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Neuroprotective Effects Of Sotagliflozin In A Rotenone-Induced Zebrafish Model Of Parkinson's Disease

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Abstract: ***Background and Objectives:*** *In this study, we investigated the neuroprotective potential of sotagliflozin, a dual SGLT1/2 inhibitor (SGLT1/2i), in a rotenone-induced zebrafish model of Parkinson's disease (PD). Rotenone, a mitochondrial complex I inhibitor, induces dopaminergic neuronal loss and replicates pathological features related to PD, including oxidative stress and neuroinflammation. As SGLT2 inhibitors have been reported to provide neuroprotection by reducing oxidative stress and inflammation, this study aimed to assess the efficacy of sotagliflozin in mitigating neurodegenerative changes and behavioral impairments related to PD in zebrafish.*

Materials and Methods: *Zebrafish embryos were initially subjected to acute toxicity tests using various concentrations of sotagliflozin to determine the safe and effective dose range. Adult wild zebrafish were subjected to 5 µg/L ROT to induce PD-like symptoms and subsequently treated with sotagliflozin at 4, 8, and 16 mg/L. A positive control group received levodopa (25 mg/L) and carbidopa (2.5 mg/L). Behavioural assessments, markers of oxidative stress, antioxidant enzyme levels, peroxidation of lipid, acetylcholinesterase (AChE) levels, apoptosis- related gene expression (BAX and BCL2), and brain histopathology were evaluated in the study.*

Results: *Toxicity assessments revealed that higher concentrations of sotagliflozin 32mg/L led to significant developmental impairments, including delayed hatching and morphological abnormalities in zebrafish embryos. In contrast, in adult zebrafish with rotenone-induced PD, sotagliflozin, particularly at 16 mg/L exerted pronounced neuroprotective effects. This high dose also significantly reduced oxidative stress, enhanced catalase and superoxide dismutase activity, restored glutathione levels, and lowered lipid peroxidation. AChE activity was improved, indicating better neurotransmitter function. Histological analysis showed reduced inflammation, edema, and cellular damage in the brain. Furthermore, sotagliflozin modulated the expression of apoptosis-related genes by reducing BAX and increasing BCL2 expression.*

Conclusion: *Sotagliflozin (16 mg/L) exhibited neuroprotective effects in a zebrafish model of PD by improving motor function, reducing oxidative stress, and modulating apoptotic pathways. These findings highlight the therapeutic potential of SGLT2 inhibitors in neurodegenerative conditions such as Parkinson's disease, supporting their role beyond glycemic control.*

Keywords: *Parkinson's disease (PD), Sotagliflozin, Rotenone (ROT), Zebrafish, Levodopa, Carbidopa.*

1. INTRODUCTION

Parkinson's disease (PD) is a gradually progressing neurodegenerative disease that leads to the loss of dopaminergic neurons in the substantia nigra. This loss of dopamine gives rise to the development of motor

function-related clinical features such as bradykinesia, tremors, rigidity, and non-motor features such as mood disturbances and cognitive decline **(1)**. Although its etiology remains largely idiopathic, contributing factors for PD include aging, genetic predisposition, and environmental toxins **(2)**. PD is the 2nd commonest NDD (Neuro Degenerative Disorder) after Alzheimer's, accounting for approximately 1% of the population above the age of 60. Its prevalence has increased significantly, with projections estimating over 12 million global cases by 2040 **(3,4)**. Exposure to pesticides, herbicides, and heavy metals is responsible for the etiopathogenesis of PD. One important risk factor is age, with neuronal loss in the substantia nigra being more pronounced in older adults **(5)**. Originally, SGLT2 (Sodium-Glucose Cotransporter 2) inhibitors were developed for glycaemic control in type 2 diabetes. However, SGLT2 inhibitors (SGLT2i) have also demonstrated neuroprotective potential by reducing oxidative stress and inflammation, and by improving insulin sensitivity. Emerging evidence suggests a correlation between SGLT2i use and a reduced incidence of neurodegenerative diseases (NDDs). Notably, these agents can penetrate the blood-brain barrier (BBB) and target SGLT1 and SGLT2 cotransporters, which are expressed in the brain parenchyma **(6,7)**. Rotenone (ROT), a neurotoxin, is widely utilized to induce PD-like pathology in preclinical models. It mimics the features of human PD, including dopaminergic neuron loss, oxidative stress, and neuroinflammation **(8)**. Zebrafish share significant genetic and neuroanatomical homology with humans, making them a valuable model for PD research. Their transparency and rapid development facilitate in vivo neurological assessments and drug screening **(9)**. Sotagliflozin is a dual SGLT1/2 inhibitor capable of crossing the BBB and regulating glucose level in the CNS. Both SGLT1 and SGLT2 are expressed in critical brain areas involved in energy balance and neuroregulation. The potential of sotagliflozin to modulate oxidative stress and mitochondrial function positions it as a therapeutic candidate for PD **(10)**. The objective of this research was to evaluate the neuroprotective effects of sotagliflozin, a dual SGLT1/2 inhibitor in ROT-induced PD of zebrafish model. Specifically, the focus was on whether sotagliflozin can ameliorate dopaminergic neurodegeneration, reduce motor function deficits, attenuate oxidative stress-induced neuroinflammation, and modulate the expression of BAX and BCL2, which are commonly associated with the pathogenesis of PD.

2. Methodology

The study was approved by the Institutional Animal Ethics Committee of the Chettinad Hospital & Research Institute (CHRI) (IAEC 3/proposal:142/A. Lr:105/Dt:28.11.2023), CARE, India.

2.1 Zebrafish (ZF) Husbandry

Adult zebrafish (Danio rerio) were maintained and handled as per the animal welfare guidelines (zfin.org). Adult zebrafish were procured and housed at 25°C, after which the fish were acclimatized for two weeks before the study. Mature females and males were selected at a ratio of 2:1 and placed in an undisturbed breeding environment the day before spawning. Viable eggs were harvested by spontaneous spawning and rinsed with distilled water in the next morning. Embryos were preserved in embryonic(E3) medium which is prepared by dissolving 1.6 g KCl, 34.8 g NaCl, 5.8 g $CaCl_2 \cdot 2H_2O$, and 9.78 g $MgCl_2 \cdot 6H_2O$ in distilled water. Damaged or coagulated eggs were discarded, and only viable embryos were used for acute embryo toxicity assessment **(11)**.

2.2 Acute Zebrafish Embryo Toxicity (ZET) Effects of Sotagliflozin

Embryotoxic effects were evaluated for sotagliflozin at varying doses of 1, 2, 4, 8, 16, and 32 µg/mL. Embryos were examined at 24-, 48-, and 72-hours post-fertilization (hpf) under a stereomicroscope **(11)**.

2.3 Experimental Groups

In this experiment, we studied six groups with 10 fish per group. Group 1 is considered as control. Group 2-ROT induced at a concentration of 5 µg/L to develop a Parkinson's neurodegeneration zebra fish model. Groups 3, 4, and 5 were the treatment groups that received sotagliflozin at 4,8 and 16 mg/L, respectively. Group 6 served as the positive control (PC)and received a combination of levodopa (25 µg/mL), and carbidopa (2.5 µg/mL) in the daily routine exposure to ROT 5 µg/L dissolved in water.

2.4 Behavioral Assessments

In this study, behavioural assessment was conducted between 8 AM and 4 PM on the 21st day of the experiment. Daily water changes were performed according to the OECD guidelines (12, 13).

2.5 Locomotion Activity

The fish were recorded for 5 min in a 25×15×15 cm polypropylene tank (5 L of water) using an advanced camera. Swimming behavior was analyzed using ANY-maze software. **(11,13)**.

2.6 Novel Tank Test (NTT)

Post 21 days of exposure to rotenone, three fish per group were placed in a designated tank (16 cm height × 28 cm top width × 23 cm bottom width × 7.5 cm width) with 1.5 Liters of water. The movement was recorded for 10 min and manually assessed **(14)**.

2.7 Light/Dark Test

A 30×22×10 cm tank was separated into equal sections (15 cm each). One half of the tank was painted black. Each fish was introduced into the light zone and observed for 5 min to record the time consumed to enter the dark zone, number of crossings, and time confined to the light zone **(15)**.

2.8 Antioxidant Assay

Antioxidant assays were performed on the harvested brain tissue following a 21-day terminal procedure **(17).** The kit methods were utilized to study the SOD (Cat # E-BC-K020-M), CAT (Cat # E-BC-K031-S) **(16),** GSH (Cat # E-EL-0026) **(17)**, GPx (Cat # E-BC-K096-S)**(18)**, MDA (Cat # E-EL-0060) **(19)** and AChE (Cat # E-BC-K174-M) **(20),** activities using the manufacturer's instructions and absorbance was recorded to determine the antioxidant effect in the experimental groups.

2.9 Histopathological Analysis:

Zebrafish were anesthetized with 0.1% tricaine, and 10% neutral buffered formalin was used to fix brain tissue, and H&E staining was carried out in the 4 μm tissue as described in a previous study **(21)**.

2.10 Gene Expression Analysis – Brain Tissue Processing

The brain tissue was homogenized, and RNA was isolated using TRIzol method (22). Primers for the zebrafish species were procured from Eurofins (Bangalore, India). Bax; Forward: 5'-GAGACACGGCATCCAGGAAA-3', Reverse:5'-TGTGTCCACGGCGGCAATCA-3'. Bcl-2; Forward:5'-TGCAGAGATGTCCAGTCAGC-3', Reverse: TGTTGACATCAGCGGTCTTCAG-3'. Loading control β-actin; Forward:5'-TGCCCCTCGTGCTGTTTT-3', Reverse:5'-TCTGTCCCATGCCAACCAT-3'. In accordance with the manufacturer's guidelines, 500 ng of total RNA were converted into complementary DNA (cDNA) using a reverse transcription kit. Gene-specific forward and reverse primers (optimized to final concentrations of 0.1–0.5 μM), cDNA template, and SYBR Green master mix were used to produce RT-PCR in a final volume of 20 μL. First, denaturation was done at 95°C for 2 minutes. This was followed by 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Melt curve was utilized to verify the specificity. The 2^(-ΔΔCt) technique was used to calculate relative gene expression, adjusting target gene expression (Bax and bcl-2) with β-actin.

2.11 Statistical analysis:

One-way analysis of variance and Bonferroni's post hoc multiple comparison test were used to collect and analyze the data. GraphPad Prism 10.0 was used to assess the significance level, and the p-values were "*$p < 0.05$, **$p < 0.01$"

3.RESULTS

3.1 Assessment of Zebrafish Embryo Toxicity with multiple doses of Sotagliflozin

In the in vivo analysis, the acute toxicity of sotagliflozin was evaluated (Figure 1). Embryos were exposed to varying doses of sotagliflozin (1 μg/ml, 2 μg/ml, 4 μg/ml, 8 μg/ml, 16 μg/ml, & 32 μg/ml). Embryos were observed at 48, 56, and 72 hpf to assess developmental toxicity. At concentrations of 16 μg/ml and above, major developmental abnormalities were evident, including delayed hatching and physical malformations, such as body axis curvature, pericardial edema, and compromised organ development. The toxic effects were dose-dependent, with more severe abnormalities observed at higher concentrations. At 16 μg/ml, the embryos exhibited a slower progression of development, including hatching delays and visible deformities. As the concentration of sotagliflozin increased beyond 16 μg/ml, the toxic effects became more pronounced, with higher levels resulting in noticeable lethality. At the maximum concentration of 32 μg/ml, almost no embryos hatched, and severe deformities were observed in the majority of the exposed embryos. The lethal dose (LD_{50}) of sotagliflozin was calculated as 16 μg/ml, as 50% of the embryos survived at this concentration. This highlights the concentration-dependent toxicity of sotagliflozin, providing important insights into its potential teratogenic effects and its lethal threshold during early zebrafish development.

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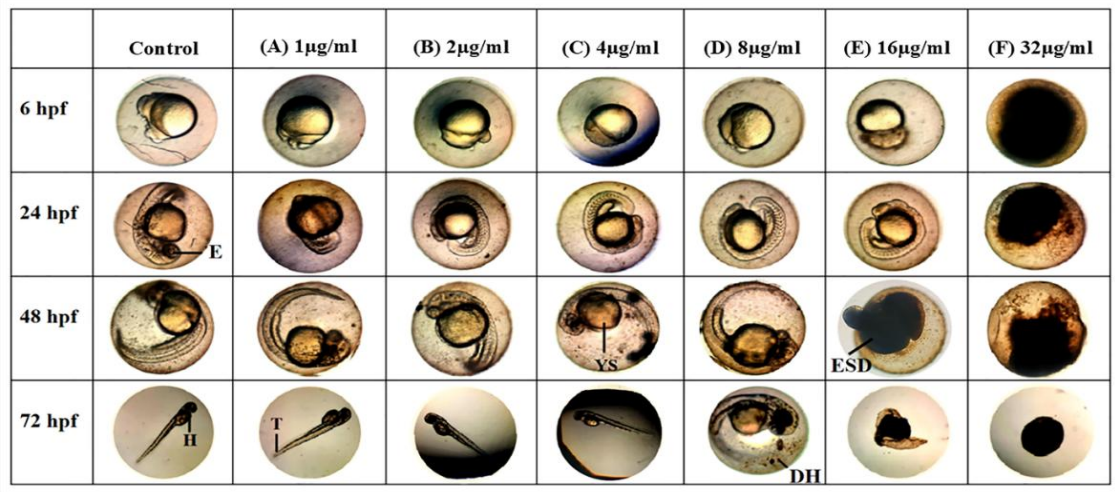

All images were taken at 4X magnification

Figure 1. Toxicological endpoints in the zebrafish embryo toxicity test of Sotagliflozin A–F at different concentrations: The zebrafish embryonic development study showed that the control group exhibited normal development. Treated groups A(1µg/ml), B (2µg/ml), and C (4µg/ml) showed normal hatching time, proper embryonic development, and normal activity, indicating no observed toxicity. Group D (8µg/ml) displayed slight developmental toxicity after 72 hpf, with delayed hatching noted. In contrast, group E(16µg/ml) shows moderate toxicity at 48 hpf, and group F (32µg/ml) shows full toxicity, with complete embryo mortality(death) observed (4X). (E- Eye, H- Heart, T- Tail, YS-Yolk Sac, DH-Delayed Hatching, ESD-Embryo Sac Deformities).

3.2 Comparison of Locomotor Activity of Zebrafish across all experimental groups

In this study, while observing locomotion tracking, there was a significant reduction in motor function in ROT-treated zebrafish, compared to the control group (Figure 2). Treatment with high-dose sotagliflozin (16 mg/L) significantly restored swimming activity, closely paralleling the positive control group (levodopa + carbidopa).

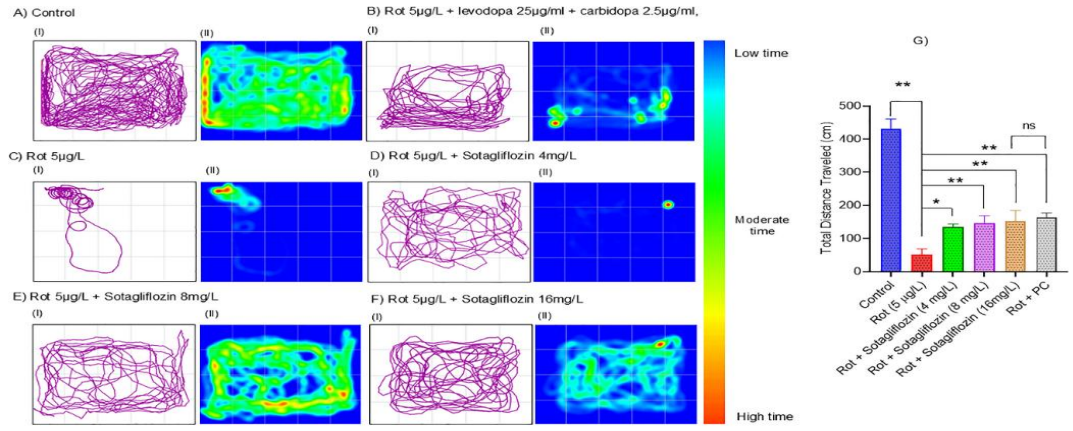

Figure 2. Locomotor trajectories of zebrafish under different conditions after the 3rd week of the experiment, with Heat maps marked as (II).

(A) Represents the control group with the respective heatmap revealing high activity and central occupancy. (B) Positive control treated with Rotenone 5µg/L, levodopa 25µg/ml, carbidopa 2.5µg/ml, exhibited moderate exploration and balanced swim pattern. (C) The Rotenone 5µg/L induced group exhibited thigmotaxis (wall-hugging), indicating anxiety-like behavior. (D) Rotenone 5µg/L induced treated with Sotagliflozin 4mg/L increased perimeter swimming with limited center crossing. (E)Rotenone 5µg/L induced and treated with the Sotagliflozin 8mg/L group shows the asymmetric distribution, suggesting disrupted

locomotion. (F) The last group shows that Rotenone 5µg/L induced and treated with Sotagliflozin 16mg/L resulted in a mixed swimming pattern, indicating partial recovery. The graph G) represents the total distance traveled by the control and induced group compared with the treated group. The colors indicate how much maximum time fish stay in a particular place: red indicates they stay for a long time, yellow indicates a moderate duration, and light green shows a very short stay.

3.2 Behavioral Assessment of Zebrafish between Experimental Groups:

Zebrafish swimming pattern was assessed using the (NTT) Figures 3A and 3B. The control group spent more time in the upper tank (65.67 ± 5.87s; P<0.001), indicating normal behavior, whereas the ROT-induced group spent only 31.33 ± 2.72s, suggesting increased anxiety. High-dose sotagliflozin significantly improved this behavior (51.33 ± 3.1s; P<0.05), indicating a potential anxiolytic effect. Similarly, the positive control group showed a notable reduction in anxiety, spending 61.67 ± 1.76s in the upper tank. Conversely, the ROT-induced group spent significantly more time in the lower tank (407.7 ± 28.66s), indicating increasing anxiety levels and impaired locomotion (Figure 3B), whereas the control group spent less time (249 ± 26.85s). High-dose sotagliflozin treatment significantly reduced the time spent in the lower tank, reflecting improved movement (P<0.05) and reduced anxiety.

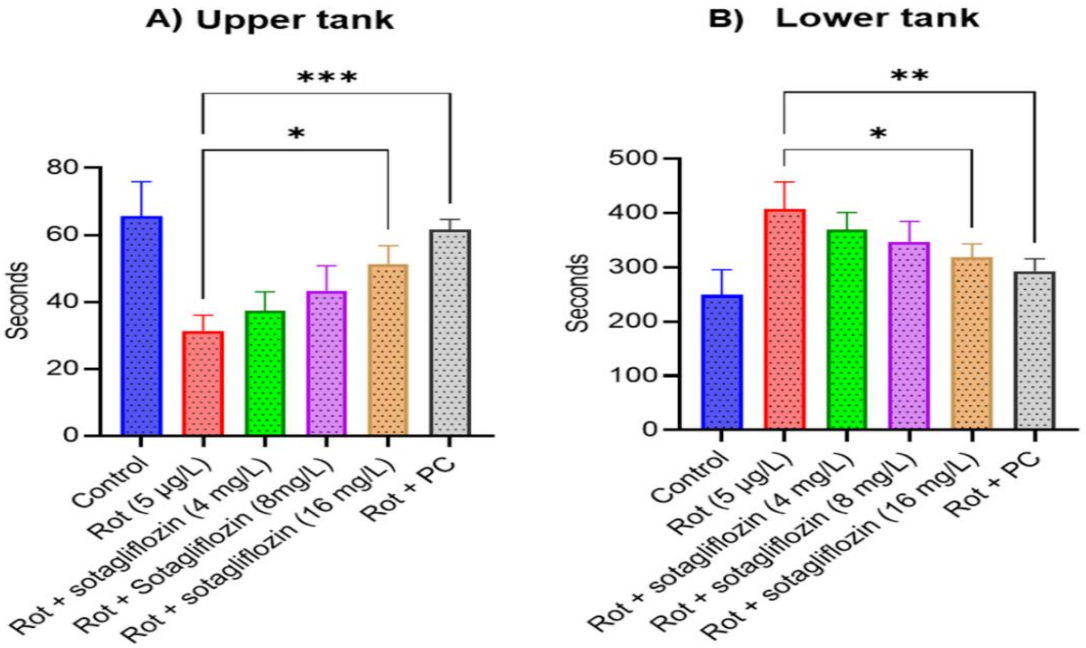

Figure 3. Novel Tank Test (A) Time spent in the upper tank zone and (B) time spent in the lower tank zone were measured in seconds across different treatment groups. Control zebrafish showed significantly higher activity in the upper zone, indicating low anxiety levels. In contrast, Rotenone (5 µg/L)-exposed fish spent significantly more time at the bottom, reflecting anxiety-like behavior. Co-treatment with Sotagliflozin at increasing doses (4, 8, and 16 mg/L) improved the behavioral phenotype in a dose-dependent manner, as evidenced by more time spent in the upper zone and less in the lower zone. The positive control (Rot + Levodopa 25 µg/mL + Carbidopa 2.5 µg/mL) showed similar improvements. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc test. $p < 0.05$ *, $p < 0.01$ *, $p < 0.001$ ***.

In the Light & Dark test (Figure 4A and 4B), the ROT-induced group spent more time in the dark (88.67 ± 11.72 Sec; P<0.05) and less time in the light (120.7 ± 13.92s), suggesting heightened anxiety. In contrast, the control group spent significantly more time in the light (239.0 ± 31.50s) and less time in the dark (31.33 ± 9.39s; P<0.05). High-dose sotagliflozin moderately improved the behavior pattern, with zebrafish spending 147.3 ± 12.44s in the light and 64.33 ± 4.63s in the dark, when compared to the ROT only group.

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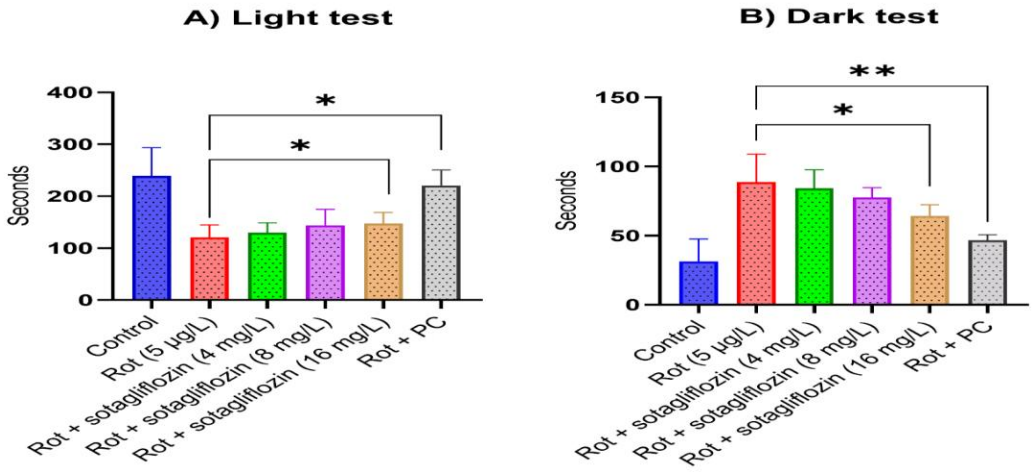
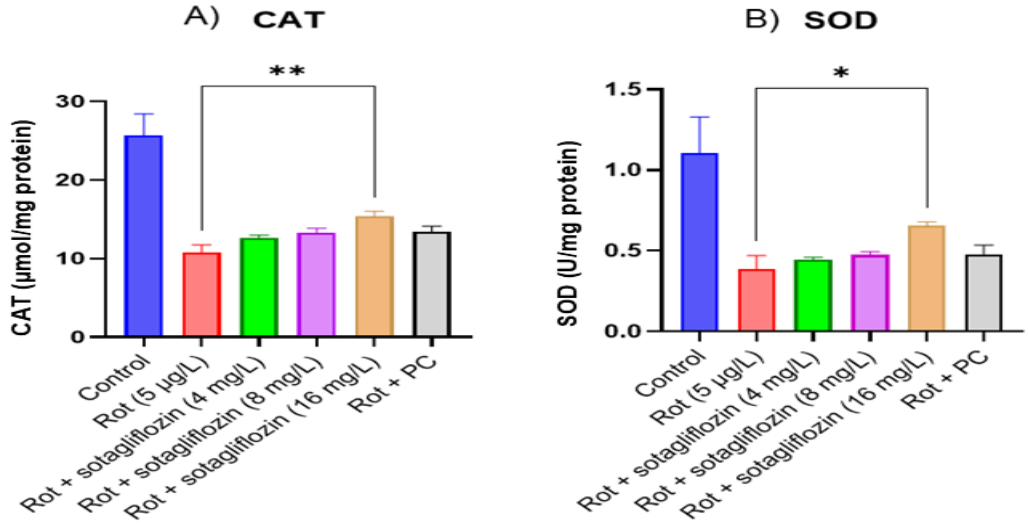


Figure 4. Zebrafish were tested for light /dark preference after treatment with Rotenone (5 µg/L) alone or with increasing doses of Sotagliflozin (4, 8, and 16 mg/L). Control fish spent more time in the light zone, indicating low anxiety, while Rotenone-treated fish preferred the dark, showing anxiety-like behavior. Co-treatment with Sotagliflozin reduced this anxiety, especially at 16 mg/L, as indicated by increased time in the light. The positive control (Rotenone + Levodopa 25 µg/mL + Carbidopa 2.5 µg/mL) also showed improved behavior. Data are presented as mean ± SEM (n = X per group), analyzed via one-way ANOVA and Tukey's test ($p < 0.05$*, $p < 0.01$ **).

Restoration of Antioxidant Enzyme level in zebrafish brain

The levels of antioxidant enzymes in zebrafish treated with ROT and sotagliflozin are shown in Figure 5. Catalase activity (Figure 5A), which is crucial for detoxifying reactive oxygen species, was significantly reduced in the ROT-induced group (10.81 ± 0.53) compared to the control group (25.66 ± 1.60; P<0.05), indicating oxidative stress. High-dose sotagliflozin significantly increased catalase levels (15.43 ± 0.34; P<0.01), suggesting partial restoration. There was also a significant reduction in SOD activity (Figure 5B), responsible for dismutating superoxide radicals, was observed in the ROT group (0.39 ± 0.04; P<0.05), whereas high-dose treatment significantly improved SOD levels (0.66 ± 0.03; P<0.05), indicating enhanced antioxidant defense. GSH levels (Figure 5C), a key free radical scavenger, were highest in the control group (1.737 ± 0.08) but were significantly reduced in the ROT group (0.45 ± 0.03; P<0.05). High-dose sotagliflozin significantly restored GSH levels (0.74 ± 0.02; P<0.01), reflecting its protective effect. GPx activity (Figure 5D), which is important for reducing hydrogen peroxide levels, was significantly lower in the ROT group (4.40 ± 0.54) than control group (6.56 ± 0.14; P<0.05), whereas high-dose sotagliflozin significantly increased GPx levels (6.10 ± 0.18; P<0.01), supporting improved oxidative stress resistance.



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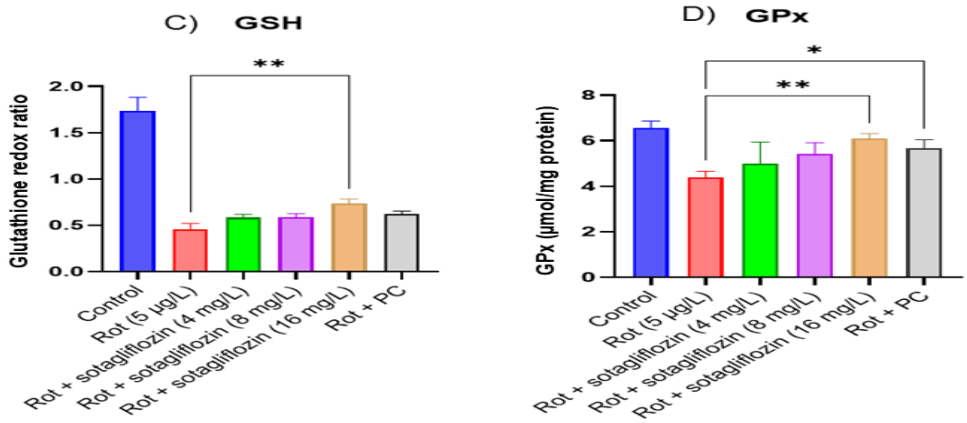

Figure 5. Antioxidant assay: This study evaluated the neuroprotective effects of Sotagliflozin against rotenone-induced oxidative damage in zebrafish. Zebrafish were exposed to 5 µg/L rotenone with Sotagliflozin at 4, 8, and 16 mg/L, while a positive control received Levodopa (25 µg/mL) + Carbidopa (2.5 µg/mL). Graph showed that (A) Catalase (CAT) activity decreased significantly in the rotenone group but was partially restored with Sotagliflozin; (B) Superoxide dismutase (SOD) levels also declined and recovered with higher Sotagliflozin doses; (C) The glutathione (GSH) redox ratio was reduced in the rotenone group, with significant restoration at 16 mg/L Sotagliflozin; (D) Glutathione peroxidase (GPx) activity followed a similar trend. Data were analyzed using one-way ANOVA and Tukey's post hoc test, with $p < 0.05$ * and $p < 0.01$ **.

3.3 Restoration of Neuro-biomarker levels (AChE and MDA) in zebrafish brain

The levels of AChE, an important enzyme in neurotransmission and neurodegeneration, were analyzed in all groups (Figure 6A). The control group showed normal AChE activity (10.64 ± 0.31), whereas the positive control group (levodopa+carbidopa-treated) had significantly reduced levels (6.64 ± 0.19; P<0.01), indicating cholinergic system modulation. Medium-dose sotagliflozin treatment resulted in AChE activity of 5.53 ± 0.17, which did not show a significant difference when compared ROT group. However, high-dose sotagliflozin significantly restored AChE activity (6.17 ± 0.14; P<0.05), suggesting a beneficial effect of the treatment. Overall, high-dose sotagliflozin improved AChE levels, although levodopa + carbidopa treatment showed a more substantial restoration. MDA levels, a marker of oxidative stress, were measured to evaluate lipid peroxidation (Figure 6B). The control group had baseline MDA levels (8.2 ± 0.17), while the ROT group showed a significant increase (12.72 ± 0.19; P<0.05), indicating oxidative damage. The positive control group (levodopa + carbidopa) also exhibited elevated MDA levels (11.67 ± 0.12; P<0.01), suggesting increased oxidative stress, however, MDA levels significantly reduced when compared to the ROT-induced group. Similarly, high-dose sotagliflozin-treated fish showed decreased MDA levels (11.92 ± 0.09; P<0.05) compared to the ROT-induced group, reflecting the protective effect of sotagliflozin.

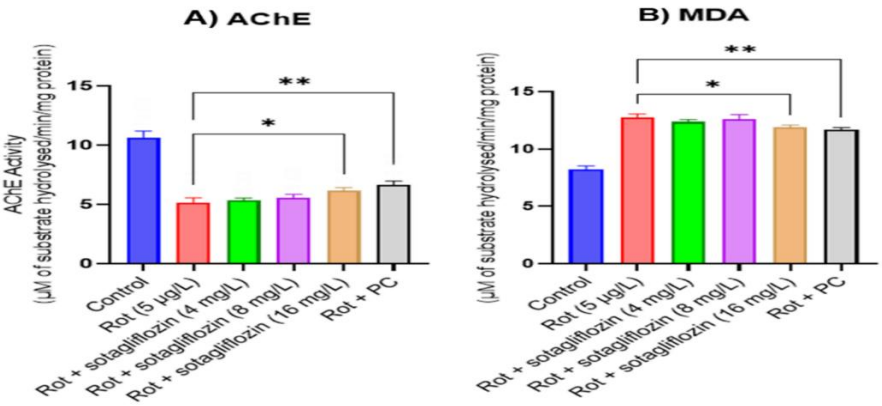

Figure 6. Neurobiomarker Assays (A) Acetylcholinesterase (AChE) activity was significantly reduced in the Rotenone (5 µg/L) group, indicating impaired cholinergic signaling. Co-treatment with Sotagliflozin (4, 8, and 16 mg/L) partially restored AChE activity in a dose-dependent manner. (B) Malondialdehyde (MDA)

levels, a marker of oxidative stress, were elevated in the Rotenone group. Sotagliflozin reduced this elevation, with the 16 mg/L dose showing a significant decrease, nearing levels in the positive control (Rot + Levodopa 25 µg/mL + Carbidopa 2.5 µg/mL). Data are mean ± SEM (n = X per group). Statistical analysis used one-way ANOVA and Tukey's post hoc test, with $p < 0.05$ *, and $p < 0.01$**.

3.4 Histopathological Evaluation of Zebrafish Brain

Histopathological analysis of zebrafish brain sections revealed significant changes following ROT induction and sotagliflozin treatment. While observing the histopathological features and changes shown in Figure 7, the control brain sections showed normal histological features, with no evidence of inflammation or pathological changes, confirming the baseline integrity of the untreated brain tissue. In contrast, ROT-induced brain sections exhibited parenchymal damage marked by dense chronic inflammatory cell infiltration, suggesting a significant inflammatory response caused by ROT. Treatment with sotagliflozin in the ROT + LD group resulted in a parenchyma with focal edema and inflammatory infiltration, indicating partial amelioration of inflammation, although localized tissue damage persisted. The ROT + MD sotagliflozin group displayed reduced inflammatory infiltration in the parenchyma, suggesting that moderate-dose treatment helped reduce the extent of inflammation compared with low-dose treatment, leading to less severe tissue damage. The ROT + PC group demonstrated a sheet-like pattern of inflammatory cell infiltration with mild stromal changes, reflecting a notable inflammatory response, although the structural damage was less pronounced than that in the ROT-only group. Finally, the ROT + HD sotagliflozin-treated brain sections showed modest cell migration and vacuole formation, indicating a mild inflammatory response.

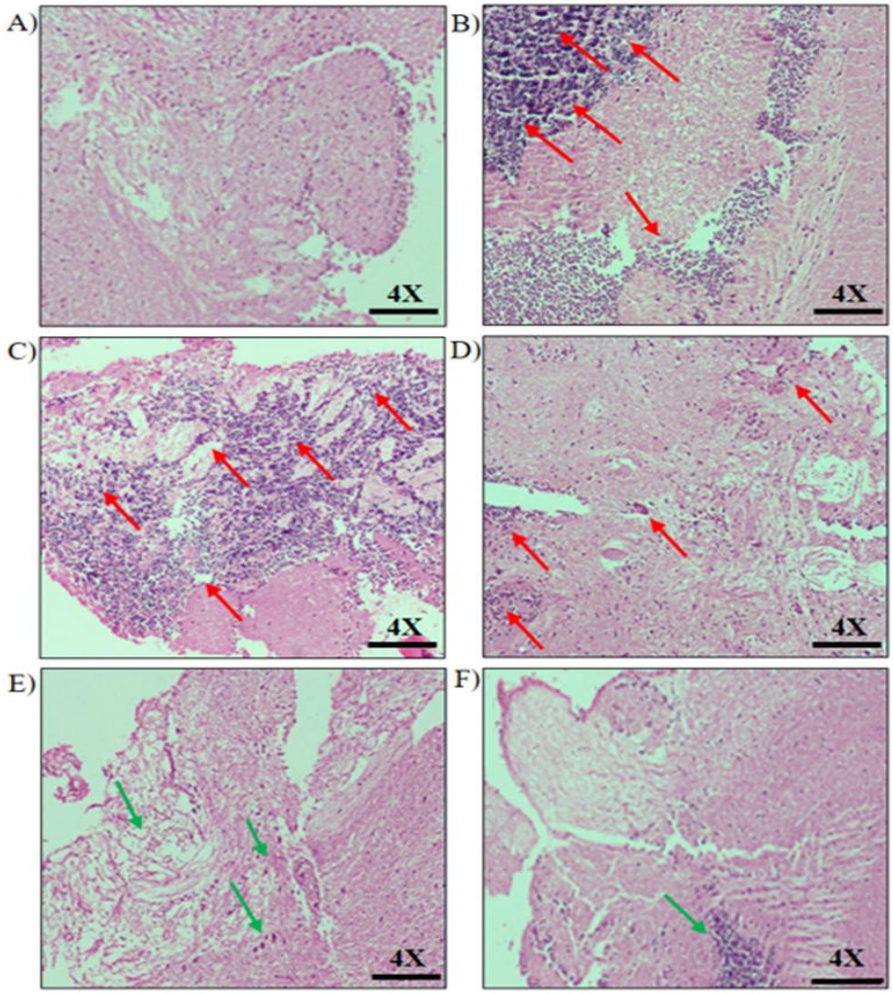

Figure 7. Histopathological images of control, ROT induced, and Sotagliflozin-treated zebrafish brains. Histological analysis of the brain tissue shows damaged regions (red arrows) and restored areas (green arrows).

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Control groups are compared to assess tissue architecture and neuronal integrity The Control Group (A) shows normal brain morphology with intact neuronal architecture and no signs of degeneration or necrosis. Positive control (B) shows significant neuronal damage, including degenerated neurons, edematous areas, vacuolation, and necrotic zones. Rotenone induced (C) resulted in more visible neuronal damage, degenerated neurons, and a greater number of vacuolation and necrosis were observed. Low-dose Sotagliflozin (D)treated Group showed mild neuronal protection, slight reduction in vacuolation, and necrosis. Medium Dose Sotagliflozin (E) shows moderate restoration of neuronal architecture with a visible decrease in inflammation and cellular degeneration. High-dose sotagliflozin (F) shows nearly normal brain architecture, minimal vacuolation, and restoration of neuronal structure.

3.5 Modulation of Apoptosis-Related Gene Expression in zebrafish brain

The graphs in (Figure 8) depicts Bax and Bcl2 expression, along with the Bax/Bcl2 ratio in response to ROT (5 µg/L) and various doses of sotagliflozin (4, 8, and 16 mg/L), with a positive control (PC) group included. ROT exposure significantly upregulated pro-apoptotic Bax compared to the control. Co-treatment with Sotagliflozin reduced expression of Bax in a dose-dependent pattern, with 8 &16 mg/L showing marked reductions (P<0.001), suggesting an anti-apoptotic effect. The PC group also exhibited significant protection. In contrast Bcl2, measures the anti-apoptotic effect. ROT exposure led to significant downregulation of Bcl2 expression (P<0.01). Sotagliflozin treatment restored Bcl2 levels in a dose-dependent manner, especially at 8 and 16 mg/L which indicates a protective response and the PC group showed similar restoration.

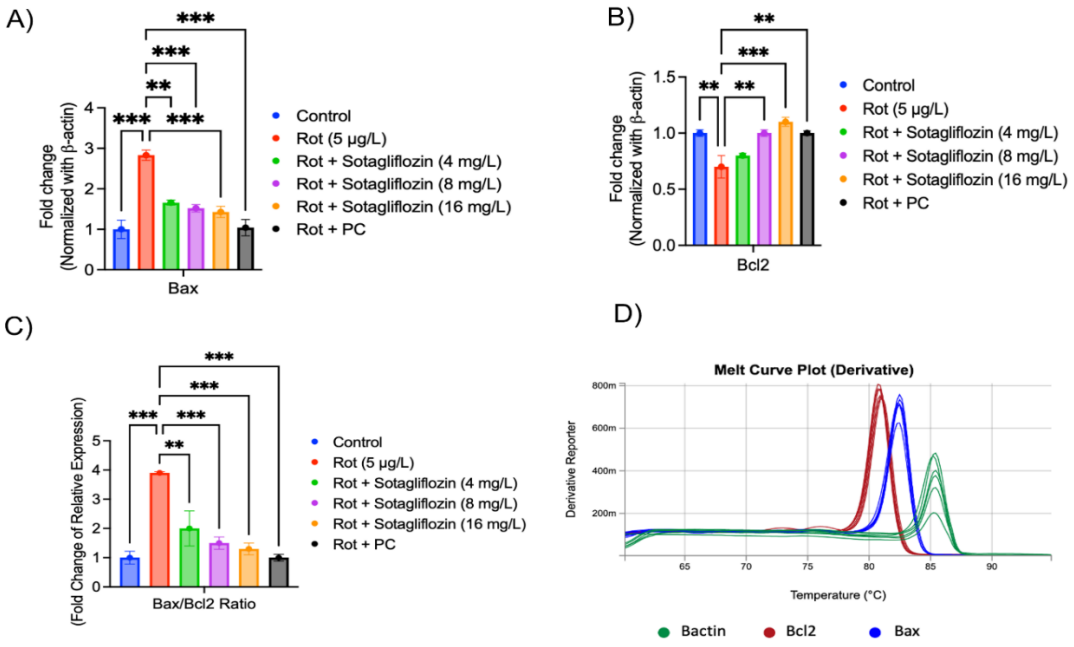

Figure 8. Gene expression analysis of BAX(A), Bcl2(B) BAX/BCl2 Ratio(C), and Melt curve plot(D) Statistics: One-way Anova with post hoc Bonferroni test and analysis was performed by GraphPad prism 10.3.1, ***P<0.001, **P<0.01, *P<0.05

4.Discussion

In this study we provided a comprehensive insight about neuroprotective potential of sotagliflozin in rotenone-induced neuronal damage in zebrafish. Acute embryo toxicity testing revealed that sotagliflozin exhibited concentration-dependent teratogenic effects, with developmental abnormalities such as pericardial edema, body curvature, and delayed hatching observed at concentrations ≥16 µg/ml, establishing the LD_{50} at 16 µg/ml (22). Behavioral assessments demonstrated that ROT exposure significantly impaired zebrafish locomotion and induced anxiety-like behavior, as evidenced by decreased time confined to the upper tank and light zones, and increased time confined to the lower tank and dark zones. High-dose sotagliflozin effectively ameliorated these behavioral abnormalities, comparable to the positive control, suggesting

anxiolytic and locomotor-restorative properties **(23)**. Biochemically, ROT-induced oxidative stress was reflected by significantly decreased catalase, SOD, GSH, and GPx levels. High-dose sotagliflozin treatment significantly restored and increased the activity of these antioxidant enzymes (Figure 5A–D), indicating a potent antioxidant effect. AChE levels, which are critical for cholinergic neurotransmission, were reduced by ROT, but significantly improved with high-dose sotagliflozin (Figure 6A). Although MDA levels, a marker of lipid peroxidation, remained elevated in the treated group (Figure 6B), the partial improvement suggests the attenuation of oxidative damage **(23)**. Histopathological analysis (Figure 7) supported these findings, showing severe inflammation and parenchymal damage in ROT-exposed brains, which were mitigated by sotagliflozin, particularly at moderate and high doses. The ROT + HD group exhibited only mild inflammation, indicating neuroprotective efficacy **(24).** Finally, gene expression studies (Figure 8) demonstrated significant upregulation of pro-apoptotic Bax and downregulation of anti-apoptotic Bcl2 expression in ROT group. Sotagliflozin reversed this pattern in a dose-dependent manner, lowering Bax/Bcl2 ratio and confirming its anti-apoptotic effect **(25, 26)**. Overall, findings highlight the potential role of sotagliflozin in improving motor deficits and alleviating neuroinflammation, oxidative stress and apoptosis in ROT-induced neurotoxicity.

5.Conclusion

To Sum up, in a zebrafish model of rotenone-induced neurodegeneration, sotagliflozin exerted potential neuroprotective effects. It improved motor function, reduced anxiety-like behaviors, and mitigated oxidative stress, particularly at higher doses. Biochemical analysis confirmed the restoration of catalase, SOD, glutathione and GPx levels. Histopathological analysis revealed reduced inflammation and tissue damage, whereas gene expression analysis revealed modulation of apoptosis-related markers. These findings suggest that sotagliflozin, a dual SGLT1/2 inhibitor, holds therapeutic potential in neurodegenerative diseases by reducing oxidative stress and regulating apoptotic pathways.

Abbreviations

AChE - Acetylcholinesterase
CAT – Catalase
GSH – Glutathione reductase
GPx – Glutathione peroxidase
MDA- Malondialdehyde
OECD - Organization for Economic Co-operation and Development
PC – Positive control
PD – Parkinson's disease
ROT- Rotenone
SGLT - Sodium-glucose cotransporter
SOD – Superoxide Dismutase
SOT – Sotagliflozin
ZFE - Zebrafish embryo
NDD – Neurodegenerative Disorder

Author contributions

RTV, AR Formal analysis, conceptualization, investigation, writing the original draft and editing, and visualization. SR, VR, RS, DPA: Formal analysis, conceptualization, data curation, and resources. TV, RKKR, PS and DMG: Formal analysis, writing the original draft, and editing.

Data availability Statement

On valid request, the data underpinning this research may be provided by the corresponding author.

Ethical Standards

The study was initiated after getting approval from the Institutional Animal Ethics Committee (IAEC 3/proposal:142/A. Lr:105/Dt:28.11.2023) Chettinad Hospital & Research Institute (CHRI), CARE, India.

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Conflict of interest

The authors declare that there are no conflict of interest that could have influenced the research.

REFERENCES

1. Miller JL. Parkinson's disease primer. Geriatric nursing. 2002 Mar 1;23(2):69-75. https://doi.org/10.1067/mgn.2002.123789
2. Kouli A, Torsney KM, Kuan WL. Parkinson's disease: etiology, neuropathology, and pathogenesis. Exon Publications. 2018 Dec 21:3-26. https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch1
3. Ball N, Teo WP, Chandra S, Chapman J. Parkinson's disease and the environment. Frontiers in neurology. 2019 Mar 19; 10:421551. https://doi.org/10.3389/fneur.2019.00218
4. Ou Z, Pan J, Tang S, Duan D, Yu D, Nong H, Wang Z. Global trends in the incidence, prevalence, and years lived with disability of Parkinson's disease in 204 countries/territories from 1990 to 2019. Frontiers in public health. 2021 Dec 7; 9:776847. https://doi.org/10.3389/fpubh.2021.776847
5. Yuan X, Tian Y, Liu C, Zhang Z. Environmental factors in Parkinson's disease: New insights into the molecular mechanisms. Toxicology Letters. 2022 Mar 1; 356:1-0. https://doi.org/10.1016/j.toxlet.2021.12.003
6. Madaan T, Akhtar M, Najmi AK. Sodium glucose CoTransporter 2 (SGLT2) inhibitors: Current status and future perspective. European Journal of Pharmaceutical Sciences. 2016 Oct 10; 93:244-52. https://doi.org/10.1016/j.ejps.2016.08.025
7. Lin KJ, Wang TJ, Chen SD, Lin KL, Liou CW, Lan MY, Chuang YC, Chuang JH, Wang PW, Lee JJ, Wang FS. Two birds one stone: the neuroprotective effect of antidiabetic agents on Parkinson disease—focus on sodium-glucose cotransporter 2 (SGLT2) inhibitors. Antioxidants. 2021 Dec 2;10(12):1935. https://doi.org/10.3390/antiox10121935
8. Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT. Mechanism of toxicity in rotenone models of Parkinson's disease. Journal of Neuroscience. 2003 Nov 26;23(34):10756-64. https://doi.org/10.1523/JNEUROSCI.23-34-10756.2003
9. Doyle JM, Croll RP. A critical review of zebrafish models of Parkinson's disease. Frontiers in Pharmacology. 2022 Mar 15;13:835827.https://doi.org/10.3389/fphar.2022.835827
10. Pawlos A, Broncel M, Woźniak E, Gorzelak-Pabiś P. Neuroprotective effect of SGLT2 inhibitors. Molecules. 2021 Nov 28;26(23):7213. https://doi.org/10.3390/molecules26237213
11. Palaniselvam S, Vijayanand R, Selvachandhran V, Manase S, Rajagopal SK, Ramachandran S. Structural characterization and toxicity effect of nano-carboxymethyl chitosan from Uroteuthis (Photololigo) sibogae (Adam, 1954) in the zebrafish model. Biomass Conversion and Biorefinery. 2024 Oct;14(20):25123-32. https://doi.org/10.1007/s13399-023-04480-7
12. Ünal İ, Üstündağ ÜV, Ateş PS, Eğilmezer G, Alturfan AA, Yiğitbaşı T, Emekli-Alturfan E. Rotenone impairs oxidant/antioxidant balance both in brain and intestines in zebrafish. International Journal of Neuroscience. 2019 Apr 3;129(4):363-8. https://doi.org/10.1080/00207454.2018.1538141
13. Wang Y, Liu W, Yang J, Wang F, Sima Y, Zhong ZM, Wang H, Hu LF, Liu CF. Parkinson's disease-like motor and non-motor symptoms in rotenone-treated zebrafish. Neurotoxicology. 2017 Jan 1; 58:103-9. https://doi.org/10.1016/j.neuro.2016.11.006
14. Surenderan S, Murkunde Y. Elucidation of acute and neurobehavioral toxicity of E-waste extracts with special reference to cognitive impairment, anxiety and stress response using zebrafish. Toxicology and Environmental Health Sciences. 2019 Mar;11:27-35. https://doi.org/10.1007/s13530-019-0385-5
15. Lin J, Xiao Y, Liu Y, Lei Y, Cai Y, Liang Q, Nie S, Jia Y, Chen S, Huang C, Chen J. Leachate from plastic food packaging induced reproductive and neurobehavioral toxicity in zebrafish. Ecotoxicology and Environmental Safety. 2022 Feb 1; 231:113189. https://doi.org/10.1016/j.ecoenv.2022.113189
16. Gonçalves CL, Vasconcelos FF, Wessler LB, Lemos IS, Candiotto G, Lin J, Matias MB, Rico EP, Streck EL. Exposure to a high dose of amoxicillin causes behavioral changes and oxidative stress in young zebrafish. Metabolic Brain Disease. 2020 Dec; 35:1407-16. https://doi.org/10.1007/s11011-020-00610-6
17. Shaw P, Mondal P, Bandyopadhyay A, Chattopadhyay A. Environmentally relevant concentration of chromium induces nuclear deformities in erythrocytes and alters the expression of stress-responsive and apoptotic genes in brain of adult zebrafish. Science of the Total Environment. 2020 Feb 10;703:135622. https://doi.org/10.1016/j.scitotenv.2019.135622
18. Cansız D, Ünal İ, Üstündağ ÜV, Alturfan AA, Altinoz MA, Elmacı İ, Emekli-Alturfan E. Caprylic acid ameliorates rotenone induced inflammation and oxidative stress in the gut-brain axis in Zebrafish. Molecular biology reports. 2021 Jun; 48:5259-73. https://doi.org/10.1007/s11033-021-06532-5
19. Huang X, Li Y, Wang T, Liu H, Shi J, Zhang X. Evaluation of the oxidative stress status in zebrafish (Danio rerio) liver induced by three typical organic UV filters (BP-4, PABA and PBSA). International Journal of Environmental Research and Public Health. 2020 Jan;17(2):651. https://doi.org/10.3390/ijerph17020651
20. Capatina L, Todirascu-Ciornea E, Napoli EM, Ruberto G, Hritcu L, Dumitru G. Thymus vulgaris essential oil protects zebrafish against cognitive dysfunction by regulating cholinergic and antioxidants systems. Antioxidants. 2020 Nov 4;9(11):1083. https://doi.org/10.3390/antiox9111083
21. Rodrigues GZ, Staudt LB, Moreira MG, Dos Santos TG, de Souza MS, Lúcio CJ, Panizzon J, Kayser JM, Simões LA, Ziulkoski AL, Bonan CD. Histopathological, genotoxic, and behavioral damages induced by manganese (II) in adult zebrafish. Chemosphere. 2020 Apr 1; 244:125550. https://doi.org/10.1016/j.chemosphere.2019.125550

22. Karpushev AV, Mikhailova VB, Klimenko ES, Kulikov AN, Ivkin DY, Kaschina E, Okovityi SV. SGLT2 inhibitor empagliflozin modulates ion channels in adult zebrafish heart. International Journal of Molecular Sciences. 2022 Aug 23;23(17):9559. https://doi.org/10.3390/ijms23179559
23. Ünal İ, Cansız D, Beler M, Sezer Z, Güzel E, Emekli-Alturfan E. Sodium-dependent glucose co-transporter-2 inhibitor empagliflozin exerts neuroprotective effects in rotenone-induced Parkinson's disease model in zebrafish; mechanism involving ketogenesis and autophagy. Brain Research. 2023 Dec 1; 1820:148536. https://doi.org/10.1016/j.brainres.2023.148536
24. Simanenkova A, Fuks O, Timkina N, Islamova A, Sufieva D, Kirik O, Korzhevskii D, Vlasov T, Karonova T. Microglia involvement into acute and chronic brain damage in diabetic rats: impact of GLP-1RA and SGLT-2i. Frontiers in Bioscience-Landmark. 2024 Jul 24;29(7):265. https://doi.org/10.31083/j.fbl2907265
25. Liang X, Yu L, Gui W, Zhu G. Exposure to difenoconazole causes changes of thyroid hormone and gene expression levels in zebrafish larvae. Environmental toxicology and pharmacology. 2015 Nov 1;40(3):983-7. https://doi.org/10.1016/j.etap.2015.10.005
26. Manigandan V, Nataraj J, Karthik R, Manivasagam T, Saravanan R, Thenmozhi AJ, Essa MM, Guillemin GJ. Low molecular weight sulfated chitosan: neuroprotective effect on rotenone-induced in vitro Parkinson's disease. Neurotoxicity Research. 2019 Apr 15; 35:505-15. https://doi.org/10.1007/s12640-018-9978-z