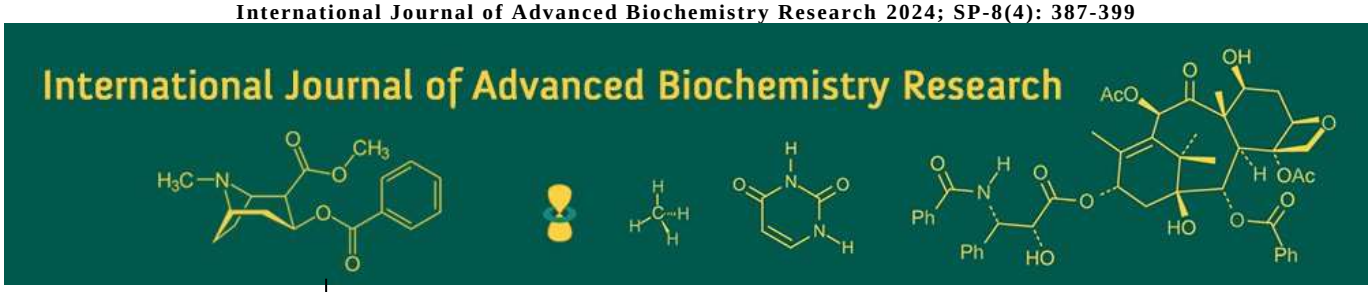

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Toxicity assessment of sub-acute silver nitrate exposure on adult zebrafish behaviour

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Abstract
Silver nitrate is used extensively in industry that can affect the health of terrestrial and aquatic animals due to contamination of environment. In the main study, adult female zebrafish *(Danio rerio)* of three toxicity groups were exposed to $AgNO_3$ concentrations of 8.75, 17.5, 35 µg/L, respectively for 28 days based on the pilot study observations, along with the control group. Zebrafish used as a model in this study to evaluate social impairing, anxiety like behaviour and swimming performance. Toxicity evaluation was done by behaviour evaluation tests like novel tank test, light-dark preference test, Social Preference test and Social Recognition test. Anxiety-like behavior, decreased locomotor activity, and altered social behaviour observed in adult female zebrafish of T_2 & T_3 groups. This study concludes that exposure for 28 days at sublethal dose might produce significant behaviour alteration which elucidate the neurotoxic potential of $AgNO_3$.

Keywords: Silver nitrate, zebrafish, behaviour, anxiety, swimming performance, social behaviour

1. Introduction
Animals as well as humans are constantly exposed to different xenobiotic chemicals at some time in their life; in fact, many medications used to cure illnesses are also considered xenobiotics. These xenobiotic substances cause harm to the body when they enter the bodies of humans or other living things in an uncontrolled way. Numerous items are produced using xenobiotics, increasing the likelihood of coming into contact with these harmful substances. A multitude of chemicals are being released into the environment, raising the possibility of environmental health risks and ultimately impacting the health of humans and animals [1].
The inorganic salt of the silver ions has a number of applications yet have the potential to seriously pollute the environment. It is acknowledged as a highly effective precursor for the synthesis of high purity compounds, specific catalysts, and materials at the nanoscale (nanoparticles, nano powders). It is applied to a skin wound to cauterize the surrounding diseased tissues. It stops the bleeding from a small skin wound by forming a scab. It is frequently used to treat wound granulation, inferior turbinate hypertrophy, and minor epistaxis [2].
It is important to explore the silver nitrate toxicity potential on behaviour of animals in animal models which can represent aquatic animals with the homology to humans as $AgNO_3$ is coming directly in contact with the animals in aquatic environment because of the spillage of industrial waste containing these ions. The study employed adult female zebrafish, as they have been shown to be an excellent model for investigating the effects of xenobiotics on a fully developed brain following exposure to varying doses of xenobiotics. Additionally, they are useful for monitoring the environment, neurodegenerative disorders, toxic heavy metals, endocrine disruptors, and organic pollutants.
Previous research has revealed that silver nitrate sub-lethal exposure for shorter duration produces neurotoxicity in adult zebrafish through behaviour study [3, 4]. But there is no available data supporting toxicity of silver nitrate in the brain after continuous exposure for 28 days or more. In view of this, the current study was planned to assess the neurotoxicity potential of silver nitrate after exposure at various concentrations in adult zebrafish for 28 days, with a focus on behaviour parameters in brain.

2. Material and Methods

2.1 Ethical statement and approval

The animals were strictly maintained and treated in Laboratory Animal House Facility of the institute according to Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines. The experiment has been approved (Approval No: KU/JVC/IAEC/SA/81/2021) by the Institutional Animal Ethics Committee, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat.

2.2 Chemicals

Silver nitrate (Purity > 99.90) (CAS No. 7761-88-8) purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India used to induce the toxicity in fish of treatment groups. All other chemicals (Purity>99.90) of analytical or molecular grade were procured from standard company.

2.3 Experimental animals and the environment

Three hundred and twelve wild type *Danio rerio* zebrafish, mature and in good condition, older than three months, were obtained from Vikrant Aqua-Culture in Mumbai, India. The typical stocking density of 5 fish/L was maintained for the zebrafish in tanks with artificial aeration provided by aerators. The experiment began 15 days before the fish were all acclimated. Fish pellets (10 mg per fish, twice daily) and Tetra bits complete®, purchased from a nearby vendor, were given to the fish. Every day, the water was replenished and routinely checked. Every day, the potential of hydrogen in the water (7 ± 0.2 pH), temperature (25 ± 3 ºC), hardness (50-100 mg/L), and electrical conductivity (500 ± 10 µS/cm) were measured in each tank. Throughout the experiment, a photoperiod cycle of 10:14 h (dark: light cycle) and light intensity of 240–260 lux was likewise maintained. Across all treatment groups, no discernible variation was seen in any of the above-mentioned environmental indicators over the exposure period.

2.4 Experiment design

Total three hundred twelve fish were randomly divided in four groups with seventy-eight zebrafish in each. Group C was served as control. Ionic silver is acutely toxic to zebrafish at concentration within the 96-hour LC_{50} range (5–70 µg/L) like for other teleost. To evaluate toxicity of silver nitrate following long term (28 days) exposure in main experiment, 35 µg/L (T_3) was decided as highest concentration which is 70 percent of the highest concentration of pilot study followed by 17.5 (T_2) & 8.75 (T_1) µg/L concentrations. Accurate weighing of silver nitrate was done using precise analytical weighing balance Mettler Toledo (MS 204S/A01 Mettler Toledo, Mumbai, India). Water with particular strength of silver nitrate in each tank was changed daily to maintain concentrations of silver nitrate. Behaviour tests performed at four different durations of 7,14,21, and 28 days. Total 24 zebrafish (6 in each group) taken for behaviour parameter evaluation.

2.5 Evaluation of Behavioural Alterations

2.5.1 Novel tank test

Fish of each group were placed in separate experimental tanks (12 x 7 x 6 inches; Length x Width x Height). Six fish from each group were used for assessment of behavioral parameters [6]. The tank was divided in to two zones by drawing two horizontal lines with marker pen [Fig. 1 (A)]. All fish of each group were habituated for 30 minutes in newer experimental tank for acclimation before starting the video recording. The videography of movement of fish was recorded in the tank from front side of the tank was done. Back side of tank was covered with white background to visualize the fish during the analysis of video. Total time in minutes spent by each fish in upper zone of the tank and no. of entries in upper zone and lower zone within 10 minutes was recorded.

2.5.2 Light-dark preference test

For assessment of anxiety-like symptoms, light dark preference test with the specially designed tank (18 x 9 x 12 inches, Length x Width x Height) was carried out [5]. The schematic diagram of the designed tank is shown in [Fig. 1 (B)]. The experimental tank was divided in two equal parts separated by a piece of glass. One side of the experimental tank was covered with thick black coloured paper sheet to avoid the light to enter in the tank from the lateral side where the main source of the light was present. This paper covered part was considered as dark side and another uncovered side was considered as light side or white side. The bottom of the tank was also covered with white paper to observe the movement of fish in the tank. The water was filled up to 10 cm height from the bottom of the tank. For the source of light, 23-watt LED lamp was placed at lateral side of the tank in a particular distance (140 cm) which provided the light to the tank [Fig. 1 (B)]. The intensity of light in the center of the light part of tank above water surface was measured thrice and the values were in the range of 240-260 lux. The intensity of light in the dark area was only 5-6 lux. Before starting the experiment, the tank was put in the dark room and the light source was kept on. The fish of each group (06) were placed in the light part of the tank and acclimation period of 30 minutes was observed prior to video recording. The glass between two partitions was then removed to allow the fish to swim freely between two compartments. The video of 10 minutes of each group was observed for documentation of time spent in dark side, no. of entries in dark side. The movement of each fish was observed by playing the video six times per group. The digital timer was used to record the time points during the movement of each fish from one compartment to another compartment. <insert figure 1 [A-B] here>.

2.5.3 Social preference test

For social preference test, a 6 L tank (50 cm ×10 cm ×12 cm; Length x Width x Height) divided into five equal cells (10 cm) by a glass divider. One zebrafish was placed at one end of the tank and the other end of the tank remained empty [Fig. 2 (A)]. To avoid bias, the zebrafish was randomly placed at the left or right end of the tank. The zebrafish being tested was introduced into the central zone of the tank. After acclimation for 5 min, the two dividers in the central zone were gently removed, and the tested fish was released to allow it to explore the tank freely for 10 min. The fish behaviors were recorded manually from the lateral site of the behavioral tank. The time spent by the fish in each area of the tank and number of entries in each area was measured.

2.5.4 Social recognition test

The social recognition test was also conducted using the same tank that was used for the social preference test. A familiar fish (the tank mate) was placed at one end of the

tank, and a stranger fish (raised in another tank) was placed at the other end [Fig. 2 (B)]. To avoid bias, the locations of familiar and stranger fish were randomly selected. The zebrafish being tested was introduced into the central zone of the tank. After acclimation for 5 min, the two dividers in the central zone were gently removed, and the tested fish was released to explore the tank freely for 10 min. The fish behaviors were recorded manually from the lateral site of the behavioral tank. The time spent by the fish in each area of the tank and no. of entries in each area was measured. <insert figure 2 [A-B] here>.

2.6 Statistical analysis

Statistical analysis of all data was carried out using Graph pad prism 9 version. Kolmogorov-Smirnov test was used to evaluate the normality of data along with Bartlett's test to confirm the equal variance. Data with normal distribution and homogeneous variance were analysed by parametric one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where $p<0.05$ (*) was considered as statistically significant and $p<0.01$ (**), $p<0.005$ (***) and $p<0.001$ (****) were considered for highly significant differences statistically. The data didn't have either normal distribution or homogeneous variance were analysed by Kruskal-Wallis test followed by Dunnett's test.

3. Results and Discussion

We evaluated behavioural alterations in adult female zebrafish on days 7, 14, 21, and 28 following $AgNO_3$ exposure at 8.75, 17.5, and 35 µg/L concentrations. The light-dark preference test and the novel tank test are used to assess the effect of silver nitrate on anxiety-like behavior, whereas the social preference and social recognition tests are used to assess the toxic effect of silver nitrate on social behaviour and interaction.

3.1 Novel tank diving test

The mean values of time spent in the upper zone and the number of entries in the upper zone on day 7, 14, 21 & 28 are graphically in Figure 3, 4, 5 & 6 respectively. We observed that zebrafish from the T_2 & T_3 group spent significantly less time in the upper zone of the tank than the control and T_1 group on day 7, 14 and 28 indicating the development of anxiety. The number of entries in the upper zone was significantly lower in the T_2 and T_3 groups compared to the control and other lower treatment group (T_1) on day 7 & 28 suggesting decreased swimming performance. The T_1 group zebrafish only spent significantly less time in the upper zone of the tank on day 14 & 28, otherwise did not show any statistically significant difference in total time spent or number of entries in the upper zone compared to the control group. <insert figure 3, 4, 5 & 6 here>.

The neurobehavioral phenomics of zebrafish is a new and promising approach that integrates behavioural phenotypes with various genetic and environmental factors [7, 8]. Toxicant-induced behavioral impairments often point to underlying physiological deficits that can be used to effectively evaluate ecological risks that affect fish survival, growth, or reproduction [9].

The novel tank test is a validated behavioural test for the assessment of "anxiety-like" behaviour and swimming performance in adult zebrafish. It is the scientifically accepted methodology to evaluate how well the zebrafish adapt to various stressors present in the environment or the effect of stressors/xenobiotics on the body which leads to stress-mediated alterations. In the novel tank test, the time spent in a particular portion of the tank (upper zone vs. lower zone) is considered the index of anxiety-like symptoms. More time spent at the bottom of the tank and less time spent at the top indicates a higher level of anxiety-like symptoms [10].

Our results showed decreased time spent and number of entries in the upper zone, suggesting decreased activity of zebrafish following $AgNO_3$ exposure. The findings of Fu et al. [4] supported our findings, who reported a significantly lower total moving distance of fish treated with 30 ppb (0.176 µM) of $AgNO_3$ compared to control, indicating the effect of silver nitrate on locomotion. While total time spent in the upper zone was not altered in any treatment group of $AgNO_3$, which is in contrast to our results in the present study, suggesting $AgNO_3$ treatment did not alter anxiety level, this disparity may be due to the longer $AgNO_3$ exposure (28 days) in our study eliciting anxiety-like behavior, implying a sub-acute toxicity effect developing over time. They also found lower endurance of the swimming behaviour by showing fatigue against lower water velocity in a swimming performance test, indicating $AgNO_3$ affected locomotion activity and reduced the physical fitness of the fish. Studies in zebrafish embryos found that significant hyperactivity was observed in zebrafish exposed to 3 ppm of silver nanoparticles. These results suggest that swimming behaviour and locomotor activity were altered through Ag^+ treatment in fish [11, 12]. Swimming behaviour and locomotor activity are combined performances of cardiovascular, neural, metabolic, and muscle function [13].

The results of the present study showed aberrant swimming patterns, i.e., arrow-like swimming, circular swimming, and finally ending in a motionless state (freezing behavior). In support of our findings, silver nitrate exposure (13, 21, 23, 25, 29, 37, and 47 µg/L) for 48 hrs in zebrafish displayed an avoidance reaction by increasing their swimming activity and trying to escape from the tank through the sensing of silver ions in the water. Reduced food consumption, food-conversion efficiency, and swimming speed were found in trout exposed to 5 ppm Ag^+ (as $AgNO_3$) for 23 days [14]. A few fish displayed jerky movements and circular swimming just before they lost equilibrium, indicating that ionic silver is also acutely toxic to zebrafish at concentrations within the 96-hour LD_{50} range (5–70 µg/L) for other teleost [3].

In fish, hypo locomotion is an indicator of motor retardation due to depression [15]. The symptoms like spontaneous movement with freezing, swimming in a group, swimming in the lower zone of the tank, forced swimming, and jerky movement observed in our study are considered typical responses to anxiogenic stimuli in adult zebrafish [16]. The symptoms observed in the study clearly confirmed the disturbed locomotor activity after exposure to different $AgNO_3$ concentrations for 28 days in the present study. In zebrafish, the relationship between higher levels of corticosteroids and depression is well characterized in zebrafish [17]. The high level of corticosteroids is responsible for the depression-like symptoms in the zebrafish following $AgNO_3$ exposure.

3.2 Light-dark preference test

The mean values of time spent in the dark side and the

number of entries in the dark side on day 7, 14, 21 & 28 are graphically in Figure 7, 8, 9 & 10. Zebrafish from the T_2 & T_3 groups spent significantly more time on the dark side of the tank when compared to the control group and the T_1 group on day 7, 14 & 28. While the number of entries on the dark side did not differ significantly between treatment and control groups on day 7 & 21. On Day 14, the number of entries on the dark side increased in T_2 & T_3 groups while only T_3 group showed this phenomenon on day 28**.** <insert figure 7, 8, 9 & 10 here>.

The light-dark preference test is one of the most commonly used tests to assess anxiety-like behaviour in animals, particularly in zebrafish. The test has the ability to assess the tendency of animals to explore or avoid zones of preference in terms of either light or dark zones. Adult zebrafish are known to be scototaxic [18]. Zebrafish have a marked preference towards the dark zone in terms of intensity of light, which is an indicator of an anxiety-like behavior, and preference towards the light zone reflects an anti-anxiety behaviour [18, 19]. The main disadvantage in relation to the open-field and the novel tank diving test is the lack of pharmacological validation. Zebrafish prefer a light environment in normal conditions, but it depends on light intensity [20]. When the light intensity is near to 200 lux, zebrafish prefer a light environment, but dark is preferred when the light intensity is increased. More entries in a light environment indicate more exploratory behavior, while more time spent in a dark environment indicates a greater tendency for anxiety-like behaviour [19]. Anxiety-like behavior is an emotional behavior due to exposure to a new environment or potential adverse stimulus [21].

Exposure to 30 ppb Ag^+ (as $AgNO_3$) for 24 h caused hyper-responsiveness to light changes in zebrafish embryos 6 days post-fertilization [22]. Furthermore, exposure to 5 ppb $AgNO_3$ and 150 ppb silver nanoparticles for 4 h was found to impair rheotaxis ability in zebrafish embryos 96 h postfertilization [23]. These studies demonstrated that exposure to silver affected swimming behaviours in fish.

3.3 Social Preference test

The mean values of time spent in the conspecific zone and the number of entries in the conspecific zone on day 7, 14, 21 & 28 are presented graphically in Figure 11, 12, 13 & 14. Adult zebrafish from the T_3 group spent significantly less time in the conspecific zone (p<.05) than the control group on observation of day 7, 14 & 28 and T_2 group only showed this phenomenon on day 21. While lower numbers of entries in the conspecific zone were observed in the T_2 and T_3 groups with different significance levels compared to the control group on day 7, 14 & 21 indicating an effect on social behavior. <insert figure 11, 12, 13 & 14 here>.

Zebrafish have a highly social phenotype. The social preference test was performed to investigate whether exposure to $AgNO_3$ has any detrimental effects on the social interaction of the zebrafish. In the present study, we found that control zebrafish spent more time in the conspecific zone in the social preference test, but as the duration and concentration of $AgNO_3$ are increased, there is a decrease in the total time spent and number of entries in the conspecific zone, showing the development of $AgNO_3$ adverse effect on abolishing social interaction towards conspecific individuals. The fish exposed to a lower $AgNO_3$ concentration (8.75 µg/L) showed altered social behaviour. In support of our findings, Fu et al. [4] observed that control fish showed a preference for a conspecific individual and spent more time staying in the conspecific area of the tank. This preference was abolished by $AgNO_3$ treatment. The durations of stay in the empty and conspecific zones of the tank were similar in the $AgNO_3$-treated fish.

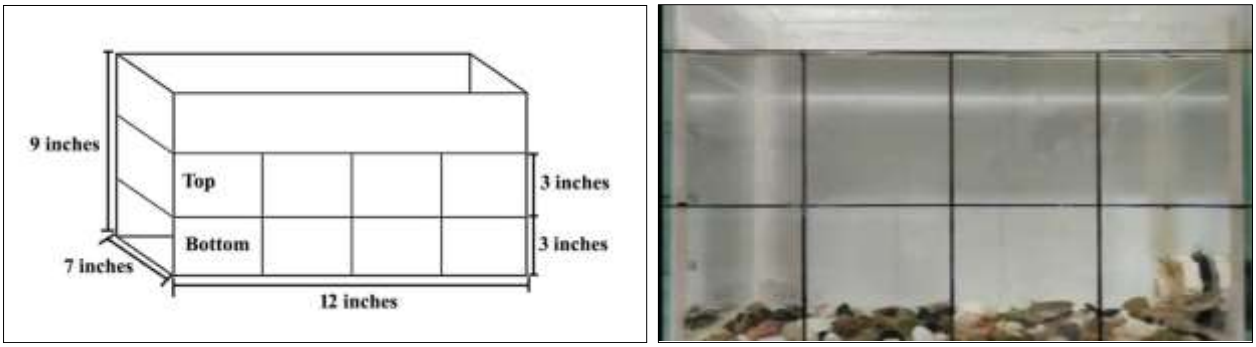
Previous studies demonstrated that exposure to sublethal concentrations of $AgNO_3$ led to an accumulation of Ag^+ in the brain and changed the social behaviour of the zebrafish. Moreover, the neural activity in the dorsal zone of the dorsal telencephalic area was reduced in $AgNO_3$-treated fish, compared with control fish [4]. Dorsal zone of the dorsal telencephalic area sends the axon to the dorsal nucleus of the ventral telencephalic area (Vd), which is a putative region corresponding to the mammalian nucleus accumbens (NAc) [24]. In mice, increased social interaction is accompanied by increased activity in the NAc, which receives input from dopamine neurons in the ventral tegmental area [25]. In the social conditional place preference assay, blockade of oxytocin and serotonin receptors in the neurons of the NAc prevented social reward and reduced the time spent in the social zone after the trial [26]. Manual and genetic lesions of the ventral telencephalon suppressed social interactions in zebrafish [27]. Thus, $AgNO_3$ treatment might affect social preference and recognition by reducing the activity of the dorsal zone of the dorsal telencephalic area.

3.4 Social Recognition test

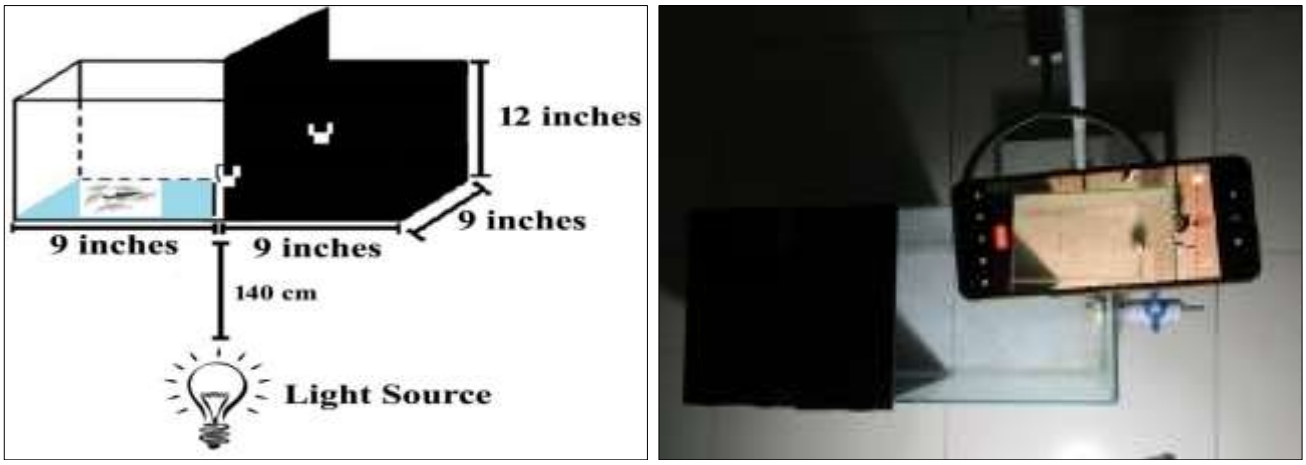
The mean values of time spent in the familiar zone and the number of entries in the familiar zone on day 7, 14, 21 & 28 are presented graphically in figure 15, 16, 17 & 18. On day 7 & 14, T_3 group zebrafish showed a significant decrease in the time spent in the familiar zone compared to control and T_1 group zebrafish; while, on day 21 & 28, T_2 & T_3 group zebrafish showed this phenomenon. The number of entries in the familiar zone also significantly decreased in T_3 group compared to control on day 7, 14 & 28, suggesting social recognition was abolished in the T_3 group. Other groups did not show significant alterations in both parameters. <insert figure 15, 16, 17 & 18 here>.

Social recognition is a critical factor for the stability of social linkage structures in animal societies [28] and is crucial for the maintenance of social hierarchy and appropriate mating choice [29]. As the $AgNO_3$ exposure duration increased, total time spent and number of entries in the familiar zone where its tankmate is placed decreased compared to unfamiliar zone (stranger fish), consequently indicating increasing toxicity of $AgNO_3$ on social recognition, which is attributed to the impaired social behavior.

Fu et al. [4] carried out a social recognition test to study whether $AgNO_3$ exposure impairs social recognition. They observed that control fish spent more time staying in the familiar area than in the novel area of the tank. By contrast, $AgNO_3$ treated fish stayed for similar lengths of time in the familiar and novel areas of the tank. These results are consistent with our results suggesting that exposure to 10 ppb of $AgNO_3$ resulted in impaired social preferences and social recognition but did not affect anxiety levels, aggressiveness, and shoaling behaviour at lower doses.



(A)



(B)

Fig 1: (A) Experimental set-up of novel tank test, (B) Experimental set-up of light-dark preference test,

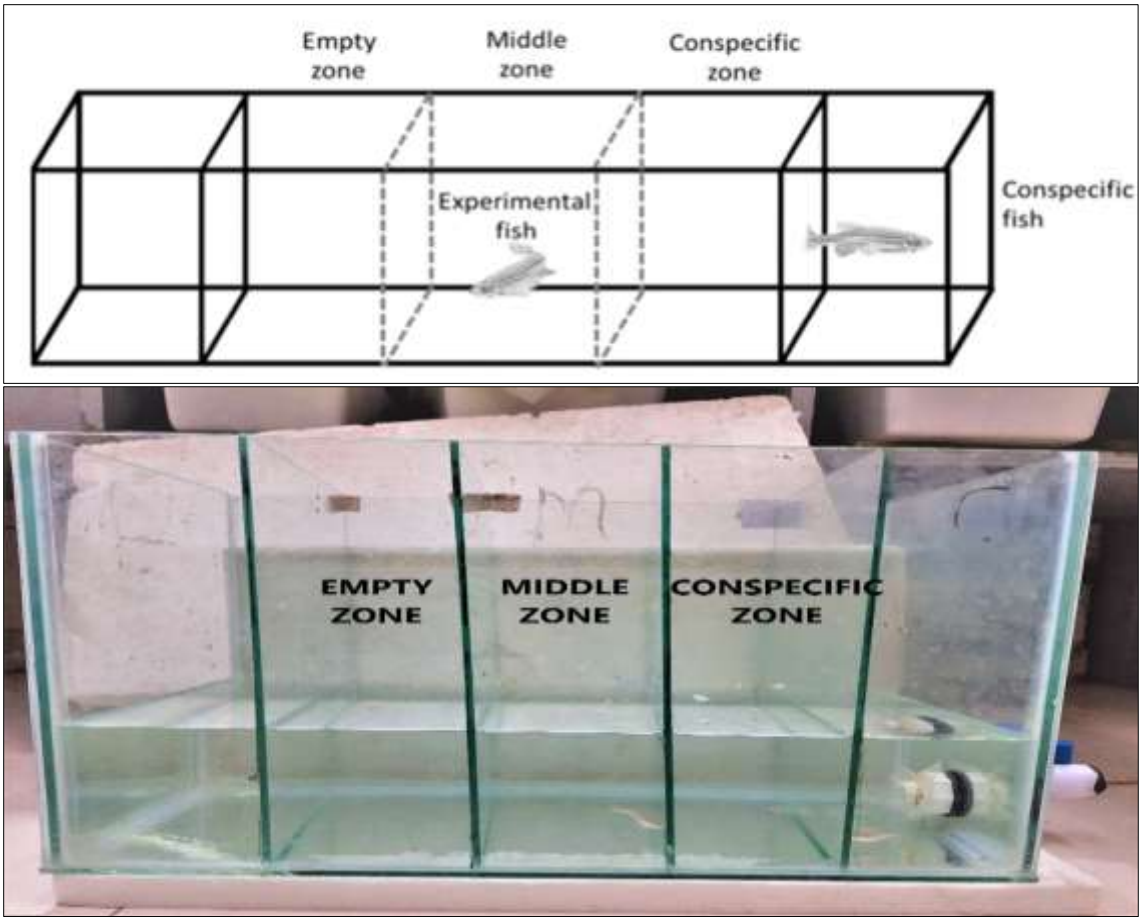


Fig 2: (A) Experimental set-up of social preference test,

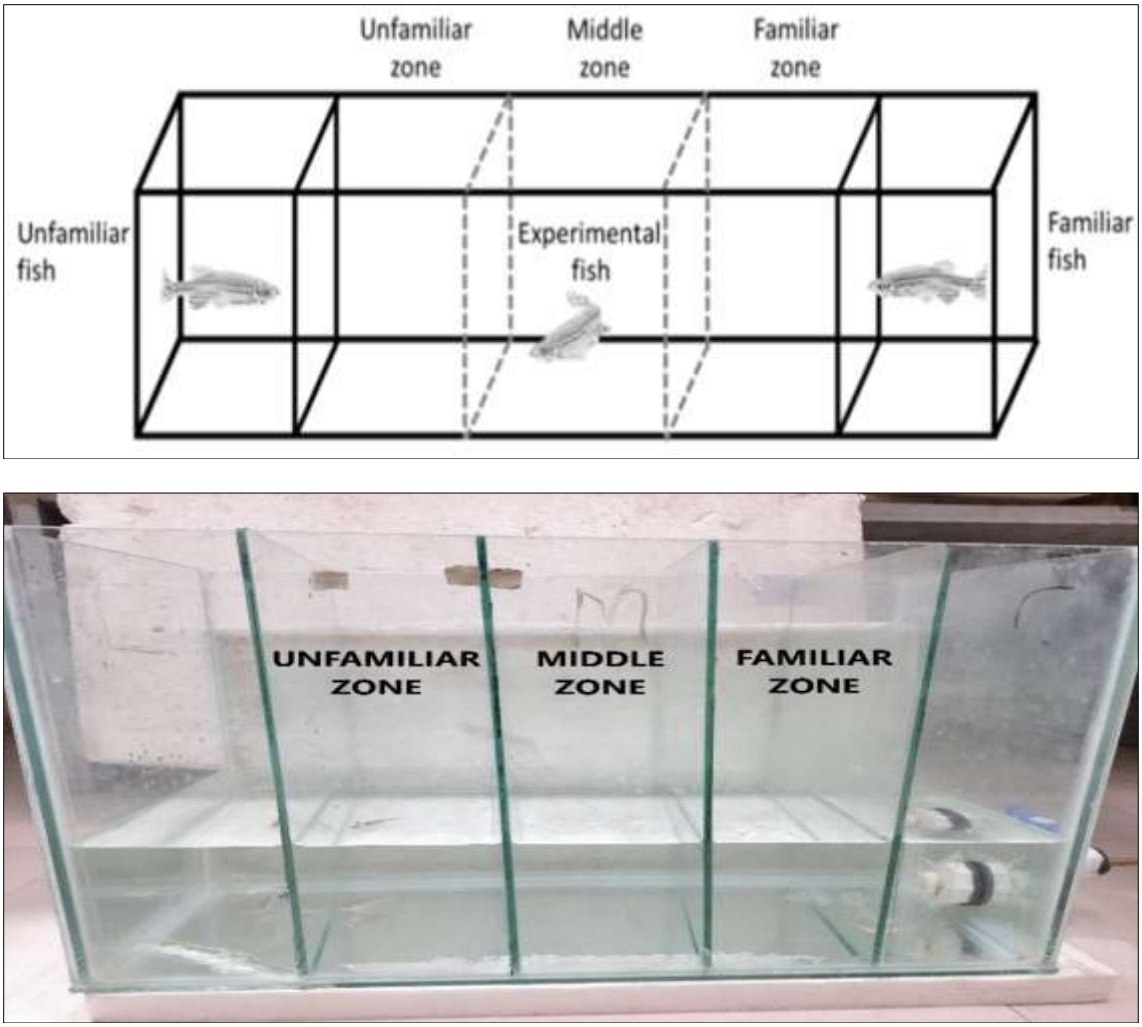
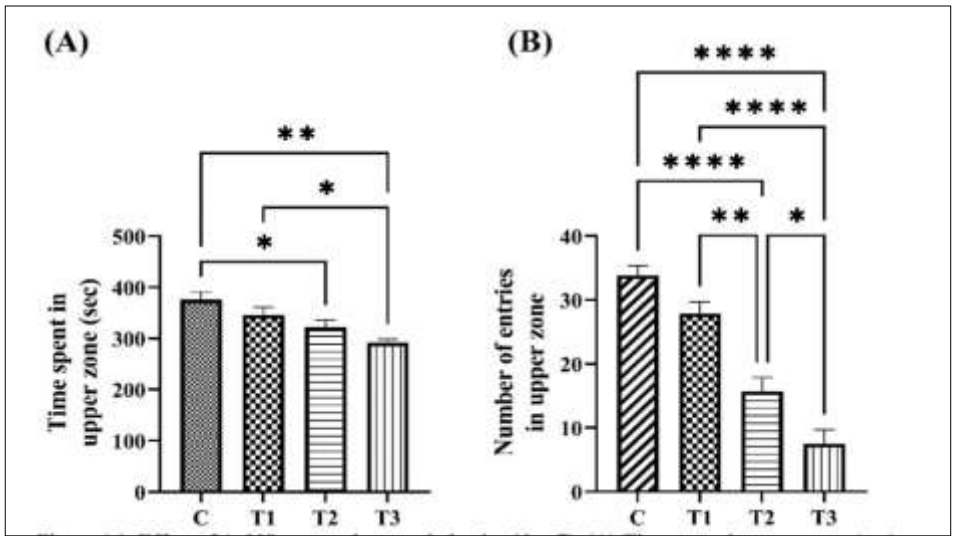
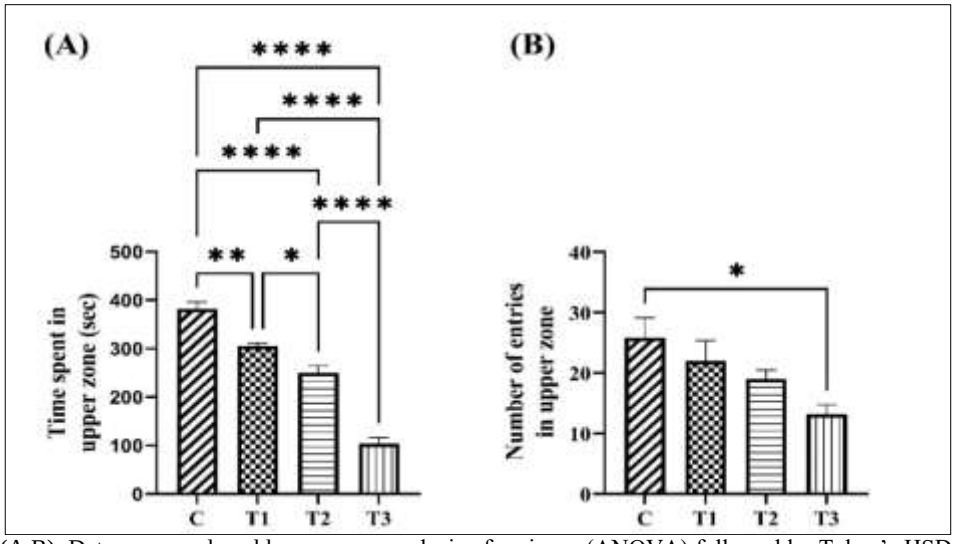


Fig 2: (B) Experimental set-up of social recognition test



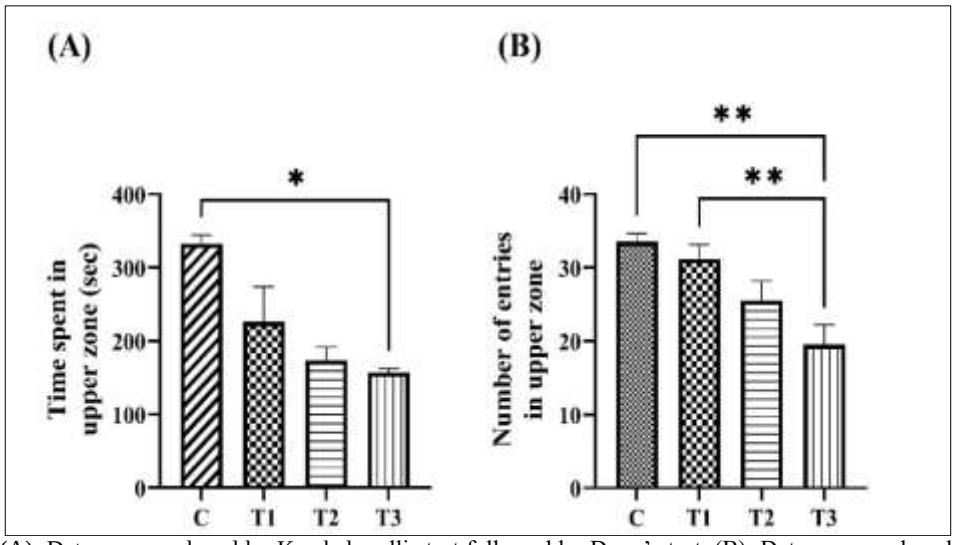
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ****p<0.001.

Fig 3: Effect of silver nitrate on exploratory behaviour (day 7). (A) Time spent in upper zone (sec) (B) Number of entries in upper zone



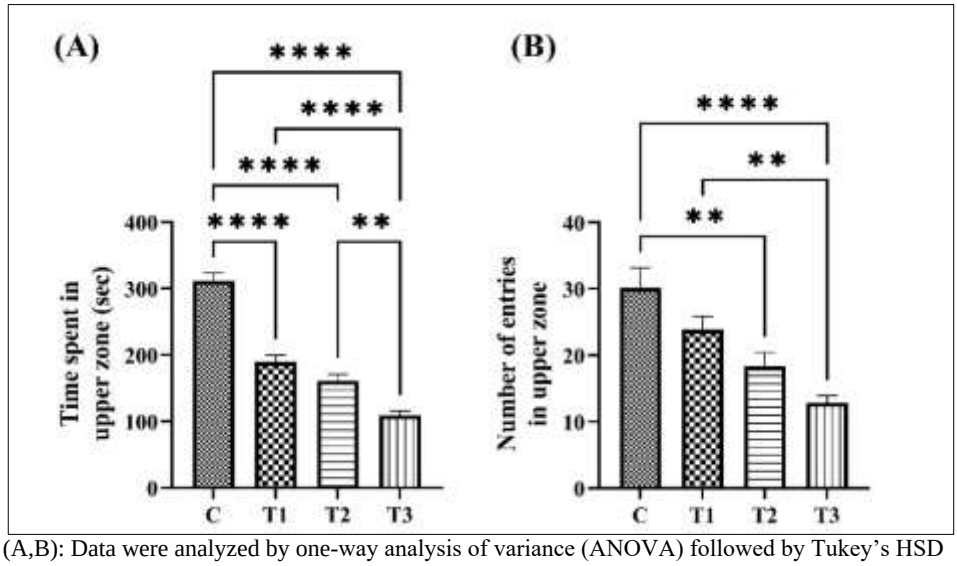
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ****p<0.001.

Fig 4: Effect of silver nitrate on exploratory behaviour (day 14). (A) Time spent in upper zone (sec) (B) Number of entries in upper zone



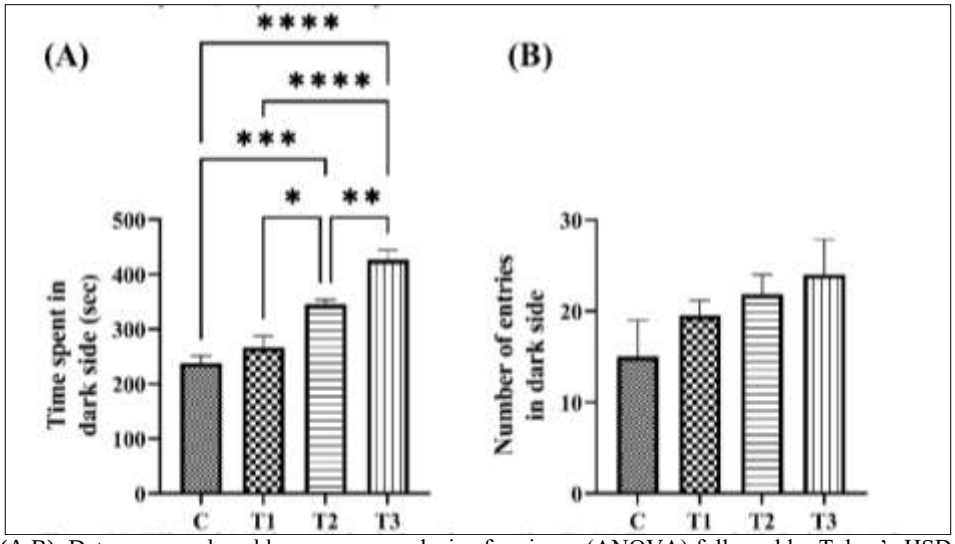
(A): Data were analyzed by Kruskal-wallis test followed by Dunn's test. (B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01.

Fig 5: Effect of silver nitrate on exploratory behaviour (day 21). (A) Time spent in upper zone (sec) (B) Number of entries in upper zone



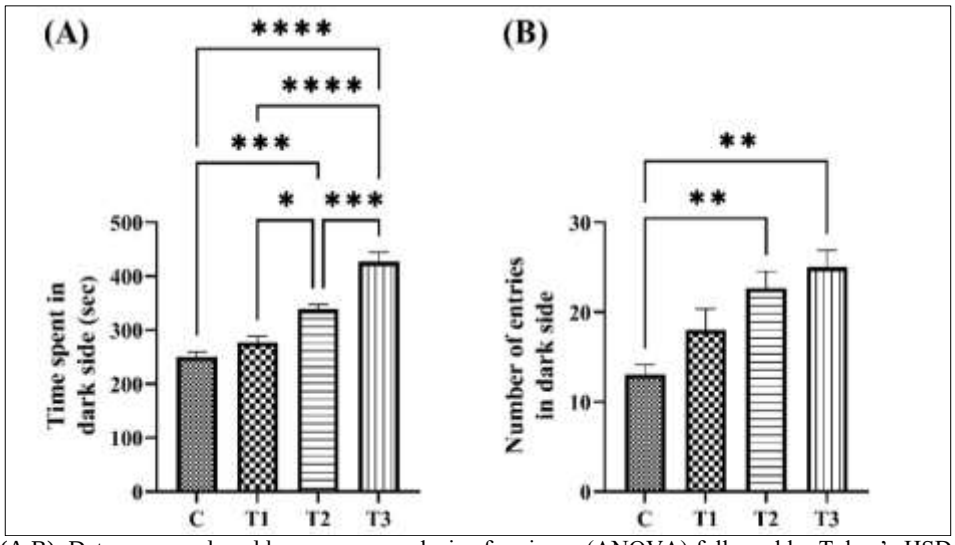
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01.

Fig 6: Effect of silver nitrate on exploratory behaviour (day 28). (A) Time spent in upper zone (sec) (B) Number of entries in upper zone



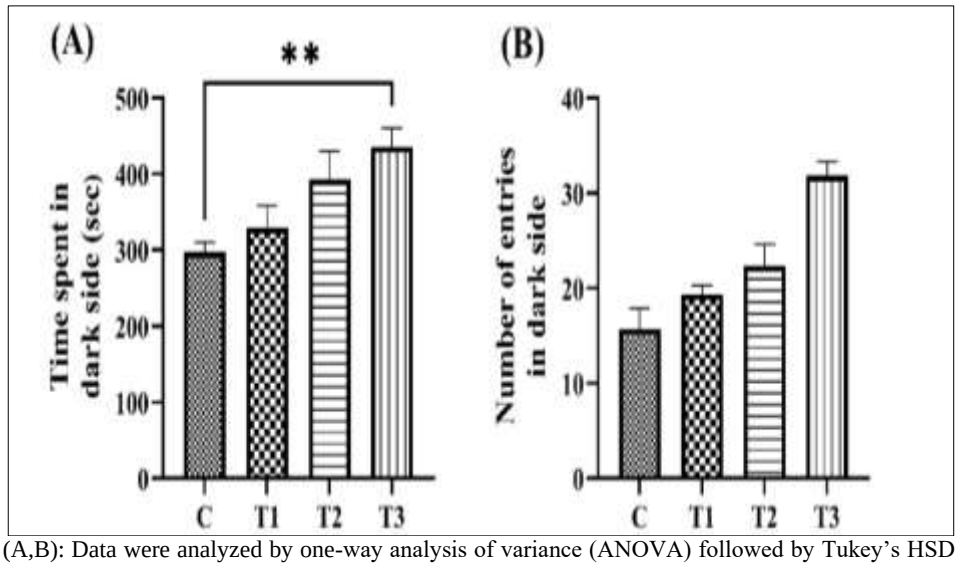
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

Fig 7: Effect of silver nitrate on light-dark preference (day 7). (A) Time spent in dark side (sec) (B) Number of entries in dark side



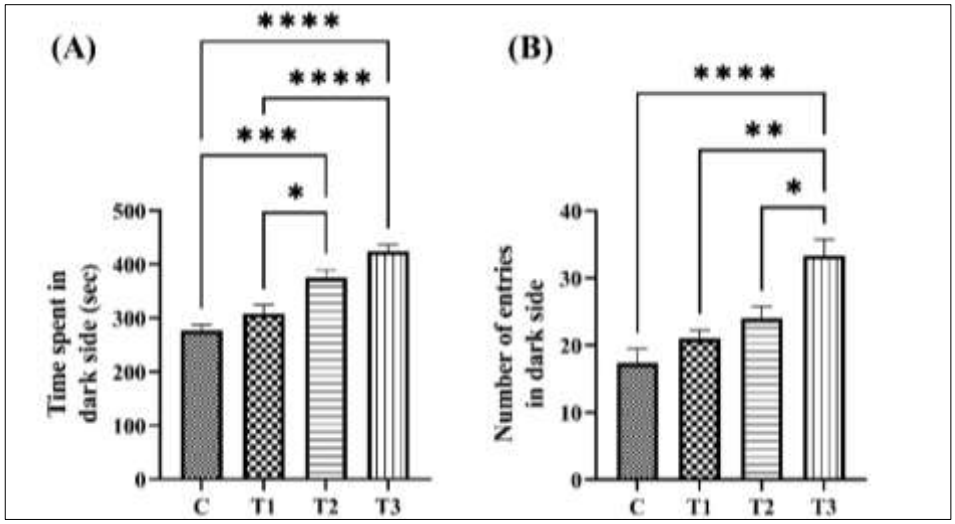
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

Fig 8: Effect of silver nitrate on light-dark preference (day 14). (A) Time spent in dark side (sec) (B) Number of entries in dark side



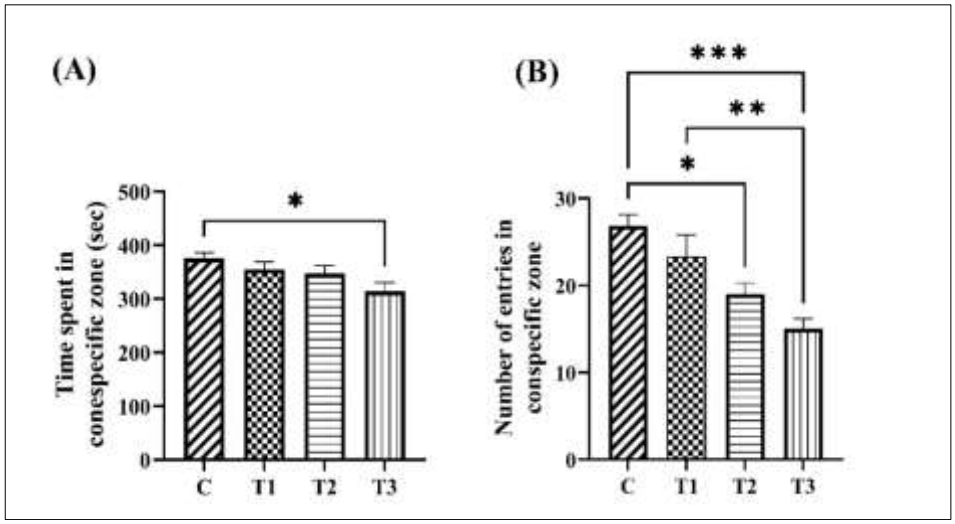
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where **p<0.01.

Fig 9: Effect of silver nitrate on light-dark preference (day 21). (A) Time spent in dark side (sec) (B) Number of entries in dark side



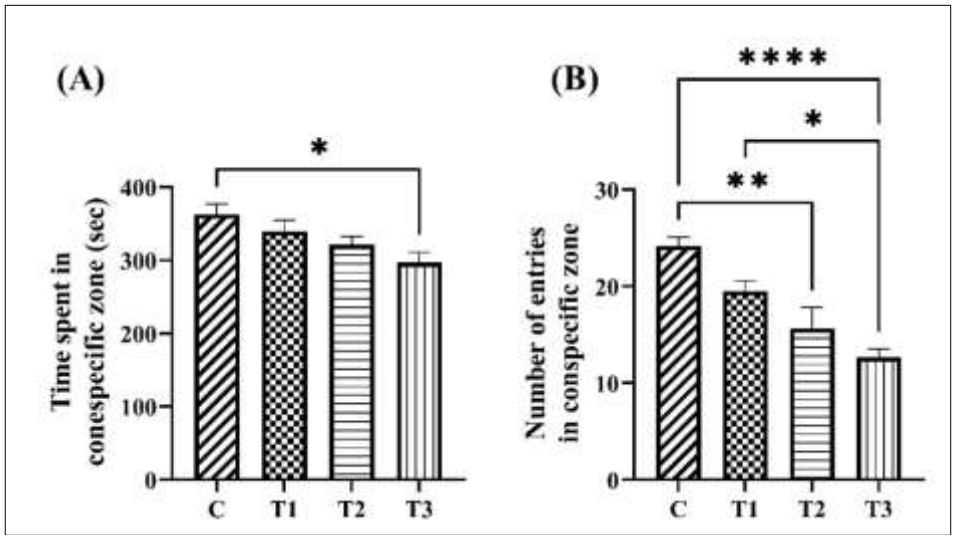
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

Fig 10: Effect of silver nitrate on light-dark preference (day 28). (A) Time spent in dark side (sec) (B) Number of entries in dark side



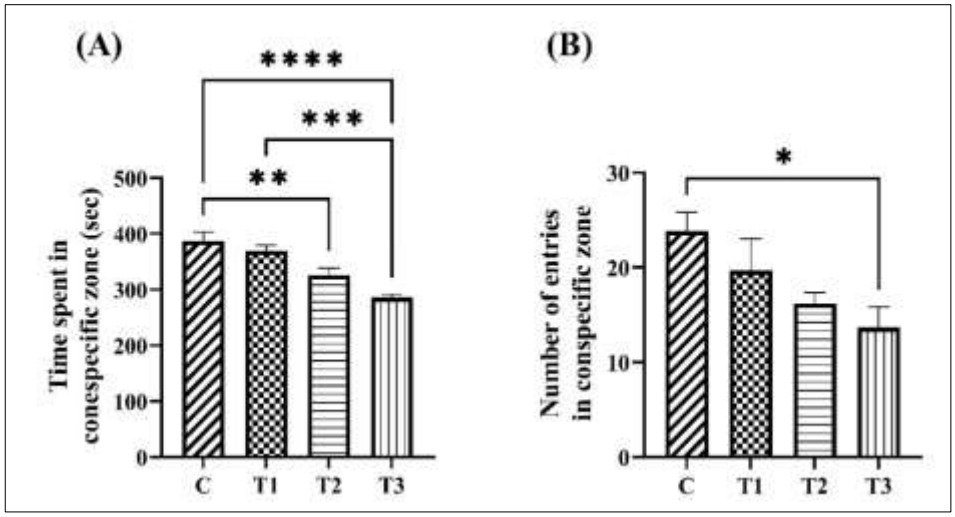
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005.

Fig 11: Effect of silver nitrate on social preference behaviour (day 7). (A) Time spent in conspecific zone (sec) (B) Number of entries in conspecific zone.



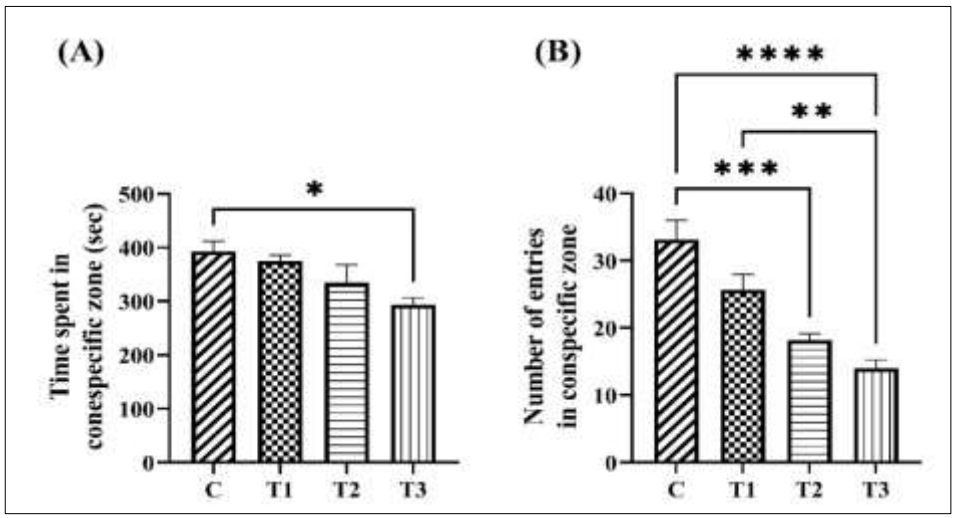
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ****p<0.001.

Fig 12: Effect of silver nitrate on social preference behaviour (day 14). (A) Time spent in conspecific zone (sec) (B) Number of entries in conspecific zone.



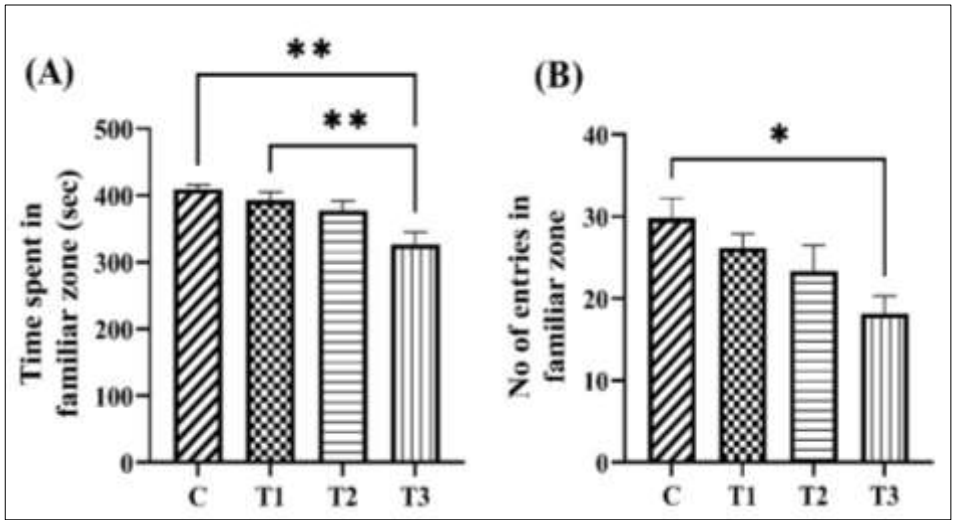
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

Fig 13: Effect of silver nitrate on social preference behaviour (day 21). (A) Time spent in conspecific zone (sec) (B) Number of entries in conspecific zone.



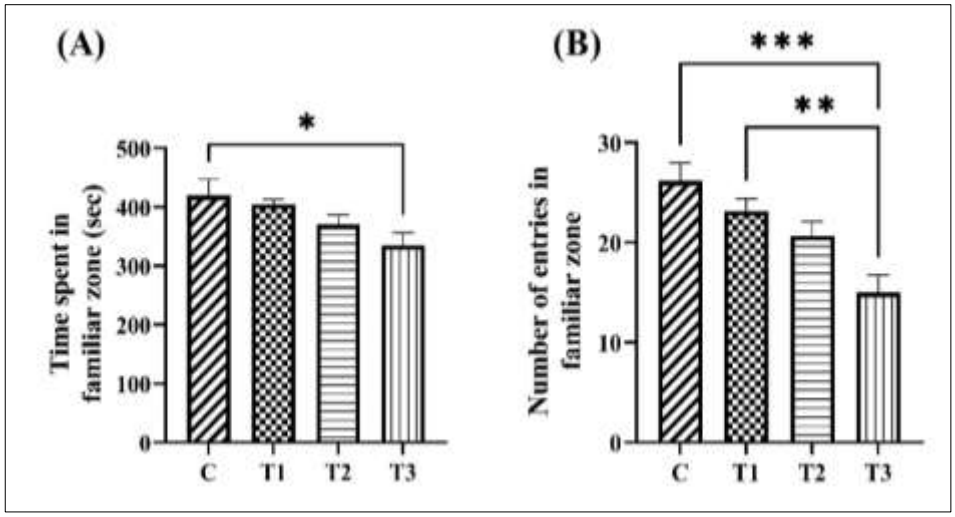
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

Fig 14: Effect of silver nitrate on social preference behaviour (day 28). (A) Time spent in conspecific zone (sec) (B) Number of entries in conspecific zone.



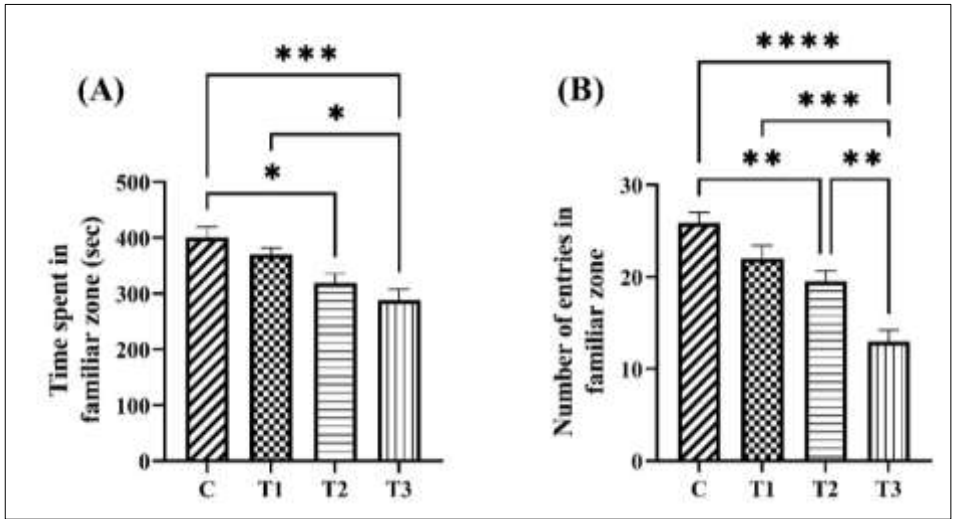
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01.

Fig 15: Effect of silver nitrate on social recognition behaviour (day 7). (A) Time spent in familiar zone (sec) (B) Number of entries in familiar zone.



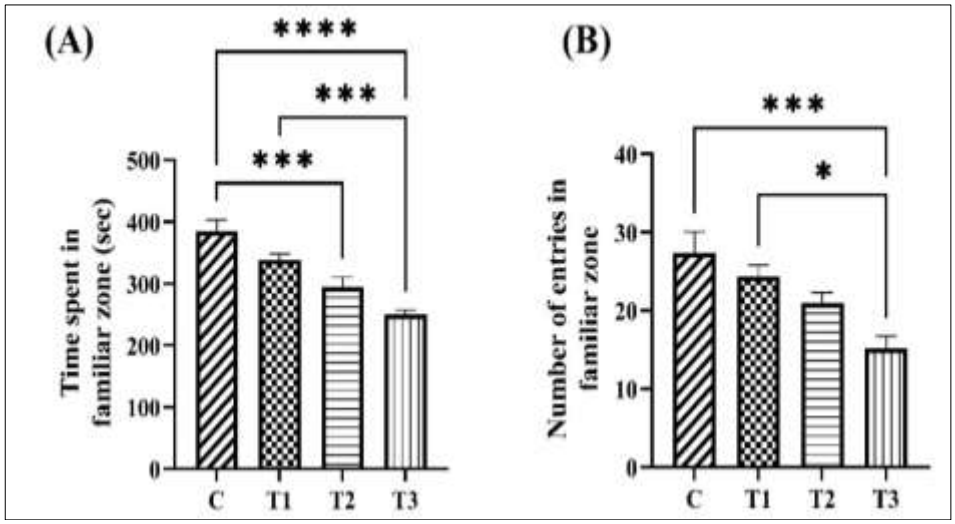
(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005.

Fig 16: Effect of silver nitrate on social recognition behaviour (day 14). (A) Time spent in familiar zone (sec) (B) Number of entries in familiar zone.



(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; **p<0.01; ***p<0.005; ****p<0.001.

Fig 17: Effect of silver nitrate on social recognition behaviour (day 21). (A) Time spent in familiar zone (sec) (B) Number of entries in familiar zone.



(A,B): Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's HSD test. Where *p<0.05; ***p<0.005; ****p<0.001.

Fig 18: Effect of silver nitrate on social recognition behaviour (day 28). (A) Time spent in familiar zone (sec) (B) Number of entries in familiar zone.

4. Conclusion

Zebrafish exposed to mid and high dose of silver nitrate exhibited anxiety-like behavior, hypo-locomotor activity, altered social behaviour indicating effects on central nervous system of adult female zebrafish. Two higher concentrations of $AgNO_3$ for 28 days were found to have more neurotoxic potential in zebrafish compared to the control and low dose group. These neurotoxic effects on the behaviour of aquatic animals can have detrimental effects on their survival.

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