



EFFECT OF ETHANOL TOXICITY ON ANXIETY BEHAVIOR AND MEMORY IN ZEBRAFISH

Nivedita Manoharan¹ and Sathya Narayanan Govindarajulu^{2*}

¹M.Sc., Physiology, Final Year, Department of Physiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600113.

²Assistant Professor, Department of Physiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600113.

***Corresponding Author: Dr. Sathya Narayanan Govindarajulu**

Assistant Professor, Department of Physiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai 600113.

Article Received on 02/11/2017

Article Revised on 23/11/2017

Article Accepted on 13/12/2017

ABSTRACT

Ethanol, is the intoxicating agent found in beer, wine and liquor. Long-term alcohol misuse leads to various disorders of heart, liver, cancer and psychiatric problems such as depression and personality disorder. Since zebrafish has 70% genetic homologue to mammals, it has been established as an eminent model for studying toxicity studies. The aim of the research is to study the effect of ethanol toxicity on anxiety behavior and memory in zebrafish using novel tank, light/dark preference tank and T-maze respectively. Fifty zebrafishes of both sexes were divided into two groups - control and ethanol exposed group (1% ethanol for 10 days). In the present study, there was significant decrease ($p < 0.05$) in number of entries to the upper portion of the tank, time spent in the upper portion and freezing, whereas there was a significant increase ($p < 0.05$) in the latency to reach the upper portion of the tank in the novel tank behavior in ethanol exposed group. In light/dark preference tank, a significant increase ($p < 0.05$) in the time taken in light and start box was observed and a significant decrease ($p < 0.05$) in dark chamber was observed in ethanol exposed group. In T-maze, no significant change in memory were observed in day 1 and 5 of ethanol exposure. Whereas a significant decrease in memory ($p < 0.05$) were observed in day 10. In the present study, there was increased anxiety behavior and memory deficit was detected when the ethanol is exposed. This may be due to the direct effect of ethanol intoxication on brain.

KEYWORDS: Ethanol toxicity, Anxiety, Memory, T-Maze.

INTRODUCTION

The term "alcohol" has been synonymous with "spirituous" liquids for the past 300 years. The history of alcohol consumption, along with codes limiting its consumption go back to 1700 B.C. There are four types of alcohol: methyl alcohol, ethyl alcohol, propyl alcohol and butyl alcohol. Ethyl Alcohol, or ethanol (C_2H_5OH), is the type used in the production of alcoholic beverages. According to the report of World Health Organization (WHO), 38% increase in alcohol consumption in India were observed (Indian Express, May 25, 2016). Alcoholism is one of the major problem, as alcohol acts through numerous molecular targets, its underlying mechanism has to be elucidated (Gerlai et al., 2008). Metabolism of ethanol to acetaldehyde produces oxidative stress and which lead to nervous dysfunction and dysmorphogenesis (Reynolds and Brein 1995). Oxidative stress which leads to increase cell death which is the underlying cause of ethanol induced nervous dysfunction (Reimer et al., 2006). The intellectual, behavioral and neuronal deficits are the most important symptoms of increased ethanol consumption. The main

target for ethanol in brain is hippocampus which plays a major role in learning and memory and in the regulation of behavior (Reynolds and Brein 1995). Chronic exposure of ethanol leads to neuronal adaptation which leads to tolerance and neurotoxicity (Dlugos and Rabin 2003).

In the present study, zebrafish has been taken as the animal model. Zebrafish is a small freshwater teleost species (Briggs 2002). Zebrafish has become the prominent vertebral model where genetic, developmental biology and physiological methods are easily applied (Nemtsas et al., 2010) and it is also an effective established model to investigate the mechanism of drug (Arenzana et al., 2006). Zebrafish models are also used to study anxiety and stress (Steenbergen et al., 2011).

Deficit in the behavioral patterns is associated with difficulty in learning and memory in both vertebrates and invertebrates (Gass et al., 1998). Zebrafish as an emerging vertebral model, its learning and memory was tested using the T-maze. Anxiety is a state of constant

fear caused by anticipation (Norton and Bally Cuif 2010). To assess anxiety, fishes were treated with ethanol in beakers for acute and in home tank for chronic effects. The relationship between anxiety and behavior has been well recognized and it is studied in zebrafish (Stewart *et al.*, 2011). The anxiety behavior of the fish is assessed by in novel tank and light/dark preference tank. Zebrafish is a diurnal species which prefers light to dark during the day in response to danger (Kalueff *et al.*, 2014). In the present study, zebrafish was exposed to a specific concentration of ethanol and the changes in its learning and memory were assessed. And also the anxiety behavioral changes were also assessed using novel tank and light/dark preference tank.

MATERIALS AND METHODS

Animals

A total of 50 adult zebrafishes of both sexes were obtained from the commercial supplier from Madurai. All fish were acclimatized for at least two weeks in the experimental room conditions by maintaining them at $28 \pm 2^\circ\text{C}$ tanks with constant aerated water. Fishes were kept in natural day and night cycle. The fishes were fed twice a day with the commercial fish feed. Zebrafish was divided into two groups: control group and ethanol group (25 fishes in each group). The fishes were approved by Institutional Animal Ethical Committee, University of Madras.

Ethanol Exposure

Adult zebrafish were exposed to water tank containing 1% ethanol for 60 min/day for 10 days. Concentrations of ethanol were chosen based on previously published data for zebrafish, as well as another cyprinid, the goldfish (Kalueff *et al.*, 2014, Dlugos and Rabin 2003).

Behavioral Protocol

1. Novel Tank

The test tank is trapezoid of about $24 \times 8 \times 20$ (length $\times$ width $\times$ height) with 15cm of water level. Fishes were tested individually during time period of 6 minutes by video recording. The back and sides of the tank were closed by sheets to avoid disturbance. The latency to reach the upper portion of the tank, number of entries to the upper portion of the tank, time spent in the upper portion of the tank, number of erratic movements, and freezing duration were recorded (Cachat *et al.*, 2010).

2. Light and Dark Preference Tank

The preference tank were 2L rectangular tank divided into three chambers. In the centre of the tank, there was a

start box that opened into both chambers. Both the light and dark sides are opened to the fish. The fish is tested individually for a time period of 15 minutes by video recording (Blaser and Penalosa 2011). Both the control group and the ethanol exposed were examined and the latency spent in dark and white portion of the tank were evaluated.

Memory Protocol

T Maze

Each zebrafish received sixteen sessions of exposure to food reward and punishment in the t-maze with the color sleeve. Each session consist of two trials, one with the right arm sealed and the one with the left arm sealed. On each trial, zebrafish was placed in the start box for about 2 minutes. The start box door was raised and lowered after the fish released from the start box. As soon as the fish reaches the blue side of the t maze the door was lowered and the food was delivered to the fish. After 30 seconds, the fish was netted and taken to the start box for the second trial. In the second trial, after the fish exit from the start box the blue side of the t-maze is lowered in order to avoid the re-entering of the fish into the blue side of the arm and the red side of the arm is punishment (Colwill *et al.*., 2005). On each trial the time taken for the fish to exit from the start box and reach the arm was recorded. Training was given for 16 days.

After the training period the zebrafish was divided into two groups: ethanol exposed group and the other control group.

RESULTS

All data were analyzed using SPSS (version 20.0). Data are expressed as bar diagram with Mean $\pm$ SD. Statistical significance between the different groups were determined by Independent Students 't' test. $p < 0.05$ was considered significant.

Anxiety Behavior

1. Novel Tank Behavior (Figure 1)

In the ethanol exposed group, zebrafishes exhibited a significant decrease ($p < 0.05$) in the number of entries to the upper portion of the tank and time spent in the upper portion when compared to the control group. In the ethanol exposed group, there was an increased latency ($p < 0.05$) to the upper portion of the tank when compared to the control group. In the ethanol exposed group, the fishes displayed a significant decrease ($p < 0.05$) in the freezing duration and a significant increase ($p < 0.05$) in erratic movements when compared to the control group.

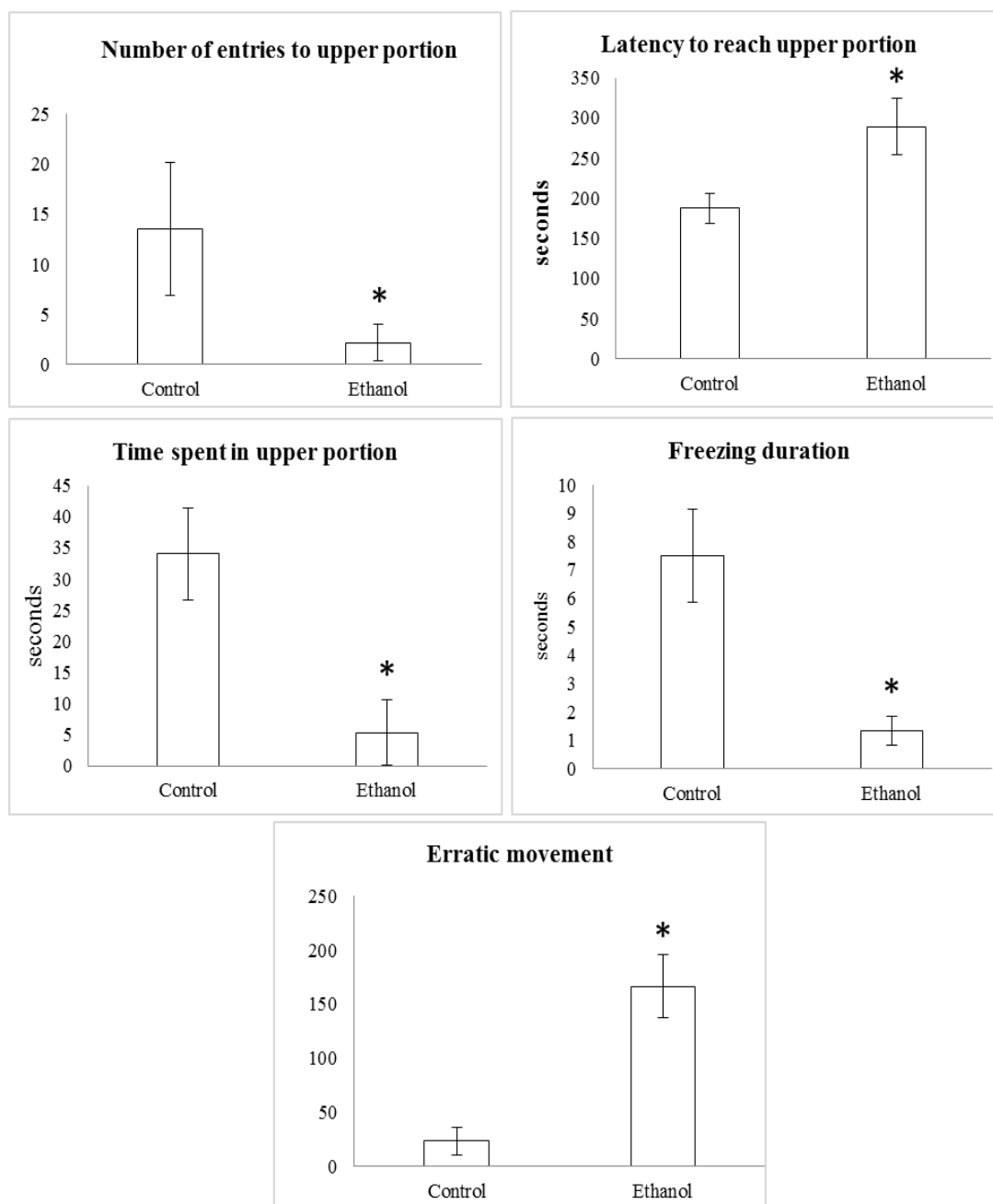


Figure. 1: Novel tank behavior. * indicates significance compared with control. $p < 0.05$ is considered significant.

2. Light and Dark Preference (Figure 2)

Ethanol exposed fishes presented a significant increase ($p < 0.05$) in the time spent in light and a significant decrease ($p < 0.05$) in the time spent in dark when

compared to the control group. In the ethanol exposed group there was a marked increase ($p < 0.05$) in the time spent at the start box when compared to the control group.

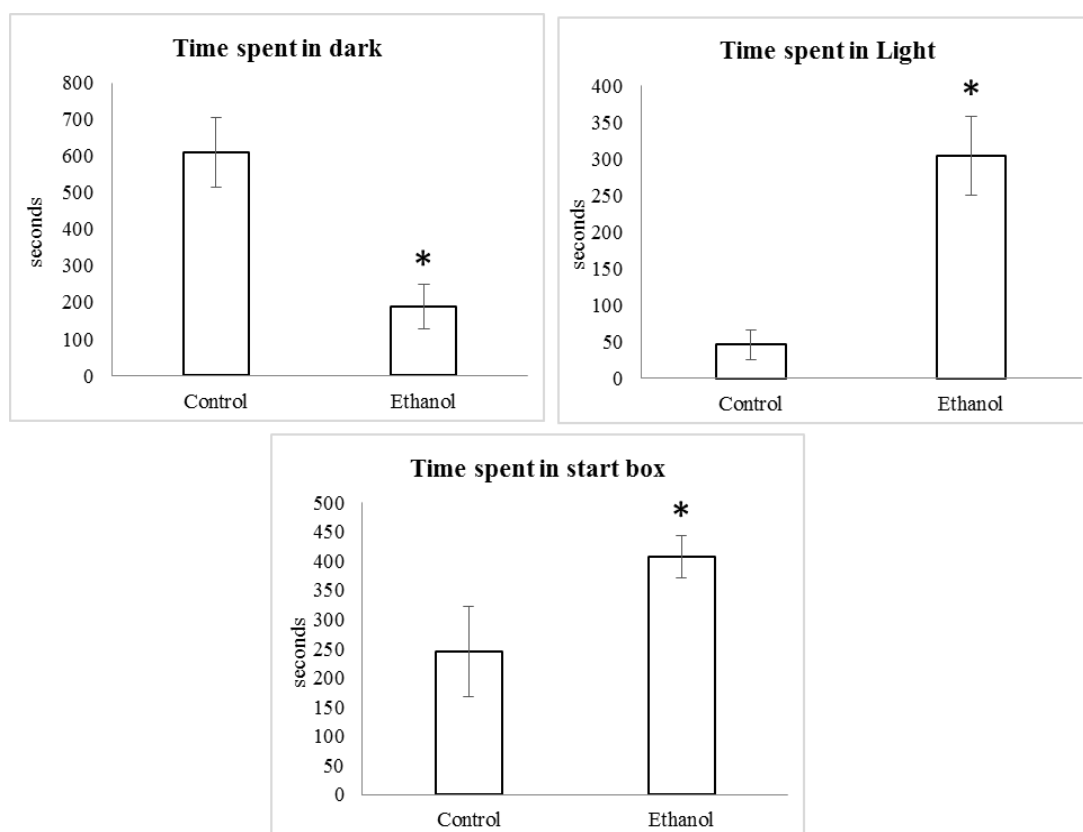


Figure. 2: Light and dark preference tank test. * indicates significance compared with control. $p < 0.05$ is considered significant.

Memory

T-Maze (Figure 3): The latency to exit the start box: there was no significant changes observed in day 1 and in day 5 in both the control and ethanol exposed group. In day 10, there was a significant change in the ethanol exposed group when compared to the control group. The latency to reach the goal box: there was a significant

change observed in day 1 in the ethanol exposed group when compared to the control group. In day 5, there was no significant changes observed in both the control and the ethanol exposed group. In day 10, there was a significant change in the ethanol exposed group when compared to the control group.

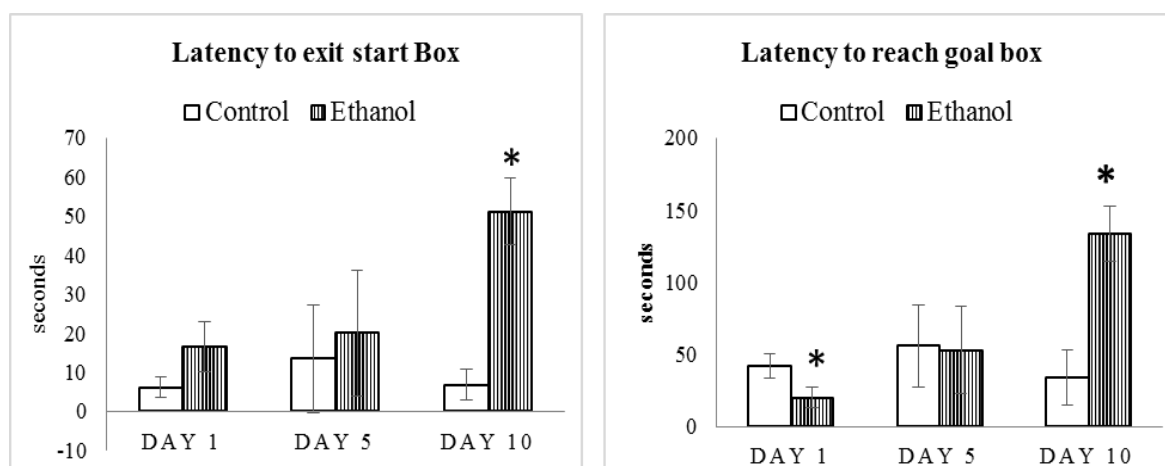


Figure. 3: T maze test * indicates significance compared with control. $p < 0.05$ is considered significant.

DISCUSSION

In the present study, the effect of ethanol toxicity is studied by the anxiety behavior and memory. Anxiety is a common neurobehavioral disorder (Stewart *et al.*, 2011). Anxiety behavior in zebrafish was evaluated by

the novel tank test and the light dark preference test. Zebrafish naturally lives on the surface of the water (Reynolds and Brein 1995), when there was chronic exposure to ethanol, behavioral alterations were observed. In novel tank behavior, significant changes

observed in zebrafish were due to stress or anxiety induced by ethanol intoxication. Zebrafish showed a longer latency to reach the upper portion of the tank, entries to the upper portion of the tank was decreased and there was decreased time spent in the upper portion of the tank whereas there was an elevated erratic movement and which reduces the freezing duration of the fish (Egan *et al.*, 2009). As there was ethanol exposure more erratic movement was observed in the fish in response to fear or anxiety (Gerlai *et al.*, 2008).

The light/dark preference test is another promising test to evaluate anxiety in the fish. In environmental discrimination of the zebrafish to the light and dark environment, Zebrafish naturally have a preference to stay in the dark environment rather than to stay in the light environment. This kind of environmental discrimination in the zebrafish is due to the aversion to danger (Serra *et al.*, 1999). When there was administration of ethanol to the fish, the fish choose to stay in the light environment. It suggests that alcohol was likely to affect central neural mechanism rather than their perception to the dark environment (Kalueff *et al.*, 2014). Zebrafish was tested individually in the preference tank, the individuals solely prefers the location where they appear because of the lack of movement or exploration of the tank which may lead to large amount of time spent in this location (start box). It was well seen in the ethanol exposed group (Steenbergen *et al.*, 2011).

In the present study, ethanol has impaired the learning and memory of zebrafish in T-maze. Ethanol exposure does not cause impairment in the initial acquisition of learning and memory but during the chronic exposure of ethanol causes impairment in the learning and memory. The main target of ethanol in brain is hippocampus which causes dysfunction of hippocampus which may have impaired the memory in the zebrafish in the present study. Chronic exposure of ethanol can also inhibit the learning and memory (Yang *et al.*, 2003, Reynolds and Brein 1995).

The toxic effect of ethanol is due to the formation of acetaldehyde which generates free radicals and causes DNA damage to cell. It has been previously reported that zebrafish has alcohol dehydrogenase enzyme which metabolize the alcohol and forms acetaldehyde (Dasmahapatra *et al.*, 2001). Ethanol induced alterations in anxiety and depression like behavior has been reported earlier in female Wistar albino rats (Stefka Valcheva-Kuzmanova *et al.*, 2013).

CONCLUSION

In the present study, zebrafishes revealed increased anxiety behavior and deficit in memory when they were exposed to ethanol. This may be due to the direct effect of ethanol on brain which affects the hippocampus in the brain.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCE

1. Arenzana, F.J., Carvan, M.J., Aijon, J., Sanchez-Gonzalez, R., Arevalo, R, Porteros, A. Teratogenic effects of ethanol exposure on zebrafish visual system development. *Neurotoxicology and Teratology*, 2006; 342–348.
2. Blaser, R.E., Penalosa, Y.M, Stimuli affecting zebrafish (*Danio rerio*) behavior in the light/dark preference test. *Physiology & Behavior*, 2011; 831–837.
3. Briggs, P.J, The zebrafish: a new model organism for integrative physiology. *Am J Physiol Regulatory Integrative Comp Physiol*. 2002; R3-R9.
4. Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Kyung Min Chung., Nadine Wu., Wong, k., Roy, S., Suciu, C., Goodspeed, J., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Tan, J., Denmark, A., Gilder, T., Kyzar, E., DiLeo, J., Frank, F., Chang, K., Utterback, E., Hart, P., Kallueff, A.V, Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nature Protocol*, 2010; 1786–1799.
5. Colwill, R.M., Raymond, M. P., Ferreira, L., Escudero, H, Visual discrimination learning in zebrafish (*Danio rerio*). *Behavioural Processes*, 2005; 19–31.
6. Dasmahapatra, A.K., Doucet, H.L., Bhattacharyya C., Carvan MJ 3rd, Developmental expression of alcohol dehydrogenase (ADH3) in zebrafish (*Danio rerio*). *Biochem Biophys Res Commun.*, 2001; 286(5): 1082-6.
7. Dlugos, A.C., A. Rabin, A. R, Ethanol effects on three strains of zebrafish: model system for genetic investigation. *Pharmacology Biochemistry and Behavior*, 2003; 471–480.
8. Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegant, M.F., Beeson, E., Elkhayat, S.I., Bartel, B.K., Tien, A.K., Tien, A.H., Mohnot, S., Beeson, E., Glasgow., Amri, H., Zukowska, Z., Kalueff, A.V, Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, 2009; 38–44.
9. Gass, P., Wolfer, D.P., Balschun, D., Rudolph, D., Frey, U., Hans-Peter Lipp., Schutz, G., Deficit in memory task of mice with CREB mutation depend on gene dosage. *Learning & Memory*, 1998; 274–288.
10. Robert Gerlai, Fahad Ahmad, and Sonal Prajapati, Differences in acute alcohol-induced behavioral responses among zebrafish populations. *Alcohol Clin Exp Res.*, 2008; 1763–1773.
11. Kalueff, A.V., Stewart, A.M., Gerlai, R., Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, 2014; 1–13.

12. Nemtsas, P., Wettwer, E., Christ, T., Weidinger, G., Ravens, U, Adult zebrafish heart as a model for human heart? An electrophysiological study. *Journal of Molecular and Cellular Cardiology*, 2010; 161–171.
13. Norton, W., Bally-Cuif, L, Adult zebrafish as a model organism for behavioral genetics. *BMC Neuroscience*, 2010; 1-11.
14. Reimers, J.M., La Du, K.J., Periera, B.C., Jack Giovanini., Tanguay, L.R, Ethanol-dependent toxicity in zebrafish is partially attenuated by antioxidants. *Neurotoxicology and Teratology*, 2006; 497–508.
15. Reynolds, D. and Brien, F, Ethanol neurobehavioural teratogenesis and the role of L-glutamate in the fetal hippocampus. *Can. J. Physiol. Pharmacol*, 1995; 73: 1209- 1223.
16. Serra, E.L., Medalha, C.C., Mattioli, R., Natural preference of zebrafish (*Danio rerio*) for a dark environment. *Brazilian Journal of Medical and Biological Research*, 1999; 1551-1553.
17. Steenbergen, P.J., Richardson, M.K., Champagne, L. D, Patterns of avoidance behaviours in the light/dark preference test in young juvenile zebrafish: A pharmacological study. *Behavioural Brain Research*, 2011; 15–25.
18. Stefkova Valcheva-Kuzmanova., Miroslav Eftimov., Krasimir Kuzmanov., An experimental model of alcohol-induced anxiety and depressive behaviour in rats. *Scripta Scientifica Medica.*, 2013; 48-52.
19. Stewart, A., Nadine Wu, Cachat, J., Hart, P., Gaikwad, S., Wong, K., Utterback, E., Gilde, T., Kyzar, E., Newman, A., Carlos, D., Chang, K., Hook, M., Rhymes, C., Caffery, M., Greenberg, M., Zadina, J., Kalueff, A.V, Pharmacological modulation of anxiety-like phenotype in adult zebrafish behavioral models. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 2011; 1421–1431.
20. Yang,S., Kim, W., Byung-Hee Choi, Hae-Young Koh and Chang-Joong Lee, Alcohol impairs learning of T-maze task but not active avoidance task in zebrafish. *Korean J Biol Sci.*, 2003; 303-307.