



IMPACT OF METHANOL TOXICITY ON STRESS AND SEXUAL BEHAVIORAL PATTERNS IN ZEBRAFISH

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ABSTRACT

Methanol is the simplest toxic alcohol similar to that of ethanol and it is highly toxic for consumption. It has been used as an adulterant in alcoholic beverages and its toxicity remains a common problem among lower socioeconomic classes. This study was conducted to analyze the effect of methanol intoxication on stress and sexual behavioral pattern in zebrafish. Sixty zebrafishes were divided into control group and methanol treated group. Methanol group fishes were subjected to 1% methanol exposure for about one hour/day for 10 days. The anxiety index of zebrafish was measured in novel and light and dark adaptation tank. In novel tank, the methanol treated group showed a significant decrease in latency to reach upper portion of the tank and time spent in the upper portion of the tank and showed a significant increase in the number of transitions to the upper portion of the tank, duration of erratic movement and freezing compared with the control group. In light and dark adaptation, methanol exposed fishes showed a significant increase in time spent in light and a significant decrease in time spent in dark. In male sexual behavior the duration of male chasing the female has no significant change between the two groups and number of times male hits the female showed significant decrease in methanol exposed group when compared with the control group. In female sexual behaviour latency period for egg laying has no significant change in methanol group when compared with the control group. Number of eggs laid showed significant decrease in methanol treated group when compared with control group. The study of novel tank test, light and dark adaptation showed an increased anxiogenicity in the methanol treatment group. Sexual behavior also showed significant alterations due to methanol toxicity.

KEYWORDS: Methanol Toxicity, Sexual Behavior, Zebrafish.

INTRODUCTION

According to WHO 30% of Indian population consumes alcohol regularly and 11% of them are heavy drinkers. Death due to alcohol consumption has reached to 3.3 million (WHO, 2014). In teen groups alcohol consumption has increased because of easy availability of liquor, access to alcohol at home, curiosity, peer pressure and its association with a certain kind of lifestyle have contributed to increased consumption of alcohol. The consumed alcohol, increases the blood alcohol concentration (BAC) and as a molecule it easily diffuses across the cell membranes and alters cell function by interacting with cellular protein functions and membranes. On chronic intake it causes slurred speech, motor impairment, confusion, memory problems, coma, breathing problems and death (National Institute of Alcohol Abuse and Alcoholism). Methanol is the simplest toxic alcohol, light, and volatile, colorless, flammable liquid with odour similar to that of ethanol (National Institute for Occupational Safety and Health, 2008). Methanol is available in the diet principally from

fresh fruits and vegetables, fruit juices, fermented beverages and diet foods and it is also a constituent of paints, shellacs, varnishes, paint thinners, cleansing solutions and antifreeze solutions (World Health Organisation, 1997). Methanol is highly toxic for consumption. Methanol when ingested gets converted to formaldehyde by alcoholic dehydrogenase and then converted to formate by aldehyde dehydrogenase (Mc Martin Kenneth et al., 1978). Previous studies have discussed that formate is toxic to central nervous system and may result in blindness, coma and death. The toxic formate inhibits cytochrome oxidase which in turn decreases ATP production (Nicholls, 1976). Cells experience high oxidative stress induced by reactive oxygen species (ROS) generated by methanol toxicity (Liesivuori and Savolainen 1991). The cell cannot withstand the increased ROS because of the reduction of antioxidants (Lien et al., 2008). Previous literature by Rajat Sandhir and Kamaljit Kaur has established the fact that acute methanol toxicity increased oxidative injury in brain that led to neurobehavioral deficits. As previously

described, oxidative injury related to methanol toxicity had been widely studied under neurological aspects. There are limited imprints on impact of methanol toxicity on gonadal dysfunction.

Therefore the purpose of the study is to establish a methanol toxicity model of zebrafish (*Danio rerio*). It has emerged as a vertebrate model well suited for studies under various aspects like basic developmental biology, toxicology and gene transfer. Human and zebrafish gene has about 86% genetic homology. It is also a model for verifying mechanisms in toxicity studies (Kimmel et al., 1995). Thus in the present study, behavioral variations induced by methanol toxicity were assessed from the tests like novel tank, light and dark adaptation and place preference. Methanol toxicity may be a vital causal factor of gonadal dysfunction and it is very scarcely understood. A female zebrafish can spawn every 2-3 days and lays 200 hundred of egg. As its fertilization is external, it is a model for embryo and larval studies where live embryos are easily accessible for research (Breder and Rosen, 1966). Spawning is based on photoperiod (Kieraten and William, 2004). In courtship behavior, male chases a female rapidly and it is active for 30 mins and continues to about an hour (Pyron, 2003). Some evidences prove the fact that female zebrafish prefer larger males (Eduardo, 2006). The above mentioned sexual behavioral patterns are well established to exhibit changes under various toxicity studies. This study is based on the methanol toxicity which induces behavioral changes especially sexual behavior in the zebrafish.

MATERIALS AND METHODS

Animals and housing

A total of 60 adult (both male and female), wild type strains of zebrafish were collected from a commercial aquarium. They were housed as control and methanol exposed groups, each group consisting of 30 fishes. They were maintained under normal room temperature (27°C) with aeration and at the pH of 7.0. All the fishes were fed with commercial pellets for about twice a day. Methanol treatment group fishes were kept separately and they were exposed to 1% methanol treatment (Caio, 2011) for one hour/day for a duration of about 10 days.

Behavior

Novel tank

Control and methanol treatment group fishes were tested in novel tank. A trapezoid shaped tank of about 24x8x20 (length x width x height) with 15cms of water level. The back and sides of the tank were closed in order to avoid disturbances. The tank rests on a level and it was divided into two equal portions marked on the outside wall. Individual fish from both the groups were tested for a time period of about 6mins. The following parameters like latency to reach upper portion of the tank; time spent in the upper portion of the tank; number of transitions to the upper portion of the tank; number of erratic movements and freezing duration were recorded.

Light and dark adaptation

The scototaxis (dark/light preference) protocol is a behavioral model for fish which is validated to assess the anxiety effect of methanol toxicity. A tank (30cmx15cmx15cm) is divided into one-half black and one-half white (Edward, 2017). Water column is about 10 cm. Each fish is dropped in the start box, which is placed at the center and a video for about 15 mins is recorded. The time spent in the light region and the time spent in the dark region were assessed.

Sexual Behavior

Male and female fishes in both methanol treated and control groups were kept separated overnight in the dark environment. Then, next day male and female fishes were transferred to the same tank in the ratio of 1:2 (male:female). Light was given as a stimulus for spawning (Breder and Rosen 1966). Male chases the female and its active for first 30 mins and then female lays eggs. This activity may last upto an hour (Kieraten and William 2004). Duration of male chasing the female; number of times the male hits the female; latency period for laying eggs and the number of eggs laid were monitored.

STATISTICAL ANALYSIS

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.

RESULTS AND DISCUSSION

Methanol toxicity produces increased stress and causes behavioral changes in the zebrafish. The following tests were done to assess the anxiety index in the methanol treated group. In this experiment, methanol treated fishes showed no significant change in the number of upper entries compared with control group (Fig. 1). In methanol treated group time spent in the upper region of the tank showed an increased significant change comparing the control group (Fig. 1). The next parameter studied is latency to reach the upper portion of the tank. In this, methanol group showed decreased significant change compared with the control group (Fig. 1). There was decreased significant change in the methanol group compared to control group in the freezing parameter (Fig. 1). Methanol treated group showed increased significant change in the erratic movements when compared with control group (Fig. 1) Novel tank test measures the anxiety level in the zebrafish. Methanol treated group shows significant changes in its behavior parameters when compared with the control group. From the above results, increased number of entries to the upper region of the tank may be due to the increased erratic movements shown by the methanol treated group. Methanol treated group spent less time in the upper region of the tank when compared with the control group. Previous literature has reported that increased nicotine administration increases the time spent in the

upper region of the tank because nicotine gives an anxiolytic effect (Edward, 2007). Previous literature on ethanol treatment showed increased freezing, erratic movements, leaping and slowing movements which was a measure of anxiety (Gerlai, 2000). Previous studies on methanol toxicity and its behavioral changes is very limited. Latency to reach upper portion of the tank in

methanol group seems to be decreased when compared to the control. This may be considered another index of anxiety. Increased erratic movement indicates stressed condition (Cachat, 1786). Similarly, increased erratic movements was observed in methanol treated group. There was no freezing found in the methanol group because of increased erratic movements.

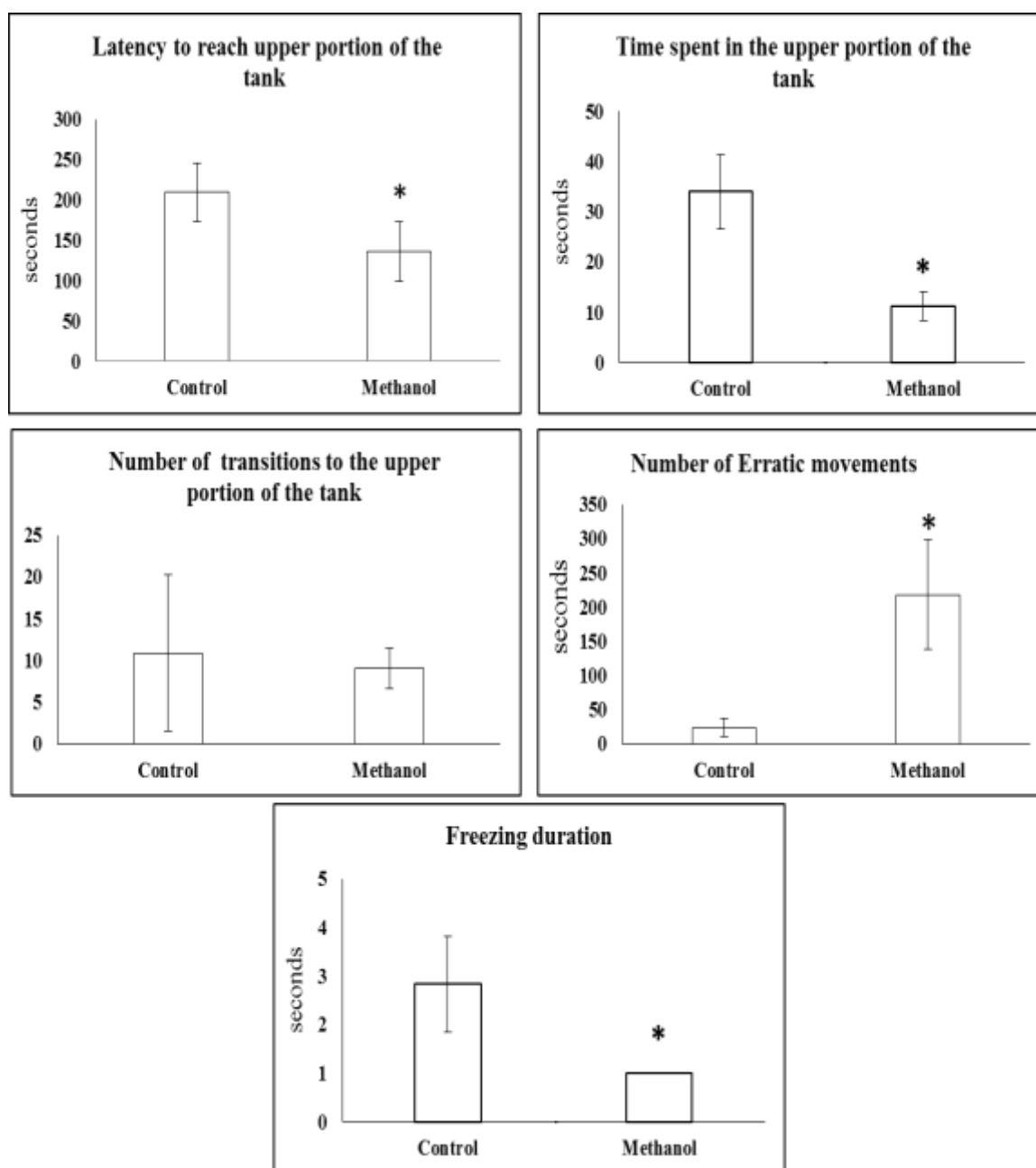


Figure 1: Novel tank task. * indicates significance compared with control. p value <0.05 is considered significant.

Time spent in the light region showed significant increase in the methanol treated group when compared to the control group (Fig. 2). Time spent in the dark showed decreased significance in the methanol group compared with the control group. Scototaxis is already studied as an anxiety index in rodents (Bourin and Hascoët 2003). Zebrafish prefers darker environment normally (Serra,

1999). From the above mentioned results, methanol treated group spent most of its time in the light and lesser time in the dark. This may be due to the fear and anxiety of the animal to stay in the dark environment. It may also be due to higher level of stress which made the animal to explore lesser.

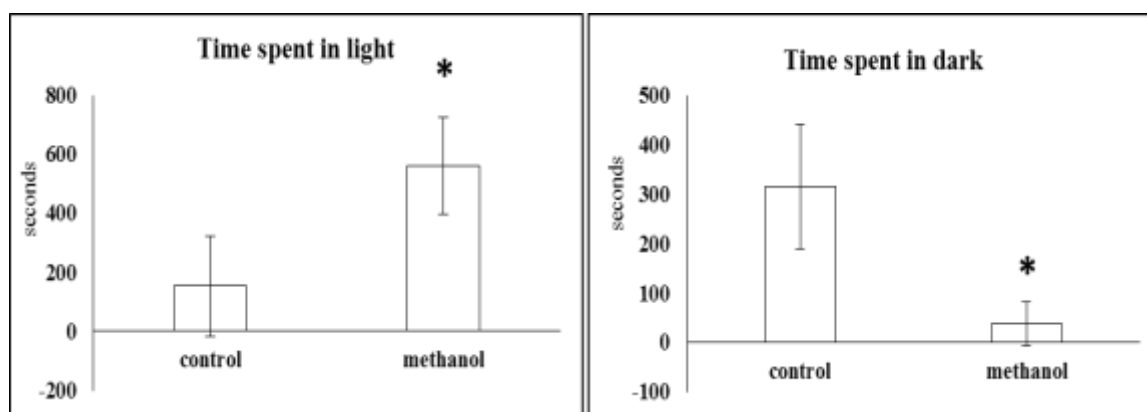


Figure 2: Light and Dark adaptation task. * indicates significance compared with control. p value <0.05 is considered significant

Methanol toxicity caused variations in the spawning of the zebrafish. In male sexual behavior, the duration of male chasing the female showed no significance in the methanol treated group (Fig. 3). The number of times male hitting female showed decreased significance in methanol treated males when compared to the control group. No significant change was observed in the latency period for laying eggs in methanol treated females. Number of eggs laid by the methanol female showed decreased significance when compared with the control group. Chronic exposure of zebrafish to methanol showed variations in the spawning episode of the zebrafish. In zebrafish spawning occurs for about an hour and the male is active for about first 20 mins and start chasing the female as soon as it releases its pheromone. Only very scarce study have been undergone in

variations in sexual behavior of methanol treated zebrafish. Previous studies on exposure to sex steroid hormones and prostaglandins showed variations in the sexual behavior in zebrafish (Ajay and Per-Erik 2015). Methanol exposed male zebrafish showed significant decrease in the chasing activity when compared to the control group. This may be due to release of formate in the gonadal cell which alters the male behavior. In previous literature, it is mentioned about the male dominance which might be a reason for the increase in the spawning success (Rowena, 2006). Number of hitting on the female showed decreased significance in methanol treated group. This may be because of the high stress, the male may not be very active in hitting the female as in the control group.

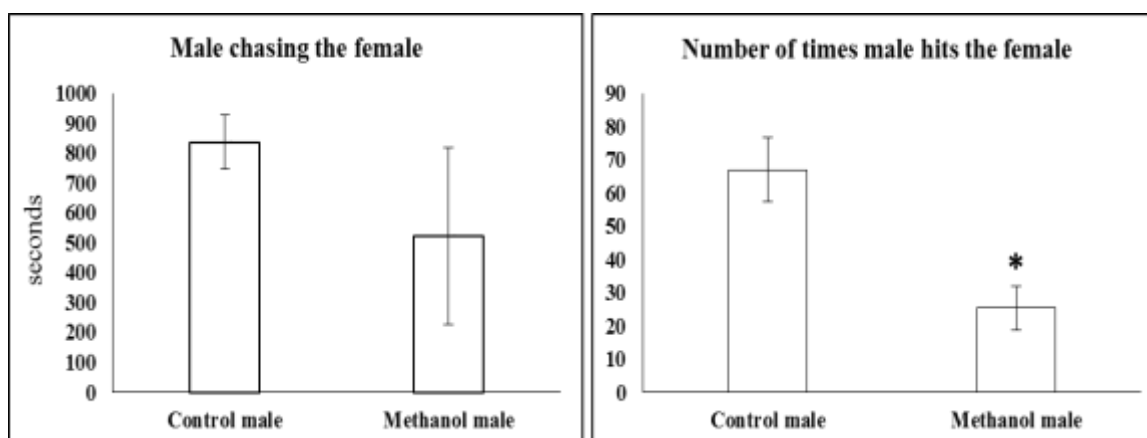


Figure 3: Male sexual behavior. *indicates significance compared with control. p value <0.05 is considered significant.

Every female zebrafish lays about 200 eggs per spawning. Female holds clutches with the male during the courtship behavior. This clutch holding may be affected in methanol administration. This may be due to that zebrafish may show lesser interest in courtship behavior and so the male circles the female and tries to clutch it. Methanol female shows no significant change in the latency period for egg laying (Fig. 4). A normal female lays egg in about 2 to 3 mins. The number of eggs

laid by the methanol female significantly decreased when compared with the control group. Previous research on sexual behavior in zebrafish showed avoidance when pheromones of other females are released (Delaney, 2002). Another literature explains that higher concentration of chemical cues from one female zebrafish showed avoidance behavior in the other female zebrafish (Golubev, 1984).

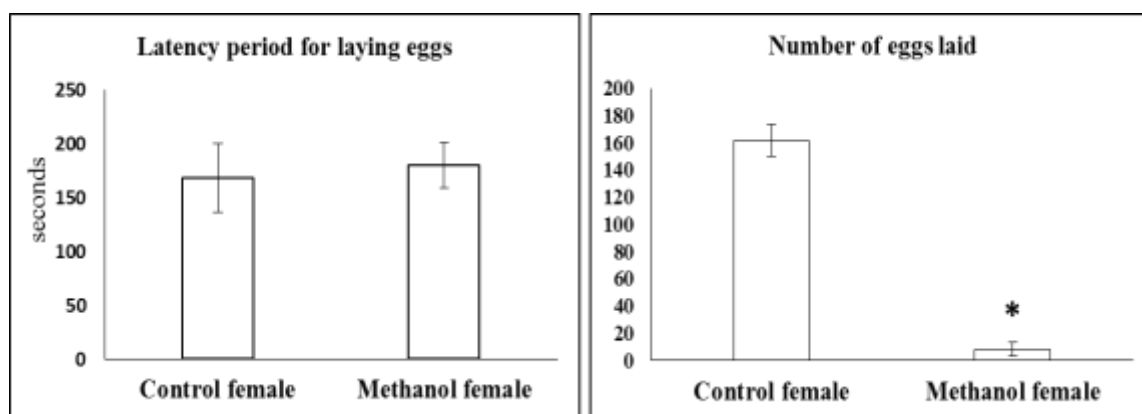


Figure 4: Female sexual behavior. *indicates significant compared with control. p value <0.05 is considered significant.

CONCLUSION

With the results of the present study, novel tank test, light and dark adaptation showed an increased anxiogenicity in the methanol treatment group fishes and they exhibited increased stressful behavior. Sexual behavior also showed significant alterations due to methanol toxicity. The main effect is on the egg laying which has been reduced drastically in methanol female fishes. So consumption of alcohol contaminated with methanol causes deleterious effects on the sexual behavior and gonadal functions and causes anxiety.

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