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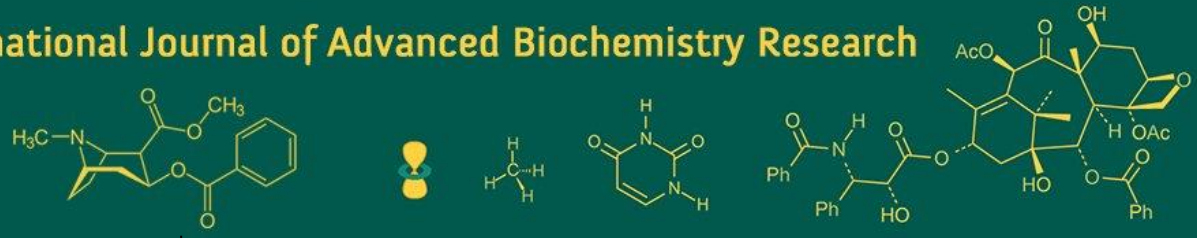

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Evaluation of protective efficacy of quercetin on Bisphenol F induced intestinal toxicity in zebrafish (*Danio rerio*)

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Abstract
The study investigated Bisphenol F (BPF)-induced intestinal toxicity in zebrafish and assessed the protective effect of quercetin on sub-acute exposure to BPF. The experiment lasted for 21 days, involving 240 zebrafish randomly assigned to six groups (n=40 per group). Group I served as the control, Group II was exposed to BPF (100 µg/L), Group III received quercetin (50 µg/L), Group IV received quercetin (75 µg/L), Group V was simultaneously treated with BPF (100 µg/L) and quercetin (50 µg/L), and Group VI was simultaneously treated with BPF (100 µg/L) and quercetin (75 µg/L). Behavioural parameters were evaluated on days 0 and 21, including line crossings, time spent in white compartments, crossings between compartments, freezing behaviour, swimming time percentage, mirror biting time percentage, and entries into the upper portion of a novel tank. The BPF-treated group (Group II) showed decreased behavioural activity compared to controls, while Groups V and VI exhibited improved parameters, indicating the protective effects of quercetin. Groups III and V showed increased time spent in the upper tank and reduced erratic movements compared to Group II. In contrast, these behaviours were less pronounced in Groups IV and VI. The results indicated that Quercetin in lower doses protect the intestine from subacute intestinal toxicity, which could potentially be used as a novel therapeutic in future.

Keywords: Bisphenol F, quercetin, zebrafish, intestinal toxicity, behavioural parameters

Introduction
Endocrine disrupting chemicals (EDCs) encompass both natural and synthetic compounds capable of mimicking, blocking, or interfering with hormones within the endocrine system (Stiefel *et al*., 2023) [23]. Exposure to these chemicals in the environment has become a significant factor contributing to various metabolic disorders, such as obesity, insulin resistance, type 2 diabetes and fatty liver disease. Additionally, EDC exposure is associated with diverse health issues, including disruptions in sperm quality and fertility, abnormalities in sex organs, endometriosis, early puberty, alterations in nervous system and immune function, certain cancers, respiratory problems, neurological and learning disabilities, among other health concerns (Ahn *et al*., 2023) [1]. Bisphenol A (BPA), a widely used epoxy material, is among the EDCs causing concern. Due to consumer apprehension about BPA, substitutes like bisphenol F (BPF), bisphenol S (BPS) and bisphenol AF (BPAF) have been developed and detected in various environmental media (Mu *et al*., 2022) [18, 19]. Numerous studies have demonstrated that BPF can elicit estrogenic, cytotoxic, genotoxic and neurotoxic effects on aquatic organisms, often comparable to or more pronounced than those induced by BPA exposure (Wang *et al*., 2021) [25]. Notably, the digestive system emerges as a sensitive target for bisphenol analogy, as evidenced by the direct impact of BPA on digestive and metabolic organs such as the liver, pancreas and intestine. Zebrafish of distinct developmental stages has become a popular model for toxicity test of environmental pollutants. Adult zebrafish has the complete intestinal structure and has been used as a powerful model to study intestinal functions and diseases (Fenero *et al*., 2016; Van Sebille *et al*., 2019) [16, 24]. The zebrafish (*Danio rerio*) has recently received attention as a powerful animal model for metabolic diseases and to investigate fundamental processes underlying

intestinal inflammation and injury, which possesses a high degree of anatomical, physiological and genomic similarity to vertebrates (Mu *et al*., 2022) [18, 19]. Zebrafish shares many physiological and genetic traits with humans, such as the brain, digestive tract, musculature, vasculature and innate immune system. Additionally, 70% of genes related to human disease genes have functional similarities with those found in Zebrafish (Khan and Alhewairini, 2018) [13]. Zebrafish also possess several advantages such as small size, inexpensive maintenance, ease of breeding, short lifecycle, high fecundity, year-round spawning, fewer legal restrictions, genetic similarities with human beings (Howe *et al*., 2013) [12]. Quercetin, a flavonoid compound is present in widely range of plants including apples, berries, brassica vegetables, capers, grapes, onions, spring onions, tea and tomatoes, as well as in many seeds, nuts, flowers, bark and leaves (Hanasaki *et al*., 1994) [11]. There is mounting evidence suggesting that quercetin has therapeutic potential for the prevention and treatment of different diseases, including cardiovascular disease, cancer and neurodegenerative disease. Mechanistically, quercetin has been shown to exert anti-oxidant, anti-inflammatory and anti-cancer activities in a number of cellular and animal models, as well as in humans by modulating the signalling pathways and gene expression involved in these processes (Ay *et al*., 2021) [3].

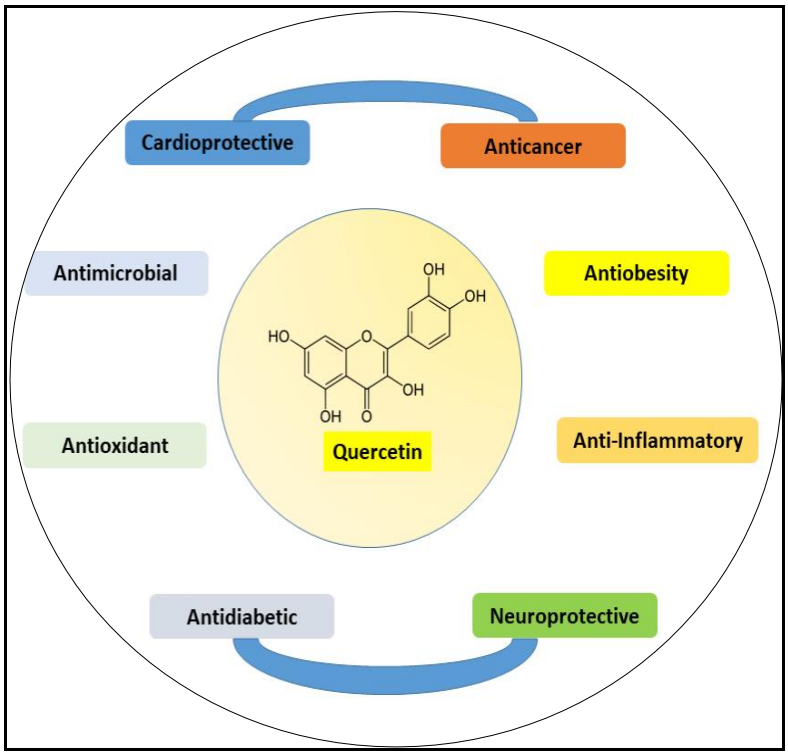

Fig 1: Showing various therapeutic properties of Quercetin

2. Materials and Methods

2.1Location of work

The proposed work was conducted at the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, N.D.V.S.U, Jabalpur, Madhya Pradesh, over a period of six months from July 2023 to December 2023.

2.2 Animals

The study utilized adult Zebrafish (*Danio rerio*) of either sex, procured from a commercial breeder at approximately three months of age. Upon arrival, the Zebrafish were housed in standard glass aquariums in groups of 40, within 90-litre capacity of glass tanks containing 80 litres of RO water with proper aeration. The fish were acclimatized to laboratory conditions under constant observation for 15 days before the commencement of the experiment. The water temperature in the tanks was maintained at 28±1 °C, with a pH range of 6.8-7.4. Oxygenation was provided by commercially available aerators. A strict light-dark cycle of 14:10 hours was observed throughout the experiment. During the study period, all fish were fed ad-libitum with Optimum micro fish pellets shown in (Figure.2).

Fig 2: Experimental model - Zebrafish (*Danio rerio*)

2.3 Chemicals

Bisphenol F (Sigma Aldrich, USA) and Quercetin (Himedia Laboratories Pvt. Ltd., Mumbai, India) was used in the present experiment.

2.4 Experimental design

The protective efficacy of quercetin on Bisphenol F induced intestinal toxicity in Zebrafish (*Danio rerio*) was evaluated

using total of 240 Zebrafish, that was divided into six groups and viz. I, II, III, IV, V, VI consisting of 40 fishes in each group as shown in (Figure.3). The fish of different groups were kept separately in standard glass aquarium and treated with different concentrations of Bisphenol F and Quercetin, as follows

Table 1: Design of experiment

Groups	No. of animals	Treatment
I	40	Normal Control
II	40	Bisphenol F (100 µg/L, in water)
III	40	Quercetin (50 µg/L, in water)
IV	40	Quercetin (75 µg/L, in water)
V	40	Bisphenol F (100 µg/L, in water) + Quercetin (50 µg/L, in water)
VI	40	Bisphenol F (100 µg/L, in water) + Quercetin (75 µg/L, in water)

2.5 Standard preparation and exposure to Bisphenol F (BPF)
A standard solution of 10mg/ml Bisphenol F was prepared in ethanol and Milli Q water at (1:4) ratio. Fish were daily subjected to water containing 100 µg/L of Bisphenol F over 21-day period.

2.6 Standard preparation and exposure to Quercetin
A standard solution of 10 mg/ml Quercetin was prepared in dimethyl sulfoxide (DMSO) and distilled water at (1:4) ratio. Fish were subjected to daily exposure to quercetin at concentrations of 50 µg/L and 75 µg/L in the water over 21-day period.

3. Behavioural Analysis
3.1 Number of line crossings
After treatment and application of the toxicant, the animal was placed in a glass petri dish (10 x 15 cm), divided into quadrants. The quantification of response based on locomotor activity, specifically the number of line crossings within a specified timeframe. A total of 10 minutes of video recording was conducted to capture and document these behavioural responses (Ohnesorge *et al*., 2021) [20].

3.2 Novel tank diving paradigm
The novel tank diving test was designed to elicit stress responses in zebrafish, enabling the comparison of anxiety-induced behaviours between experimental and control groups. This quick and sensitive test involved observing and recording the time each fish spent in the upper and lower zones of the tank over a 10-minute period, as well as specific behaviours such as the number of entries into the upper portion, average entry duration, and number of erratic movements, as described by (Collier *et al*. 2017) [8, 9]. The efficacy of Quercetin was assessed by meticulously recording these behaviours using slow-motion video analysis.

3.3 Mirror aggression test
The mirror aggression / biting test is a well-established method for studying zebrafish boldness and aggression. Aggressive behaviour of zebrafish was recorded by mirror aggression test as described by (Audira *et al*., 2018) [2]. Aggressive episodes were characterized by the erection of fins accompanied by biting towards the mirror, undulating body movements and fast swimming. The mirror biting zone was designated at the area within 5 cm from mirror (Plate 04A).

3.4 Dark light test
In zebrafish, scototaxis, or the avoidance of bright areas, serves as a behavioural indicator for anxiety, where an increased duration in dark regions is associated with enhanced anxiety levels. The assessment of anxiety levels in zebrafish, post-exposure to Bisphenol F and the protective efficacy of quercetin was determined by recording the time spent in the white area, observing freezing behaviour in the white region and noting the total number of crossings between the dark and light compartments (Midttun *et al*., 2020) [16].

4. Statistical Analysis
Statistical analysis was done using One-way Anova described by (Snedecor and Cochran 1994) [22].

5. Results and Discussion
5.1 Behavioural Parameters
Behavioural parameters in adult zebrafish were systematically assessed after exposure to various treatments, including Quercetin at concentrations of 50 µg/L and 75 µg/L, Bisphenol F (BPF) at 100 µg/L alone and combinations of BPF with Quercetin. The evaluations were conducted on both Day 0 and Day 21 of exposure

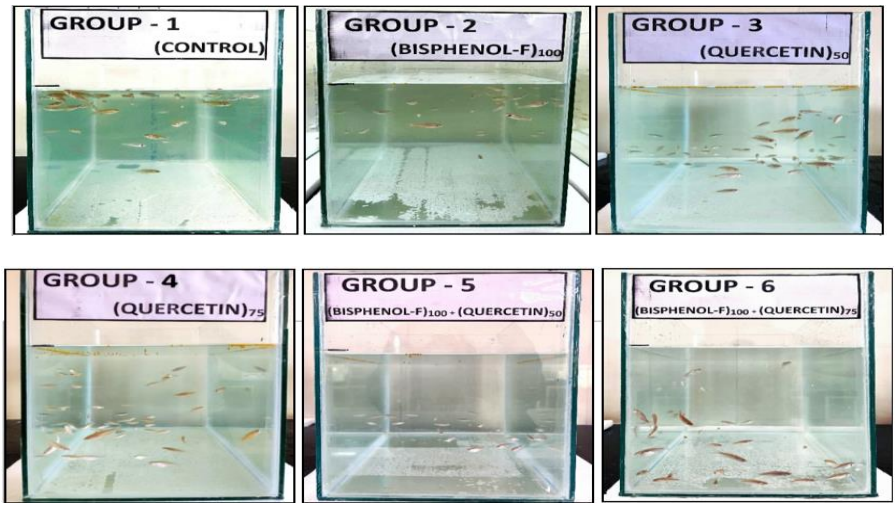

Fig 3: Experimental groups for evaluation of protective efficacy of Quercetin on Bisphenol F induced intestinal toxicity in zebrafish (*Danio rerio*)

5.1.1 Number of Line Crossing by Zebrafish

The average number of line crossings by zebrafish in different treatment groups were assessed at Day 0 and Day 21. The mean value for number of line crossing by zebrafish at Day 0 and Day 21 is presented in Table. 2, graphically depicted in Figure.4 and shown in (Figure.5).

Table 2: Efficacy of quercetin on the number of line crossing by zebra fish in Bisphenol F-induced intestinal toxicity.

Groups	Number of Line Crossing		
	Treatment	Day 0 (Mean±S.E.)	Day 21 (Mean±S.E.)
I	Normal Control	163.83±3.1	150.83^{a}±3.9
II	Bisphenol F (100 µg/L; in water)	212.16±7.8	53.33^{b}±1.7
III	Quercetin (50 µg/L; in water)	211.16±6.3	106.66^{a}±3.4
IV	Quercetin (75 µg/L; in water)	162.16±1.0	83.66^{c}±2.1
V	Bisphenol F (100 µg/L; in water) + Quercetin (50 µg/L; in water)	214.50±8.3	98.32^{a}±2.3
VI	Bisphenol F (100 µg/L; in water) + Quercetin (75 µg/L; in water)	196.33±3.2	47.16^{c}±1.0

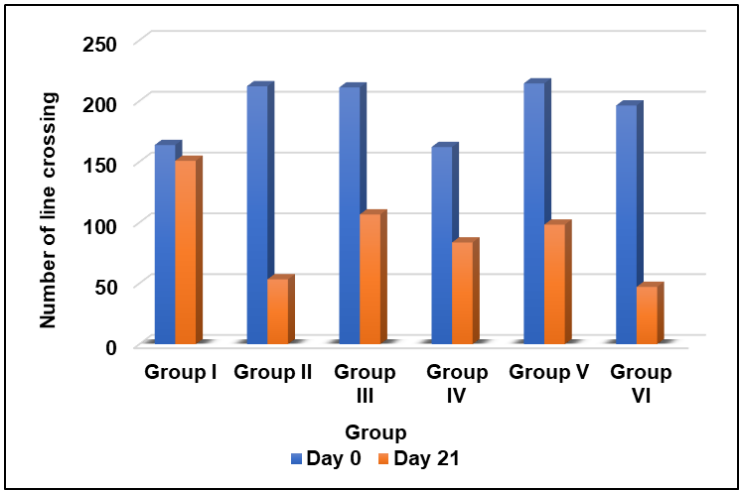

Fig 4: Effect of quercetin on number of line crossing after subacute toxicity of bisphenol F in zebrafish

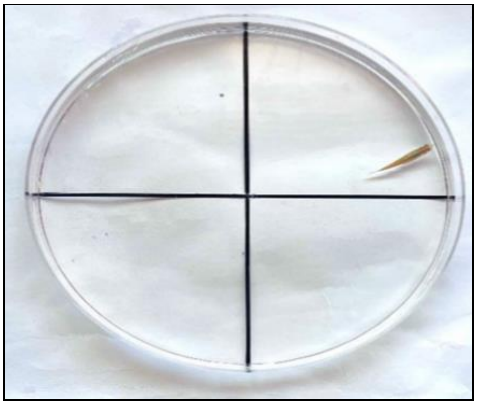

Fig 5: Evaluation of behavioural parameters of protective efficacy of quercetin on Bisphenol F induced intestinal toxicity in zebrafish by Number of line crossing

The results showed a significant decrease in locomotor activity, indicating stress in the BPF alone treated group, i.e., in Group II also Group VI, which was treated with BPF along with a higher dose of Quercetin (Moreira and Carolina 2022; Kim *et al*. 2023) [17, 14]

5.1.2 Novel Tank Diving Paradigm

Novel tank test was carried out to evaluate various behaviour parameters at day 0 and day 21 in zebrafish exposed to BPF and Quercetin and treatment with two different concentrations of BPF + Quercetin for 0-10 minutes and compared with control group. The mean values of time (sec) spent in upper zone, entries into upper portion of tank, average entry duration (sec), number of erratic movements, number of freezing bouts, freezing duration by zebrafish of Group I, Group II, Group III, Group IV, Group V and Group VI at Day 0 and Day 21were calculated. The mean value for various behavioural parameters of zebrafish is presented in Table 3, graphically depicted in Figure 6 to 11 and shown in (Figure.12).

Table 3: Efficacy of quercetin on the novel tank diving test by zebra fish in Bisphenol F-induced intestinal toxicity.

Novel tank diving paradigm													
Groups	**Treatment**	**Time spent in upper portion of tank (sec) (Mean±S.E)**		**Number of entries in upper portion of tank (Mean±S.E)**		**Average entry duration (sec) (Mean±S.E)**		**Number of erratic movements (Mean±S.E)**		**Number of freezing bouts (Mean±S.E)**		**Freezing duration (sec) (Mean±S.E)**	
		Day 0	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**
I	Normal Control	324.83±3.9	349.66^{b}±6.9	27.83±0.7	28.16^{a}±1.3	11.72±0.3	12.59^{b}±0.7	1.83±0.7	01.66^{c}±0.6	1.50±0.3	01.16^{d}±0.4	1.66±0.3	1.16^{d}±0.4
II	Bisphenol F (100 µg/L; in water)	329.16±4.7	98.83^{e}±1.7	21.83±1.2	6.50^{de}±0.9	15.07±0.8	17.07^{a}±2.5	3.16±0.4	14.16^{a}±1.0	1.66±0.3	14.33^{b}±0.7	2.33±0.4	341.50^{a}±6.0
III	Quercetin (50 µg/L; in water)	359.50±3.1	365.5^{a}±8.1	28.83±0.7	24.20^{b}±2.2	12.46±0.3	15.92^{a}±1.5	2.33±0.7	02.33^{c}±1.1	1.50±0.4	0.83^{d}±0.3	2.10±0.5	1.50^{d}±0.5
IV	Quercetin (75 µg/L; in water)	344.16±8.1	135.16^{d}±5.1	25.63±0.9	8.33^{d}±0.4	13.39±0.5	16.34^{a}±0.4	2.83±0.7	06.66^{b}±1.1	1.83±0.6	10.66^{c}±1.1	2.83±0.8	156.50^{b}±4.9
V	Bisphenol F (100 µg/L; in water) + Quercetin (50 µg/L; in water)	353.33±6.9	194.20^{c}±3.1	29.50±2.7	13.83^{c}±0.7	11.97±0.5	14.19^{ab}±0.7	2.66±0.4	05.33^{b}±0.6	1.33±0.4	9.33^{c}±0.6	1.66±0.4	23.16^{c}±2.0
VI	Bisphenol F (100 µg/L; in water) + Quercetin (75 µg/L; in water)	348.83±5.0	59.33^{e}±1.1	26.33±0.6	03.66^{e}±0.4	13.26±0.2	16.21^{a}±0.8	2.53±0.6	16.33^{a}±0.6	1.16±0.4	17.50^{a}±0.4	1.83±0.6	349.33^{a}±4.8

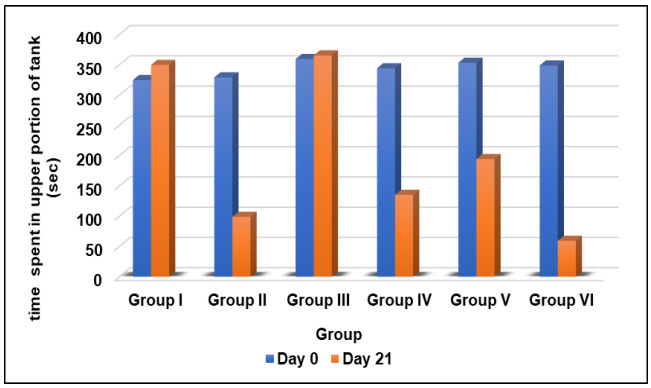

Fig 6: Effect of quercetin on time spent in upper of tank (sec) after subacute toxicity of bisphenol F in zebrafish

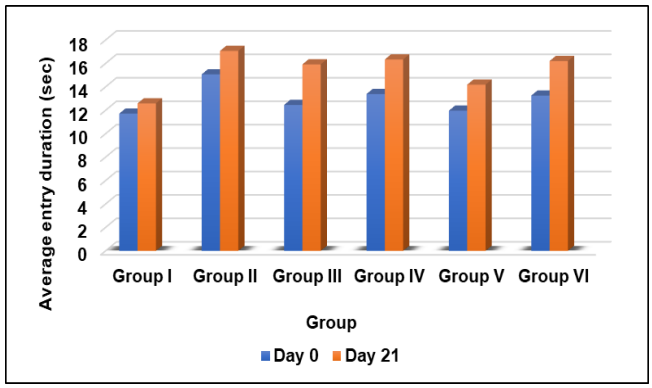

Fig 8: Effect of quercetin on average entry duration (sec) after subacute toxicity of bisphenol F in zebrafish

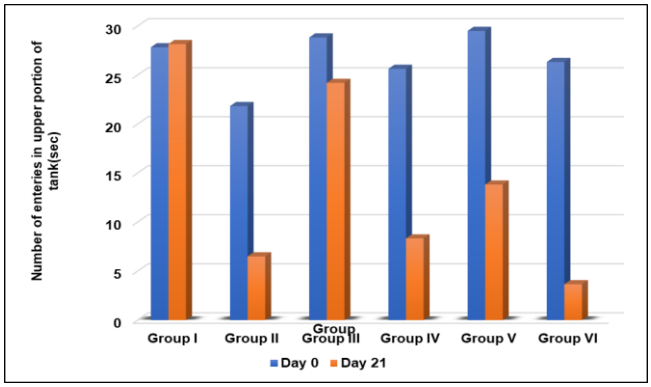

Fig 7: Effect of quercetin on number of entries in upper portion of tank after subacute toxicity of bisphenol F in zebrafish

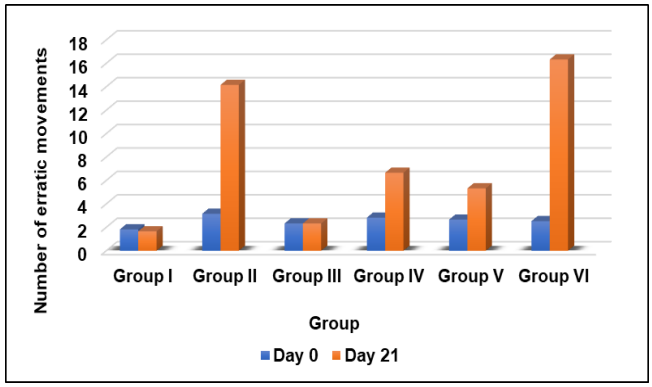

Fig 9: Effect of quercetin on number of erratic movements after subacute toxicity of bisphenol F in zebrafish

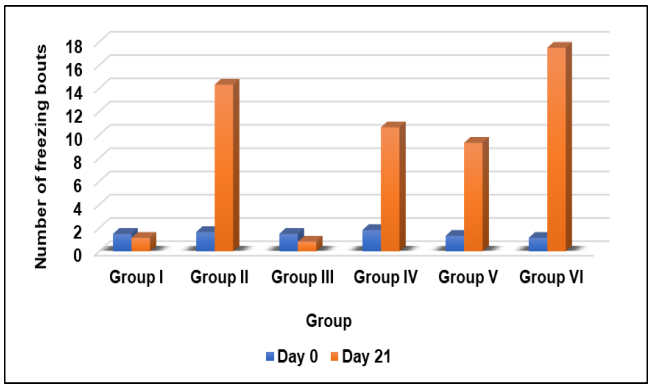

Fig 10: Effect of quercetin on number of freezing bouts after subacute toxicity of bisphenol F in zebrafish

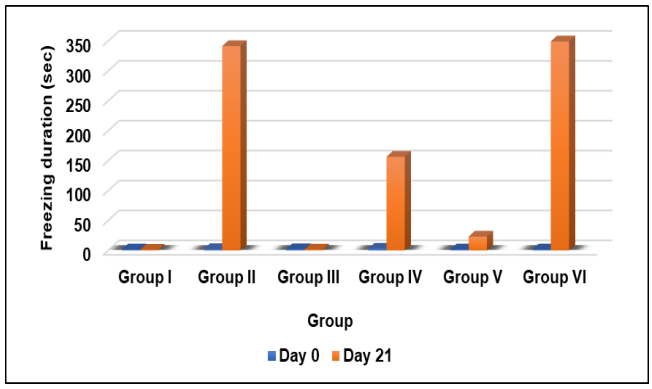

Fig 11: Effect of quercetin on freezing duration (sec) after subacute toxicity of bisphenol F in zebrafish

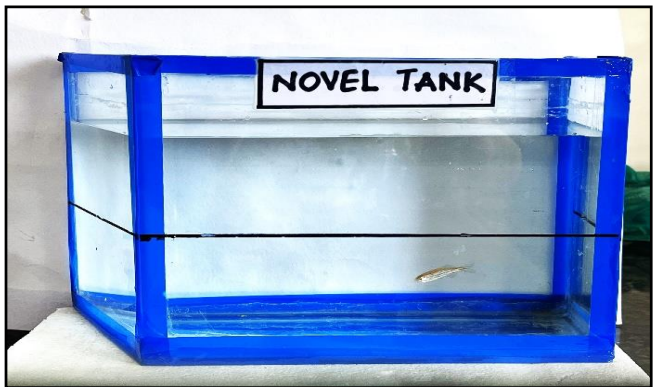

Fig 12: Evaluation of behavioural parameters of protective efficacy of quercetin on Bisphenol F induced intestinal toxicity in zebrafish by Novel tank diving paradigm

The novel tank test serves as a well-established method for assessing "anxiety-like" behaviours in adult zebrafish. This test measures anxiety in zebrafish by observing their tendency to initially stay at the tank's bottom, gradually exploring higher areas over time. Spending more time at the bottom indicates higher anxiety, whereas increased vertical exploration suggests reduced anxiety (Collier *et al*, 2017) [8, 9], increased frequency of freezing bouts and freezing duration (sec) indicate increased anxiety and are generally higher in stressed zebrafish (Cachat *et al*., 2011) [5]. As a result, showed fish exposed to BPF alone showed increased bottom-dwelling, longer freezing, and higher average entry duration, indicating elevated anxiety compared to controls. Similar anxiety responses were seen with high-dose Quercetin alone or combined with BPF, indicating increased toxicity. However, fish treated with BPF and a lower dose of Quercetin spent more time in the upper zone, exhibited fewer and shorter freezing bouts, and had reduced average entries, suggesting Quercetin's protective effect against BPF-induced toxicity (Moreira and Carolina, 2022) [17]

5.1.3 Mirror Aggression Test

Mirror aggression test was carried out to evaluate various behaviour parameters at day 0 and day 21 in zebrafish exposed to BPF and Quercetin and treatment with two different concentrations of BPF + Quercetin. Fish was allowed to acclimatize for 3 minutes and for 5 minutes movement was recorded. The mean value of Freezing time (%), Swimming time (%), rapid time movement (%), mirror biting time (%) of Group I, Group II, Group III, Group IV, Group V and Group VI at Day 0 and Day 21 by zebrafish were calculated. Various behavioural parameters of zebrafish are presented in Table 4, graphically depicted in Figure 13 to 16 and shown in (Figure.17).

Table 4: Efficacy of quercetin on the mirror aggression test by zebra fish in Bisphenol F-induced intestinal toxicity.

Mirror aggression test									
Groups	**Treatment**	**Freezing time (%) (Mean±S.E)**		**Swimming time (%) (Mean±S.E)**		**Rapid time movement (%) (Mean±S.E)**		**Mirror biting time (%) (Mean±S.E)**	
		Day 0	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**	**Day 0**	**Day 21**
I	Normal Control	19.50±1.1	18.11^{c}±1.1	77.00±1.3	79.29^{c}±1.1	3.50±0.7	2.60^{a}±0.2	24.78±0.8	25.21^{a}±0.9
II	Bisphenol F (100 µg/L; in water)	20.03±0.6	33.33^{a}±2.3	76.90±0.9	66.22^{d}±2.3	3.05±0.3	0.44^{c}±0.1	20.09±1.1	7.49^{d}±0.9
III	Quercetin (50 µg/L; in water)	18.44±1.0	12.94^{d}±0.9	78.40±1.0	84.05^{b}±0.9	3.15±0.2	2.99^{a}±0.2	21.22±1.1	29.53^{a}±2.4
IV	Quercetin (75 µg/L; in water)	20.05±0.8	24.66^{b}±1.3	76.05±0.9	74.39^{c}±1.5	3.88±0.3	0.94^{ab}±0.3	22.03±1.2	17.69^{b}±1.0
V	Bisphenol F (100 µg/L; in water) + Quercetin (50 µg/L; in water)	16.94±1.8	19.94^{c}±0.8	79.01±2.2	78.38^{c}±0.6	4.05±0.4	1.66^{a}±0.3	21.55±1.2	11.01^{c}±1.1
VI	Bisphenol F (100 µg/L; in water) + Quercetin (75 µg/L; in water)	21.44±1.1	39.61^{a}±1.6	75.41±1.2	60.38^{d}±1.6	3.16±0.2	0^{c}±0	19.03±0.8	5.97^{d}±0.9

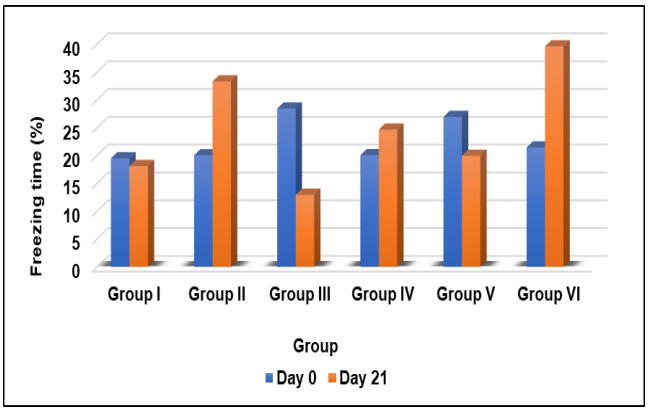

Fig 13: Effect of quercetin on freezing time (%) after subacute toxicity of bisphenol F in zebrafish

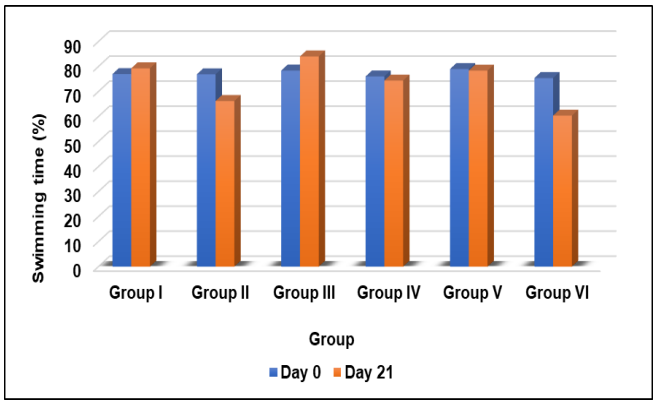

Fig 14: Effect of quercetin on swimming time (%) after subacute toxicity of bisphenol F in zebrafish

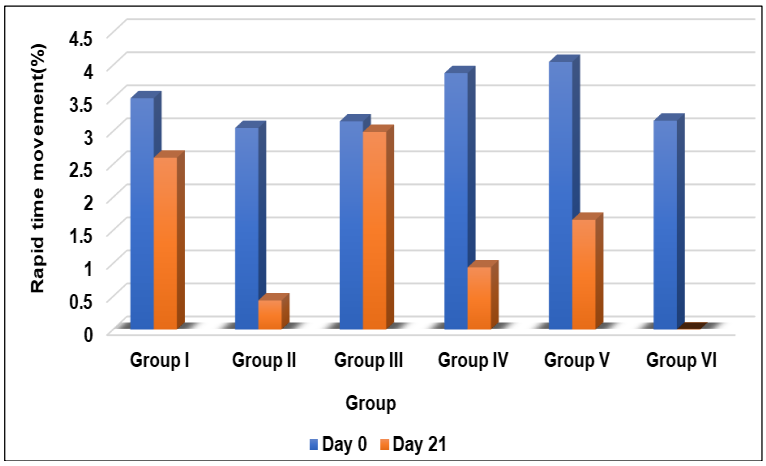

Fig 15: Effect of quercetin on rapid time movement (%) after subacute toxicity of bisphenol F in zebrafish

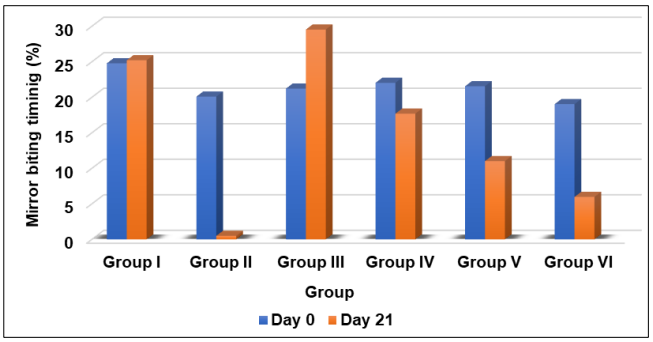

Fig 16: Effect of quercetin on mirror biting time (%) after subacute toxicity of bisphenol F in zebrafish

Fig 17: Evaluation of behavioural parameters of protective efficacy of quercetin on Bisphenol F induced intestinal toxicity in zebrafish by Mirror aggression test

The mirror biting test is a well-established method for studying zebrafish boldness and aggression. In present study, aggressive behaviour of zebrafish was assessed using the mirror aggression test, following the protocol described by (Audira *et al*., 2018) [2] Fish with reduced aggression were seen as lacking social behaviour. In this study, the BPF-treated group showed significantly increased freezing time and decreased swimming, rapid movement, and mirror biting times compared to controls. The group treated with BPF and a high dose of Quercetin also exhibited non-significantly increased freezing time and similarly reduced swimming, rapid movement, and mirror biting times, indicating heightened anxiety and decreased social behaviour.

5.1.4 Dark Light Test

Dark Light Test was carried out to evaluate various behaviour parameters at day 0 and day 21 in zebrafish exposed to BPF and Quercetin and treatment with two different concentrations of BPF + Quercetin. Fish was allowed to acclimatize for 5 minutes between removable door and for 10 minutes movement was recorded after removing the door. The mean value time spent in white portion of tank, freezing in the white portion of the tank, total number of crossings between the dark and light compartments by zebrafish of Group I, Group II, Group III, Group IV, Group V and Group VI at Day 0 and Day 21 by zebrafish were calculated. Various behavioural parameters of zebrafish are presented in Table 5, graphically depicted in Figure 18 to 20 and shown in (Figure.21).

Table 5: Efficacy of quercetin on dark light test by zebra fish in Bisphenol F-induced intestinal toxicity

Groups	Dark light test						
	Treatment	Time spent in the white (sec) (Mean±S.E)		Freezing in white (sec) (Mean±S.E)		Total number of crossings between the dark and light compartments (Mean±S.E)	
		Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
I	Normal Control	97.83±3.7	97.5^{b}±2.6	5.83±0.8	1.16±0.8	23.83±3.8	21.05$^{ab±}$2.5
II	Bisphenol F (100 µg/L; in water)	95.33±3.3	2.33^{e}±0.4	0.83±0.5	0.00±00	20.16±3.9	1.33^{d}±0.2
III	Quercetin (50 µg/L; in water)	99.30±4.6	165.33^{a}±6.3	0.66±0.6	4.62±1.1	19.10±1.1	27.16^{a}±3.5
IV	Quercetin (75 µg/L; in water)	97.16±4.3	45.03^{d}±5.1	0.00±00	3.50±3.5	21.16±2.9	7.83^{cd}±0.9
V	Bisphenol F (100 µg/L; in water) + Quercetin (50 µg/L; in water)	91.66±3.5	67.66^{c}±2.4	0.51±0.3	0.00±00	16.96±3.0	17.51^{bc}±1.3
VI	Bisphenol F (100 µg/L; in water) + Quercetin (75 µg/L; in water)	101.69±5.5	1.11^{e}±0.8	0.59±0.4	0.33±0.3	18.66±2.8	00.66^{d}±0.4

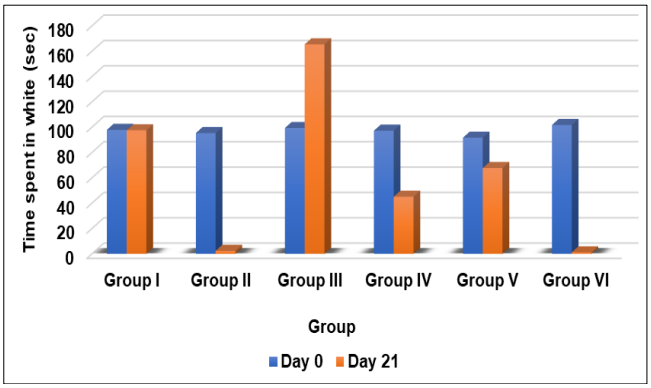

Fig 18: Effect of quercetin on time spent in white (sec) after subacute toxicity of bisphenol F in zebrafish

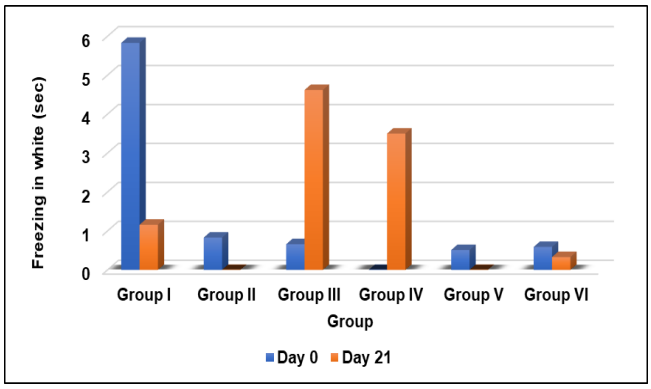

Fig 19: Effect of quercetin on freezing in white (sec) after subacute toxicity of bisphenol F in zebrafish

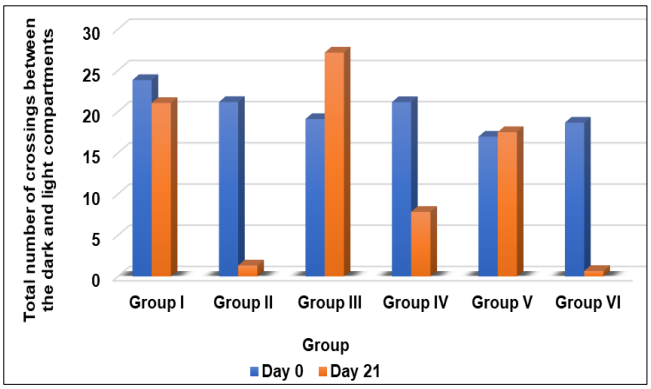

Fig 20: Effect of quercetin on total number of crossings between dark and light compartment after subacute toxicity of bisphenol F in zebrafish

Fig 21: Evaluation of behavioural parameters of protective efficacy of quercetin on Bisphenol F induced intestinal toxicity in zebrafish by Dark light test

The light-dark preference test assesses anxiety in zebrafish by measuring their preference for light or dark zones. Zebrafish prefer dark zones, indicating anxiety, while a preference for light zones reflects anti-anxiety behaviour (Serra *et al*., 1999) [21]. The BPF alone and Quercetin (75 µg/L) groups showed a significant decrease in crossings between dark and light compartments compared to controls. The group treated with BPF and Quercetin (75 µg/L) also had reduced crossings, but not significantly different from the BPF alone group. However, BPF with Quercetin (50 µg/L) did not reduce crossings, showing no significant difference from the BPF alone group. In this study, zebrafish treated with BPF alone or BPF with Quercetin (75 µg/L) showed increased preference for the dark zone, indicating anxiety-like behaviour This preference can be considered as anxiety-like behaviour as suggested by (Maximino *et al*. 2010) [15]. Conversely, the Quercetin (50 µg/L) group and the BPF with Quercetin (50 µg/L) group had more line crossings and spent more time in the white zone, suggesting lower toxicity and anti-anxiety behaviour.

In alignment with the previous reports, our findings advocate the behavioural parameters indicate the intestinal toxicity in zebra fish. These findings align with the explanations of another study that suggests, Stress can cause notable alterations in the structure of the intestinal mucosa and bring about changes in the composition of intestinal mucus which leads to the behavioural change in the zebrafish (Butt and Volkoff, 2019) [4]. Stress results in a substantial reduction in the number of goblet cells and an increase in the presence of vacuoles within the intestinal tissue causes change in behavioural pattern (Cheng *et al*.,

2022) [7]. The presence of BPA in aquatic animals has been reported to affect physiological homeostasis and intestinal wall even at environmentally relevant concentrations (Canesi and Fabbri, 2015) [6].

Conclusion

This investigation proposes the possible protective efficacy of low dose of quercetin against BPA-induced stress-mediated toxicity in zebrafish. The results of this study revealed that animals with BPF alone and BPF with high-dose Quercetin had reduced locomotor activity, decreased entries into the upper portion of the tank, increased freezing, and exhibited less social behaviour, indicating heightened anxiety and toxic effects. In contrast, groups treated with Quercetin alone or BPF with low-dose Quercetin showed increased locomotor activity, more entries to the tank's upper portion, reduced anxiety, and enhanced social behaviour. The dark-light test indicated higher anxiety in BPF and BPF with high-dose Quercetin groups, while Quercetin and BPF with low-dose Quercetin groups spent more time in the light portion, indicating reduced anxiety. Freezing in white showed no significant differences. Bisphenol F @ 100 µg/L in water, daily for 21 days in zebrafish showed significant toxicity as evidenced by alteration in behavioural parameters. Quercetin @ 50 µg/L in water, i.e., in lower dose protected the intestinal toxicity of Bisphenol F as shown by behavioural parameters. Based on the findings of this study, low dose of quercetin might be an effective intervention against BPA-induced behavioural alteration and intestinal toxicity. In future, a better understanding of the signalling cascades causing intestinal toxicity could provide new insights into novel approach against BPA-induced intestinal toxicity and their alternative treatment for the aquatic animals.

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References

1. Ahn C, Jeung EB. Endocrine-disrupting chemicals and disease endpoints. International Journal of Molecular Sciences. 2023;24(6):5342.
2. Audira G, Sampurna BP, Juniardi S, Liang ST, Lai YH, Hsiao CD. A versatile setup for measuring multiple behaviour endpoints in zebrafish. Inventions. 2018;3(4):75.
3. Ay M, Charli A, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG. Quercetin. In: Nutraceuticals. Academic Press; c2021. p. 749-755.
4. Butt RL, Volkoff H. Gut microbiota and energy homeostasis in fish. Frontiers in Endocrinology. 2019;10:429202.
5. Cachat JM, Canavello PR, Elkhayat SI, Bartels BK, Hart PC, Elegante MF, Beeson EC, Laffoon AL, Haymore WA, Tien DH, Tien AK. Video-aided analysis of zebrafish locomotion and anxiety-related behavioural responses. Zebrafish Neurobehavioural Protocols. 2011;1-14.
6. Canesi L, Fabbri E. Environmental effects of BPA: focus on aquatic species. Dose Response. 2015;13(3).
7. Cheng JH, Li Y, Shen MH, Yang ZR, Zhang WJ, Zhang K, *et al*. Hypoxia stress and recovery on intestinal tissue of hybrid yellow catfish "huangyou-1" (Pelteobagrus vachelli♂× Pelteobagrus fulvidraco♀). Acta Hydrobiologica Sinica. 2022;46(11):1598-1608.
8. Collier AD, Kalueff AV, Echevarria DJ. Zebrafish models of anxiety-like behaviours. The Rights and Wrongs of Zebrafish: Behavioural Phenotyping of Zebrafish. 2017;27:45-72.
9. Collier AD, Kalueff AV, Echevarria DJ. Zebrafish models of anxiety-like behaviours. The Rights and Wrongs of Zebrafish: Behavioural Phenotyping of Zebrafish. 2017;27:45-72.
10. Fenero CIM, Flores AAC, Câmara NOS. Inflammatory diseases modelling in zebrafish. World Journal of Experimental Medicine. 2016;6(1):9.
11. Hanasaki Y, Ogawa S, Fukui S. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. Free Radical Biology and Medicine. 1994;16(6):845-850.
12. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Teucke M. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013;496(7446):498-503.
13. Khan FR, Alhewairini SS. Zebrafish (*Danio rerio*) as a model organism. Current Trends in Cancer Management. 2018;27:3-18.
14. Kim Y, Kim SS, Park BH, Hwang KS, Bae MA, Cho SH, Kim S, Park HC. Mechanism of Bisphenol F affecting motor system and motor activity in zebrafish. Toxics. 2023;11(6):477.
15. Maximino C, Marques de Brito T, Dias CAGDM, Gouveia Jr A, Morato S. Scototaxis as anxiety-like behavior in fish. Nature Protocols. 2010;5(2):209-216.
16. Midttun HL, Vindas MA, Nadler LE, Overli O, Johansen IB. Behavioural effects of the common brain-infecting parasite Pseudoloma neurophilia in laboratory zebrafish (*Danio rerio*). Scientific Reports. 2020;10(1):8083.
17. Moreira ALP, Luchiari AC. Effects of oxybenzone on zebrafish behaviour and cognition. Science of The Total Environment. 2022;808:152101.
18. Mu X, Liu J, Wang H, Yuan L, Wang C, Li Y, Qiu J. Bisphenol F impaired zebrafish cognitive ability through inducing neural cell heterogeneous responses. Environmental Science and Technology. 2022;56(12):8528-8540.
19. Mu X, Qi S, Liu J, Yuan L, Huang Y, Xue J, Qian L, Wang C, Li Y. Toxicity and behavioural response of zebrafish exposed to combined microplastic and bisphenol analogues. Environmental Chemistry Letters. 2022;1-8.
20. Ohnesorge N, Heinl C, Lewejohann L. Current methods to investigate nociception and pain in zebrafish. Frontiers in Neuroscience. 2021;15:378.
21. Serra EL, Medalha CC, Mattioli R. Natural preference of zebrafish (*Danio rerio*) for a dark environment. Brazilian Journal of Medical and Biological Research. 1999;32:1551-1553.
22. Snedecor GW, Cochran WG. Statistical Methods. 8th ed. The Iowa State University Press; c1994. p. 491.
23. Stiefel C, Stintzing F. Endocrine-active and endocrine-disrupting compounds in food–occurrence, formation and relevance. NFS Journal. 2023.

24. Van Sebille YZ, Gibson RJ, Wardill HR, Carney TJ, Bowen JM. Use of zebrafish to model chemotherapy and targeted therapy gastrointestinal toxicity. Experimental Biology and Medicine. 2019;244(14):1178-1185.
25. Wang K, Deng Y, Zhang J, Cheng B, Huang Y, Meng Y, Zhong K, Xiong G, Guo J, Liu Y, Lu H. Toxicity of thioacetamide and protective effects of quercetin in zebrafish (*Danio rerio*) larvae. Environmental Toxicology. 2021;36(10):2062-2072.