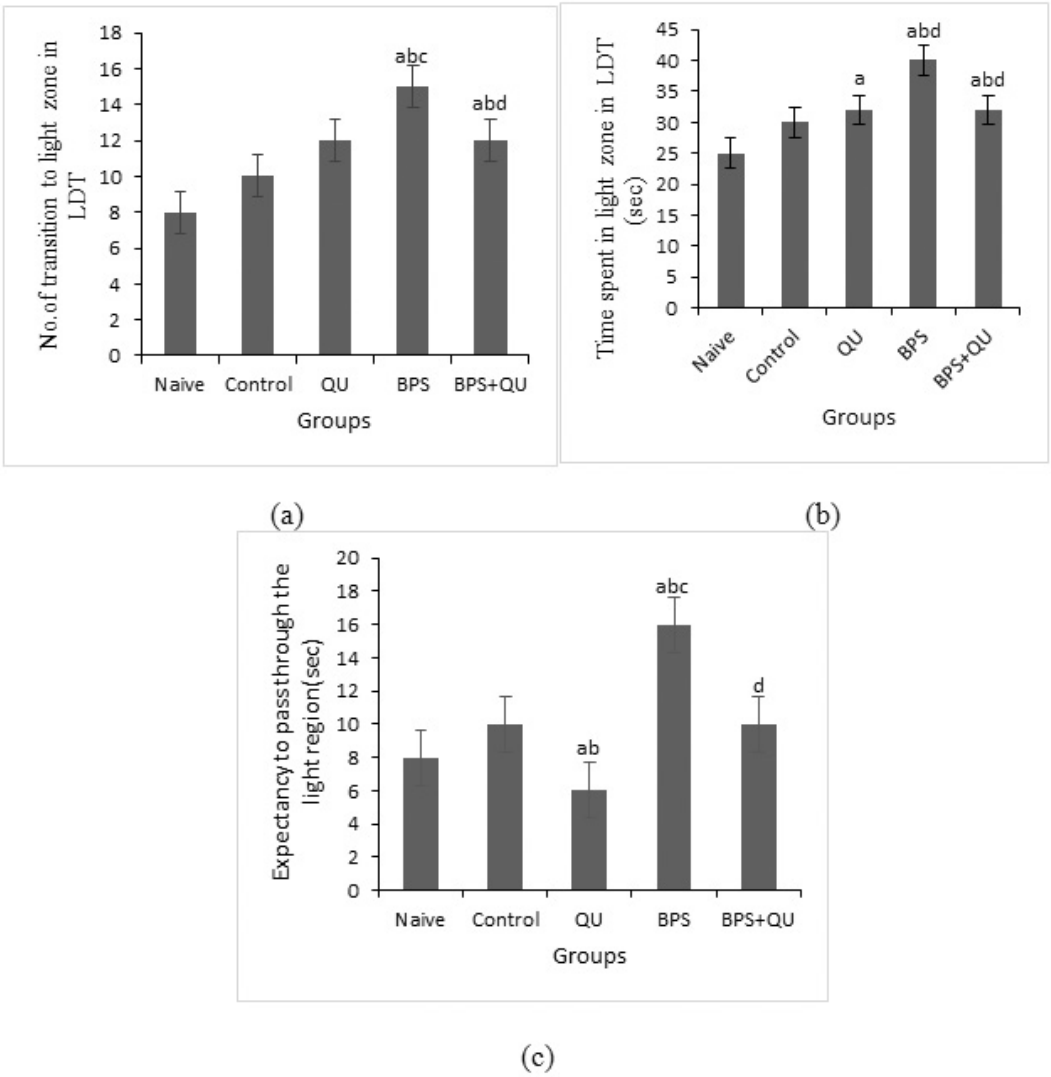

Fig. 1. Test of light and dark (LDT). Graphs showing the frequency of transitions to the bright zone, the length of time spent there, and the likelihood that they will occur after quercetin supplementation and BPS exposure. The values are expressed using the mean and standard deviation. When compared to the naive, control, quercetin, and BPS groups, the letters a, b, c, and d signify $P>0.05$

Quercetin co-supplementation can undo glutathione synthesis alterations brought on by BPS.

In comparison to the naive and control zebrafish groups, the levels of BPS significantly decreased the GR and superoxide dismutase (SOD) actions in the zebrafish brain (Fig. 5a, 5b, 5c, and 5d). The current work suggests that BPS induces oxidative stress in zebrafish brains, which alters antioxidant levels compared to naive and control groups. The protective impact of flavonoids in restoring neuronal redox balance against oxidative stress has been validated by earlier investigations. The function of quercetin as a viable method of action contrary to BPS-induced toxicity has also been hypothesized, along with a standard dose

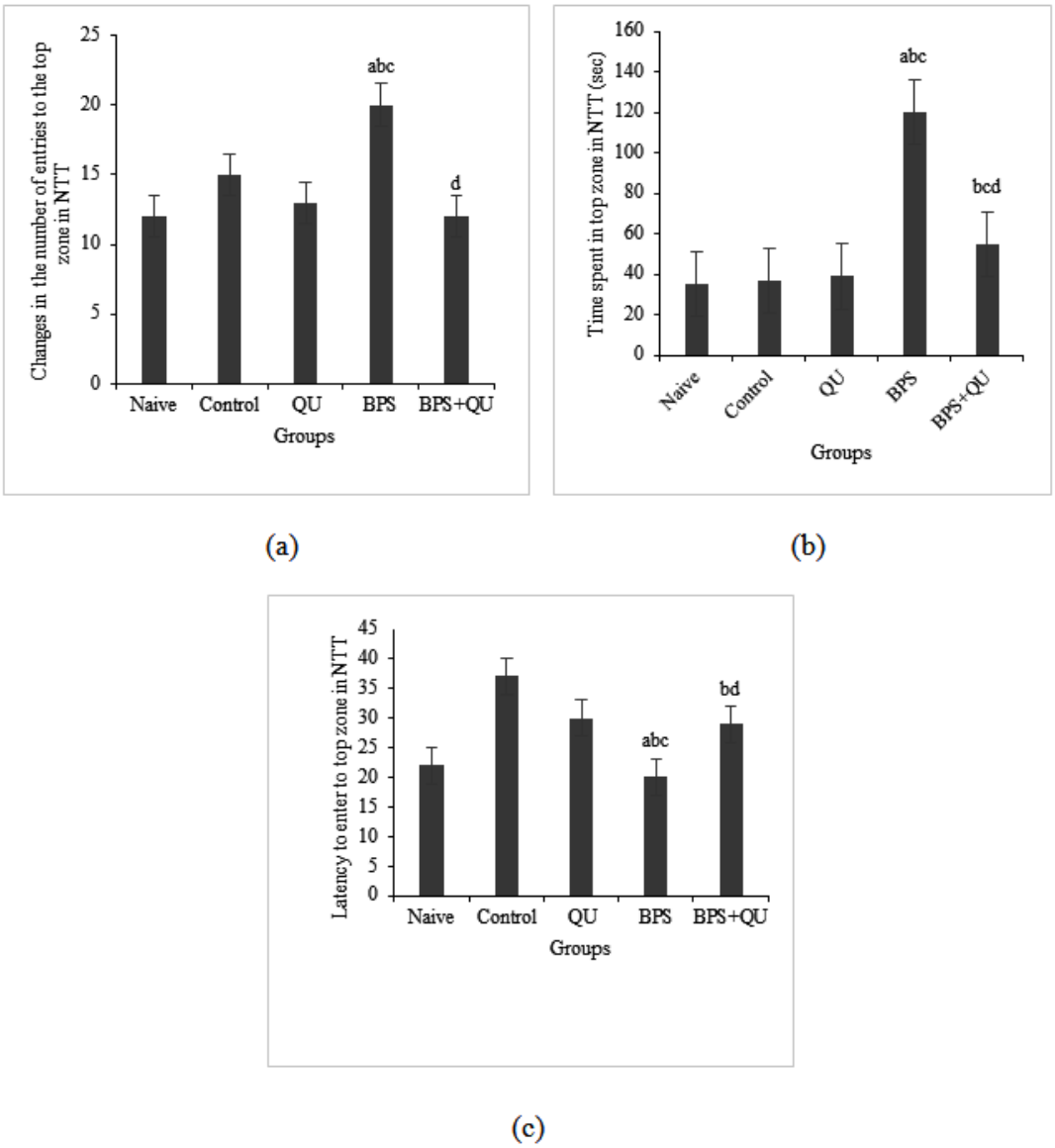

Fig. 2. Test of a novel tank (NTT). [a] Graphs representing deviations in the number of full zone statements, [b] time spent there, and [c] the probability of doing so while being exposed to BPS and quercetin. A, B, C, and D represent p0.05 compared to the naive, control, quercetin, and BPS groups. The values are shown as mean SD

of quercetin for waterborne complementation. According to our research, BPS significantly affects how antioxidant-rich the zebrafish brains are. As a result, it has been demonstrated that quercetin acts as a preventative supplement against oxidative stress brought on by BPS by boosting antioxidants and free radical scavenging enzymes in the cellular environment.

DISCUSSION

The BPS in the surrounding can pose a substantial threat to people's well-being by leading to the emergence of serious health issues. The majority of BPS is a man-made poison. Determining the toxicity of BPS and the protective impact of quercetin on BPS toxicity is the goal of the current investigation. The concentration of BPS chosen

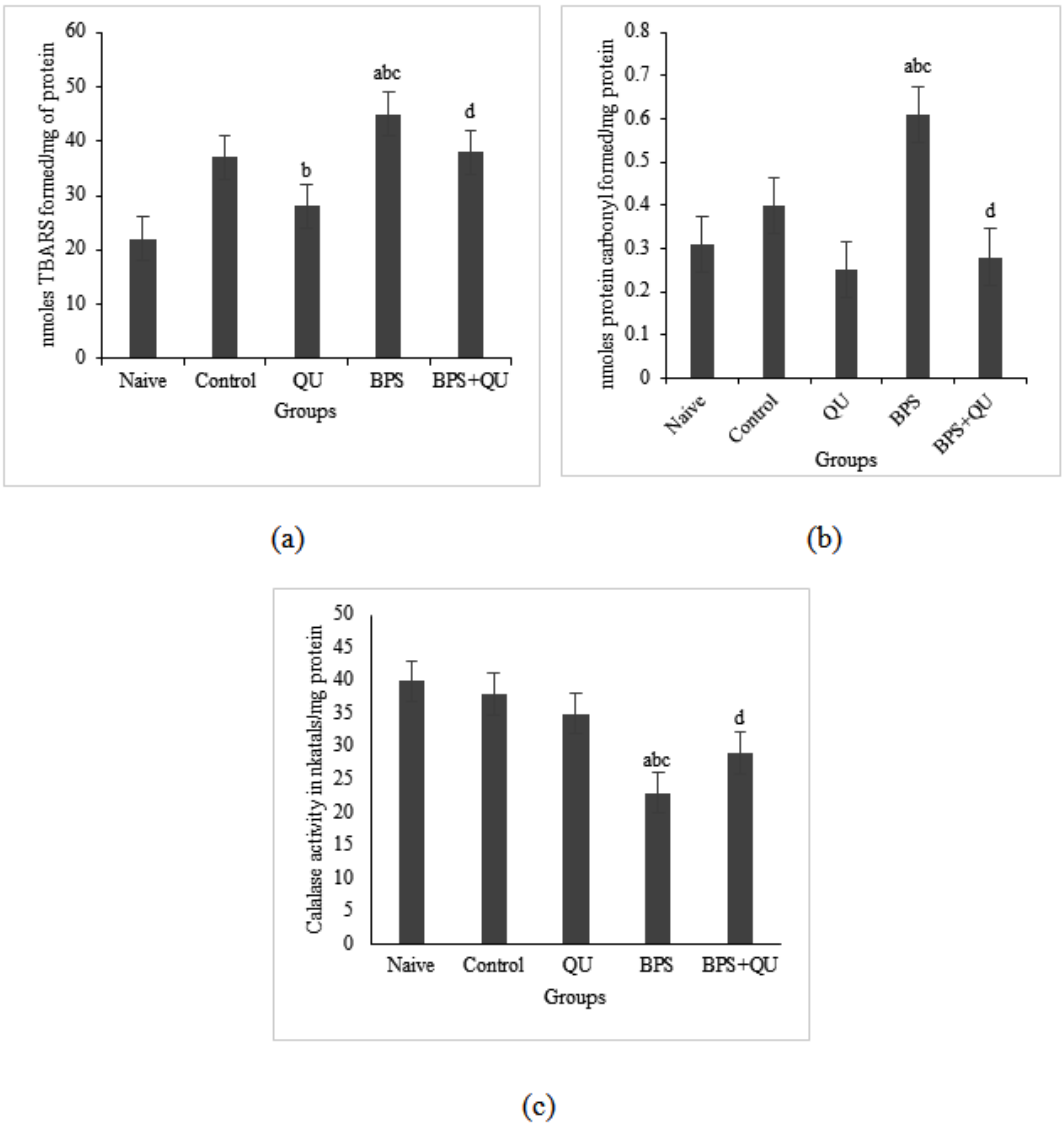

Fig. 3. Check the oxidative stress metrics. Graphs showing the changes in TBARS levels, protein carbonyl content, and catalase activity in the zebrafish brain after BPS and quercetin supplementation. A, B, C, and D represent p0.05 when compared to the naive, control, quercetin, and BPS groups, correspondingly. The values are shown as mean SD

for this investigation was 20.52 M, substantially higher than the environmental significance threshold in water bodies, to examine the effects of high BPS load on zebrafish brains. Additionally, the protective effectiveness of quercetin against BPS-induced toxicity was investigated at a concentration of 2.96 M. The current research shows that quercetin has both enhancing and protective effects on BPS-induced behavioural changes and toxicity (Kang et al., 2006, Kang et al., 2007, Kawato et al., et al., 2004, Kelly et al., 1998, Kuo et al., 2017 and Lorber et al., 2015). Finally, we found that quercetin reduces oxidative damage caused by BPS and restores scototaxis and bottom-dwelling behavior in zebrafish. When compared to the other groups, NTT exposed that co-supplementing quercetin after waterborne BPS exposure dramatically altered the bottom-dwelling behavior of zebrafish. As seen by a significant reduction in the frequency of migration to the highly lighted zone and the amount of time spent in the light zone in LDT, the quercetin administered

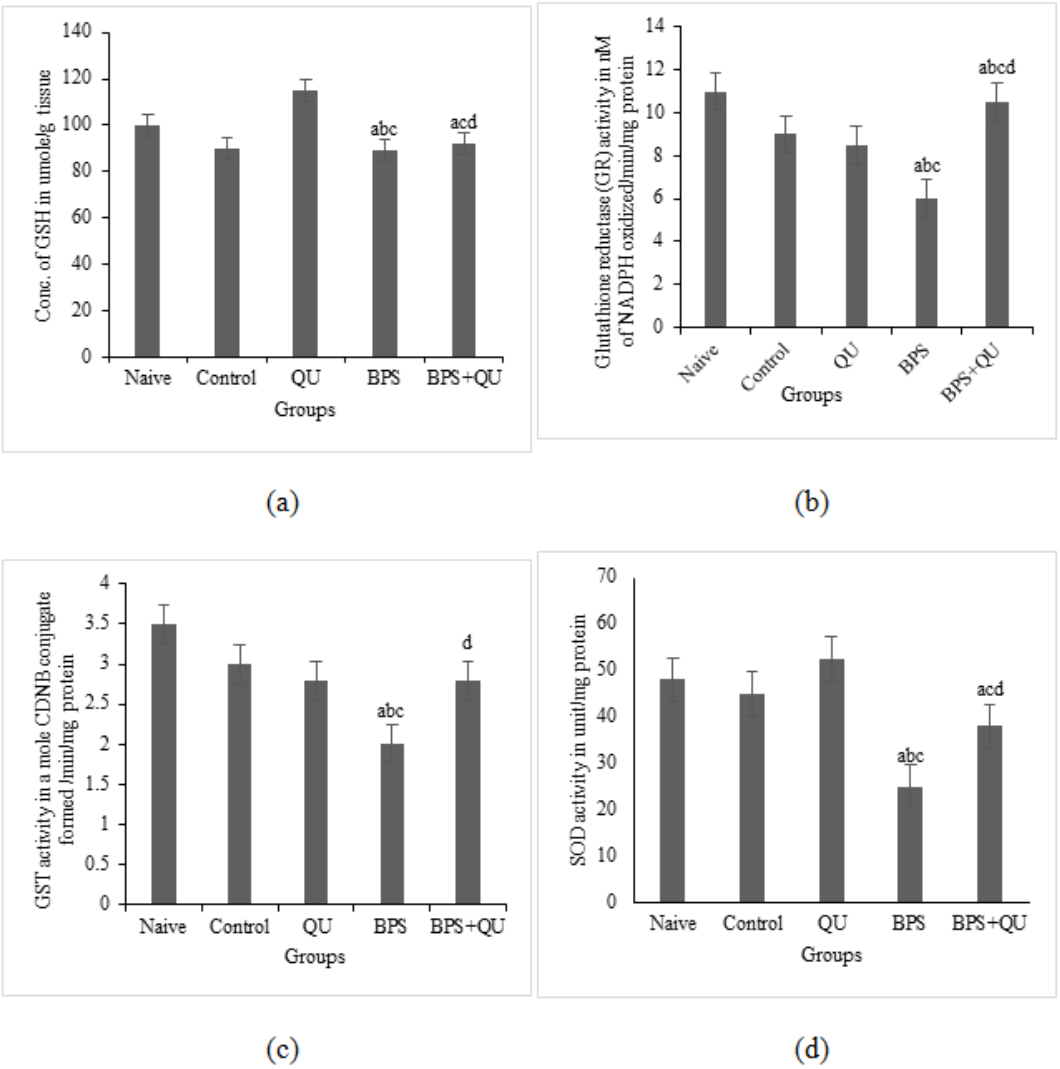

Fig. 4. Check the oxidative stress metrics. Graphs showing the changes in TBARS levels, protein carbonyl content, and catalase activity in the *Danio rerio* brain after exposure to BPS and quercetin. A, B, C, and D represent p0.05 compared to the naive, control, quercetin, and BPS groups. The values are shown as mean SD

group significantly reversed the altered behavioral changes caused by BPS treatment as compared to the other groups (Murata et al., 2018, Nagel et al., 2013, Negri-Cesi et al., 2015 and Nishikawa et al., 2010). It is clear that BPS has the potential to be neurotoxic to zebrafish by altering their scototaxis and bottom-dwelling behavior, as well as from the preliminary evidence (Rennekamp et al., 2015 and Rochester et al., 2013). We monitored the levels of several oxidants and antioxidant enzymes in the zebrafish brain to validate that the BPS-induced reformed behavioural response is due to increased oxidative stress and determine if quercetin may defend against BPS-induced oxidative stress. When given quercetin, it decreased ROS and lipid peroxidation in the zebrafish brain (Sangai et al., 2014). (Sangai et al., 2014). The results and further research revealed that this substance counteracts BPS-induced antioxidant levels and the free radical scavenging enzyme system reducing oxidative stress in the zebrafish cerebral cortex (Winston et al., 1991, Wong et al., 2017 and Xu D et al., 2019). According to this study, long-term ingestion of BPS increased the amount of free radicals produced in the brains of zebrafish while also impairing glutathione reductase activity. Low GSH was present in the zebrafish brain. A proper balance between glutathione synthesis and oxidation is necessary to maintain the cell environment. Glutathione reductase is therefore essential for controlling oxidative stress. The regulation of GSH levels in the zebrafish brain and other tissues may be affected by quercetin's ability to lessen the effects of BPS on glutathione reductase activity in the zebrafish brain (redox balance). The ability of quercetin to control the amount of cytosolic GSH, which is accountable for its protective actions, is assumed to increase its antioxidant potential. According to earlier studies (Eid et al., 2015), our findings indicate that quercetin significantly lowers the rise in LPX in the zebrafish brain, possibly by raising GSH levels and superoxide dismutase activity. Therefore, it follows that instantaneous stimulation of GPX and CAT activity is necessary for oxidative stress defense, which is compatible with SOD's role in superoxide radical detoxification. According to the initial claim, our findings show quercetin significantly reduced the BPA-induced downregulation of CAT activity in the zebrafish brain (Yamazaki et al., 2015; Zhou et al., 2017 and Zimmers et al., 2014). According to our findings, quercetin might be an effective supplement for zebrafish experiencing oxidative stress brought on by BPA, which results in altered behavioral responses. Additional research on the subject was conducted to determine the effectiveness of quercetin as a preventative measure and to learn more about the behavior of scototaxis. According to our research, quercetin co-supplementation significantly lessened the behavioral alterations brought on by BPS exposure. In a nutshell, human populations in developing and underdeveloped nations have recently developed an indiscriminate use of plasticizers (microplastics, including BPA). According to the results of the present investigation, quercetin is more effective and protective against the behavioral modifications, toxicity, and oxidative stress brought on by BPS. We discovered that quercetin decreases oxidative stress brought on by BPS, restores scototaxis in zebrafish, and modifies behavioral alterations. Compared to naive and control groups, the NTT has shown a considerable development in the bottom-life habit of zebrafish (Serra et al., 1999 and Rahal et al., 2014). It is clear from the abrupt decrease in the number of light zone transitions and duration spent in the light zone after BPS treatment that the changed scototaximization behavior in LDT produced by BPS was dramatically inverted by the quercetin exposure. These results provide significant evidence in favor of the theory that quercetin can reduce the harmful potential of BPS in zebrafish by changing their behavior.

CONCLUSION

In conclusion, zebrafish quercetin protects against the brain's toxicity brought on by BPS. A recent study found that zebrafish responded differently to elevated oxidative stress. Quercetin has been demonstrated to successfully scrounge ROS and hydroxy radicals after protracted marine contact with BPS; it is also beneficial when used medically. According to the study's conclusions, quercetin may be utilized to treat oxidative stress and behavioural alterations brought on by BPS. Future studies on signaling cascades may reveal novel therapeutic strategies for treating BPS-induced propensity to severe diseases.

ACKNOWLEDGMENT

The authors would like to acknowledge the input and study design from the corresponding author.

Conflict of interest

The authors declared no conflict of interest.

Funding

This study was not supported by any funding source.

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