Pre-Treatment with Quercetin Prevents Dementia in Streptozotocin Treated Zebrafish via Modulation of Neuroinflammation and Oxidative Stress

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Research Article

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Abstract

Aim: To examine the protective outcome of quercetin against streptozotocin induced dementia in adult zebrafish.

Materials and method: In this study, adult zebrafish (weighing 470-530 mg) were subjected to the streptozotocin administration (300 mg/kg; *i.p*) followed by a quercetin (50 & 100mg/kg *i.p*) pre-treatment before 24 hr of streptozotocin administration followed by blood glucose level measurement, behavioral parameters (light-dark test, novel diving test, open field test and T-maze test), biochemical parameters (lipid peroxidase, reduced glutathione, nitrite and AChEs activity), molecular and histopathological analysis.

Results: In light-dark test, streptozotocin treated zebrafish shown their preference in dark compartment as compared to normal group. In novel diving tank, streptozotocin zebrafish spent less time and decrease in total number of entries to the top zone when compared to the normal group. In the T-maze test, streptozotocin treated zebrafish has shown significantly more time spent in unfavorable zone and less time spent in favorable zone with increase in total latency as compared to normal group. However, in the open field test no significant effect was seen among any group. Moreover, pre-treatment with quercetin (50 mg/kg and 100 mg/kg) significantly ameliorated the memory deficits in all the behavioral, biochemical and molecular parameters as compared to animals treated with streptozotocin. Additionally, results of histopathology studies showed less disruption in neuronal cells in quercetin treated group when compared to STZ treated animals.

Conclusion: Pre-treatment with quercetin ameliorates streptozotocin induced dementia in adult zebrafish by preventing oxidative stress and neuroinflammation.

Introduction

Dementia is a clinical syndrome, which is mainly characterized by rapid or progressive destruction in energy metabolism and learning and memory function as well. Reduced level of cerebral glucose utilization that ranges from 10 % to 40 % has been reported in numerous conditions of memory dysfunction [1]. Although, impaired insulin receptor signal transduction has been also reported in the hypothalamus and hippocampus region of the brain [2]. Impaired insulin signaling is associated with increased level of oxidative stress and mitochondrial and endothelial dysfunction in brain cells [3].

Streptozotocin (STZ) is a wide-spectrum antibiotic also containing anti-neoplastic activity, produced by the gram-positive bacteria *Streptomyces achromogenes [4].* It is used commonly for inducing the hyperglycemic condition because it is a very potent toxicant for beta cells of pancreas [5]. It causes memory dysfunction by reducing the levels of various neurotransmitters such as acetylcholine (ACh) which further leads to homocysteine accumulation and by altering the metabolic condition [6]. It also causes memory impairment by causing mitochondrial and endothelial dysfunction which is maintained by low levels of nitric oxide [7].

Now-a-days, zebrafish (*Danio rerio*) is also used as an emerging model for various neurodegenerative disorders [8,9]. The major neurotransmitter system accountable for neurodegenerative disorders such as Alzheimer disease, Parkinson disease, dementia in mammals are widely conserved in zebrafish and the pharmacological and genetic characteristics of zebrafish are analogous to those of vertebrates including mammals [8]. This model is well accepted and suitable for the study of dementia due to 70% similarity of genome and neurological functions to human beings. It possesses numerous genes which are involved in dementia [9].

Quercetin is a bioflavonoid which is obtained from various sources like apples, honey, raspberries, onions, red grapes, cherries, citrus fruits, and green leafy vegetables [10] it contains antioxidant, anti-inflammatory and anti-neoplastic activity [11]. Several studies suggested that it has been a potent scavenger of reactive oxygen species and reactive nitrogen species and well accepted to exert neuroprotection [12–14]. Additionally, quercetin is also known to enhance the insulin sensitivity; reduce diabetes related complications including neuropathy, nephropathy, retinopathy and depression [15–18].

Therefore, based on these findings, we hypothesized that pre-treatment with Quercetin could help to protect against the STZ induced dementia 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 objectives of our study were to identify the outcome of Quercetin pre-treatment in STZ induced dementia and the mechanisms underlying its neuroprotective effect, i.e., through amelioration of inflammation, and oxidative stress.

2. Material And Methods

2.1 Animals and Housing

In this study, wild- type adult zebrafish of mixed sexes less than 4-5 months old, weighing 470-530 mg were used. This wild type species of zebrafish were obtained from Aquarts, 26B K Komedanbagan lane, Kolkata, India. For maintenance zebrafish were housed in experimental room maintained with 12L: 12D cycle. Temperature of the system was maintained at 26-27^{o}C by automatic thermostat. Fishes were fed twice daily with commercially available diet Tetrabits. All experiments were conducted in accordance with Institutional Animal Biosafety Committee (IBSC) with approval number ISFCP/IBSC/M 1 /2021/09.

2.2 Chemicals and Drug

STZ and Galantamine were purchased from Sigma-Aldrich (St Louis, MO, India). Quercetin was purchased from natural remedies, Bangalore, India. The chemicals used for biochemical analysis were purchased from Himedia and SRL ltd. The rest of reagents were of analytical grade.

2.3 Study design

Before start of the experiment, fishes were separated in 3L tank prior to habituation in behavioral tests. Total no. of108 adult zebrafish of both the sexes were used for this experimental study. The animals were divided into different groups as (n=12 in each group) as shown in Table 1. The study was carried out for duration of 4 days, detailed experimental design has been shown in Figure 1.

Table 1

Experimental Group/ Treatment group

S.No.	Group	Treatment	No. of animals
1.	Control	Normal	12
2.	STZ (300)	Streptozotocin (300 mg/kg; i.p) for1 day	12
3.	DMSO	1 % Solution	12
3.	Gal (4)	Galantamine (4mg/kg, i.p) for 1day	12
4.	Q (50)	Quercetin (50mg/kg, i.p) for 1 day	12
5.	Q (100)	Quercetin (100mg/kg, i.p)	12
6	STZ (300) + Gal (4)	Pre- treatment with galantamine (4mg/kg, i.p) 24 hr before STZ administration	12
7	STZ (300) + Q (50)	Pre- treatment with Quercetin (50mg/kg, i.p) 24 hr before STZ administration	12
8	STZ (300) + Q (100)	Pre- treatment with Quercetin (100mg/kg,i.p) 24 hr before STZ administration	12

Treatment Schedule

For administration, quercetin (50 mg/kg and 100 mg/kg) and galantamine (4 mg/kg), were dissolved in (1%) DMSO, the doses were chosen based on former studies [19-22]. All the treatments were given via intraperitoneal (*i.p*) route. For STZ administration we have chosen 300 mg/kg as a dose which causes

memory dysfunction. So, we have done preliminary studies where we have used different doses of STZ starting from 50, 100, 200, 300, 400, 500 and 600 mg/kg. And we have done glucose estimation and behavioral analysis and found that 300 mg/kg is the dose that start causing memory dysfunction (data in supplementary file)

Intraperitoneal STZ administration

STZ and drug treatment were injected *i.p* (into the abdominal cavity dorsal to the pelvic girdle) with a 10µl Hamilton syringe (Stewart *et al.,*. 2011a). Briefly, each fish was anesthetized by immersing it in a tricaine 100 mg/LMS-222 solution until it showed loss of motor coordination and lowered respiratory rate. Then, fish was taken out from the solution and placed in a petri dish on a soft sponge with a 20 mm height that had been wet with water (Stewart *et al.,* 2011). A cut was made on the sponge of about 10-15mm deep for holding the fish for injection. Then, *i.p.* injections were administered by means of a 31G Ultra-Fine Hamilton Syringe (Himalaya Scientific, Chandigarh, India) according to the protocol formerly described. The needle was injected into the spines posterior to the pectoral fins in the midline of the abdomen. The whole injection process should not take more than 10 s to ensure animal safety and immediately afterwards the injection the animals were transferred in a different tank with unchlorinated water to enable the recovery of animals from the anesthesia.

After 24 hr of STZ administration the blood glucose level of zebrafish was measured with the help of blood glucometer. Blood collection was performed by tail ablation method in zebrafish [23]. In this method, the zebrafish was anesthetized by immersion in a100 mg/L MS-222 solution and placed on a soft sponge that was soaked with water, fixed into a Petri dish. A small cut was made at the tail of zebrafish by using a surgical scissor and then the blood was collected directly on the strip of blood glucometer and the reading of blood glucose was measured.

2.4 Behavioral Analysis

After 24 hr of STZ administration, behavioral studies that is T-maze test, light and dark test, novel diving test and open field test were performed to test the memory dysfunction in the normal group and test groups. We have used ANY maze video tracking software version 7.00 (Stoelting Co., USA) for the analysis.

2.4.1 T-maze test

The T-maze test used to assess the memory functions in zebrafish was described by Colwill *et al.,* [24] with some modification of Kim *et al.,* [25]. It is a transparent Plexiglas apparatus. Briefly; it consists of one long arm (28×12×5 cm) attached with starting zone (12×12×6 cm) and two shorter arms (12×12×12 cm) attached with two differently (red and green) colored zone. Red colored zone is

considered as unfavorable zone or danger zone because fishes were disturbed using glass rod (30×1 cm) and green color zone is considered as favorable zone or reward zone (12×12×12 cm) because reward in the form of food pellet were provided. Plexiglas doors were used to close the entry from starting zone into the long arm and from the short arm into the two test zones. The water in the maze and pH was maintained at and 7.3 and a height of 8 cm were used to fill the maze. All of the equipments were custom-designed and built by the Puri glass house, Moga, India.

During training session of T-maze for 3 days before STZ administration, 3 trials were given per day. During each trial zebrafish were initially habituated for 2 min to explore the starting zone and the long arm with doors leading to test zone remains closed.

During test trial, each fish was placed in the starting zone ,after which all gates were opened and the time required for each fish to reach in the favorable zone was recorded in the 5 min which is termed as transfer latency (TL) [26]. Apart from this parameter, time spent in favorable zone (TSFZ) and unfavorable zone (TSUZ) was recorded [26-27].

*In STZ treated group, this test was carried out 24 hr later to the STZ administration for the analysis of memory impairment.

2.4.2 Light and dark test

This test was used to analyze spatial memory functions in adult zebrafish as explained by Dubey *et al.,* [27]. Briefly, this apparatus is made up of Plexiglas and having dimensions 30 cm length, 16 cm width and 15 cm height. The tank is filled up with water up to the level of 10 cm and it is separated into two equal halves of 15 cm length each. One half is black in color and second half is transparent or white in color. Generally, when animal is introduced in novel environment it initially prefers darker side and after some seconds it moves towards the lighter side of the chamber and if didn't do so then this indicates poor spatial memory. If the animal prefers light chamber than this specifies normal or improved memory functions. The study was performed for 7 minutes, animals were habituated for 2 minutes, and we have measured time spent in light and dark zone (TSLZ and TSDZ) and number of entries in light zone.

2.4.3. Novel diving tank test

The novel diving is 1.5 L trapezoidal tank with dimension (19cm X 11cm X 22cm). Briefly, the tank was filled up to its maximum height with water and was divided into two equal halves horizontally, with the help of marker on the outside walls. The area above the division is regarded as the "Top zone" and area below the division is termed as "Bottom zone" [28]. The test was carried out for 7 minutes, 2 minutes for acclimatization and 5 minutes for recording. The time spent in the top zone (TSTZ), and total no. of entries to the top zone were evaluated using ANY maze video tracking software version 7.00 (Stoelting Co., USA).

2.4.4 Open field test

The open field test (OFT) was utilized to analyze the swimming behavior and locomotor activity of adult zebrafish as described by Sattaa *et al.,*[29]. The OFT is made up of plexiglass (dimensions 30 m height X 30 cm width X10 cm height) containing 5L of water at 28◦C. Tests were performed in a controlled environment. Each fish was carefully transferred into the apparatus, the animals were first acclimatized for 2 minute and then behavioral activity was recorder for 5 minutes and the total distance travelled by the fish was evaluated by using ANY maze video tracking software version 7.00 (Stoelting Co., USA).

2.5 Biochemical estimations

2.5.1 Tissue preparation

After the behavioral parameters performed, the zebrafish were anaesthetized by using ice cold water at 4^{o}C till the movements of gills is stopped and euthanized. Then, the brains were isolated instantly by using micro-dissecting tools and forceps and freeze dried at -4°C. After this all the samples were homogenized in a homogenizing tube with 0.1 M phosphate buffer solution [30]. After centrifugation for 15 minutes at the speed of 10,000 g, the supernatant were collected and used for estimation of various biomarkers such as, lipid peroxidation assay (LPO), reduced glutathione (GSH), Nitrite and acetylcholinesterase (AChEs) activity levels.

2.5.2 Estimation of LPO

The LPO level was estimated by the method as described by Wills, 1966 [31]. Briefly, 0.5 ml homogenate and 0.5 ml of Tris HCL (pH 7.4) were incubated at 37°C for 2 hr. Then 1ml of TCA (10%) was mixed and centrifuged for 10 minutes at the speed of 1000 g. After centrifugation, 1ml of supernatant and 1 ml of thiobarbituric acid (0.067%) were mixed and the centrifugation tubes were kept in hot water for 10 min and the quantity of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid at 532 nm with the help of Perkin Elmer Lambda 20 spectrophotometer and the values were determined using the chromophore's molar extinction coefficient (1.56 X 105(mol/l)-1 cm-1).

2.5.3 Estimation of GSH

The amount of GSH was measured by the process explained by Ellman method [32]. Briefly, the homogenate was mixed with 1ml of sulfosalicylic acid (4%) and were stored for an hour at 4°C. Samples were centrifuged at 4°C for 5 min at the speed of 1200 g. Then after centrifugation the 1ml supernatant, 0.2ml of 5,5- dithiobis-(2-nitrobenzoic acid) (DTNB) and 2.7 ml of phosphate buffer (0.1M, pH 8) were

added to test tube. The variation in absorbance was measured at 412 nm by means of Perkin Elmer Lambda 20 spectrophotometer. The values were denoted as µmol of GSH/mg protein.

2.5.4 Estimation of Nitrite

To measure the amount of nitrite present in the sample Green *et al.,* method was used. Briefly, a mixture of the equal volume of the supernatant and Greiss reagent (0.1% Naphthyl ethylene diamine dihydrogenchloric acid, 1% Sulphadiazine in 5% phosphoric acid) were incubated at ambient temperature for 5 minutes, the variation in absorbance was estimated at 546 nm by means of Perkin Elmer Lambda 20 spectrophotometer. The values were expressed as µmol/mg protein [33].

2.5.5 AChEs activity

The activity of AChEs was assessed by Ellman method [34]. Briefly, the mixture of assay included 0.05 ml of supernatant, 3ml of 0.01M Sodium phosphate buffer (pH 8), 0.10 ml of ACh iodide and 0.10 ml of 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) (Ellman reagent). The variation in absorbance was measured for duration of 2 min at 30 s interval at 412 nm by the use of Perkin Elmer Lambda 20 spectrophotometer. Outcomes were expressed as micromoles of acetylthiocholine iodide hydrolyzed/min/mg protein.

2.5.6 Protein estimation

The protein content was determined by the Biuret method and bovine serum albumin was taken as a standard [35]. Briefly, 0.1ml of tissue homogenate supernatant, 2.9 ml NaCl and 3 ml working biuret reagent were mixed and incubated at room temperature for 10 minutes. The absorbance was measured at 536 nm by means of Perkin Elmer Lambda 20 UV spectrophotometer.

2.6 Estimation of tumor necrosis factor- α (TNF-α) activity

The concentration of cytokines (TNF-α) was determined by using the fish TNF-α Detection Kit (ELK Biotechnology, Cat no. ELK8512 Fish TNF-α Kit). 3 brains of zebrafish as a pool from each group were homogenized and the absorbance was read on a microtiter plate reader at 450 nm wavelength [36].

2.7 Histopathological Analysis by Hematoxylin and Eosin Staining (H&E staining)

Zebrafish were sacrificed immediately after the completion of last behavioral test. Briefly, the brains were isolated and transferred into formalin (10 % v/v). Serial coronal sections were taken and fixed tissue dehydrated in increasing grades of ethanol, cleaned in xylene, embedded in paraffin wax blocks and 3

mm thick sections were made with the help of rotatory microtome. The tissue sections were flattened in warm water and mounted onto glass slides. H & E staining was performed according to the staining protocol [37,36].

2.8 Statistical Analysis

Graph Pad Prism (version 5.0, Graph Pad Software, San Diego, CA) was used for all the statistical analysis. The result values were expressed as mean ± SD. The behavioral evaluation data and the biochemical estimations were analyzed by one-way ANOVA. Post-hoc comparisons between groups were made using Tukey's test. The P-value<0.05 was considered significant.

3. Result

3.1 Effect of quercetin treatment on Blood glucose in STZ treated zebrafish

STZ (300 mg/kg) produces a significant raise in the blood plasma glucose levels as compared to normal group (Fig. 2) whereas pre-treatment with galantamine (4 mg/kg) and quercetin with doses 50 and 100 mg/kg produced a significant decline in the blood glucose level as compared to STZ (300mg/kg) administered group. No significant rise or decline in the level of blood glucose was observed in the vehicle (DMSO), and *per se* treatment groups (galantamine 4mg/kg, quercetin 100mg kg and quercetin 50mg/kg).

3.2 Effect of quercetin in STZ induced memory deficit in T-maze apparatus

Figure 3.A show that STZ (300 mg/kg) produced a significant decrease ($p < 0.001$) in the TSFZ as compared to the normal group. However, vehicle (DMSO) and *per se* treatment with galantamine (4 mg/kg), quercetin (50 mg/kg, 100 mg/kg) did not show any significant change when compared to the normal animals. Further pre- treatment with galantamine (4mg/kg) significantly ($p < 0.001$) increased the TSFZ as compared to the STZ (300mg/kg) administered group. Moreover, pre-treatment with quercetin (50mg/kg and 100mg/kg) significantly ($p < 0.001$) reversed the effect produced by the STZ by increasing TSFZ as compared to the STZ treated animals and it was found that quercetin (100mg/kg) showed better ($p < 0.05$) effect than quercetin (50 mg/kg).

Figure 3.B shows that STZ (300 mg/kg) significantly altered the zebrafish behavior, as TSUFZ was significantly ($p < 0.001$) more as compared to normal animals. No significant changes were detected in the *per se* (galantamine 4 mg/kg), quercetin (50 mg/kg, 100 mg/kg) and vehicle treated animals. However, pre-treatment with galantamine (4 mg/kg) significantly ($p < 0.001$) decreased the TSUFZ.

Further, treatment with quercetin (50 mg/kg, 100 mg/kg) significantly reversed the effect produced by STZ (300 mg/kg) by lowering the TSUFZ significantly ($p < 0.001$) as compared to the animals who received STZ treatment. Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly greater in ameliorating the TSUFZ when compared to the effect of quercetin (50 mg/kg) ($p < 0.05$).

Figure 3C shows that STZ (300 mg/kg) significantly affected the zebrafish behavior, as TL was significantly ($p < 0.001$) enhanced as compared to normal animals. Whereas, DMSO and *per se* treatment with galantamine (4 mg/kg) and quercetin (50 mg/kg, 100 mg/kg) didn't show any significant difference in TL when compared to the normal group. However, pre-treatment with galantamine (4 mg/kg) significantly ($p < 0.001$) reversed the effect produced by the STZ by decreasing TL as compared to STZ treated animals. Further, a significant ($p < 0.001$) decline in TL was found in the animals, who received the pre- treatment of quercetin (50 mg/kg, 100 mg/kg) as compared to STZ administered animals. Moreover, the outcome of treatment with quercetin (100 mg/kg) was found to be significantly greater in ameliorating TL when compared to quercetin (50 mg/kg) ($p < 0.01$).

3.3 Effect of quercetin on STZ induced memory impairment in Light and Dark chamber test

Figure 4A shows that STZ produced significant decrease in memory functions in adult zebrafish. It was seen that zebrafish treated with STZ (300 mg/kg) show a significant decline in TSDZ ($p < 0.001$) as compared to normal animals. However, DMSO and *per se* treatment with (galantamine 4 mg/kg) and quercetin (50 mg/kg, 100 mg/kg) didn't produce any significant result as compared to normal group. However, zebrafish pre- treated with galantamine (4 mg/kg) reversed this effect produced by STZ by significantly ($p < 0.001$) increasing the TSLZ when, compared to the STZ treated group. Further, pre-treatment with quercetin (50 mg/kg, 100 mg/kg) significantly ($p < 0.001$) decreased the TSLZ as compared to the animals who received STZ (300 mg/kg). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly greater in improving this effect when compared to quercetin (50 mg/kg) ($p < 0.001$).

Figure 4B shows STZ produced significant decrease in memory functions in adult zebrafish. It was seen that zebrafish treated with STZ (300 mg/kg) show significant increase in TSDZ ($p < 0.001$) as compared to normal animals. However, DMSO and *per se* treatment with (galantamine 4 mg/kg) and quercetin (50 mg/kg, 100 mg/kg) didn't show any significant effect as compared to normal group. However, zebrafish pre-treated with galantamine (4 mg/kg) reversed this effect produced by STZ by significantly ($p < 0.001$) decreasing the TSDZ when, compared to the STZ treated group. Further, pre-treatment with quercetin (50 mg/kg, 100 mg/kg) significantly ($p < 0.001$) decreased the TSDZ as compared to the animals who received STZ (300 mg/kg). Moreover, the effect of treatment with quercetin (100 mg/kg) was found to be significantly greater in improving this effect when compared to quercetin (50 mg/kg) ($p < 0.001$)

3.4 Effect of quercetin on STZ induced memory impairment in Novel diving tank test

Figure 5.A, B shows that STZ (300 mg/kg) significantly altered the behaviour of zebrafish, as TSTZ and no. of entries to the top zone were significantly ($p < 0.001$) less when compared to normal animals. However, no significant change in TSTZ and no. of entries to the top zone were found in *per se* treatment with (galantamine 4 mg/kg) and quercetin (50 mg/kg, 100 mg/kg) and DMSO treated group as compared to the normal animals. Further pre-treatment with galantamine (4 mg/kg) significantly ($p < 0.001$) reversed the effect produced by STZ and it was found that TSTZ and no. of entries to the top zone was increased when compared to the STZ treated animals. However, pre- treatment with quercetin (50 mg/kg, 100 mg/kg) significantly ($p < 0.001$) ameliorated the TSTZ and no. of entries to the top zone as compared to the STZ treated group. Moreover, it was found pre-treatment with quercetin (100 mg/kg) was significantly more effective as compared to quercetin (50 mg/kg) ($p < 0.05$).

3.5 Effect of quercetin on STZ induced memory dysfunction on exploratory behavior in open field test

In the open field test, a definite parameter of zebrafish swimming movement was investigated. The *i.p.* injections had no discernible effect, and they did not obstruct data processing because all of the zebrafish were subjected to the same conditions. As shown by the total distance travelled, no significant variations in locomotor activity were identified in animals administered with any of the treatments as compared to the normal animals (Fig. 6A and B).

3.6 Effect of quercetin treatment on biochemical marker in STZ treated zebrafish

Figure 7A), B), C) shows that STZ (300 mg/kg) administration produced a significant elevation ($p < 0.001$) in the concentration of LPO, nitrite, and AChEs as compared to normal group. However, vehicle (DMSO) treatment and *per se* treatment with galantamine (4 mg/kg), quercetin (50 mg/kg, 100 mg/kg) didn't produce any significant effect as compared to normal group. Further, pre-treatment with galantamine significantly ($p < 0.001$) reduced the concentration of LPO, nitrite, and AChEs activity when compared to the STZ administered animals. Moreover, pre-treatment with quercetin (50 mg/kg and 100 mg/kg) significantly ($p < 0.001$) attenuated this level in comparison with STZ treated group.

Additionally, Fig. 7D shows treatment with STZ (300 mg/kg) produced a significant ($p < 0.001$) decline in the concentration of GSH. Further, no significant effect was produced by vehicle (DMSO) treated and *per se* treatment with galantamine (4 mg/kg), quercetin (50 mg/kg, 100 mg/kg) group when compared to the normal animals. However, pre-treatment with galantamine (4 mg/kg) significantly ($p < 0.001$) increased the concentration of GSH as compared to the STZ administered animals. Pre-treatment with quercetin

(100 mg/kg, 50 mg/kg) significantly ameliorated the concentration of GSH as compared to the STZ administered group. Moreover, quercetin (100 mg/kg) was found to be significantly ($p < 0.001$) more effective than quercetin (50 mg/kg).

3.7 Effect of Quercetin treatment on the level of brain inflammatory cytokine

Figure 8 shows, in zebrafish STZ administration significantly elevated the expression of TNF-α level when compared to the control groups ($p < 0.001$). Whereas, DMSO and *per se* treatment with galantamine (4 mg/kg), quercetin (50 mg/kg, 100 mg/kg) produced no significant effect as compared to the normal group. However, pre-treatment with galantamine (4 mg/kg) significantly ($p < 0.001$) decreased the concentration of TNF-α when compared to STZ administered group. Further, pre-treatment with quercetin (50 mg/kg, 100 mg/kg) significantly ($p < 0.001$) ameliorated the level of TNF-α as compared to the STZ administered group. Moreover, quercetin (100 mg/kg) was found to be significantly ($p < 0.001$) more effective in showing anti-inflammatory action as compared to quercetin (50 mg/kg)

3.8 Neuromorphological study of zebrafish brain by H & E staining

Histopathological analysis of brain sample was performed under light microscopy. In the histopathological study, the brains of normal, vehicle and *per se* treated groups showed undamaged neuronal cells. STZ (300 mg/kg) administration causes disruption of several cell layers as well as the loss of pyramidal neuronal cell was found which was significant when compared to the normal group. However, treatment with quercetin significantly attenuated the damage of neuronal cell density as compared to STZ treated animals (Fig. 9). *Per se* treatment with galantamine (4 mg/kg), quercetin (50 mg/kg, 100 mg/kg) did not show any significant outcome as compared to normal group.

4. Discussion

In the current study, we have investigated the possible protective action of the bioflavonoid quercetin to prevent the STZ induced memory impairments in adult zebrafish. It was seen that hyperglycemic condition was developed after STZ administration because it is a potent toxicant which damages the pancreatic β-cells. However, after pre-treatment with quercetin and galantamine the blood glucose level was significantly declined. In line with former experiments [38,39], our findings have described that STZ induced memory deficit in the T-maze, light-dark and novel tank diving test. Interestingly, it was found that pretreatment with single *i.p.* injection of 50 mg/kg and 100 mg/kg of quercetin 24 hour before the STZ treatment prevented STZ induced memory deficit in zebrafish and it also reduced the level of biochemical markers (LPO, Nitrite, AChEs) and inflammatory marker (TNF-α). Quercetin significantly improved the level of oxidative stress by enhancing the antioxidant (GSH) concentration. It was evident from the

histopathological analysis that quercetin exerts neuroprotective action as neuronal damage produced by STZ was reduced.

STZ is responsible for memory dysfunction by causing insulin receptor desensitization in brain cells [40]. However, STZ cause neuronal damage due to increased level of oxidative stress as a result of impaired energy and glucose metabolism and increased level of reactive oxygen species (ROS) in brain responsible to cause neurodegeneration [41]. STZ also inhibits activity of tyrosine kinase enzyme of insulin receptors by secreting nitric oxide (NO) in the cell. Excessive NO can react with ROS and form ONOO−, which can cause oxidative damage and lead to the aggregation of cytotoxic substances [42]. These cytotoxic substances can harm mitochondrial functions so that biological energy unable to meet the demand of neuronal functions. Eventually, this damage promotes the inflammatory cascade and exacerbates neurovascular dysfunction that ultimately leads to cognitive impairment [42].

In our study, it has been observed that in the T-maze test STZ (300 mg/kg) administration produced a significant decline in TSFZ and TL as compared to the normal animals. Whereas, TSUFZ was significantly increased, indicating impairment in memory. In the light and dark test, TSLZ was significantly declined and TSDZ was increased as compared to the normal group. In novel tank diving test, TSTZ and no. of entries to the top zone were also found to be significantly decreased in comparison with the normal animals, indicating altered behaviour. However, there are a lot of discrepancies related to effect of STZ in the locomotion. To discourse this issue, we performed a general analysis on zebrafish locomotor activity using open field test. There were no significant changes in total distance travelled by animals of STZ treated group, indicating no locomotor impairment.

It was found that STZ administration significantly elevated level of LPO, nitrite which are measure of oxidative stress. The increased level of inflammatory cytokine (TNF-α) and AChEs activity after administration in zebrafish resulted into memory impairment. Histopathological analysis of the STZ treated group was more evident to show damage to the pyramidal neuronal cells and disarrangement of cell layers as compared to the normal group.

Galantamine is an alkaloid, mainly present in the bulbs and flowers of the Caucasian snowdrop (Voronov's snowdrop) and Lycoris radiate (Red Spider Lily) and related species [43]. Galantamine, a centrally acting acetyl-cholinesterase (AChEs) inhibitor, commonly used to treat Alzheimer's disease (AD) and to ameliorate cognitive deficit [44]. Galantamine enhances the release of acetylcholine (ACh), a neuro transmitter in the hippocampus, which plays very crucial role in learning and memory [45,46]. Hence it was taken as a standard treatment in our study. It has been observed that pre- treatment with galantamine (4 mg/kg) reversed the effect produced by STZ (300 mg/kg). It increased the TSFZ, decreased the TSUFZ and TL in the t- maze test indicating restoration of memory. In the light and dark test, it was observed that pre- treatment with galantamine (4mg/kg), TSLZ was significantly declined and TSDZ was increased when compared to the STZ administered group. In the novel diving test, TSTZ and no. of entries to the top zone were also significantly increased in comparison with the STZ administered group, indicating reversal of altered behavior. No significant change in the total distance travelled was

found in OFT. However, pre- treatment with galantamine (4mg/kg) significantly lowered the level of LPO, nitrite which are measure of oxidative stress. The level of inflammatory cytokine (TNF-α) and AChEs activity also decreased significantly. Galantamine was also found to increase the level of antioxidant enzyme (GSH).

Quercetin is a natural compound generally obtained in diet, have been studied for a very long time and known to show numerous physiological activities (37). The activity of quercetin on cognitive functions have been associated to their capacity to interact with the possible cellular or molecular functions, involved in learning and memory, such as synaptic potentiation and neuronal plasticity (38). Quercetin is also identified to have anti-oxidant property, efficiently protecting nerve cells against neurotoxins, attenuating neuroinflammation, and increasing neuronal activity (39, 40). We found that in T-maze test pre-treatment with quercetin (50 mg/kg, 100 mg/kg) ameliorated the learning and memory of zebrafish by increasing TSFZ and decreasing TSUFZ and TL significantly as compared to STZ treated animals. In the light and dark test, quercetin was found to be effective to restore the memory, as it was observed that TSLZ was significantly increased and TSDZ was declined significantly when compared to the STZ administered group. Pre-treatment with quercetin (50 mg/kg and 100 mg/kg) was found to be effective in restoring the normal behaviour by increasing the number of entries to the top zone and TSTZ. There were no significant changes produced in total distance travelled in animals who received the pre-treatment of quercetin (50 mg/kg, 100 mg/kg). Further, quercetin significantly improved the level of oxidative stress by decreasing the level of LPO and Nitrite and by increasing the level of antioxidant enzymes (GSH) significantly in comparison with the STZ treated group. The elevated level of inflammatory cytokine (TNF-α) and AChEs activity after STZ administration was further reduced significantly due to pre-treatment with quercetin (100 mg/kg, 50 mg/kg).

The results of histopathological analysis suggested that STZ treated group were more evident to show damage to the pyramidal neuronal cells and disarrangement of cell layers, whereas, pre-treatment with galantamine reduced the neuronal damage. Quercetin (50, 100 mg/kg) also recovered the neuronal damage, which depicts its neuroprotective effect.

According to our findings, administration of quercetin prevented STZ- induced memory impairment in adult zebrafish, by antioxidant and anti-inflammatory potential suggesting that this flavonoid might act as a protective therapy against the advancement of dementia. However, in our present study, we have found that administration of quercetin (100 mg/kg) was more effective than quercetin (50 mg/kg) in the STZ model of memory dysfunction.

Conclusion

This study therefore highlighted the pharmacological justification for use of quercetin for streptozotocin induced memory dysfunction. STZ is reported to cause hyperglycaemia, oxidative stress and neuronal damage that ultimately lead to memory deficit. Therefore, neuroprotective effects of quercetin may be attributed to its attenuation of neuroinflammation, oxidative stress and cognitive deficits induced by STZ.

Declarations

Funding

This project is not supported by any funding source but Institute provide research facility to perform the work.

Conflicts of interest/Competing interests

Authors wish to assure that there was no conflict of interest related to this publication and there has been not any significant financial help for this study that would influence its outcome.

Availability of data and material

Data can be made available as when required.

Code availability (software application or custom code)

Not available

Authors' contributions

Authors participated in forming the design: AS, Interpretation of the research and evaluation of the data: NP, Materials: NP, BD, DS, Drafted the manuscript: NP, Critical evaluation and finalizing the manuscript content: CS and AS.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Not applicable

Ethics approval

This research work is approved by IBSC committee.

Consent to participate

Not applicable

Consent for publication

All authors give full consent to publish.

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Figures

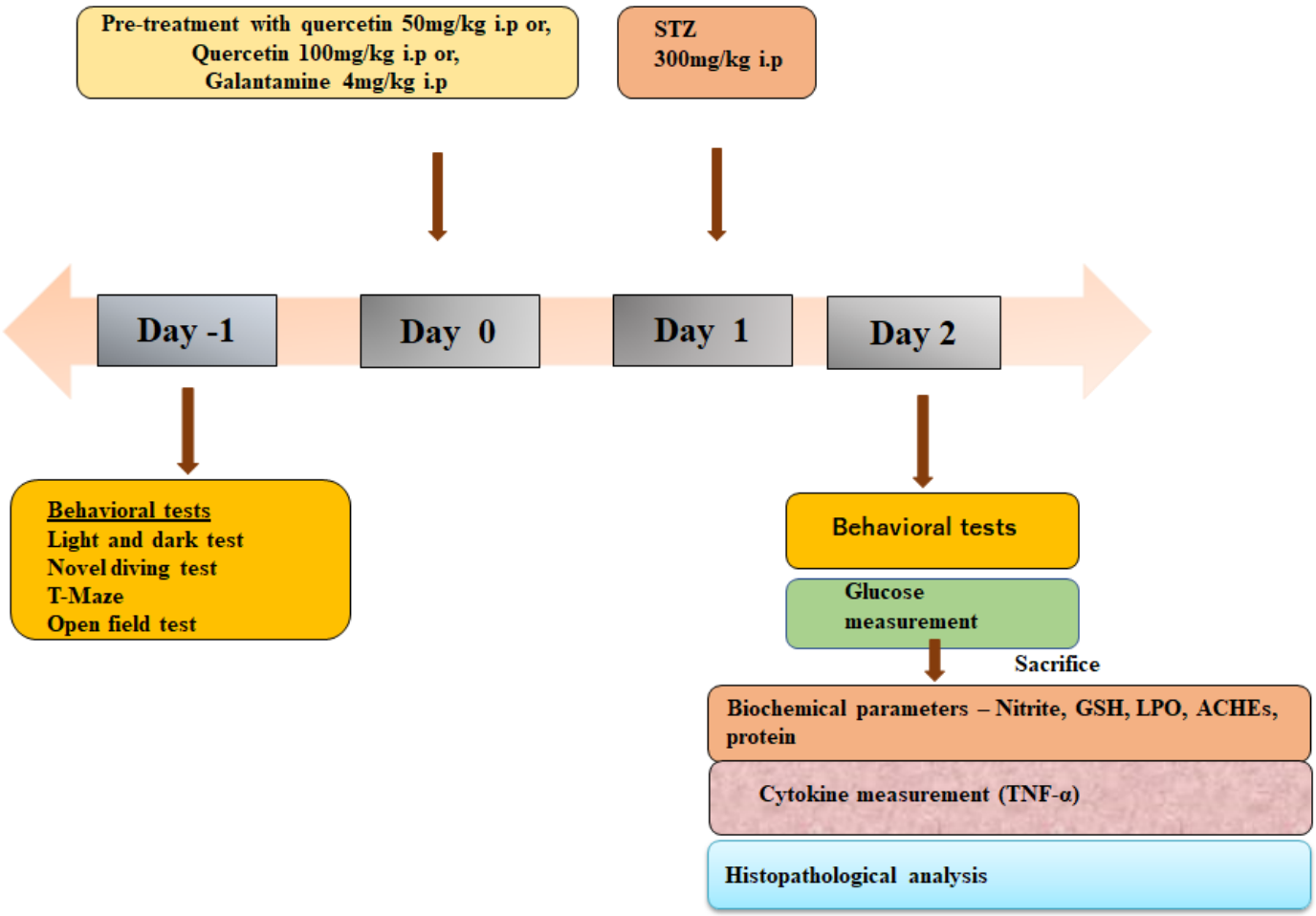

Figure 1

Experimental protocol (STZ: streptozotocin; LPO: lipid peroxidation; AChEs: acetylcholinesterase; GSH: reduced glutathione)

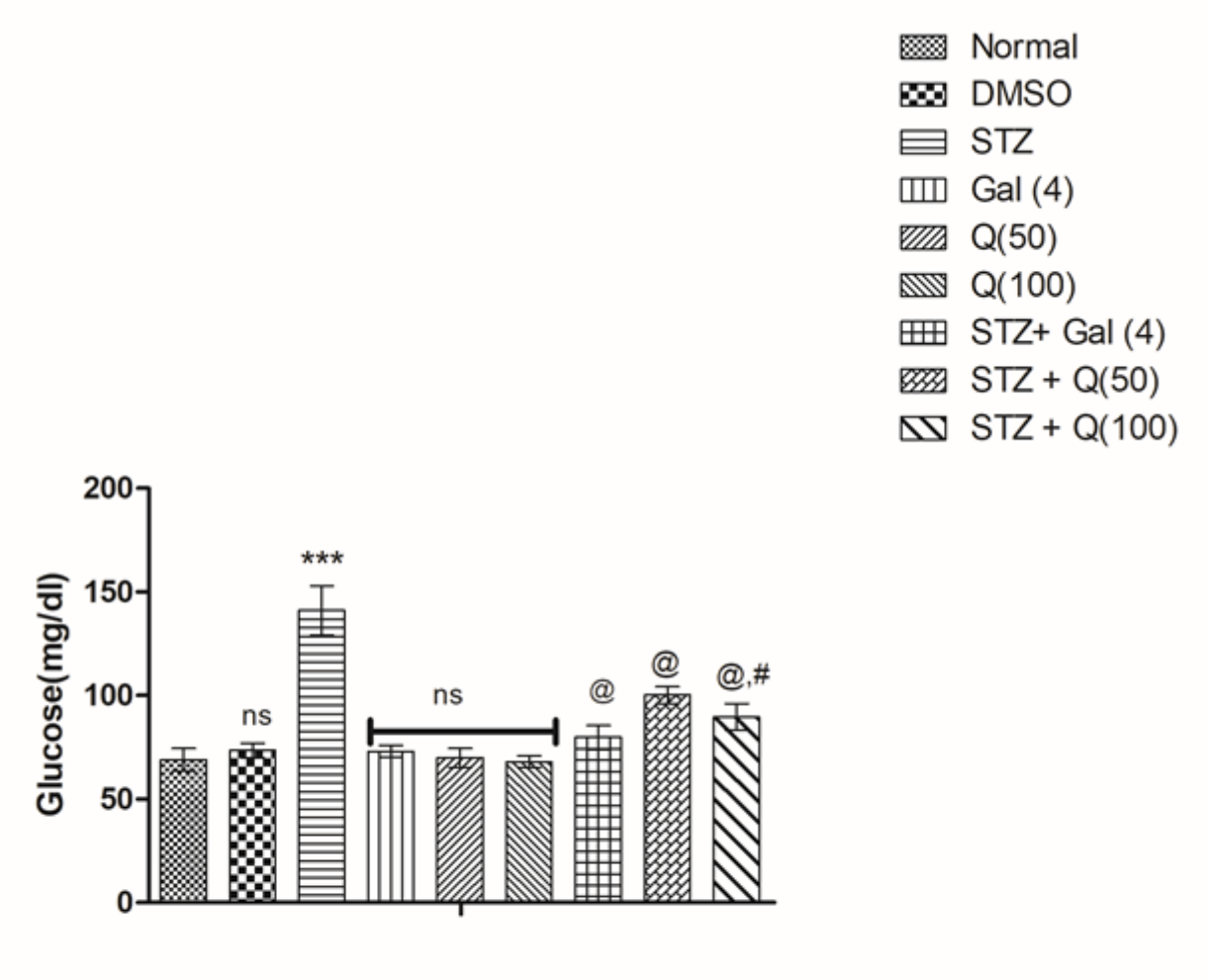

Figure-2

Figure 2

Quercetin prevented STZ induced hyperglycemia. Data is stated as mean ± SD denoted by columns and bars. ***p < 0.001 vs NC; @p < 0.001 vs STZ; #p < 0.05 vs Quercetin (50 mg/kg, i.p.). Statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. [Abbreviation: STZ-Streptozotocin; Q (50): Quercetin 50mg/kg ; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg)]

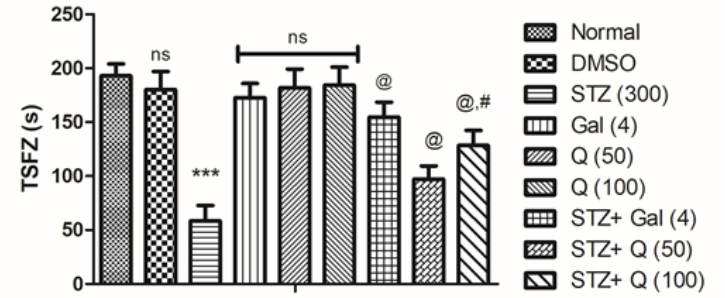

Figure 3A

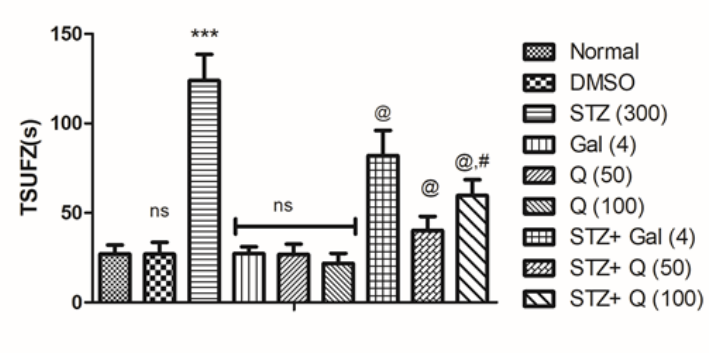

Figure 3B

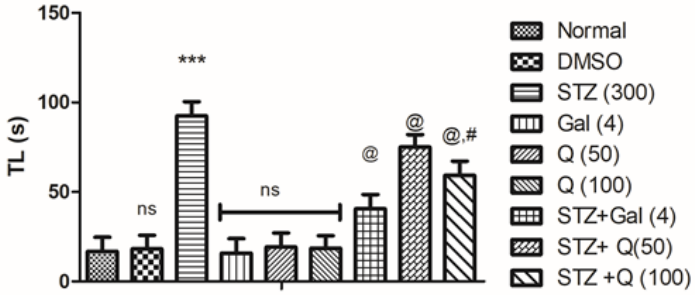

Figure 3 C

Figure 3

Quercetin prevented STZ induced memory deficit of adult zebrafish in T-maze apparatus. A) TSFZ B) TSUFZ C)TL. Zebrafish were pre-treated before 24 hr of STZ administration with quercetin. Data were expressed as the mean ± SD,***p < 0.001 vs NC; @p < 0.001 vs STZ; #p < 0.05 vs Quercetin (50 mg/kg, i.p.). (One way Anova followed post test Tukey) [STZ: Streptozotocin (50): Quercetin 50mg/kg ; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg; TSFZ: Time spent in favorable zone; TSUFZ: Time spent in unfavorable zone;); TL: Transfer latency to enter in favorable zone;]

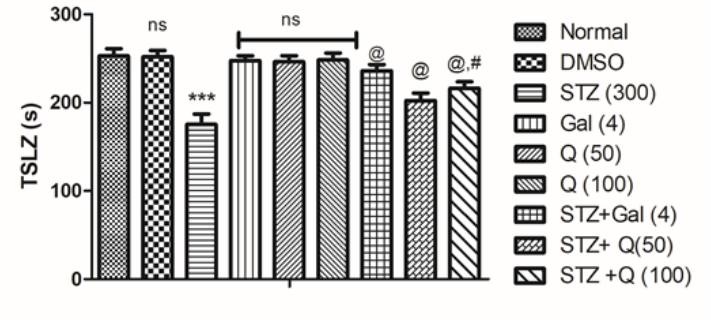

Figure 4A

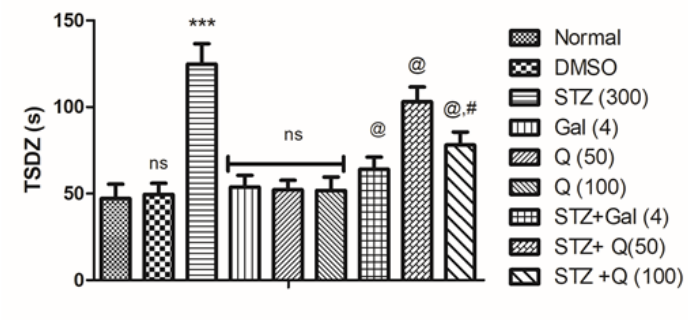

Figure 4 B

Figure 4

Quercetin prevented STZ induced memory impairment of adult zebrafish in Light and Dark chamber test. A) TSLZ B) TSDZ. Data is expressed as mean ± SD represented by columns and bars. ***p < 0.001 vs NC; @p < 0.001 vs STZ; #p < 0.01 vs Quercetin (50 mg/kg, i.p.). Statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. [Abbreviation: STZ-Streptozotocin; Q (50): Quercetin 50mg/kg ; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg); TSLZ: Transfer spent in light zone; TSDZ: Time spent in dark zone]

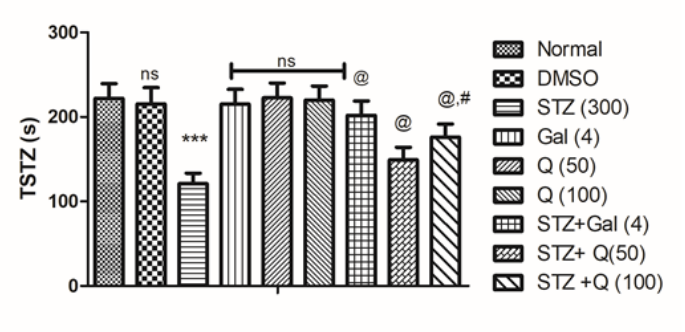

Figure 5 A

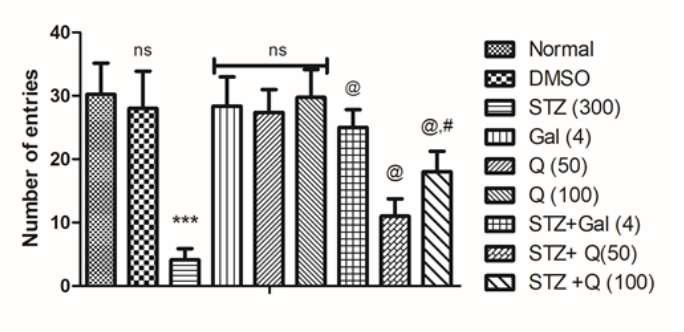

Figure 5 B

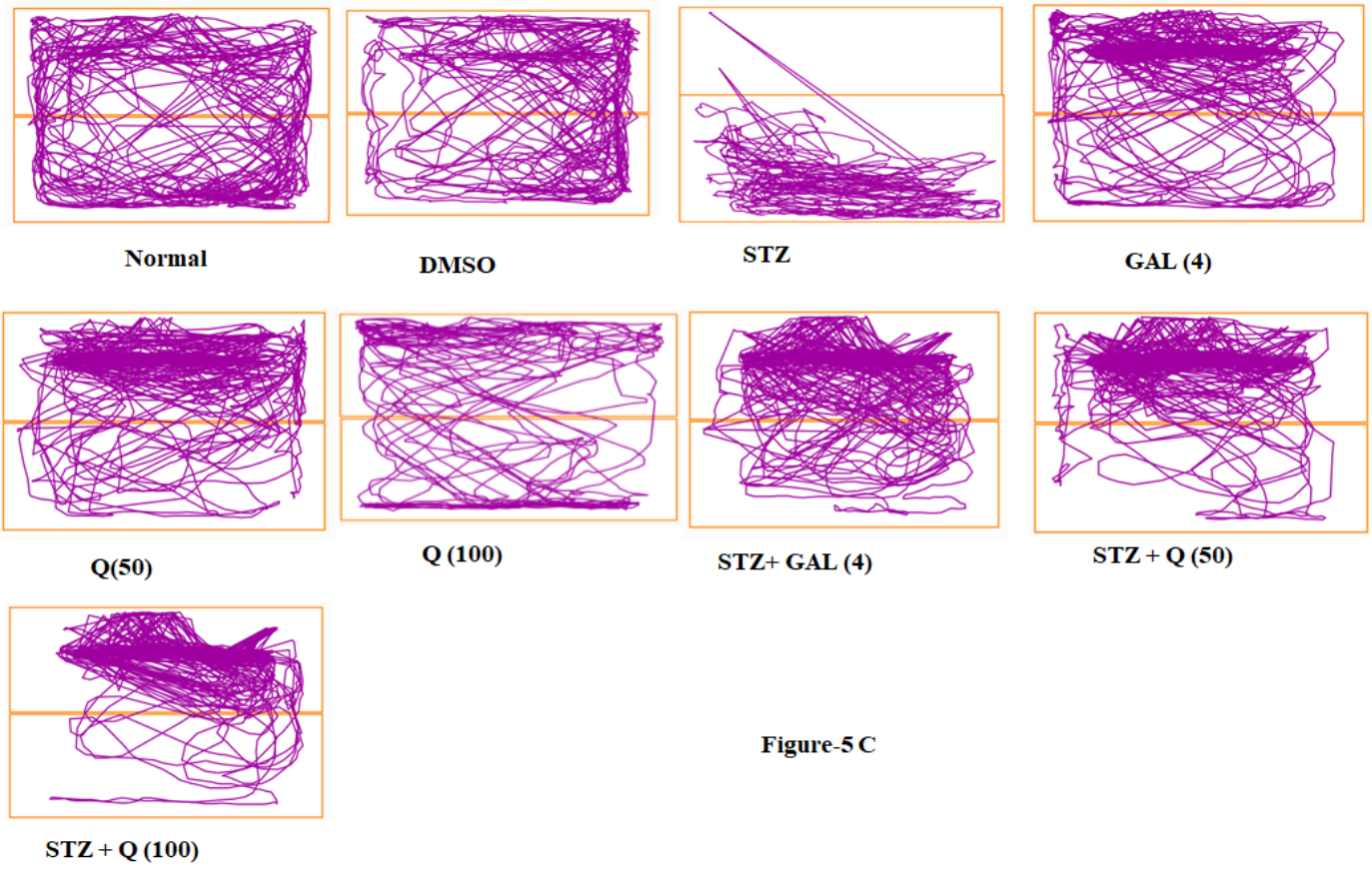

Figure-5 C

Figure 5

Quercetin and STZ effects on Novel diving tank test. a) TSTZ b) Number of entries c) Representative track plots of NTDT. Data is expressed as mean ± SD represented by columns and bars. ***p < 0.001 vs NC; @p < 0.001 vs STZ; #p < 0.01vs Quercetin (50 mg/kg, i.p.). Statistical analysis performed by one-way

ANOVA followed by Tukey's post hoc test. [Abbreviation: STZ-Streptozotocin; Q (50): Quercetin 50mg/kg ; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg); TSTZ: Transfer spent in top zone]

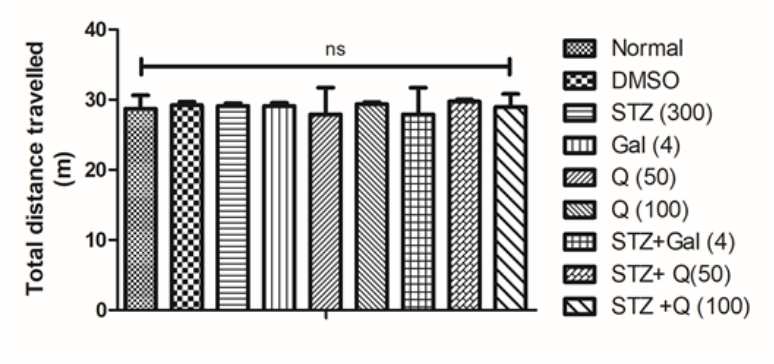

Figure-6 A

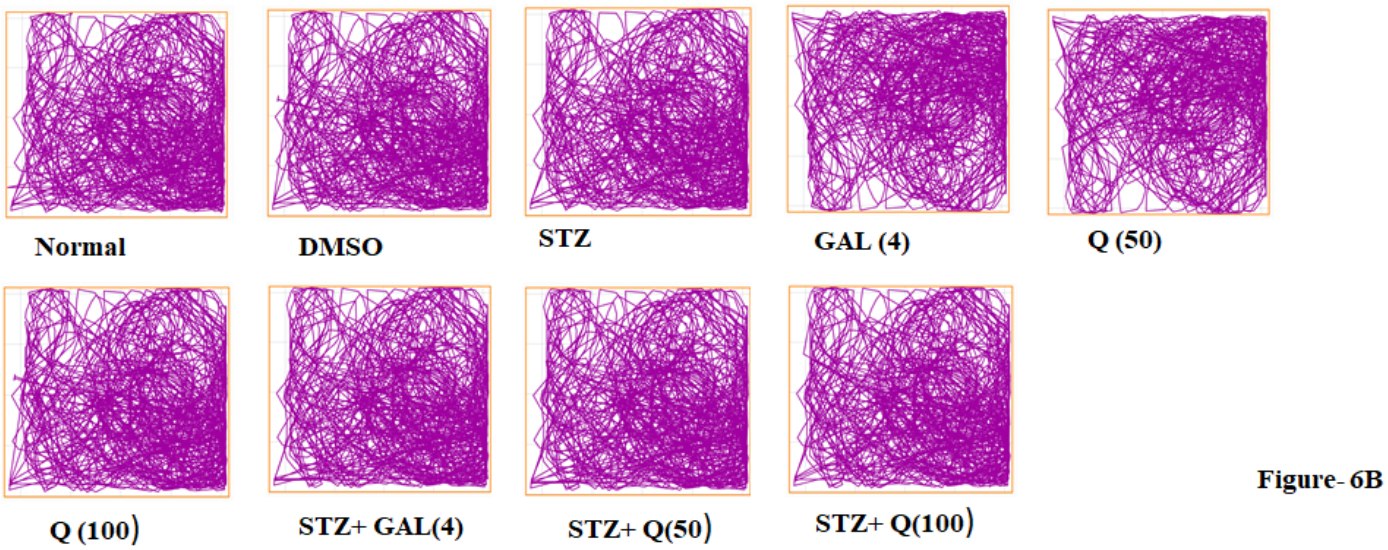

Figure- 6B

Figure 6

Quercetin and STZ effects on exploratory assessment. a) Total distance travelled b) Representative track plots of Open field test. Data is expressed as mean ± SD represented by columns and bars..). Statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. [Abbreviation: STZ-Streptozotocin; Q (50): Quercetin 50mg/kg ; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg)]

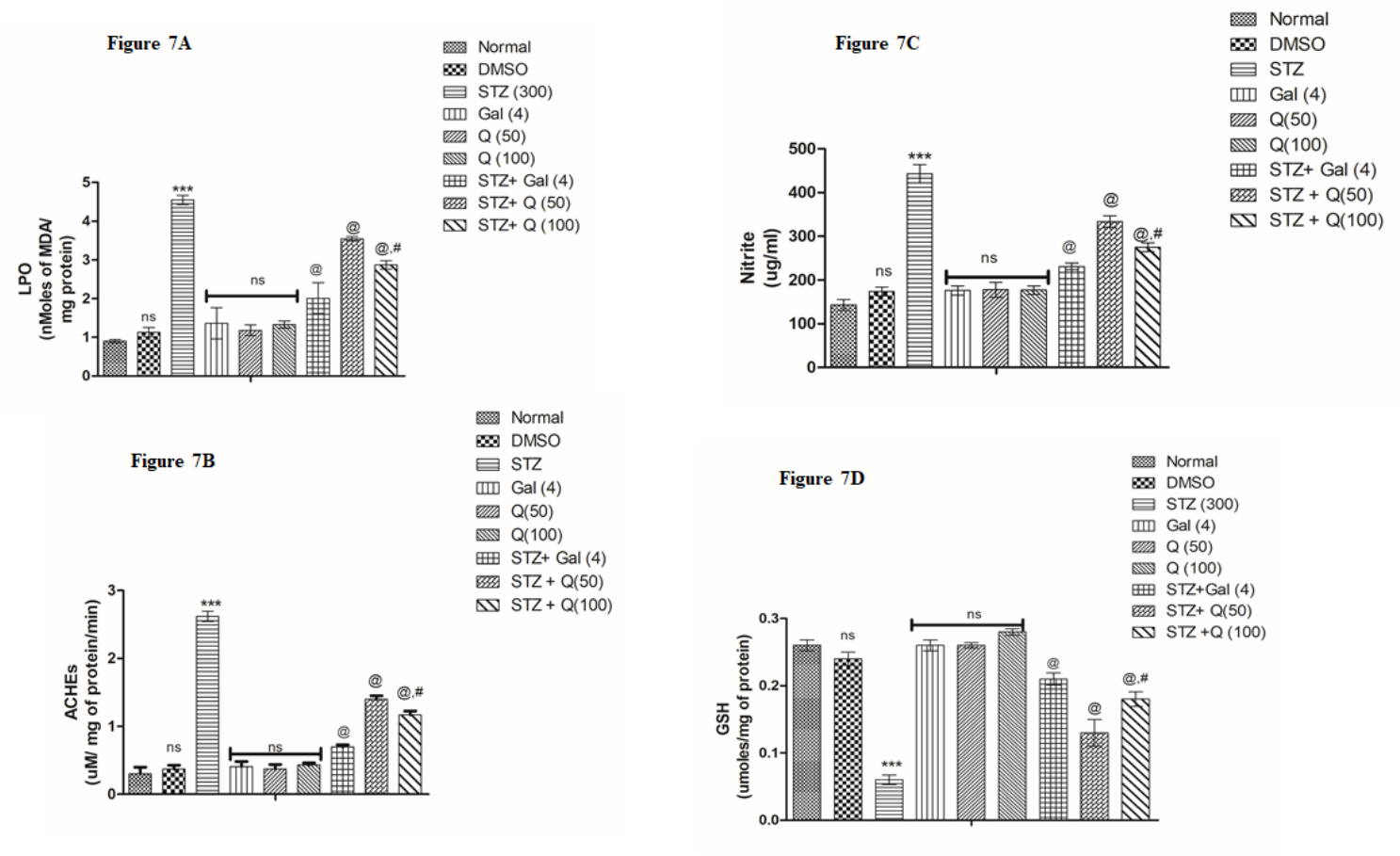

Figure 7

Quercetin effects the oxidative stress biomarkers induced by STZ. A) LPO B)AchE C) Nitrite D) GSH. Data is expressed as mean ± SD represented by columns and bars. ***p < 0.001 vs NC; @p < 0.001 vs STZ; #p < 0.05 vs Quercetin (50 mg/kg, i.p.). Statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. [Abbreviation: STZ-Streptozotocin; Q (50): Quercetin 50mg/kg ; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg); LPO: lipid peroxide; GSH; reduced glutathione; AChE: Acetylcholinesterase]

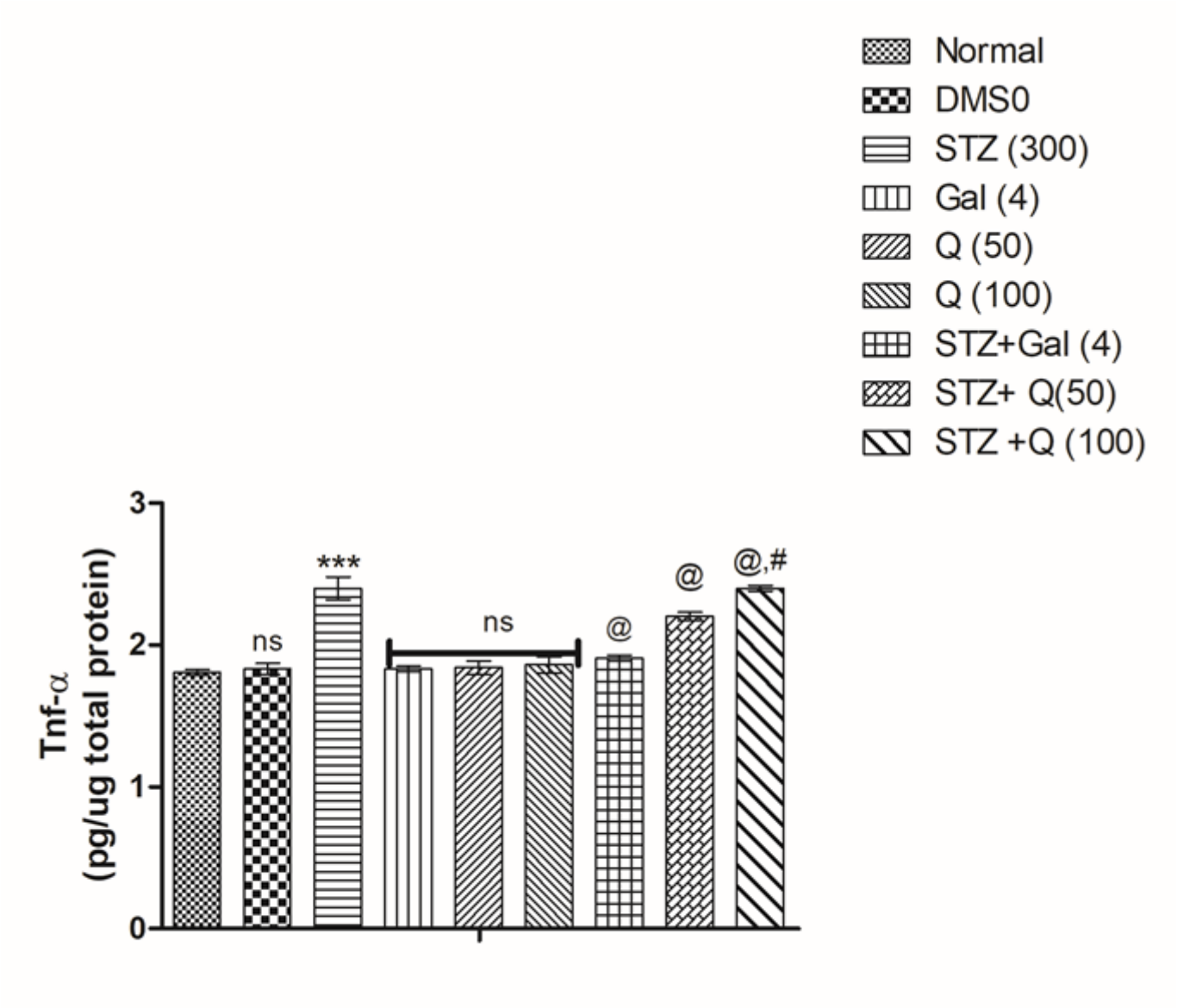

Figure 8

Figure 8

Quercetin attenuates the level of brain inflammatory cytokine (TNF-α). Data is stated as mean ± SD denoted by columns and bars. ***p < 0.001 vs NC; @p < 0.001 vs STZ; #p < 0.05 vs Quercetin (50 mg/kg, i.p.). Statistical analysis performed by one-way ANOVA followed by Tukey's post hoc test. [Abbreviation: STZ-Streptozotocin; Q (50): Quercetin 50mg/kg; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg)]

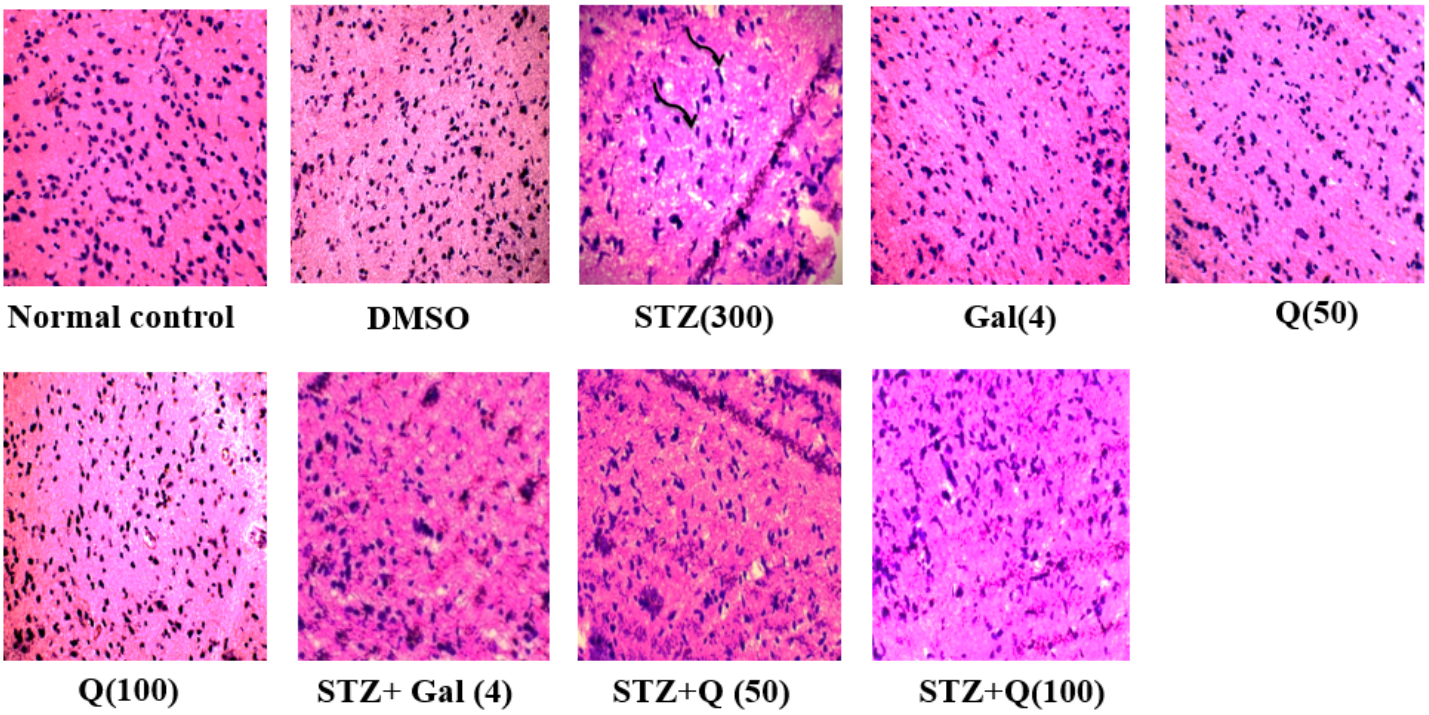

Figure-9

Figure 9

Neuromorphological study of zebrafish brain by Hematoxylin and eosin staining. (i) Respective image of whole brain cross sectional view after hematoxylin and eosin staining [a]. curved arrowsdepict pyknotic cells.; magnification 40X, scale 20µm.[Abbreviation: STZ-Streptozotocin; Q (50): Quercetin 50mg/kg ; Q (100): Quercetin 100mg/kg ; Gal (4): Galantamine (4mg/kg)].

Supplementary Files

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