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REVIEW ARTICLE OF LIVER DAMAGE IN ZEBRA FISH INDUCED BY ALCOHOL

Tharunkumar. A. S[1], Vinothkumar. C[2]

[1,2]Department of Biotechnology, V.S.B. Engineering College, Karur, India.

ABSTRACT

The zebra fish has appeared as a suitable vertebrate model for examining liver related disorders. Liver impairment is a pivotal problem in the procedure of drug development. Zebra fish have demonstrated to be an essential tool in high throughput screening of drugs for hepatotoxicity. In spite of construction of zebra fish liver varies from mammals, the elementary physiological functions, genetic evolution and much resemblance. The major advantage of the zebra fish is the larval transparency which is helpful in hepatic studies by real time imaging. The bio conversion of drugs which includes oxidation, reduction has identical pathways as present in mammals due to the presence of cytochrome p450 enzymes. In the present review, the effects in the liver due to long term exposure to ethanol is evaluated.

KEYWORDS: Ethanol, Zebra Fish, Hepatic steatosis, liver damage.

1. INTRODUCTION

The zebra fish contains functional resemblance to 70% of normal genes 84% of diseased genes in mammals besides many physiologic and genetic compatibility[1]. The liver carryout many essential functions like production of bile, absorption of bilirubin, supporting blood clots storage of vitamins etc.[2] In many cases, congenital and acquired diseases arise by interference in liver functions. Zebra fish patient derived xenograft models are a better choice for rapid clinical interventions. The life cycle of zebra fish is very small and the reproduction rate is high.[3] The damage created because of ethanol consumption is a global problem. In the world, about 2 billion people intake ethanol in which 50% of the people are prone to alcoholic liver disease. [4]The different methods of ethanol induced liver injury were mentioned below.

2. METHODOLOGY

Wild type, Adult Zebra fish (Danio rerio) both male and females were purchased 2 weeks before the experiment for acclimate to laboratory facilities. Animal protocols were designed to reduce the discomfort for the fishes. Well aerated water was used to grow the fish in experimental tanks[5]. The temperature was maintained at 28^{o} C. The PH should be maintained at 6.8 - 7. The photo period cycle is 12/12 light/dark hours. The biochemical parameters like PH, acidity, alkalinity, chlorine, hardness, dissolved oxygen and biological oxygen demand and quality of water were monitored. The food was given twice a day. The fishes were separated in two groups such as ethanol group and control group[6]. For the ethanol group, ethanol of about 0.5% (v/v) was directly added into the fish tank. The water in the fish tanks were changed every 2 days. At 2^{nd} and 4^{th} week, fishes were killed by hypo thermal shock and the livers are removed and molecular and histological analysis were done.[7]

The transgenic strain of zebra fish larvae after 96 hpf (hours post fertilization) were incubated with 2% ethanol up to 32 hours at the temperature of 28^{o} C[8]. The fish will start to behave abnormally. Further, it is treated with 2% ethanol for 32 hours.

The adult zebra fish were purchased and maintained in optimal environmental conditions. Ethanol concentration of about 0.2% were added to the fish water directly. The water is changed regularly to avoid impurities. The fish should be treated with ethanol continuously for 4 weeks[2]. After 4 weeks, the fishes were euthanized and the liver is removed and analyzed.

In this method, transgenic zebra fish larvae (lfabp10a:eGFP) were used. Before the treatment with ethanol, the larvae were treated with quercetin at different concentrations (100µm, 50µm, 25µm) for 48 hours after 3 dpf (days post fertilization)[4]. Then the larvae were soaked in 350 mmol/L ethanol for up to 32 hours. After 32 hours, the fishes were treated with 1mM ATP for 30 minutes. After this, the liver is removed from the fish for examination[9].

3. HISTOLOGICAL ANALYSIS

The dissected livers of zebra fish at 2^{nd} and 4^{th} week were carefully stained with either eosin and hematoxylin or oil red[10]. By using 10% formalin solution, the livers were fixed and embedded in paraffin wax. They were sectioned into thin slices (5µm) and stained with hematoxylin and eosin[11]. These sectioned slices were treated with xylenol and optimum PH is maintained. Finally, the slices were assessed for fatty droplet accumulation[12].

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TABLE 1: Effects of ethanol caused in zebra fish

	% Of ETHANOL	TIME PERIOD	ZEBRA FISH TYPE	LIPID & FAT DEPOSITION IN LIVER	SEVERITY OF HEPATIC STEATOSIS
METHOD 1	0.5	4 weeks	Adult	Yes	High
METHOD 2	2	32 hours	Larvae	No	High
METHOD 3	0.2	4 weeks	Adult	Yes	High
METHOD 4	1.6	32 hours	Larvae	No	Very low

In method 1 and method 3, due to the continuous exposure of ethanol for 4 weeks, high amount of lipid and fat accumulation were identified in the liver. The severity of hepatic steatosis and hepatomegaly is also high[13].

In method 2, there will be no fat accumulation because of the acute exposure of ethanol. Even though it is acute, the severity of hepatic steatosis is high because the fish is in the larval condition[14].

In method 4, before treating the larvae with ethanol it is exposed to quercetin in different concentrations. In many in-vivo and in-vitro studies, it is proved that quercetin prevents hepatic injury caused by drugs and alcohol[15]. Because of the quercetin exposure to larvae, the severity of hepatic steatosis is very low compared to other methods.

4. CONCLUSION

Ethanol induced liver damage in zebrafish is a promising area of research that has already yielded valuable insights into the mechanisms of liver injury and the potential for therapeutic intervention. However, it is important to note that the zebrafish model is not perfect and may not fully reflect the human experience of liver injury due to alcohol consumption. For example, the rate of ethanol metabolism in zebrafish may be different from humans, and the model may not capture the complex interactions between liver injury and other organs, such as the brain and the gut. As our understanding of liver injury continues to grow, the zebrafish model is likely to play an increasingly important role in the discovery of new treatments and therapies for this debilitating condition.

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