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Effects of perchlorate (rocket fuel) on liver and gonad tissues of a freshwater fish *Rasbora dandia* (Valenciennes, 1844)

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ABSTRACT

Perchlorate (Rocket fuel) is an oxy-anion (ClO_4^-) and emerging persistent environmental contaminant reported to have different toxicity effects on plants and animals. Perchlorate (ClO_4) is an emerging pollutant which is widely reported from ground and surface waters near military sites and manufacturing units. The reported perchlorate toxicity studies have been confined to labs in experimental conditions on plants or animals. In the present study, we have identified a natural pond heavily contaminated with perchlorate. The toxicity study was initiated in the laboratory to understand the effect of environmentally relevant perchlorate on the predominant freshwater fish present in the pond. Histological observation of the liver of the fishes exposed at environmentally relevant concentrations (10, 12 and 14 mg/l) showed significant changes such as the pyknotic nucleus, necrosis, haemorrhage, cytoplasm, and vacuolar degeneration. The changes observed in liver tissue varied with increasing concentration. These results suggest that ClO_4^- is a hepatotoxic compound in *Rasbora dandia.* Histological observation of testes of the fishes exposed at higher concentrations (14 mg/L) showed multiple germinal epithelium layers, less spermatozoa and oedema. The present study was limited to only one species and may extend up to other aquatic life of an ecosystem

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1. Introduction

Environmental contamination of perchlorate (ClO_4^-) is becoming a serious health concern due to its widespread distribution in both ground and surface waters and its impairment of thyroid gland functioning (Isobe *et al.,* 2012). Perchlorate is a naturally occurring and man-made compound, whereas anthropogenic source are the leading cause of contamination of the aquatic ecosystem (Trumpolt *et al*., 2005; Morrison *et al*., 2006).

Perchlorate is highly soluble, mobile and very stable in water (ITRC, 2005). Due to high solubility and low reactivity, ClO_4^- is predicted to transport readily in surface water and through the soil to groundwater (Sparks, 1995). In an aqueous medium, perchlorate anion readily dissociates from its various cations and remains stable for a long period under normal environmental conditions (Urbasky, 1998). Perchlorate compounds are mainly found in solid salts at ambient temperatures and consist of white or clear crystals. Common salts of ClO_4^- are NH_4ClO_4, $NaClO_4$, $Ba(ClO_4)_2$, $CsClO_4$, $FClO_4$, $LiClO_4$, $Mg(ClO_4)_2$, $KClO_4$, $RbClO_4$ and $AgClO_4$. Due to its relatively low density, perchlorate does not form complexes with metals (Urbansky, 2002).

Most of the naturally occurring sources of perchlorate appear to be geographically limited to arid environments. It occurs naturally in low levels in arid regions, while high concentration was reported in northern Chile and West Texas (Dasgupta *et al.,* 2005) Perchlorate, known as rocket fuel is widely used in ammunition industries in many parts of the world. The major source of environmental contamination of perchlorate has been its manufacturing, storage, testing, disposal sites and military installations (Urbansky *et al*., 2002; Morrison *et al*., 2006; ATSDR, 2008). The U.S. Department of Defence (DoD) is the largest user of perchlorate as rocket propellant and ammunition. Perchlorate is commonly used as an oxidiser in solid propellants, munitions, fireworks, vehicle airbag initiators, matches and signal flares (ITRC,2005). Perchlorate contamination generated considerable concern for human health due to its endocrine disruption property. The main mechanism of perchlorate toxicity is associated with the thyroid gland and thyroid hormone production. The thyroid gland secretes two hormones, thyroxin (T_4) and triiodothyronine (T_3), iodine is a key component of both. Thyroxin (T_4) and triiodothyronine (T_3) regulate tissue growth, maturation and cell respiration.Thyroid tissues selectively concentrate iodide from blood. The molecule responsible for the transport of iodide into the thyroid is called sodium – iodide symporter (NIS) (Dohan *et al.,* 2003). Perchlorate act as a strong inhibitor of the sodium iodide symporter (NIS) and it reduces the production of thyroid hormones (Clark, 2000; Yu *et al*., 2002).

Wolff (1998) reported that perchlorate has a greater affinity (30-fold greater) to sodium – iodide symporter than Iodine. Besides thyroid the NIS appears in the mammary gland, salivary gland, gastric mucosa and placenta (Tazebay *et al*., 2000). Perchlorate in food and water is a primary pathway for human exposure (ATSDR, 2008). Perchlorate is absorbed through the gastrointestinal tract and has a 6 to 8 hours half-life in humans. Approximately 95% of perchlorate is excreted within 72 hrs through urine (Eichler, 1929). Perchlorate is mainly adsorbed through oral exposure and migrating from the stomach and intestines to the bloodstream (ATSDR, 2008).

Due to the solubility and persistent nature of perchlorate salts it is stable in the aquatic environment and aquatic organism are more susceptible to perchlorate (Flowers and Hunt, 2000; Morrison *et al.*, 2006). In fishes, perchlorate exposure is through the gills, integuments, and gastrointestinal tract (Theodoraki *et al*., 2006). Due to toxicological and potential effects of perchlorate, numerous states and agencies proposed enforceable standards and guidance levels for perchlorate. In 1998 perchlorate was added to the Contaminant Candidate List (CCL) for drinking water by USEPA (USEPA, 1998). In 2002 the USEPA published a reference dose (RfD) of perchlorate in drinking water level (DWEL) of approximately 1µg/L (USEPA, 2008). In 2005, U.S. EPA established an oral reference dose (RfD) of perchlorate was 0.0007 milligrams per kilogram body weight per day and a drinking water equivalent level (DWEL) of 24.5 µg/L. According to USEPA (2008), ClO_4^- is set as 15µg/L based on the reference dose recommended by the National Academy of Sciences (NAS).The current health advisory level for ClO_4^- based on the reference dose recommended by the National Academy of Sciences is 56µg/L (USEPA, 2019).

Among fishes perchlorate toxicity study was mainly carried out on Zebra fish (*Danio rerio*), Fathead minnows (*Pimephales promelas*), Eastern mosquito fish (*Gambusia holbrooki*), threespine stickle back (*Gasterosterus saculeatus)* and rare Chinese minnows (Bernhardt and Von Bernhardt *et al.,* 2011; Furin *et al*., 2015; Petersen *et al*.,2016). These studies reported that perchlorate causes morphological deformities (Mukhi *et al*., 2005; Mukhi and Patino., 2007; Bernhardt *et al*., 2011), reproductive abnormalities (Park *et al*., 2006; Bernhardt and Von Hippel., 2008), altered thyroid hormone production and disruption of thyroid follicles in fishes (Bradford *et al.,* 2005; Schmidt *et al*., 2012; Petersen *et al*., 2016).

Histological studies have been considered a tool for evaluating the toxic effect of fishes (Wester and Canton 1991, Schwaiger *et al*., 1992; Dutta, 1996). Histology can also detect health effect that is not readily discernable with gross visual inspection and provide early warning signals for secondary diseases (Couillard *et al*., 1988). Histopathological examination was widely recognised as a reliable method for disease diagnosis and for assessing the acute and chronic effects of toxicants at the cellular level in marine and fresh water species (Ferguson, 1989; Hinton *et al.,* 1992). Pathological alternations found in fishes are the net result of physical and biochemical changes in an organism (Schlacher *et al*., 2007). The pathological changes in histological structure can impair the function of organs or tissue of fish (Couch and Fournie., 1993).

Among perchlorate-induced toxicity studies, histological analysis was mainly focused on the thyroid gland of fish (York *et al*., 2001; Goleman *et al*., 2002; Mukhi and Patino., 2007; Furin *et al.,*2015). These previous studies reported that ClO_4^- causes hypertrophy, colloid depletion, angiogenesis, hyperplasia in thyroid gland of Zebrafish (Mukhi and Patino. 2007; Patino *et al*., 2003; Liu *et al*., 2008). Similarly, another study on three spine sticklebacks reported that ClO_4^- exposed fishes showed histomorphological changes such as follicle proliferation, reduced follicle area, colloid depletion and hypertrophy in thyroid tissue (Gardell *et al*., 2016).

In earlier reports, most of the histological studies were carried out only in the thyroid gland of the perchlorate-treated fishes. At the same time, only a few reports are available on the histological changes in liver and gonads of fish due to ClO_4^- toxicity. For example, studies conducted on Zebra fish (*Danio rerio*) exposed to perchlorate, showed delayed spermatogenesis and reduced fecundity (Mukhi *et al.,* 2007; Sharma and Patino, 2013). Similarly, perchlorate reduced primodial germ cell number, decreased oocyte maturation, disruption of reproductive behaviour, and induced hermaphroditism in threespine sticklebacks (Bernhardt *et al*., 2006; Petersen *et al*., 2014; Petersen *et al.,* 2016). The present study was mainly focused on evaluating the effect of environmentally relevant concentrations of perchlorate in the liver and gonads of fish (*Rasbora dandia)* and to finding out the histological changes in tissues due to perchlorate toxicity. Hence, the present study was mainly focused on the histological changes in gonads and the liver of the perchlorate- treated fishes.

2. Materials and Methods

2.1 Test organism

The fish selected for toxicity study was *Rasbora dandia* (Valenciennes, 1844). It is a fresh -water fish that occurs in different habitats such as ditches, ponds, canals, streams and rivers and in inundated fields. *Rasbora dandia was* selected for the toxicity study since it is commonly found in the freshwater ecosystem, has easy availability and is the dominant species present in the contaminated-perchlorate pond (Divya and Benno periera., 2020).

2.2 Collection and maintenance of test organism

Healthy and adult *Rasbora dandia* were collected from a local fish collector for toxicity studies. The fish were transported to the lab in live condition with care to avoid mechanical injury. They were kept in an aquarium tank with a 2000 L capacity filled with de- chlorinated tap water and provided continuous aeration. Prior to the experiment, fish were acclimatised to laboratory conditions for two weeks. During the acclimatisation period, fish were fed with commercial fish food (Aqua mix pellet) twice daily. The physico - chemical parameters of the water were monitored daily using a standard procedure (APHA, 2005) and maintained constant conditions during the acclimatisation period. The acclimatization was done at room temperature. Fishes free of any deformities, diseases or lesions and showed good health conditions were selected for the experiments.

2.3 Chronic exposure study

For the preparation of stock solution, potassium perchlorate ($KCLO_4^-$) purchased from Sigma Aldrich was used. It is a white coloured crystal with molecular weight 138.55 g/mol. A stock solution (10000mg/L) was prepared with potassium perchlorate as per the standard procedure (APHA, 2005) prior to the experiment. Test solutions were made by diluting the stock solution to produce the desired perchlorate concentration for each treatment.

Long term exposure studies (Chronic exposure study) were carried out in five rectangular glass aquaria with 500 L capacity filled with 300 L ClO_4^- solution. The aquaria were labelled from T1 T2, T3, T4 and T5 and filled with different concentrations of ClO_4^- solution (6, 8, 10, 12 and 14 mg/L) respectively along with a control. The concentration selected for the toxicity study has been based on the average perchlorate concentration (6 to 14 mg/L) reported from the contaminated pond (study site). Healthy adult fishes, irrespective of sex, having uniform size were randomly divided into 6 groups (6.1 ± 2.4 cm of total length,4.2±1.6 g of weight) and introduced into each aquarium (30 fishes per aquarium). Fishes were fed with commercial food (Aqua mix pellet) daily during the experiment period. During the experimental period 75 % of the perchlorate solution was replaced with fresh solution twice a week for 90 days. A control aquarium was kept (500L capacity) under the same condition without perchlorate. The experiment was conducted in triplicate for both (test and control). During the experiment at regular intervals (two weeks) water samples were collected from each aquarium for perchlorate and physico chemical analysis. The total exposure period (duration of the experiment) was 90 days. At the end of the experiment, fish from each aquarium were collected. Blood samples and tissues from gills, liver and muscles from six groups of fish were carefully collected and used for hormonal, enzymatic, biochemical and histopathological studies.

After 90 days of ClO_4^-exposure, five fish from each tank (experimental and control) were collected and sacrificed. For histological studies, the liver and gonads (testes and ovary) were dissected out from each fish through a trans-spinal dissection and gently washed with normal saline solution (0.9% NaCl) to remove the blood and adhering debris. The tissues were immediately immersed in a fixative (Glacial acetic acid, formaldehyde and ethanol- 1: 3: 7) for 48 hrs. Then the fixed tissues were dehydrated through a graded alcohol series (70 to 100%) and finally cleared with chloroform. Then the tissues were embedded in paraffin wax (58°C). After embedding the blocks were subjected to sectioning at 5µ thickness by using a rotary microtome (Leica Rotary model). The sections were stained with Harris hematoxylin and eosin stains. The stained slides were treated with xylene and mounted with DPX. These slides were photographed under a light microscope attached to a digital camera (Olympus, Japan).

3. Results and Discussion

During the experiment perchlorate and physico chemical analysis of water samples collected from each aquarium were reported in Table 1 and Table 2. The histological changes observed in the liver and testes due to perchlorate exposure were illustrated in Fig 1 and Fig 2. The histological observation of the ovary does not show any remarkable changes in exposed fishes compared to the control group. In comparison, several changes were observed in the testes and liver of the ClO_4^-exposed fish compared to control. The following observations were found in ClO_4^- exposed - fish compared to control.

3.1 Histological Changes in Liver

The histological examination of the liver of the control fish does not show any pathological changes. Hepatocytes are radially arranged around central sinusoids and hepatic sinusoids are lined with endothelial cells. The hepatocytes are polygonal in shape with a spherical and centrally placed nucleus and contain homogenous cytoplasm. Hepatopancreas was present and consisted of a central portal vein. Histological observation of liver of the fishes exposed to lower concentration of ClO_4^- (6, 8 mg/L) does not show any significant changes compared to control.

However, fishes exposed at higher concentration (10, 12 and 14 mg/L) showed significant changes such as the pyknotic nucleus, necrosis, hemorrhage, cytoplasmic and vacuolar degeneration. The changes observed in liver tissue were varied with increasing ClO_4^- concentration.

Liver tissue of the fishes exposed at 10mg/L perchlorate showed hepatocytes with cytoplasmic degeneration and mild nuclear pyknosis. Some of the hepatocytes became swollen and degenerated cytoplasmic vacuoles were present. Hepatocytes contain amorphous and granular cytoplasm. While the fishes exposed at 12 mg/L of perchlorate concentration showed hepatocytes with moderate nuclear pyknosis, cellular necrosis and appearance of haemorrhage. However, liver of the fish exposed at the highest concentration (14 mg/L) showed intensive haemorrhage. Moreover, the hepatocytes with severe cell degeneration and cellular necrosis were prominently observed in fishes exposed to the highest concentration (14 mg/L).

The liver is a highly sensitive organ to environmental contaminant and the significant changes present in the liver were considered for the evaluation of the health status of fish (Myers *et al.,* 1992; Thophon *et al*., 2003). The liver is one of the most important organs affected by pollutants present in water, and liver metabolism is a potential target for the toxic action of chemicals (Rodrigues and Fanta, 1998; Hinton *et al*., 2001). Due to its position, function and blood supply the liver is a major organ for detoxification and biotransformation processes (Vander Oost *et al*., 2003). The monitorisation of histological changes in the fish liver is a compassionate way to assess the effect of the xenobiotic compound in field and experimental studies (Figueire-do- fernandes *et al*., 2007). The histopathological abnormalities observed in *Rasbora dandia* exposed to perchlorate revealed that the environmentally relevant high concentration of perchlorate could impair the normal liver function.

Only one previous report is available on the effect of perchlorate on the liver tissue of a fish. A study conducted by Brucu *et al* (2009) reported that, molly fish (*Poecilia sphenops*) were exposed to perchlorate at different concentrations (1, 5, 25 and 125 mg/L) for 10 days showed remarkable changes in the liver such as steatosis, fibrosis, hyperemia and necrosis. However, the most distinctive changes such as hepatocellular breakdown, nuclear polymorphism, hyperemia and necrosis were only exhibited in fishes exposed at higher concentrations (25 and 125 mg/L). Similarly in the present study distinctive changes were observed in fishes exposed to perchlorate at high concentrations (10 mg/L, 12 mg/L, and 14 mg/L). In previous report, intensive steatosis, hepatic vacuolisation and fibrosis were observed in fishes exposed at lower concentrations (1 and 5 mg/L) (Brucu *et al*., 2009). The present study observed no remarkable changes at lower concentrations (6 and 8 mg/L).

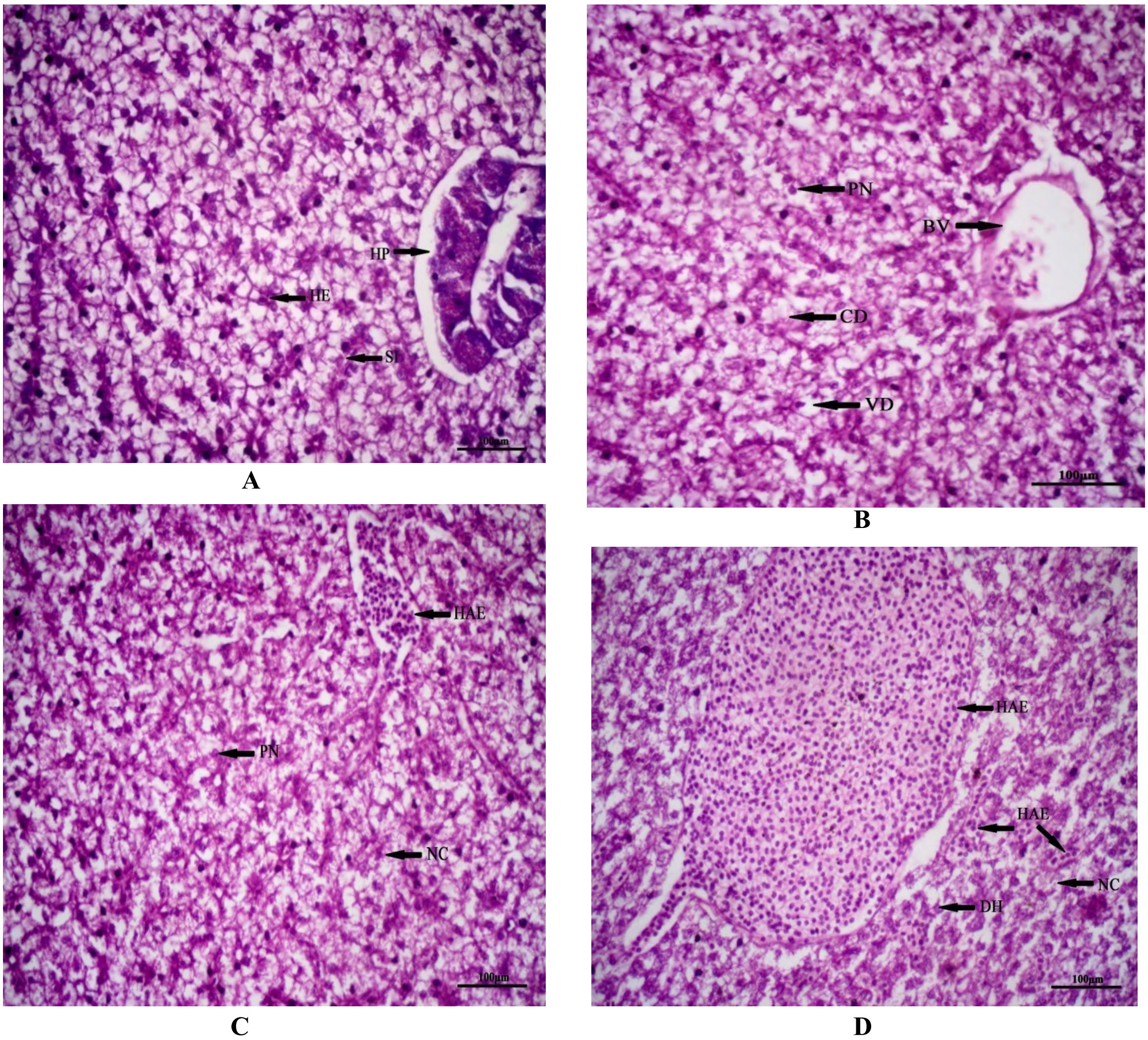
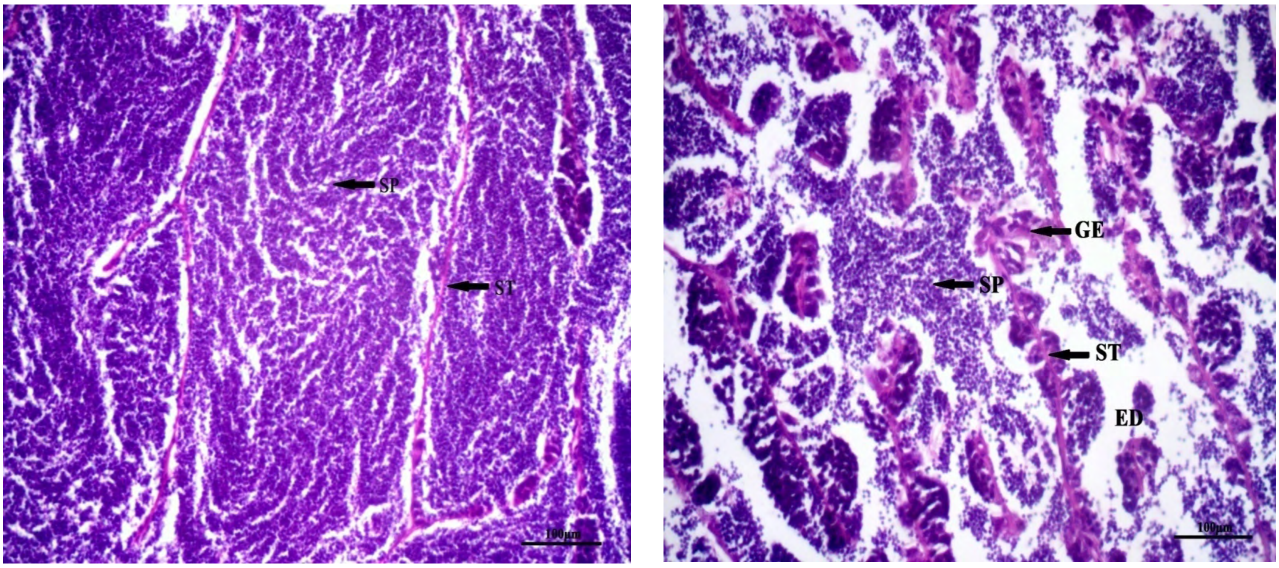


Fig. 1. Histology of the liver of *Rasbora dandia* exposed to different concentrations of perchlorate.

(A). Liver of *Rasbora dandia* treated as control for 90 days showing normal Haepatopancreas (HP), Hapatocytes(HE) and Sinusoids (SI). **(B).** Liver of *Rasbora dandia* exposed to 10 mg/L perchlorate for 90 days showing blood vessel (BV), Pyknotic nucleus (PN), Cytoplasmic degeneration (CD) and Vacuolar degeneration (VD). **(C)**. Liver of *Rasbora dandia* exposed to 12 mg/L perchlorate for 90 days showing Pyknotic nucleus (PN), Hemorrhage (HAE) and Necrosis (NC) **(D).** Liver of *Rasbora dandia* exposed to 14 mg/L perchlorate for 90 days showing Hemorrhage (HAE), Necrosis (NC) and degenerated hepatic cells (DH). Magnification 40x, Scale bar -100µm).

In the present study, the duration of the experiment was 90 days, and the concentration used for the toxicity study was the average concentration reported from the study site. The histological changes observed in the liver of *Rasbora dandia* were not only associated with the concentration of the perchlorate but also with the duration of exposure. The present study reveals that hepatic cells of *Rasbora dandia* were damaged due to chronic exposure to perchlorate, and this result is consistent with the previous study.

In the present study, pyknotic nucleus, cytoplasmic and vacuolar degeneration, necrosis and haemorrhage were observed in liver tissue of *Rasbora dandia* exposed to perchlorate. Similar observations were reported from various studies in fish due to different toxicants. A study conducted in *Clarius gariepinus* exposed to fenvalerate showed cytoplasmic vacuolisation of hepatocytes, necrosis and blood vessel congestion (Sakr and Jamal Al Lail., 2005). Das and Mukherjee (2000) reported that necrosis, and swellings of hepatocytes were observed in the liver of *Labeo rohita* exposed to hexachlorocyclohexane. Similarly, a study conducted in *Tilapia mossambica* reported that degeneration of hepatocytes, rupture of blood vessels and appearance of blood cells among hepatocytes and pyknotic nucleus were present in fenvalerate exposed fishes. A study in a fresh - water fish *Garra mullya* (Sykes) reported that fish exposed to sub lethal concentrations of Dimethoate showed different histopathological changes such as vacuolisation, necrosis, fibrosis, nuclear pyknosis and degeneration of hepatocytes leading to tumour (Borane., 2016). Hepatocytes swelling, Pyknotic nuclei and lipid vacuoles were also observed in *Oreochromis niloticus* exposed to Alachlor (Peebua et al., 2008). Loganathan et al., 2006 reported that *Labeo rohita* were exposed to Zinc metal (5 and 10 mg/ L) showed severe necrosis, haemorrhage, pyknotic nuclei and vacuolation. Pal et al., 2012 reported that *Cyprinus carpio* exposed to chlorpyrifos (1 and 100 µ/l) for 14 days showed pyknotic nucleus, cytoplasmic vacuolation, necrosis, and nuclear degeneration in hepatic tissue. A study conducted on rainbow trout (*Oncorhynchus mykis*) exposed to oxytetracycline showed haemorrhage, an increase in



A B

Fig. 2. Histology of the Testes of *Rasbora dandia* exposed to different concentrations of perchlorate.

(A). Testes of *Rasbora dandia* treated as control for 90 days showing seminiferous tubule (ST) with normal spermatozoa (SP) (**B**) Testes of *Rasbora dandia* exposed to perchlorate for 90 days showed seminiferous tubules (ST) contain less number of spermatozoa (SP), multiple layers of germinal epithelium (GE) and Edema (ED). Magnification 40x, Scale bar -100µm.

sinusoidal space, pyknotic nucleus, vacuolisation and hepatocellular degeneration in the liver (Rodrigues et al., 2017).

3.2 Histological Changes in Gonads

The histological observation of the gonads (testes and ovary) revealed that fish exposed at lower concentrations (6mg/L to 12 mg/L) showed no significant changes. While fishes were exposed to high concentrations (14 mg/L), remarkable changes were observed in the testes. The histological observation of the testes of the control fish showed that testes enclose seminiferous tubules, lined with a single layer of germinal epithelium. Interstitial cells and connective tissues are present in between the seminiferous tubules.The seminiferous tubules consist of primordial germ cells, spermatocytes, spermatids and abundant spermatozoa.

Testes of the fishes exposed at the highest concentration (14 mg/L) showed multiple layers of germinal epithelium. In the exposed fishes, the spermatogonia were not differentiated into spermatozoa. The lumens of the seminiferous tubules contain less number of spermatozoa. This may be due to the maturation arrest of spermatids due to perchlorate toxicity, leading to the total arrest of spermatogenesis. This permanent testicular damage and reduction of spermatids may lead to delayed spermatogenesis. In addition, testicular inflammation was observed in treated fish.

This study elucidates that prolonged exposure to perchlorate at the environmentally relevant concentration (14mg/L) may leads to testicular damage in *Rasbora dandia*. Previous studies reported that perchlorate causes different developmental and reproductive abnormalities in fishes. Bernhardt et al. 2006 reported that three spine stickle back was chronically exposed to perchlorate (100mg/L) and showed hypertrophy and ovotestis formation. Mukhi and Patino (2007) reported that prolonged exposure to ClO_4^- at high concentrations (10 and 100mg/L) suppressed fecundity in Zebrafish. Furin et al. (2015) reported that ClO_4 exposed three spine stickle-back showed delayed gonadal maturity. These previous reports were consistent with the findings of the present study.

Sharma and Patino, (2013) reported that spermatogenesis was delayed in Zebra-fish treated with goitrogens. A recent study reported that ClO_4^- exposure (100mg/L) at high concentration reduce primordial germ cell number in female threespine stickleback (Petersen *et al.,* 2016). In the present study perchlorate showed an inhibitory effect of spermatogenesis on testes. However, there are some reports available on the stimulatory effect of perchlorate on

Table 1. Perchlorate concentration (mg/L) measured from water samples collected from the experiment tank during the exposure period**.**

SI. NO	Nominal concentration (mg/L)	Actual concentration (mean ± S.D) (mg/L)		
		30 days	60 days	90 days
1	6	5.8 ± 0.78	5.2 ± 0.24	4.6 ± 0.51
2	8	7.3 ± 0.65	6.9 ± 0.64	6.2 ± 0.45
3	10	9.4 ± 0.32	8.8 ± 0.28	7.8 ± 0.62
4	12	11.08 ± 0.45	10.22 ± 0.48	9.92 ± 0.52
5	14	13.6 ± 0.14	12.91±0.69	11.02 ± 0.49

Table 2. Physico chemical parameters measured from water samples collected from the experiment tank during the exposure period.

SI .NO	Parameters	mean ± S. D
1	Temperature (°C)	27.6 ± 1.3
2	PH	7.3 ± 0.4
3	Dissolved oxygen(mg/L)	4.63 ± 0.38
4	Free CO_2 (mg/L)	1.24 ± 0.12
5	Total alkalinity (mg/L)	5.92 ± 1.4
6	Total hardness (mg/L)	24 ± 1.8

gametogenesis in fishes (Bernhardt *et al*., 2006; Petersen *et al*., 2015). In this study perchlorate does not shows any stimulatory or inhibitory effect on ovary of *Rasbora dandia*. This report is consistent with other studies in Zebra fish. Patino et al. 2003 reported that an environmentally relevant concentration of perchlorate (18mg/L) does not affect egg volume and rate of fertilisation in Zebrafish *(Danio rerio)*. Similarly, this study also suggests that high concentration of ClO_4^- (677 mg/L) suppressed spawning activity in Zebrafish. Previous studies reported a negative correlation between the reproductive effect and thyroid hormone (Cyr and Eales, 1988). Another study reported that thyroid hormones affect the secretion of reproductive hormones (Cyr and Eales, 1996; Parhar et al., 2000). The present study has significantly varied thyroid hormone due to perchlorate exposure. This may be the probable reason for the impairment of spermatogenesis in *Rasbora dandia.*

Testicular inflammation is one of the common responses in aquatic animals exposed to environmental toxicants (Sokal *et al*., 1985; Ruby *et al*., 1987). A study in *Heteropneustes fossilis* reported that fishes exposed to alkyl benzene sulphonate showed damage of the germinal epithelium, inflammatory response and reproductive impairment leading to delayed gonadal maturity (Ruby *et al*., 1986). Another study in *Clarius batrachus* exposed to chromium showed deformation of seminiferous tubules, disorganisation of spermatogonia, spermatocytes and spermatids with cytoplasmic vacuolization and nuclear pyknosis (Johnson *et al*., 2016).

4. Conclusion

This study concluded that prolonged perchlorate exposure at environmentally relevant concentrations could leads to hepatic and reproductive damage in *Rasbora dandia*. This study obviously suggests that perchlorate is a hepatotoxic compound in *Rasbora dandia,* and it affects fish spermatogenesis. Therefore, the study highlights that perchlorate contamination in a natural ecosystem is harmful to the fish population and may be adversely affected in the in natural ecosystem.

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