Administration of Quercetin Ameliorates Lipopolysaccharide Induced Neuroinflammation and Oxidative Stress in Adult Zebrafish

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Research Article

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Abstract

Background: Quercetin is a natural flavonoid which is known to have numerous pharmacological activities such as antioxidative, anti-inflammatory and neuroprotective effects against various neurological disorders. Lipopolysaccharide (LPS) is a potent endotoxin, reported to cause various neurological disorders such as Alzheimer's Disease (AD), Parkinson's Disease (PD), Multiple Sclerosis (MS), Amyotrophic Lateral Sclerosis (ALS), Stroke (Brain Attack), Meningitis.

Aim: The present study was designed to investigate the possibility thatquercetin ameliorates LPS induced oxidative stress and neuroinflammation in adult zebrafish.

Materials and methods: Zebrafish (weighing 470-530 mg) were treated with single injection of LPS (1 mg/kg) intraperitoneally (*i.p.*) followed by post treatment for 7 days with quercetin (50 and 100 mg/kg; i.p.). After sacrificed, brain was harvested and subjected for biochemical, molecular and histological analyses.

Results: Results revealed post treatment with quercetin was able to ameliorate the behavioral abnormalities as in novel diving test- time spent in top zone (TSTZ), and number of entries in top zone was significantly more as compared to time spent in bottom zone (TSBZ). In light-dark chamber test- time spent in light zone (TSLZ), and number of entries in light zone was significantly more as compared to time spent in dark compartment (TSDC). Additionally, results of histopathology (H & E stain) studies showed less disruption in neuronal cells as compared to LPS treated group. Moreover, results of molecular analysis implies that quercetin treatment significantly decrease TNF-α and IL-1β level as compared to LPS treated animals. Further, results of biochemical analysis reveal that quercetin reduce the level of LPO, nitrite, AChEs and increases anti-oxidant GSH.

Conclusion: Quercetin treatment helps to prevent oxidative damage and neuroinflammation in LPS treated adult zebrafish.

1. Introduction

The word "neuroinflammation" as employed here relates to the central nervous system's (CNS) intrinsic cellular reaction to neurodegeneration. The inflammatory response in the CNS is primarily mediated by microglial cells and astrocytes [1]. Microglial cells are the CNS resident macrophages. They represent around 10–12 % of the CNS population [2]. They are important not only for neurogenesis, neuronal plasticity and regeneration but also as a first line of immune defence in any type of brain injury. They have the capability to phagocytose toxic products, releasing cytotoxic factors and act as antigen presenting cells.Microglial cells are in a "resting" condition in the absence of exogenous stimuli, yet their spidery processes are continually examining the immediate surroundings for changes in the brain milieu without interfering with neurons and neuronal activity [1, 3]. When activated by a brain injury, they alter morphologically, with the ramified processes becoming amoeboid, and they may migrate toward the lesion site [4]. In our ageing society, brain injuries and neurodegenerative diseases like Alzheimer's

disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), are growing increasingly widespread, posing a substantial social and economic burden. Adult mammalian CNS have only a limited capacity for regeneration, which includes the replacement of lost neurons (*de novo* neurogenesis) and the repair of damaged axons (axonal regeneration), traumatic lesions and neurodegeneration drastically reduce quality of life (QoL) and result in severe and frequently fatal impairments [5, 6]. AD is the most well-known cause of dementia, affecting around 50 million people globally [7], whereas PD affected 6.1 million people worldwide in 2016 [8]. The estimated number of persons living withMS globally grew from 2.1 million in 2008 to 2.3 million in 2013 [9], while the global yearly incidence of Amyotrophic lateral sclerosis (ALS) is around 1.9 per 100,000 [10].

Lipopolysaccharide (LPS) is employed in a variety of neurodegenerative diseases, including AD, PD, ALS, and MS, in experimental in vitro and in vivo models of neuroinflammation and amyloidosis that cause systemic inflammation. LPS is a structural component of Gram-negative bacteria's outer membrane that is localised to the outer layer of the bacterial cell wall and released from the bacterial cell surface in non-capsulated strains. It is made up of three parts: lipid A, a core oligosaccharide, and an O side chain [11, 12]. Several studies have shown that LPS causes neuroinflammation by activating microglial cells, which subsequently produce pro-inflammatory cytokines (such as IL-1, TNF-, IL-6, IL-18, and COX-2) [13]. The essential role of LPS in inflammatory responses is first recognised by the TLR-4/CD14 receptor complex expressed in microglia in the CNS, which then stimulates the TLR4/NF-B signalling pathway, which eventually contributes to the secretion of pro-inflammatory cytokines, neuroinflammation, and neurodegeneration [14].

The utility of zebrafish (*Danio rerio*) as a popular laboratory model for genetics, gene function, and development biology is growing worldwide [15]. Despite the obvious distinctions between fish and mammals, zebrafish and humans are genetically and physiologically similar [16].The zebrafish genome contains orthologs of 71% of human genes, as well as a high degree of functional conservation in many of the encoded proteins [17]. The chemistry, cellular populations, and basic anatomical structure of the zebrafish and human nervous systems are all evolutionarily conserved [18, 19]. Thus, zebrafish is considered as a suitable model for studying neuroinflammation.

Quercetin is a common natural polyphenolic flavonoid and it is found in a variety of vegetables and fruits [20]. It has been reported to be used as a nutritional supplement and has anti-inflammatory and antioxidant properties, as well as neuroprotective action [21, 22]. Quercetin has been demonstrated to scavenge reactive oxygen radicals and decrease oxidative DNA damage and LPO in many cell-free experimental systems [23, 24]. In previous studies clearly demonstrated that the quercetin decreased the expression of proinflammatory cytokines (TNF-α and IL-1β) [25].

Therefore, according to previous studies, we hypothesized that *i.p.* injection of quercetin could help to protect against the LPS induced neuroinflammation in adult zebrafish. This study further hypothesized that this protective effect could be mediated via decreased in oxidative stress, inflammation and neuronal damage in the brains of adult zebrafish. Thus, the aims of this study were to identify the effect of

quercetin in LPS induced neuroinflammation and the mechanism underlying its neuroprotective effect, i.e., through amelioration of inflammation, and oxidative stress.

2. Materials And Methods

Animals

Adult zebrafish (3 months old, weighing 470–530 mg) were brought from Aquarts, 26B K Komedanbagan lane, Kolkata, India. Animals were kept in aquarium (94.7L) having temperature 26–27°C with constant aeration and pH (6–7) and were acclimatized by maintaining the experimental room condition. All the animals were kept on a 12 hrs light/dark cycle and were feed twice in a day with commercially available diet (tetrabits). All experiments were conducted in accordance with Institutional Biosafety Committee (IBSC) with approval number ISFCP/IBSC/M1/2020/11.

Chemicals and drug

LPS and Quercetin were purchased from Sigma-Aldrich (St Louis, Mo, India). ELISA kits of Zebrafish TNF-α and IL-1β were purchased from ELK Biotechnology Cat no ELK8512 (Wuhan, China). All other chemicals for biochemical analysis were procured from Himedia and SRL Lmt. All other reagents were of analytical grade and prepared freshly.

Study Design

Before start the experiment, zebrafish were separated in 1L tank with proper aeration and temperature. Total no. of 84 adult zebrafish of both the sexes were used in the study and the animals were divided into different groups as shown in the table (Table 1) with each group having number of animals 12 (n = 12). The seven different groups are (I) Normal group, (II) Vehicle (1% DMSO), (III) LPS treated group, (IV) Quercetin (50 mg/kg) group, (V) Quercetin (100 mg/kg) group, (VI) LPS + Quercetin (50 mg/kg) group, (VII) LPS + Quercetin (100 mg/kg) group. Detailed experimental protocol is shown in the Fig. 1.

Table 1
Animal grouping

S.No.	Groups	Treatment	No. of animals
1.	Control	Normal	12
2.	Vehicle	1% DMSO; *i.p.*	12
3.	LPS	Lipopolysaccharide (1mg/kg; *i.p.*)	12
4.	Quercetin	Quercetin (50mg/kg; *i.p.*)	12
5.	Quercetin	Quercetin (100mg/kg; *i.p.*)	12
6.	LPS + Quercetin	Post treatment quercetin (50mg/kg; *i.p.*) for 7 days	12
7.	LPS + Quercetin	Post treatment quercetin (100mg/kg; *i.p.*) for 7 days	12

On the day of the experiment, the adult zebrafish was treated with single *i.p.* injection of LPS (1 mg/kg) dissolved in 1% DMSO at the 0 day. The fishes in normal group were maintained in normal water under identical conditions. In drug treated group, after LPS exposure, the fishes were treated with quercetin, dissolved in 1% DMSO in concentration of 50 mg/kg and 100 mg/kg for 7 days.

Intraperitoneal injection of LPS and quercetin in Zebrafish

Briefly, each fish was anesthetized by immersion in a tricaine MS-222 solution 100 mg/L until the animal shows lack of motor coordination and reduced respiration rate. Then after the fish was taken out from the solution and placed on a soft sponge of 20 mm height which was saturated with water and set into a petri dish [26]. A cut was made on the sponge of about 10–15 mm deep for holding the fish for injection. Then, *i.p.*, injections were conducted using a 31G Ultra-Fine Hamilton Syringe (Himalaya Scientific, Chandigarh, India) according to the protocol previously described. The needle was inserted into the spines posterior to the pectoral fins in the midline of the abdomen. The whole injection procedure should not take more than 10 sec to ensure animal safety and immediately after the injection the animals were placed in a separate tank with unchlorinated water to facilitate the animal recovery from the anaesthesia. After the injection of LPS and Quercetin, the fish was transferred into a separate tank immediately.

Evaluations of behavioral parameters

Noval diving tank test

The noval diving tank is used to evaluate anxiety and depression types of behaviour in zebrafish which consisted of 1.5 L trapezoidal tank with dimension 19 x 11 x 22 cm (height x length x breadth). The tank was maximally filled with water and divided into two equal virtual horizontal portions by using a marker on the outside walls [27]. The test was performed for a total of 15 minutes, including a 5-minute

acclimatization period and a 10-minute for recording. In this test, we have examined the time spent in top zone (TSTZ), time spent in bottom zone (TSBZ), and the number of entries in the top zones during the experiment.

Light-dark chamber test

The light-dark chamber test is used to analyse spatial memory functions in adult zebrafish[28, 29] whichis made up of Plexiglass with dimension 30 x 16 x 15 cm(length x width x height) and is separated into two equal parts, one part of which is black while the other half is transparent or white in colour. The apparatus was filled with water up to a height of 10cm, and fish were placed in it individually. The test was performed for 15 minutes, with the fish being acclimatised for 5 minutes in the dark and 10 min for recording. In this test, we have measured the time spent in the light zone (TSLZ), time spent in dark compartment (TSDC) and the number of entries in light zone. The light and dark chamber test has been identified as a promising behavioural assay for analysing anxiety-like behaviour in adult zebrafish [30].

Evaluations of biochemical parameters

Tissue preparation

After the behavioural parameters were performed, the zebrafish were anaesthetized with ice cold water at 4^{o}C until the gill movements stopped and they were euthanized. Furthermore, the skull was removed with the help of forceps and micro-dissecting tools. After that, the fish's brains were dissected outand homogenised with 5 ml of 0.1 M phosphate buffer at pH 7.2 in a homogenizing tube [31]. After the homogenate the sample was centrifuged for 10 minutes at 10,000 rpm at 4°C and supernatant was used for further analysis.

Estimation of lipid peroxidation

The lipid peroxidation (LPO) was measured using a procedure previously described by Wills, 1966 [32].Briefly, 0.5ml homogenate and add 0.5ml of Tris HCL were incubated for 2 hours at 37°C. 1 mL 10% tricholroacetic acid (TCA) was added to the incubated solution, followed by 10 minutes of centrifugation at 1000 g. 1 ml of 0.67 percent thiobarbituric acid (TBA) were added to 1 ml supernatant, and tubes were put in a boiling water bath for 10 minutes before adding 1 ml of double-distilled water, and the level of LPO was measured at 532 nm absorbance using a Shimadzu spectrophotometer. The final values were calculated using the chromophore's molar extinction coefficient 1.56 x 10^{5}M-1cm^{-1} and expressed as nmole of MDA per mg protein.

Estimation of Glutathione

Reduced glutathione activity was estimated by a method described by Ellman et al., 1959 [33]. The homogenate was mix with 1 mL of 4% sulfosalicylic acid, then after the sample were centrifugation at 1200 g for 5 minutes at 4°C. After centrifugation, 1 mL of supernatant was added to a test tube with 0.2 mL of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) and 2.7 mL of phosphate buffer (0.1M, PH 8) and absorbance was measured by Shimadzu spectrophotometer at 412 nm. The enzyme activity was expressed in µmol per mg protein.

Estimation of Nitrite

Nitric oxide production was determined in the brain homogenate supernatant based on Greiss reagent [0.1% Naphthylethylene diamine dihydrogenchloric acid, 1% Sulphanilamide in 5% phosphoric acid], and mixed and kept at room temperature for 5 minutes and absorbance was measured by Shimadzu spectrophotometerat 540 nm. The concentration of nitrite was expressed as µmol/mg protein.

Estimation of protein

Protein estimation was done by Biuret method (Gornall et al., 1949). 0.1ml of tissue homogenate supernatant, 2.9 ml NaCl and 3 ml biuret working reagent were added and kept at room temperature for 10 minutes. The absorbance was measured by Shimadzu spectrophotometer at 536 nm [34].

Measurement of AChEs activity

The AChEs enzyme activity was estimated by Ellman method [35]. The assay mixture contained 0.05ml of supernatant, 3ml of 0.01M Sodium phosphate buffer (pH 8), 0.10ml of ACh iodide and 0.10ml of 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman reagent). The change in absorbance was measured immediately at 412nm using Shimadzu spectrophotometer. Results were expressed as micromoles of acetyl thiocholine iodide hydrolyzed/min/mg of protein.

Estimation of pro-inflammatory cytokines (TNF-α and IL-1β) levels

The pool of 3 brain tissues was homogenized and prepared in PBS (mM) (50 NaCl, 18 Na2HPO4, 83 NaH2PO4.H2O, pH 7.4), containing 1 mM EGTA and 1 mM PMSF, followed by centrifugation at 1000 ×*g* for 5 min at 4°C. This assay was carried out in 100 µL of supernatant, by using TNF-α and IL-1β ELISA kits according to the manufacturer's protocol (ELK Biotechnology Cat no ELK8512 (Wuhan, China)[36].

Histopathological analysis

Animals were sacrificed by decapitation immediately after the last behavioral test. The brain were removed and transferred to formalin (10 % v/v). The brain tissues were embedded in paraffin blocks and sectioned into 3 mm thickness with the help of a microtome. The brain sections (5–10 µm) thick were de-waxed and stained with H & E [37]. The stained sections were examined at 40X under a fluorescence microscope (Model: 102 M, Motic microscope, China).

Statistical Analysis

GraphPad Prism 5.0 software for Windows (GraphPad Software, San Diego, CA, USA), was used to analyse all data, which was presented as mean ± SEM.Values were expressed as the mean ± SD. The behavioural, biochemical and neuroinflammatory assessment data was evaluated using one-way analysis of variance. Post hoc comparisons between groups were made by using Tukey's test. A value of $p < 0.05$ was considered statistically significant.

3. Results

1. Effect of quercetin treatment on novel diving tank in LPS treated zebrafish

The normal, vehicle and quercetin (50, 100 mg/kg) per se group didn't show any significant effect in novel diving test.Figure 2 A shows that single injection of LPS (1 mg/kg) significantly affected the zebrafish behaviour, as TSTZ was significantly ($p<0.001$) less as compared to normal group. However, treatment with quercetin (50 mg/kg and 100 mg/kg) significantly reverse the effect produced by the LPS by spending more time in top zone as compared to LPS treated animals ($p<0.001$). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly greater in ameliorating the TSTZ when compared to the effect of quercetin (50 mg/kg) ($p<0.01$).

Figure 2 B shows that single injection of LPS (1 mg/kg) significantly affected the zebrafish behaviour, as TSBZ was significantly ($p<0.001$) more as compared to normal group. However, treatment with quercetin (50 mg/kg and 100 mg/kg) significantly reverse the effect produced by the LPS by spending less time in bottom zone as compared with LPS treated group ($p<0.001$). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly attenuated the TSBZ when compared to the effect of quercetin (50 mg/kg) ($p<0.01$).

Figure 2 C shows that single injection of LPS (1 mg/kg) significantly affected the zebrafish behaviour, as number of entries in the top zone was significantly ($p<0.001$) less as compared to normal group. However, treatment with quercetin (50 mg/kg and 100 mg/kg) significantly reverse the effect produced by the LPS by increasing number of entries in top zone as compared with LPS treated animals ($p<0.001$). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly greater in ameliorating this effect when compared to quercetin (50 mg/kg) ($p<0.01$).

2. Effect of quercetin treatment on light-dark chamber test in LPS treated zebrafish

The normal, vehicle and quercetin (50, 100 mg/kg) per se group didn't show any significant effect in light and dark chamber test. Figure 3 A shows that single *i.p.* administration of LPS (1 mg/kg) significantly affected the zebrafish behaviour, as TSLZ was significantly ($p<0.001$) less as compared to normal group. However, treatment with quercetin (50 mg/kg and 100 mg/kg) significantly reverse the effect produced by the LPS by spending more time in light zone as compared to LPS treated animals ($p<0.001$). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly greater in ameliorating the TSLZ when compared to the effect of quercetin (50 mg/kg) ($p<0.01$).

Figure 3 B shows that single *i.p.* injection of LPS (1 mg/kg) significantly affected the zebrafish behaviour, as TSDC was significantly ($p<0.001$) less as compared to normal group. However, treatment with quercetin (50 mg/kg and 100 mg/kg) significantly reverse the effect produced by LPS by spending less time indark compartment as compared to LPS treated animals ($p<0.001$). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly reduce in ameliorating the TSDC when compared to the effect of quercetin (50 mg/kg) ($p<0.01$).

Figure 3 C shows that single injection of LPS (1 mg/kg) significantly affected the zebrafish behaviour, as number of entries in light zone was significantly ($p<0.001$) less as compared to normal group. However, treatment with quercetin (50 mg/kg and 100 mg/kg) significantly reverse the effect produced by the LPS by increasing number of entries in light zone as compared with LPS treated animals ($p<0.001$). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly greater in ameliorating this effect when compared to quercetin (50 mg/kg) ($p<0.01$).

3. Assessment of oxidative stress markers

3.1 Effect of quercetin treatment on LPO level in LPS treated zebrafish

Figure 4 A shows, the level of LPO in normal, vehicle and quercetin (50, 100 mg/kg) per se group didn't show any significant effect. Single injection of LPS (1 mg/kg) treated adult zebrafish showed significantly increased oxidative stress due to increase in LPO level as compared with normal group ($p<0.001$). However, treatment with quercetin (50, 100 mg/kg) for 7 days significantly reduced LPO level as compared to LPS treated group ($p<0.001$). Moreover, quercetin (100 mg/kg) showed significantly reduced the level of LPO as compared with quercetin (50 mg/kg) treated group ($p<0.01$).

3.2 Effect of quercetin treatment on nitrite level in LPS treated zebrafish

Figure 4 B shows, in normal, vehicle and quercetin (50, 100 mg/kg) per se group didn't show any significant effect on nitrite level. Single *i.p.* administration of LPS (1 mg/kg) treated brain of adult zebrafish showed significantly raised in nitrite level as compared with normal group ($p<0.001$). Moreover, treatment with quercetin (50, 100 mg/kg) for 7 days significantly reduced the level of nitrite as compared with LPS treated group ($p<0.001$). Furthermore, quercetin (100 mg/kg) showed significantly reduced nitrite level as compared with quercetin (50 mg/kg) treated group ($p<0.01$).

3.3 Effect of quercetin treatment on AChEs level in LPS treated zebrafish

Figure 4 C shows, the level of AChEs in normal, vehicle and quercetin (50, 100 mg/kg) per se group didn't show any significant effect. Single injection of LPS (1 mg/kg) showed increased the level of AChEs as compared with normal group ($p<0.001$). Increased AChEsis associated with memory impairment. After treatment with quercetin (50 and 100 mg/kg) for 7 days significantly reduced memory impairment as decrease in AChEs level as compared to LPS treated group ($p<0.001$). Moreover, quercetin (100 mg/kg) showed significantly decreased AChEs level as compared with quercetin (50 mg/kg) treated group ($p<0.01$).

3.4 Effect of quercetin treatment on GSH level in LPS treated zebrafish

Figure4 Dshows, the GSH level in normal, vehicle and quercetin (50, 100 mg/kg) *per se* group didn't show any significant effect. The GSH level in the brain of adult zebrafish treated with a single intraperitoneal injection of LPS (1 mg/kg) was significantly lower as compared to normal group ($p<0.001$). Furthermore, compared to the LPS-treated group, treatment with quercetin (50, 100 mg/kg) for 7 days significantly raised the level of GSH ($p<0.001$). Furthermore, quercetin (100 mg/kg) showed significantlyincreased GSH level as compared with quercetin (50 mg/kg) treated group ($p<0.01$).

4. Effect of quercetin treatment on TNF-α, IL-1β level in LPS treated zebrafish.

Figure 5 shown, in normal, vehicle and quercetin (50, 100 mg/kg) per se group didn't show any significant effect on TNF-α andIL-1β. Figure 5 A shown, Single injection of LPS (1 mg/kg) in zebrafish brain showed a significant increased level of TNF-αin adult zebrafish brain as compared with the normal group ($p < 0.001$). However, treatment with quercetin (50 and 100 mg/kg) has significantly reduced the level of TNF-

αas compared with the LPS treated group ($p < 0.001$). Moreover, quercetin (100 mg/kg) showed significantlydecreased level of TNF-α as compared withquercetin (50 mg/kg) treated group ($p<0.01$).

Figure 5 B shown, Single injection of LPS (1 mg/kg) in zebrafish brain showed a significant increased level of IL-1β in adult zebrafish brain as compared with the normal group ($p < 0.001$). However, treatment with quercetin (50 and 100 mg/kg) has significantly reduced the level of IL-1β as compared with the LPS treated group ($p < 0.001$). Moreover, quercetin (100 mg/kg) showed significantly decreased level of IL-1β as compared withquercetin (50 mg/kg) treated group ($p<0.01$).

5. Histopathological analysis- Effect of quercetin treatment on histopathological damagesin LPS treated zebrafish.

Figure 6 shows, histopathological evaluation of brain tissue was carried on under light microscopy. In the histopathological study, the brains of normal, vehicle and *per se* treated groups showed undamaged neuronal cells. However, treatment with LPS (1mg/kg) causes disarrangement of various cell layers as well as the pyramidal neuronal cell loss was found which was significant as compared to the normal group. However, treatment with quercetin (50 and 100 mg/kg) significantly attenuated the loss of neuronal cell density as compared to LPS treated animals.

4. Discussion

In the present study, we have evaluated the potential preventive role of quercetin to attenuate LPS induced neuroinflammation in adult zebrafish. In accordance to previous studies showed that LPS is an endotoxin, found in outer membrane of gram-negative bacteria. It is potent activator of immune system. Immunocytes detect LPS through the TLR4, which it binds to with high affinity and activated microglia. Microglia, which are resident innate immune cells in the CNS, play an important role in the inflammatory process [38]. The activation of microglia generates various pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, iNOS, COX-2), causing damage to surrounding neurons and eventually inducing neurodegeneration [39, 40], in macrophages LPS activated NFkB and mitogen-activated protein kinases (MAPKs) [41]. Sickness behaviour, impaired memory, and depression-like behaviour are all symptoms of increased neuroinflammation. These findings showed that LPS enhanced oxidative stress and neuroinflammation, which can lead to neurological diseases such as anxiety, sadness, and memory loss.

According to the literature review, the Noval Diving test and the Light-Dark test are used to evaluate anxiety, depression, and memory functions. When normal zebrafish are exposed to a new environment, they swim to the bottom and progressively explore the top compartment of the novel tank apparatus, increasing the time spent and the number of entries in the top zone. Normal zebrafish prefer the light chamber as compared to the dark compartment in a light-dark test. Anxiolytic, locomotor, and memory functions are all assessed using the light-dark test[42, 43]. In our study, we have developed the model of LPS induced neuroinflammation in adult zebrafish and our findings revealed that a single injection of

LPS (1 mg/kg; i.p.) on day 0 of the procedure caused significant behavioural and biochemical alterations. In a novel diving test, the LPS-treated group significantly increased TSBZ while dramatically reducing TSTZ and the number of entries into the top compartment as compared to the normal group. The enhanced TSBZ was the measure of anxiety. In the light dark test, the LPS treated group showed significantly less TSLZ as well as reduced number of entries in the light zone and greater the preference in dark compartment as compared with the normal group. The increased TSDC and number of entries was the measure of depression and anxiety like state.

According to the previous studies, quercetin is a natural flavonoid, found in various vegetables, fruits, leaves and grains [44]. Quercetin is a natural antioxidant with anti-inflammatory properties, as well as the ability to prevent pro-inflammatory cytokine production and microglia activation in vivo and in vitro [45]. Various investigations have shown that it can function as a scavenger of reactive oxygen species, protecting tissues from free radicals [46]. Quercetin is significant to aquaculture since its beneficial qualities have been explored as a possible addition in fish diet [47]. In our work, different doses of quercetin (50 and 100 mg/kg; *i.p.*) were used to check the antioxidant potential. Our results showed, both doses reversed the preferences as compared to the LPS treated groups in novel diving test and light-dark chamber test. In novel diving test, post-treatment group of quercetin (50 and 100mg/kg) explore both zone showing more preference in top zone thus significantly increasing the TSTZ and the no. of entries to the top. Reduced behaviour sickness is shown by the treatment group's return to normal exploratory behaviour. The light-dark compartment test revealed that the quercetin (50 and 100 mg/kg) post-treatment group favoured the light compartment, which resulted in significantly more TSLZ and number of entries in the light compartment. The TSDC is an indication of depression and anxiety like state which is significantly decreased in the treatment groups.

As previously mentioned, the oxidative stress parameter can be used to indicate anxiety and depression-like behaviour [48]. The level of LPO assessed in biochemical estimates is used to represent increased oxidative stress and enhanced the production of nitrite level [49]. In addition, GSH is an antioxidant, that reduced its level in neuroinflammation [50]. Keeping the above cascade in mind we also found significantly increased the level of LPO, nitrite and decreased level of GSH in LPS treated zebrafish brain as compared with normal group. On the other hand, quercetin is an antioxidant potentially [51, 52]. We found that post-treatment with quercetin (50 and 100 mg/kg) significantly reduced oxidative stress, LPO, nitrite level and increased in GSH level.

Acetylcholinesterase (AChEs) levels were measured to see whether LPS caused memory impairment. The activity of acetylcholinesterase is altered by LPS, which suppresses the activity of acetylcholine, which is responsible for memory and learning [53, 54]. our investigation, the LPS-treated group had higher levels of AChEs as seen against normal group, indicating memory impairment. Whereas quercetin (50 and 100 mg/kg) treated group significantly decreased the level of AChEs in zebrafish brain.

Through previous studies, LPS increased the level of inflammatory cytokines such as TNF-α and IL-1β in the brain via activation of microglia and astrocytes [55, 56]. Our study also showed similar results

according to previous studies, in the LPS treated group increased level of TNF-α and IL-1β as compared with the normal group. However, treatment with quercetin (50 and 100 mg/kg) decreased the level of TNF-α and IL-1β as compared with LPS treated group.

Histopathological evidence revealed damaged morphology in the LPS administration group which was confirmed by the pyramidal neuronal cell loss and also disarrangement of various cell layers. Moreover quercetin (50 and 100 mg/kg) attenuate the above given morphological damage.

Post treatment with quercetin (50 and 100 mg/kg) significantly reduced in the anxiety and depression like behaviour and improved memory functions by changing the behavioural and biochemical parameters performed and attenuated proinflammatory cytokines. Quercetin (100 mg/kg) showed marked reduction by altering above the given parameters as compared with quercetin (50 mg/kg). Based on the favourable results, it was determined that quercetin had a protective effect on LPS-induced behaviour illness by decreasing lipid peroxidation and its products, as well as delaying the neuroinflammation caused by LPS (figure 7).

Conclusion

In this study, we investigated the neuroprotective effect of quercetin against LPS induced neuroinflammation in adult zebrafish. It was concluded that the beneficial effects of quercetin on brain like anxiety, depression, locomotor activity and memory as illustrated by novel diving and light-dark chamber test may be due to its neuroprotective potential which can correlated with its antioxidant and anti-inflammatory activities. Various mechanisms may be working in the CNS and induce neurological changes in neuroinflammation. However, further studies on basic cellular and molecular grounds are required to understand the deficits induced by LPS.

Abbreviations

LPS : Lipopolysaccharide

AD : Alzheimer Disease

PD : Parkinson Disease

MS : Multiple Sclerosis

ALS : Amyotrophic Lateral Sclerosis

TSTZ : Time spent in top zone

TSBZ : Time spent in bottom zone

TSLZ : Time spent in light zone

TSDC : Time spent in dark compartment

TNF- α : Tumour necrosis factor

IL-1β **:** Interleukin 1 beta

LPO : Lipid peroxidation

AChEs : Acetylcholinesterase

GSH : Glutathione

CNS : Central nervous system

QoL : Quality of life

BBB : Blood- brain barrier

DMSO : Dimethlsulfoxide

TCA : Tricholroacetic acid

TBA : Thiobarbituric acid

Declarations

Author contribution

Sukhdev Singh did the research, Kuleshwar Sahu and Lakshay Kapil helped in data collection, Charan Singh helped in editing the manuscript, Arti Singh design the layout and critically revise the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Conflict of interest

All authors confirm and declare no competing financial interests.

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Figures

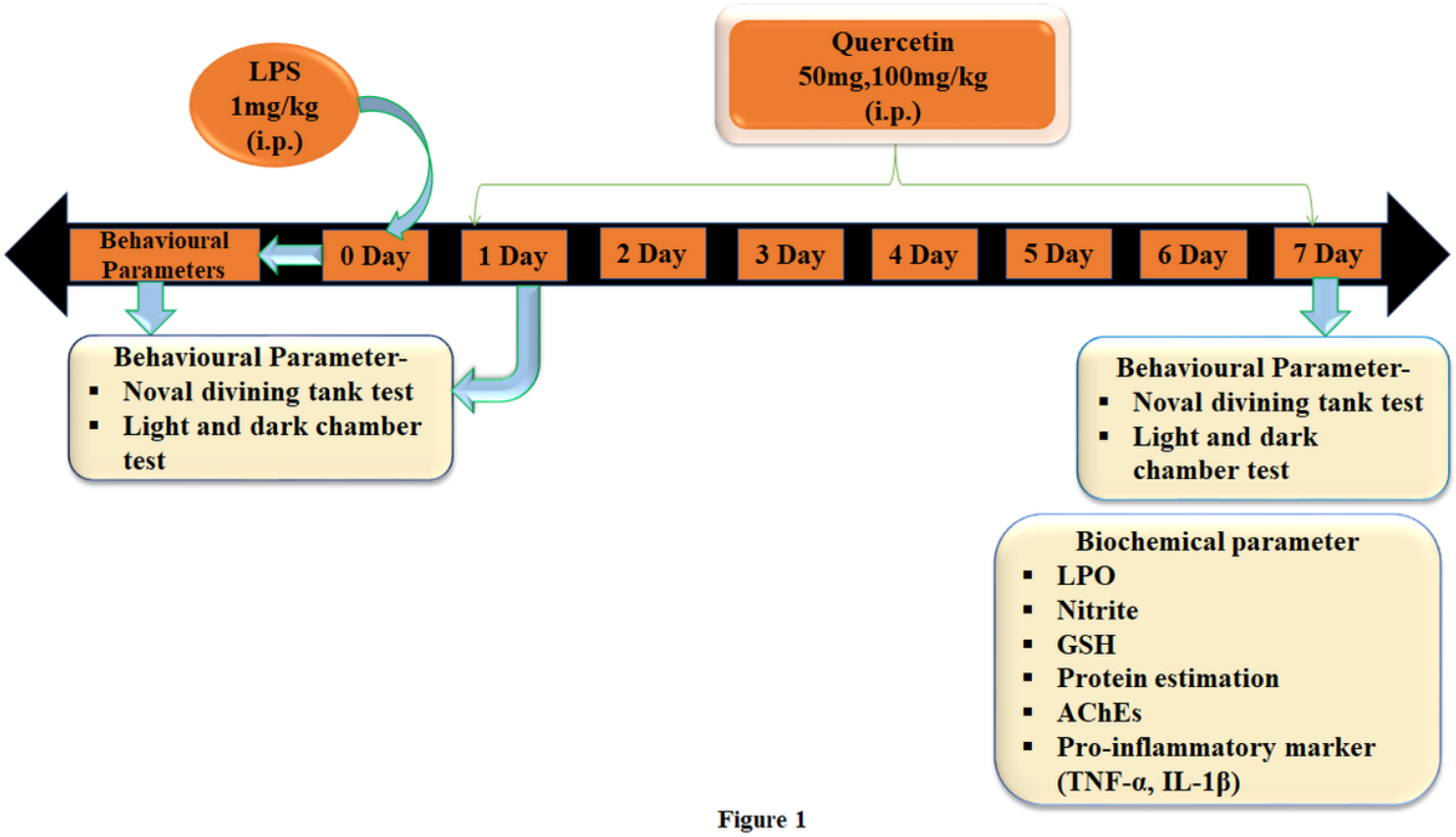

Figure 1

Figure 1

Experimental protocol.

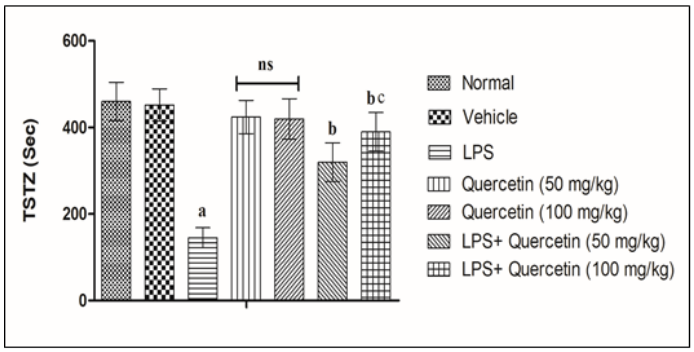

Figure 2A

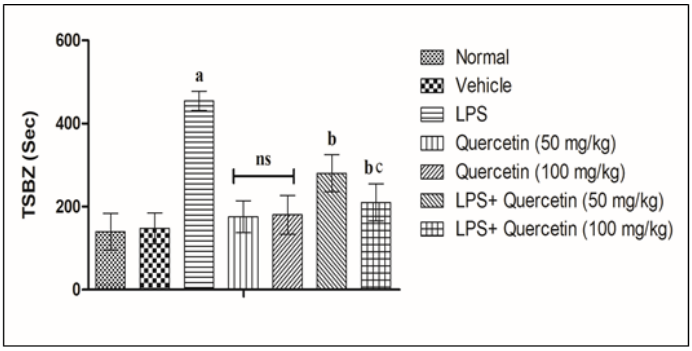

Figure 2B

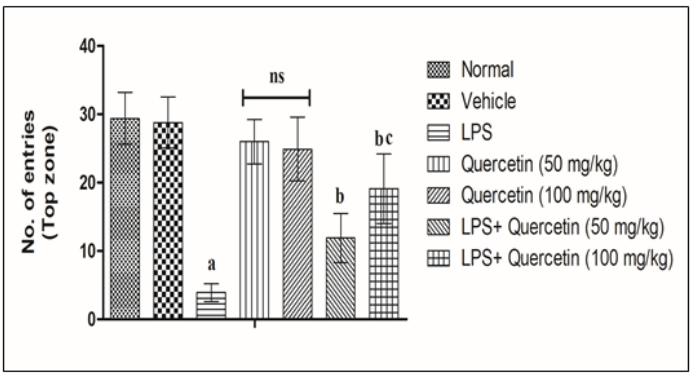

Figure 2C

Figure 2

Effect of Quercetin treatment on novel diving tank in LPS treated zebrafish. (A) TSTZ (B) TSBZ (C) Number of entries in top zone. Zebrafish were post treated with different doses of quercetin (50 & 100 mg/kg) for 7 days. Data are expressed as the mean ± SD. ap<0.001 vs Normal, bp<0.001 vs LPS, cp<0.01 vs LPS+ Quercetin (50mg/kg), nsp>0.05 vs normal group. Statistical analysis performed by one-way ANOVA followed by post test Tukey multiple comparison. [LPS: lipopolysaccharide; TSTZ: time spend in top zone; TSBZ: time spend in bottom zone].

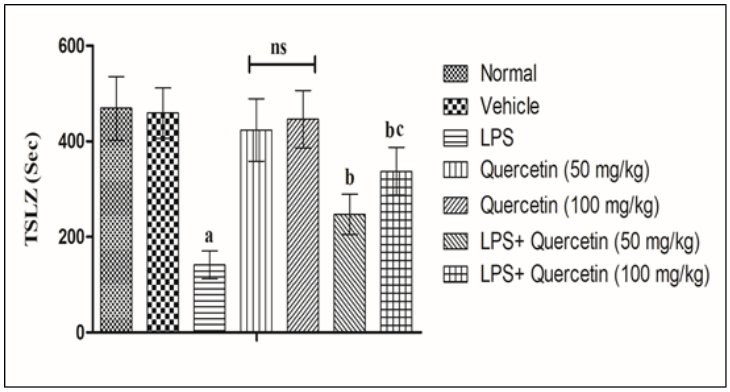

Figure 3A

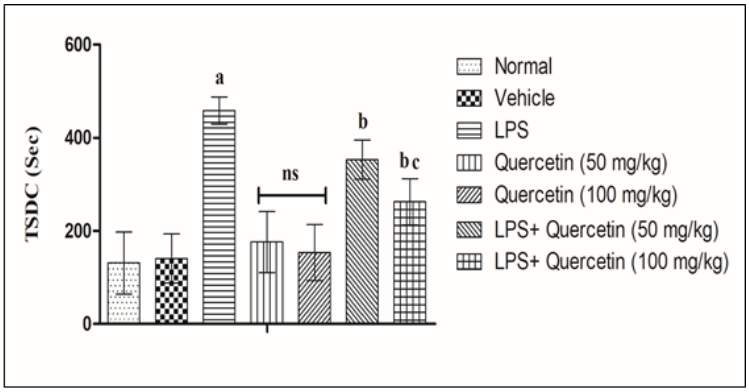

Figure 3B

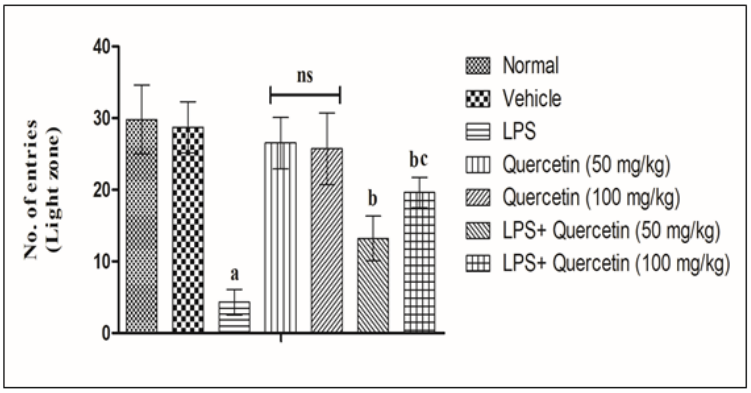

Figure 3C

Figure 3

Effect of quercetin treatment on light-dark chamber test in LPS treated zebrafish. (A) TSLZ (B) TSDC (C) Number of entries in light zone. Zebrafish were post treated with different doses of quercetin (50 & 100 mg/kg) for 7 days. Data are expressed as the mean ± SD. ap<0.001 vs Normal, bp<0.001 vs LPS, cp<0.01 vs LPS+ Quercetin (50mg/kg) nsp>0.05 vs normal group. Statistical analysis performed by one-way ANOVA followed by post test Tukey multiple comparison. [LPS: lipopolysaccharide; TSLZ: time spend in light zone; TSDC: time spend in dark compartment].

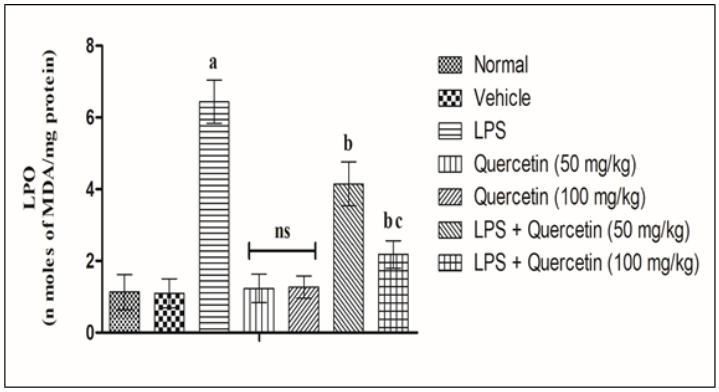

Figure 4A

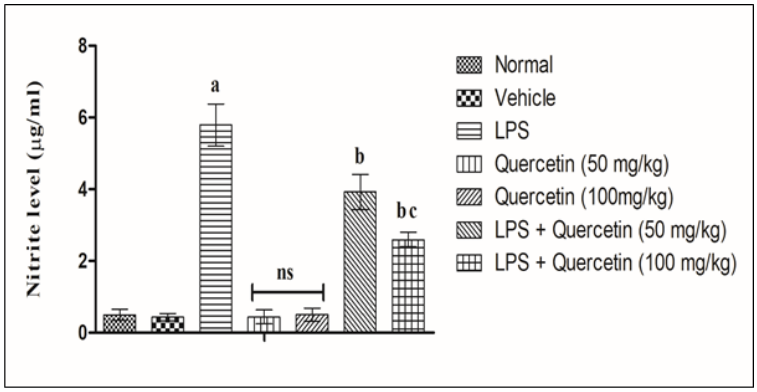

Figure 4B

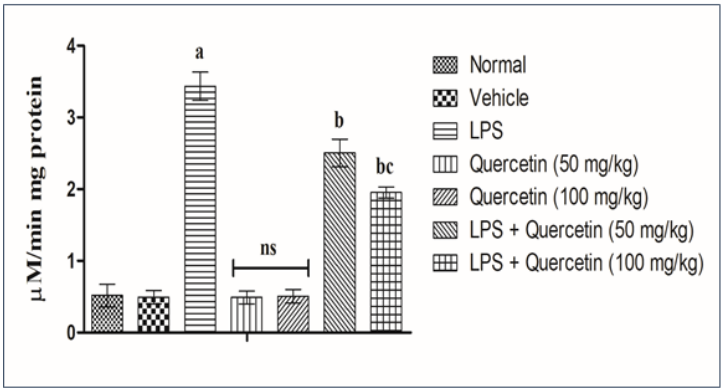

Figure 4C

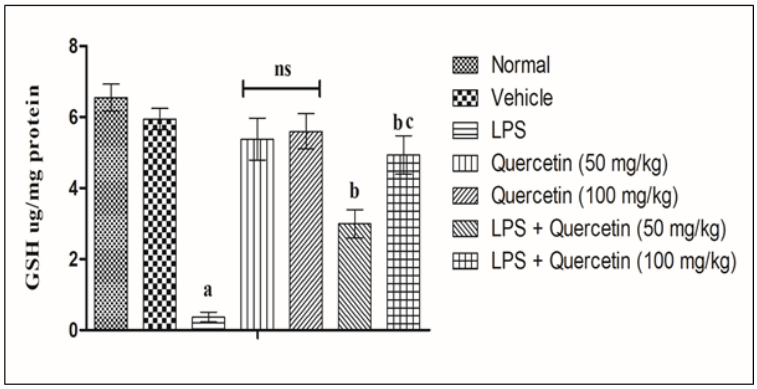

Figure 4D

Figure 4

Assessment of oxidative stress markers. (A) LPO (B) nitrite (C) AChEs and (D) GSH. Zebrafish were post treated with different doses of quercetin (50 & 100 mg/kg) for 7 days. Data are expressed as the mean ± SD. ap<0.001 vs Normal, bp<0.001 vs LPS, cp<0.01 vs LPS+ Quercetin (50mg/kg) nsp>0.05 vs normal group. Statistical analysis performed by one-way ANOVA followed by post test Tukey multiple comparison. [LPS: lipopolysaccharide; LPO: lipid peroxidation; AChEs: Acetylcholinesterase; GSH: glutathione].

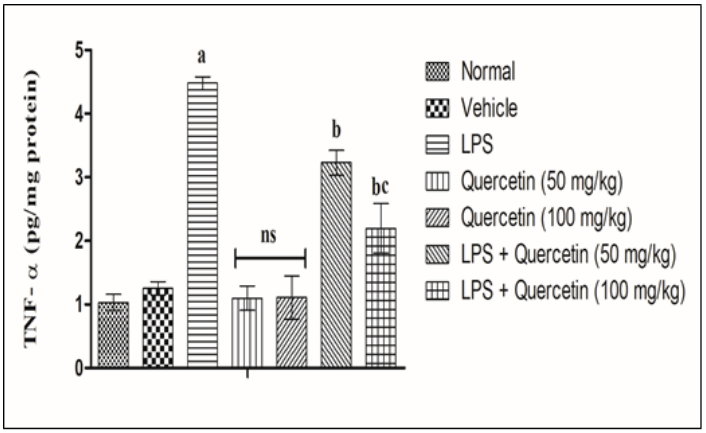

Figure 5A

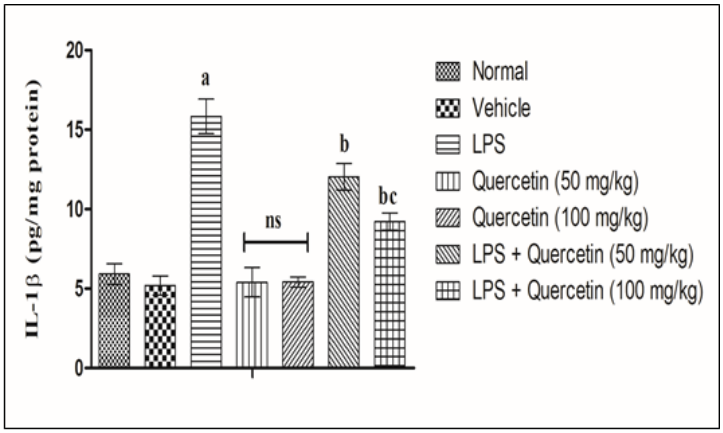

Figure 5B

Figure 5

Effect of quercetin treatment on TNF-α, IL-1β level in LPS treated zebrafish. (A) TNF-α (B) IL-1β. Zebrafish were post treated with different doses of quercetin (50 & 100 mg/kg) for 7 days. Data are expressed as the mean ± SD. ap<0.001 vs Normal, bp<0.001 vs LPS, cp<0.01 vs LPS+ Quercetin (50mg/kg), nsp>0.05 vs normal group. Statistical analysis performed by one-way ANOVA followed by post test Tukey multiple comparison. [LPS: lipopolysaccharide; TNF-α: Tumour Necrosis Factor- α; IL-1β: interleukin-1β].

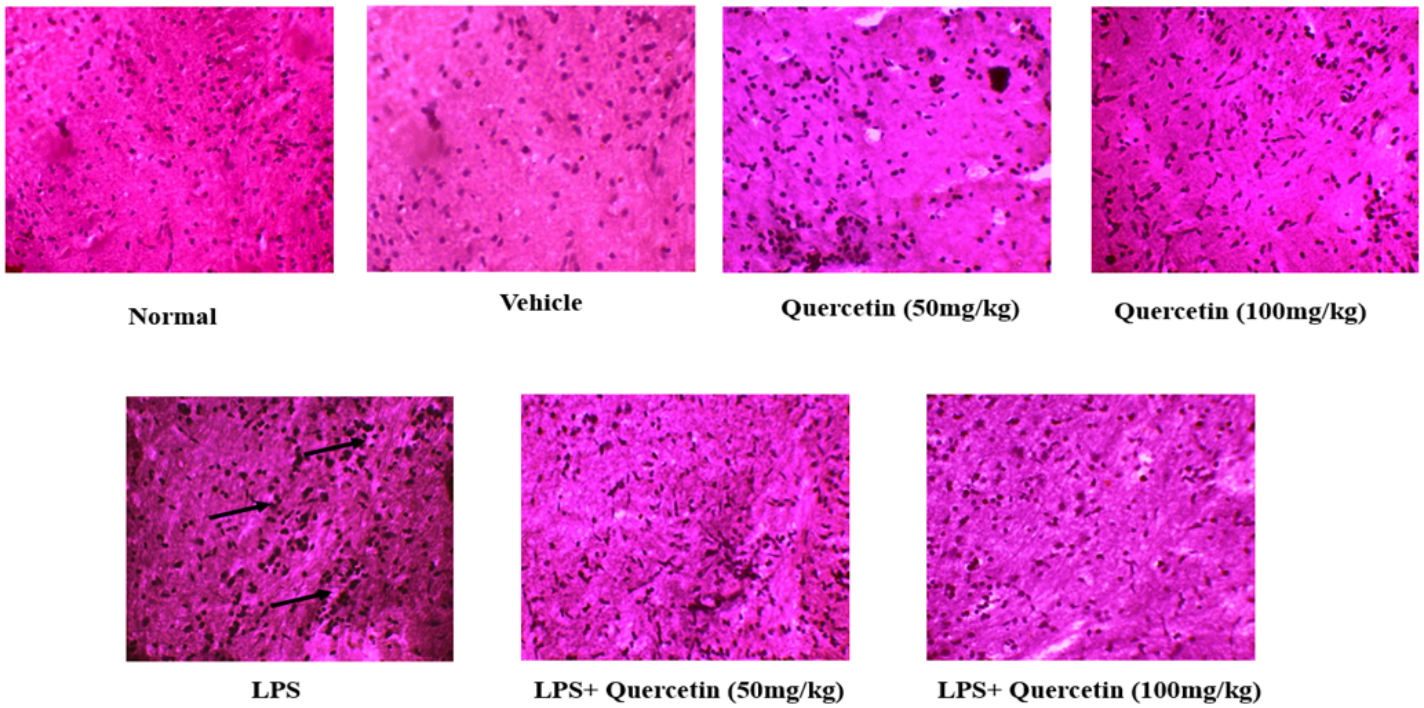

Figure 6

Figure 6

Effect of quercetin treatment on histopathological damagesin LPS treated zebrafish. Photomicrograph brain of normal, vehicle and quercetin (50 and 100 mg/kg) zebrafish showing normal neuronal cells, LPS treated group showed disarrangement of various cell layers and pyramidal neuronal cell loss (straight arrow) as compared to normal group. However, quercetin (50 and 100 mg/kg) treatment altered the effects as seen against LPS group.

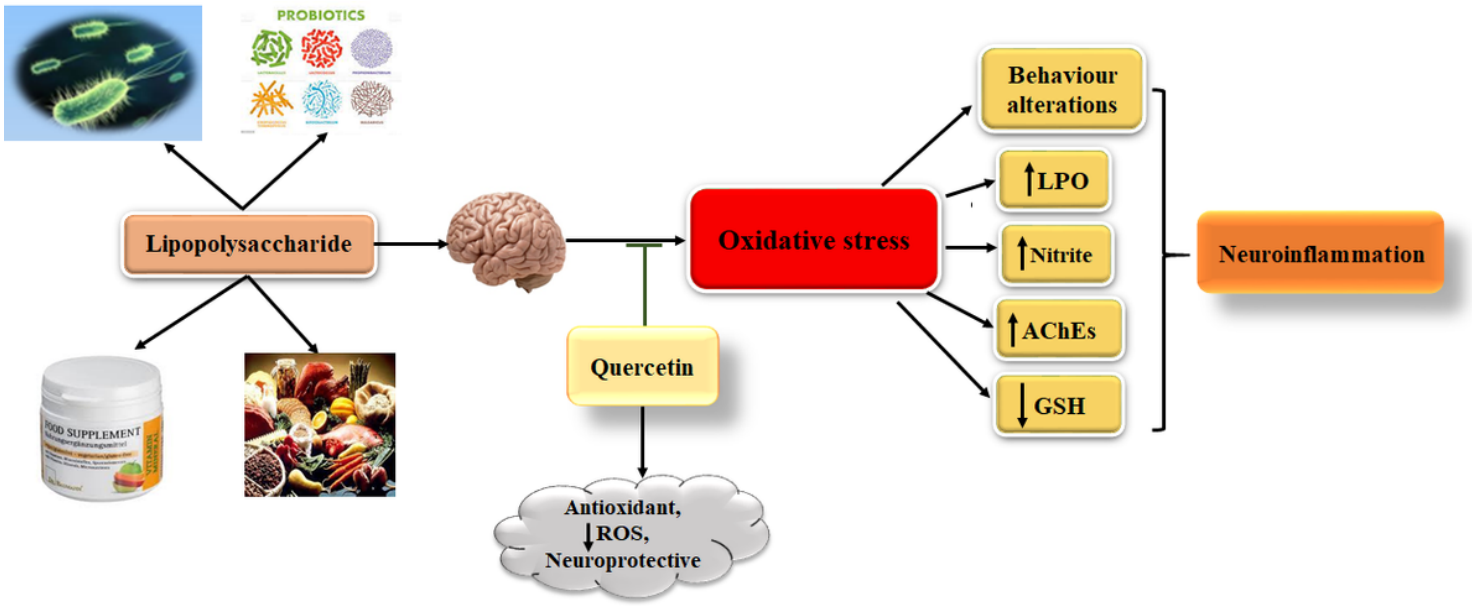

Figure 7

Figure 7

Mechanism of quercetin in reducing neuroinflammation.