

Study Evaluating the Toxicity of Arsenic in Biochemical, Hematological and Histological Parameters of *Etroplus suratensis* (Bloch, 1790), on Exposure to Various Concentrations Found in the Coastal Waters in Chennai, India

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ABSTRACT

Arsenic contamination in the aquatic environment is a worldwide concern due to its ubiquity and potential health effects on humans. Taiwan, Chile, Mexico, China, Bangladesh, India, and Argentina are considered hotspots for arsenic contamination. A large number of people in India, Bangladesh, and China are affected by groundwater poisoning and arsenic-contaminated food. Arsenic pollution in marine environments affects the environment and the local population. Fish are regarded as highly vulnerable to arsenic toxicity as they are constantly exposed to it through their gills and consume arsenic-contaminated food. Arsenic, once accumulated, may have deleterious effects on the behavioural, physiological, and structural composition of organisms. Consumption of arsenic-contaminated fish in humans may affect the skin, gastrointestinal system, and nervous system and cause anaemia, skin cancers, and liver diseases. The US Food and Drug Administration has demonstrated that marine fish and other seafood contribute to 90% of the total exposure to humans through ingestion. This study is focused on evaluating the toxicity of arsenic on biochemical, haematological, and histological parameters of *Etroplus suratensis*. In this study, sodium arsenate displayed a decline in the levels of Hb, RBC, MCV, glucose, and triglycerides and increased production of WBC, MCH, and MCHC, protein, and cholesterol in the treated groups. As it has negative impacts on the liver, gonads, and gills of the fish as toxicity increases with its increasing concentration. It is also noted that as rapidly accumulates in all tissues exposed to sodium arsenate. From this study, it is clear that as has deleterious effects on biochemical, histopathological, and hematological characteristics. More studies have to be done on risk assessment and its control in the marine environment.

Key words: Arsenic, Biochemical, Histopathological, and Haematological, Atomic absorption spectrophotometer

Introduction

Globally, approximately, 300 million people are affected by Arsenic contamination. Arsenic contamination in the aquatic environment is a worldwide concern due to its ubiquity and potential health ef-

fects on humans (Kumari *et al*, 2016). Taiwan, Chile, Mexico, China, Bangladesh, India, and Argentina are considered hotspots for Arsenic contamination (Murcott, 2012; Kumar *et al.*, 2021). Arsenic is formed by natural processes such as volcanic eruption weathering, arsenic-containing rocks, and cer-

tain biological activities, as well as anthropogenic processes such as mining, electroplating, smelting and use of fertilizers and pesticides in aquatic environments (Williams *et al.*, 2009; Zhang *et al.*, 2022). Sources of arsenic contamination are categorized as Anthropogenic, Geogenic, Volcanogenic, Coal and Petroleum-related (Kumar *et al.*, 2021). Arsenic compounds exist in the environment as organic arsenic compounds, inorganic arsenic compounds, and arsenic gas. Organic arsenic compounds include arsanilic acid, methylarsonate (MMA), dimethylarsinate (DMA), tetramethyl arsine (TMA), Trimethylarsine oxide (TMAO), arsenocholine (AsC), arsenobetaine (AsB), thiolated arsenic, arsenosugars (As-Sug) and arsenolipids. Inorganic arsenic species include arsenite (As III); arsenic trioxide, sodium arsenite and arsenic trichloride and arsenate (As V); arsenic pentoxide, arsenic acid, lead arsenate and calcium arsenate. Arsenic (III and V) is considered as group 1 carcinogen (WHO, 2000, Zhang *et al.*, 2022). They are the dominant forms of inorganic arsenic in marine ecosystems and one of the most hazardous substances released in the aquatic environment (ATSDR, 2002).

Arsenic contamination in food and water is reported as a global concern. Large number of people in India, Bangladesh and China are affected by groundwater poisoning and arsenic contaminated coal used for heating homes and drying foodstuffs etc. Though at low concentration upon long term exposure arsenic causes several diseases, at high concentration it is lethal to the organisms (Chowdhury *et al.*, 1999; Hall, 2002; Hughes, 2002). Arsenic enters into an organism through water, food, and air. Inhaled arsenic is deposited in the respiratory tract and gets absorbed into the bloodstream. Intake of well water with high concentration of Arsenic and consumption of marine food and arsenic pesticides treated fruits and vegetables also cause heavy metal accumulation in the body.

The US Food and Drug Administration has demonstrated that marine fish and other seafood contribute to 90% of the total As exposure to humans through ingestion (FDA, 1993). Arsenic pollution in marine environments affects the environment and the local population. Fishes are regarded as highly vulnerable to arsenic toxicity as they are constantly exposed to it through their gills and consume arsenic-contaminated food. Any changes in the environment is reflected in the body of fish and it can be considered as a bioindicator of aquatic environmen-

tal pollution. Besides direct absorption across the gills or skin, indirect absorption through consumption of prey and inorganic, methylated and other organic arsenicals (WHO, 2000; Rahman *et al.*, 2012) is also a pathway for the entry of arsenic into the body. Consumption of arsenic contaminated fish affects skin, gastrointestinal system, nervous system and causes anemia, skin cancers, liver diseases (ATSDR, 2006; Lavanya *et al.*, 2011)

Arsenic bioaccumulation in aquatic organisms is potentially influenced by a variety of biological factors such as organism species, body size, and age as well as environmental factors such as phosphorus, pH, salinity, and dissolved organic matter (DOM) levels. Further, ingestion rate, gut passage time, gut environment, living prey / food composition and density and the presence of iron oxides influence arsenic assimilation from food. Several physiological functions, including excretion, moulting, and reproduction, contribute to arsenic removal from the body. (Zhang *et al.*, 2022). Bioaccumulation of arsenic depends on assimilation and excretion of arsenic in any organism. Therefore, bioaccumulation of arsenic is influenced by all the above factors. Changes in the blood parameters would be a sign of fish-physiological response against environmental stress (Vosyliene, 1996, Maurya *et al.*, 2019). Hematological indicators are a crucial tool for assessing fish health as this technique has been used to assess the oxygen carrying capacity of the blood cells as well as an indicator of metal pollution in aquatic environments (Shah and Altindag, 2005; Kumar and Banerjee 2016). Blood chemistry parameters like glucose, triglycerides and total protein can be used as an early indicator of essential alterations in stressed fish to detect the health status of the fish, to detect cellular damage, deficiencies, metabolic disorders, target organs affected by toxicants and to measure the response of fish to metals. (Oner *et al.*, 2008, Kumar and Banerjee, 2016, Bahmani *et al.*, 2011). Hematological parameters such as hemoglobin, RBC, WBC, MCV, MCH, MCHC were studied to evaluate the changes in the fish health (Javed and Usmani, 2013). Heavy metal exposure in fish stimulates many histopathological changes in various organs in the fish and affects physiological systems. In this way histopathological investigations are helpful in determining the impacts of contaminations on the specific organs, (Ahmed *et al.*, 2013; Rabbaneet *et al.*, 2022;). Consumption of arsenic contaminated fish in humans may affect skin, gastrointestinal system,

nervous system and cause anemia, skin cancers, liver diseases (ATSDR, 2006; Lavanya *et al.*, 2011). Arsenic once accumulated may have deleterious effects on behavioral, physiological and structural composition of the organisms, hence this study is focused on evaluating the toxicity of arsenic in biochemical, hematological and histological parameters of *Etroplus suratensis*.

Etroplus suratensis, is a brackish water fish found in the coastal regions of the Indian States of Tamil Nadu, Kerala, Pondicherry, and Odisha. This tropical euryhaline fish can survive in both fresh and brackish water, and has a high level of adaptive capacity, allowing it to withstand a wide range of salinity and temperature conditions (Taju *et al.*, 2012; Joy *et al.*, 2017; Mandal *et al.*, 2017; Sebastian *et al.*, 2018). Therefore, it was chosen as the candidate species for the study.

Materials and Methods

Chemical

Arsenic standards for the study were freshly prepared by dissolving laboratory standards Sodium arsenate in cleaned autoclaved glass and bottles by serial dilution of stock solution. Stock solution was prepared by dissolving 1g of laboratory standard Sodium Arsenate in 1L of water.

Test organism

Wild caught live *E. suratensis* adults of average mean length 14.47 ± 1.77 cm in length and 96.14 ± 16.75 grams in weight were acclimatized for a period of 15 days in well aerated water maintained in cement tanks. Fishes were fed with artificial food during acclimatization.

Procedure

Acclimatized live fish of 20 numbers each were maintained for 21 days in 4 tanks: Tank-1 maintained as control with no concentration of arsenic in water, tank-2 with EPA permitted concentration of Arsenic i.e. 0.03 mg/L in water, Tank-3 with concentration of Arsenic equal to the average concentration all the 5 locations along the coast of Chennai, Tamil Nadu i.e. 2.9 mg/L and Tank 4- with the maximum concentration of arsenic detected among the 5 locations along the coast of Chennai, Tamil Nadu i.e. 5.5mg/L. During the experiment, 20 % water was exchanged daily. Artificial feed was given according

to the body weight of the fish and the feeding was stopped one day prior to the termination of the experiment. The specimens were anesthetized using ethylene glycol monophenyl ether and washed in running water. Gills, liver, gonads, intestine and muscle were dissected out using autoclaved forceps, scissors, petri plates.

Gonadosomatic and Hepatosomatic index

Gonads and liver were dissected out intactly from adult female fish, to compare the weight of the gonads and liver with the body weight. The weight of the ovary and of the liver were measured, GSI was calculated using weight of ovary with that of the body, HSI was calculated using weight of liver with body weight.

Biochemistry

Increase or decline in the level of carbohydrate, protein and lipid profile of fish (Javed and Usmani, 2014) may be caused by heavy metal pollution. Biochemical estimation of serum glucose, total protein and cholesterol are performed by colorimetric method (Glucose- GOP/POD method, Protein- Biuret Method, Cholesterol- Enzymatic CHOD/PAP method) using Commercial kits (Autospan Liquid Gold Test Kits, India Arkray Healthcare Pvt. Ltd.)

Hematological

Blood samples were drawn using a heparinized syringe, from the caudal vein of 3 fishes from control and each treatment. The blood samples were collected to measure hemoglobin, WBC, RBC, MCV, MCH, MCHC using standard procedures. Hemoglobin (Hb) was determined using the cyanmethemoglobin method as described by (Dacie and Lewis, 2001; Kumar and Banerjee 2016). Red Blood Cells (RBC) and White Blood Cells (WBC) were counted using an improved Neubaurhaemocytometer (Darmady and Davenport, 1954; Shah and Altindag, 2005; Kumar and Banerjee, 2016) The other hematologic indices like MCV, MCH, MCHC calculated using standard formulae.

MCV is the average size of the RBC in a given sample of blood represented in cubic microns.

$$MCV = \frac{PCV \times 10}{RBC}$$

MCH is the average weight of the haemoglobin contained in each RBC in a given volume of the blood, represented in picograms (pg).

$$\text{MCH} = \frac{\text{Haemoglobin (g/dL)} \times}{10\text{RBC}}$$

MCHC is the average concentration of the haemoglobin in the RBC in a given volume of the blood.

$$\text{MCHC} = \frac{\text{Haemoglobin (g/dL)}}{\text{PCV}} \times 100$$

Histology

The tissues of gills, liver and gonads were fixed separately in 10% neutral buffered formalin. Later the tissues were washed under running tap water and dehydrated with ascending grades of alcohol. The tissues were cleaned twice in xylene for 30 minutes, embedded in paraffin blocks and sections were prepared using a rotatory microtome. The sections were stained with haematoxylin- eosin and mounted on glass slides. The histopathological reading was observed under a light microscope

Arsenic in Fish

The specimens were washed with distilled water and their organs (liver, intestine, gills, gonads, and muscle) were dissected using a sterile knife. The samples were pooled to obtain a sufficient amount of tissue, then labeled and preserved at -20°C. The preserved samples were lyophilized, then ground in a clean mortar and pestle, and stored at -20°C until heavy metal analysis. The lyophilized samples were transferred to digestion flasks and digested with perchloric acid, nitric acid, and sulfuric acid in a 1:5:1 ratio on a hot plate until all the tissues were dissolved. The heavy metals in the tissues were analyzed using a calibrated atomic absorption spectrophotometer with recovery rates above 90%.

Results and Discussion

GSI and HSI

High GSI & female HSI values in control are clear indicators of reproductive potential due to the increase in the number of mature oocytes and female HSI are related to vitellogenin synthesis, which facilitates gonadal development. (Schalk *et al.*, 2014; Passos *et al.*, 2021). In this study, the mean GSI of Control, Treatment-1, Treatment-2 & Treatment-3 are 1.18 ± 0.3 , 1.25 ± 0.37 , 0.97 ± 0.25 & 0.24 ± 0.50 respectively; mean HSI of Control, Treatment-1, Treatment-2 & Treatment-3 are 1.49 ± 0.37 , 1.19 ± 0.49 , 1.37 ± 0.55 & 1.52 ± 0.47 . As the concentration of ar-

senic increased from control to treatment, a lowering trend of GSI and a rising trend of HSI were detected (Fig. 1) showing the deleterious effects of As on the development of gonads.

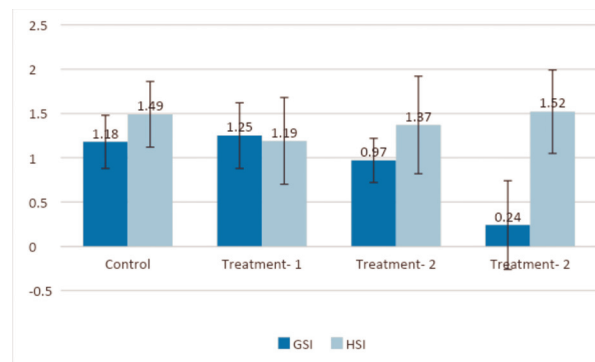


Fig. 1. Gonadosomatic and Hepatosomatic index

Biochemical composition

Total protein concentration increased gradually in treatments when compared to controls (2.17 ± 0.15 mg/L). And the highest concentration was observed in Treatment-3 (Fig. 2). The changes in the level of protein may be due to liver damage, reduced absorption and protein loss. Proteins are considered as important structural components, biocatalysts and hormones, controlling growth and a variety of other functions. Metal accumulation in gills can cause structural damage to gills and liver thereby decreasing in oxygen consumption, reduction in metabolic rate, decreased protein absorption and protein loss (Oner *et al.*, 2008; Yacoub and Gad, 2012). The rise in total protein may be the result of increased protein synthesis for the purpose of fulfilling the high energy demand (Javed and Usmani, 2014). The concentration of Glucose decreased from control (165.77 ± 9.90) to treatments and the lowest concentration of glucose was observed in Treatment-3 (59.49 ± 3.59 mg/l). The depletion in the glu-

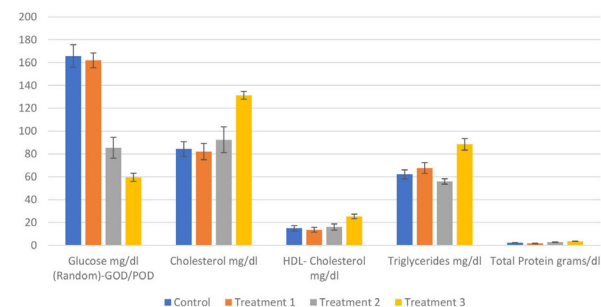
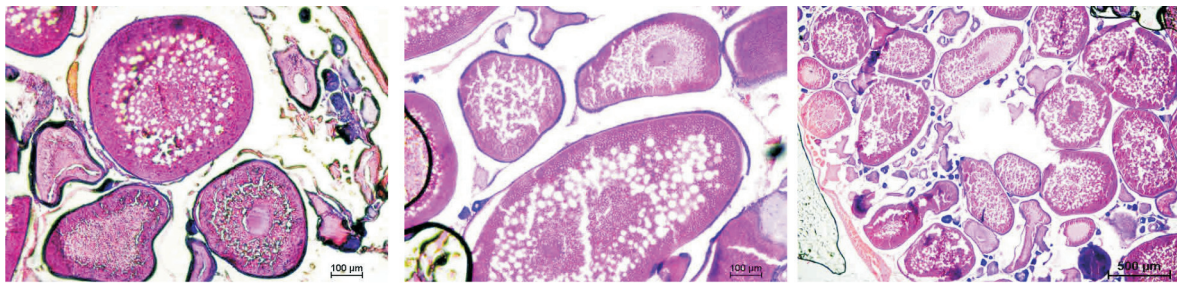


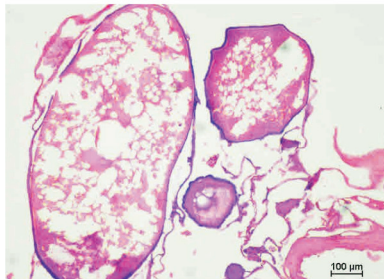
Fig. 2. Biochemical composition of *Etroplus suratensis*

cose level might be due to extra energy demand in the metabolism which may reflect an increased glucose level in serum (Canli, 1995). Alterations in the glucose level can also be related to renal inquiry, liver damage, and lack of nutrients, Metals may influence the way carbohydrates are metabolized, and this can lead to a rise in serum glucose levels due to glycogenosis and the production of glucose from extrahepatic tissue proteins and amino acids. (Oner *et al.*, 2008). The concentration of Triglycerides decreased from control (62.07 ± 3.94 mg/dl) to in treatment 1 (67.58 ± 4.75 mg/dl) and in treatment 2 (55.93 ± 2.37 mg/dl) and increased in the treatment-3 (88.31 ± 5.01 mg/dl). Triglyceride concentration is essential for assessing lipid metabolism, and higher

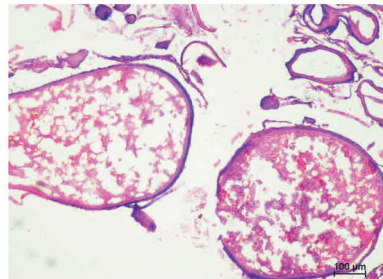
levels can be seen among individuals with nephritic syndrome and impaired glycogen storage. Triglycerides are largely responsible for supplying cellular energy and can be utilized as a sign of dietary alterations in treated samples (Oner *et al.*, 2008). Triglycerides, phospholipids, and cholesterol combine to make total lipids. Lipid molecule helps to overcome the stress (Javed and Usmani, 2014). The decrease in lipid levels could be caused by the stress induced release of catecholamine and corticosteroids into the blood, which results in an enhanced metabolic rate and a consequent drop in metabolic reserves (Yacoub and Gad, 2012). The Cholesterol concentration in the metal exposed treatments increased when compared to controls 84.31 ± 6.47 mg/dL. The



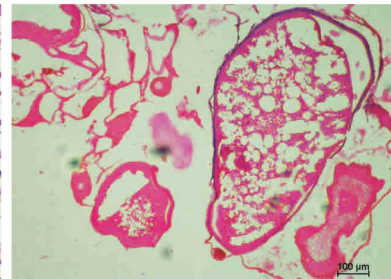
Control- Proper sized oocytes with all developmental stages



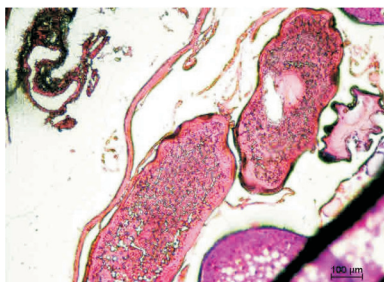
T-2 Deformed late pre-vitellogenic oocytes



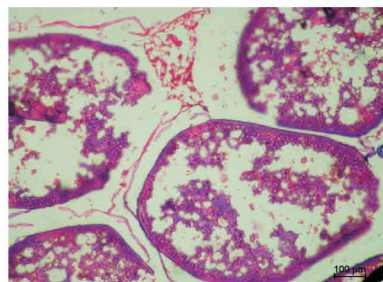
T-2 Atretic oocytes and degenerated follicles



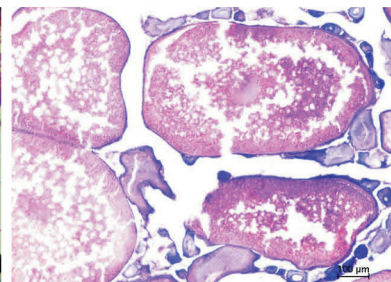
T-3 Oocytes with folded Zona pellucida



T-3- increased vacuolization in specific areas of ovaries



T-3 Atretic oocytes with broken membrane and vacant space in the ovary



T-3 Centrally aggregated nucleoli in the disintegrating nucleus

Fig. 3A. Histology of ovaries

highest concentration was observed in T-3 (131.37 ± 3.52 mg/dL). Similarly HDL-Cholesterol concentration in the metal exposed treatments also increased when compared to that of controls. The interaction of arsenic with the enzymatic pool of cholesterol metabolism might be responsible for the increased level of cholesterol. Kidney and liver failure can raise cholesterol concentration and result in the increased release of cholesterol into the blood. On the other hand, heavy metals are known to have harmful effects on cell structure, particularly on the membranes, consequently, elevating the levels of cholesterol. Cholesterol is a precursor to all steroid hormones and a crucial structural component of cells (Oner *et al.*, 2008)

Haematology

Etroplus suratensis exposed to three different concen-

trations of As heavy metals showed reduction in the level of haemoglobin, RBC, MCV & MCH from control to treatment (Table 1), the lowest mean concentration of haemoglobin was observed in T-3 (6.47 ± 0.42). The reduction of hemoglobin affects the oxygen binding capacity and reduced oxygen level indicates an anemic condition in fish which may be due to the stress related haemolysis. The decrease in haemoglobin content in fishes exposed to metals could be due to destruction of haemoglobin or due to decreased synthesis of haemoglobin (Kumar and Banerjee, 2016). The above findings supported the present observation that the reduction of hemoglobin due to toxicants leads to significant decrease in haemoglobin and final reduction in the oxygen binding capacity of fishes. The erythrocyte counts of control showed a mean value of $2.37 \pm 0.45, 10^6 \text{mm}^3$ and that of T1, T2 and T3 $2.93 \pm 0.53, 1.83 \pm 0.37,$

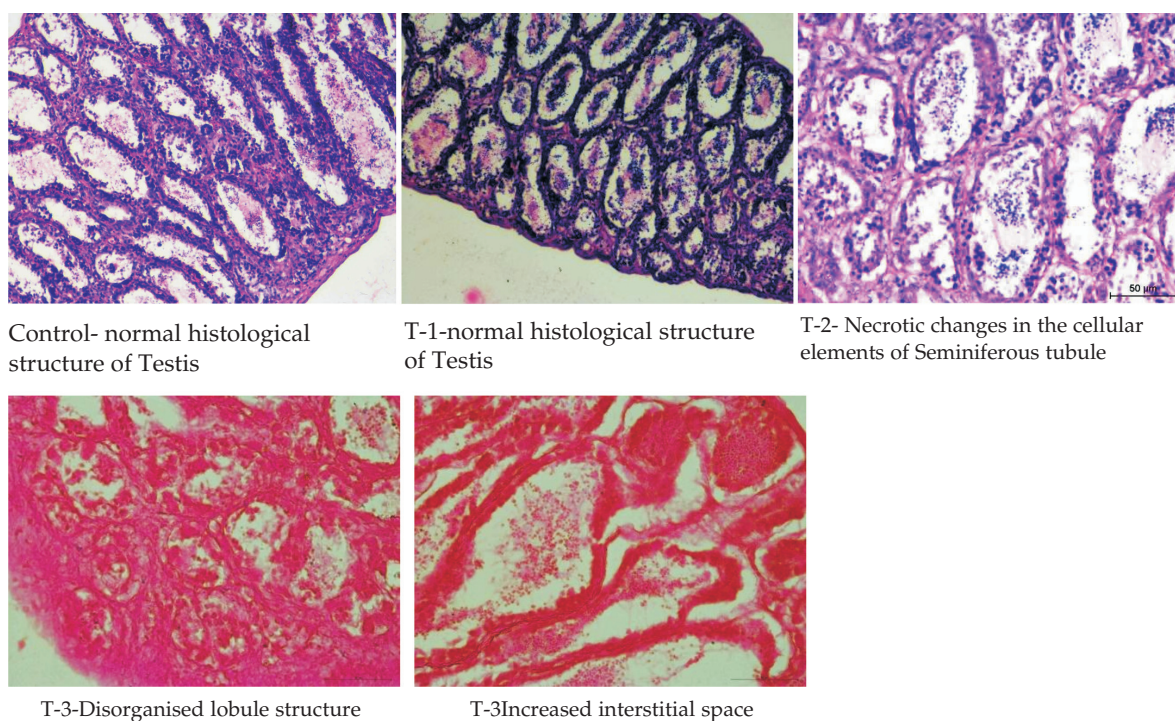


Fig. 3B. Histology of Testis

Table 1. Haematological parameters (mean $\pm$ SD) of *Etroplus suratensis* exposed to various concentration of Sodium arsenate for 21 days

	Hemoglobin	RBCs	Total WBC	MCV	MCH	MCHC
Control	10.97 ± 0.29	2.37 ± 0.45	5.63 ± 0.91	139.56 ± 4.92	40.95 ± 1.44	26.92 ± 1.36
Treatment 1	10.77 ± 0.40	2.93 ± 0.53	6.04 ± 0.90	134.87 ± 6.58	41.98 ± 2.20	24.63 ± 1.31
Treatment 2	8.43 ± 0.70	1.83 ± 0.37	7.99 ± 1.28	122.33 ± 2.93	36.27 ± 0.79	34.27 ± 3.09
Treatment 3	6.47 ± 0.42	1.30 ± 0.37	9.89 ± 1.30	115.40 ± 3.07	35.07 ± 1.27	28.91 ± 1.23

1.30±0.37, respectively. Prominent reduction of red blood cell (RBC) was observed with respect to increased concentration of arsenic. The decrease in RBC in the study might be resulted from the inhibition of RBC production or due to the accumulation of effluents in the gill region, causing damage to the structure of the gill resulting in hemolysis (Nte *et al.*, 2011; Malik *et al.*, 2015). The decline in RBC may also have been brought on by the damaging impact of heavy metal on peripheral red cells and reduction in the number of Red Blood Cells in fish exposed to pollutants have been noted in several studies (Maheswaran *et al.*, 2008; Ali *et al.*, 2018; Maurya *et al.*, 2019). The WBC count of fish exposed to three different concentration of As showed a mean value (5.63 ± 0.91 , 6.04 ± 0.90 , 7.99 ± 1.28 , $9.89 \pm 1.30 \times 10.4/\text{mm}^3$) ranged from controls to T1, T2 & T3. The increase in WBC count can be correlated with an in-

crease in antibody production, which helps in survival and recovery of the fish exposed to sub-lethal concentrations of pesticide (Malik *et al.*, 2015). The initial rise in leucocyte count is an adaptation mechanism used by animals to deal with stress, and it's an immunological defence mechanism. Due to the presence of toxic substances, the following drop in leucocyte count denotes immune system deterioration resulting from the series of changes in immunological setup as a result of stress caused by high concentration of metals (Malik *et al.*, 2015; Maurya *et al.*, 2019). Hematological parameters like MCV, MCH, MCHC provides information on size, relationship, shape and Haemoglobin constants of erythrocytes (Ali *et al.*, 2018). MCV, MCH, MCHC showed noticeable variations among control and treatments, the mean values of MCV, MCH, MCHC (139.56 ± 4.92 , 134.87 ± 6.58 , 122.33 ± 2.93 , 115.40 ± 3.07),

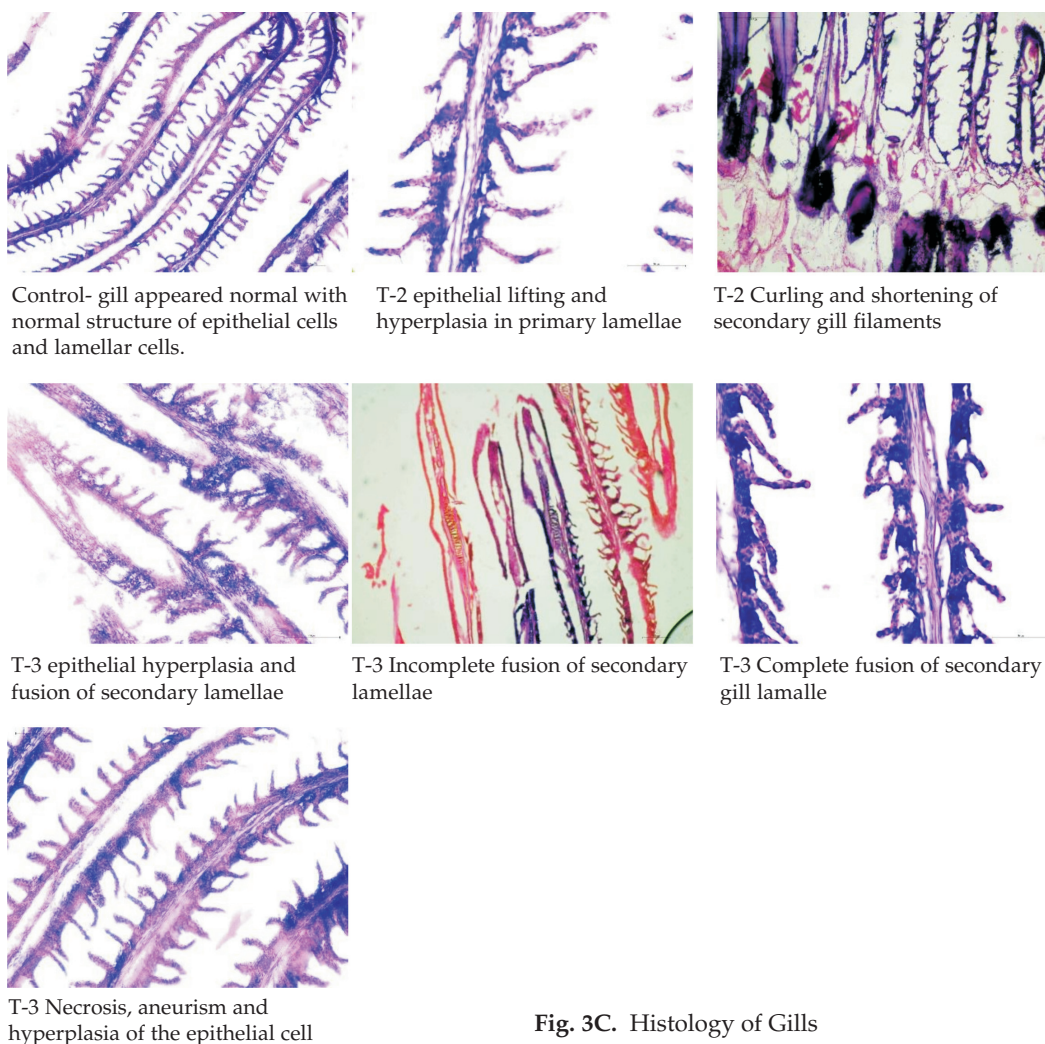


Fig. 3C. Histology of Gills

(47.90 ± 8.26 , 38.22 ± 8.29 , 47.127 ± 5.56 , 54.81 ± 17.92) & (34.23 ± 5.12 , 28.28 ± 5.52 , 38.47 ± 4.02 , 47.14 ± 14.28) ranging from controls to treatments 1, 2 & 3 respectively. MCV showed a decline pattern whereas, MCH and MCHC increased in T2 and T3. The variations in MCHC in Treatments may be related to RBC swelling caused by arsenic uptake (Kumar *et al.*, 2016). According to studies, the cells discharged from the spleen may have decreased the MCV value (Ololade and Oginni, 2010). The low MCV and MCH could be attributed to the high proportion of immature red blood cells in circulation (Nte *et al.*, 2011). The MCV represents the size of the red blood cells and reflects the cell division during erythropoiesis, the MCH and MCHC is a diagnostic method to evaluate the degree of RBC swelling (Kumar *et al.*, 2016; Ali *et al.*, 2018).

Histology

The alternations in the histopathological structure of gills, liver, gonads are represented in Fig-3A to 3D. The present study showed histopathological alterations in T2 & T3, while T3 showed devastating changes, no alterations were observed in T1 and control. The detailed changes observed in the treatments are discussed below. Gills of the fish in the control and T-1 group appeared normal with nor-

mal structures of pillar cells, epithelial cells and lamellar cells. However, on exposed groups T-2 and T-3 atrophy of gill structures were observed. While T-2 groups showed epithelial lifting, hyperplasia in primary lamellae, dilation in marginal channels, T-3 groups showed alterations in gills such as necrosis, lifting of the lamellar epithelium, incomplete fusion of secondary lamellae, aneurism, hyperplasia of epithelial cells, fusing of secondary lamella, and curling and shortening of secondary gill filaments. The major target tissues for toxicants to effect are the gills (Ahmed *et al.*, 2013). Because of gill damage brought on by pollution, fish may use less oxygen and have problems with their osmoregulatory systems, leading to eventual death. Arsenic exposure reduces the oxygen consumption and disrupts the osmoregulatory functions of the fish (Ahmed *et al.*, 2013). Gills are the most susceptible, thus the first organs to be impacted by environmental changes because they perform respiratory, osmoregulatory, and excretory processes while being in constant contact with water (Kumar *et al.*, 2014).

In control and T-1 the histopathology of the liver appeared normal and systematically arranged with uniform cytoplasm and centrally placed nucleus. In contrast to this the T-2 showed vacuolated cytoplasm, enlarged nuclei, increased intercellular

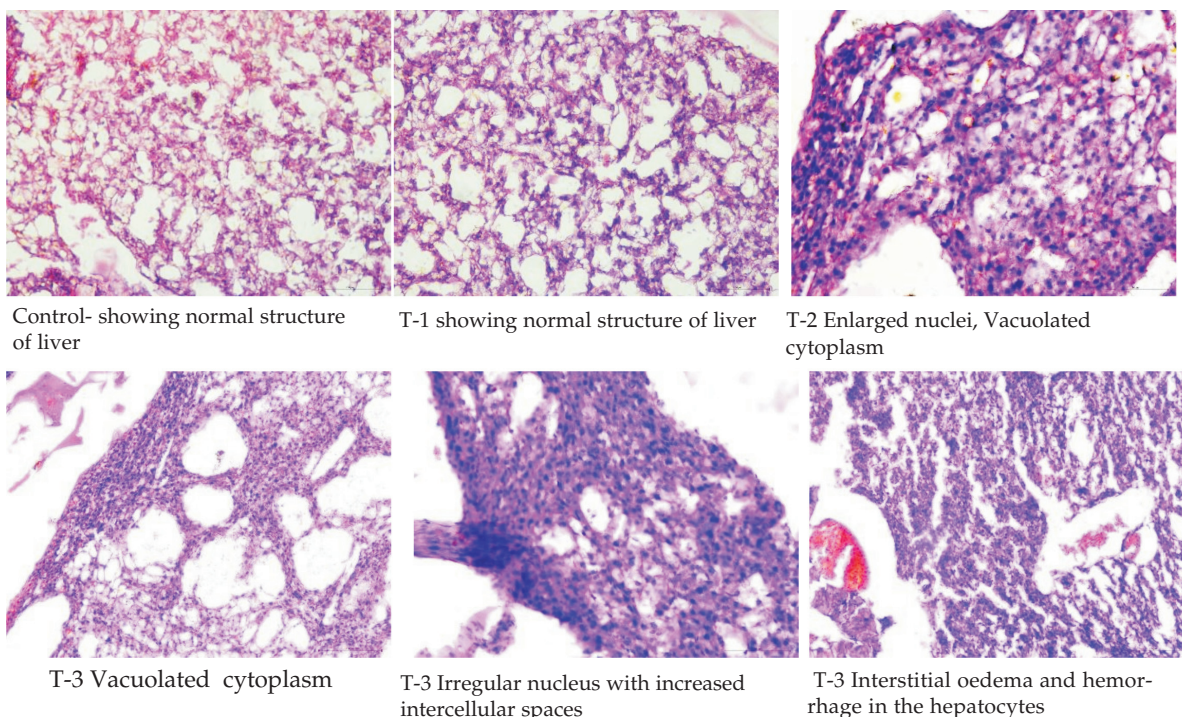


Fig. 3D. Histology of Liver

spaces and T-3 showed hepatocyte with irregular nucleus, intercellular spaces, necrosis, nuclear degeneration vacuolated cytoplasm, interstitial odema and hemorrhage in the hepatocytes (Kumar *et al.*, 2019; Ahmed *et al.*, 2013). In addition to that, increased areas of inflammation, delicate tissues were also observed in liver tissues (Lam *et al.*, 2006) of T-3 groups. Liver is the primary organ for detoxification and important for the metabolic pathway involved in synthesis of components of blood, glycogen storage and release of glucose in the blood (Brusle and Andadon, 1996). Any changes in the histology of the liver affects the protein synthesis. The formation of large vacuoles may be brought on by the inhibition of protein synthesis, energy exhaustion, and microtubule disaggregation (Hinton and Lauren, 1990).

Histopathological alterations in the ovary showed different degrees of severity in treatments when compared to controls. Control and T-1 group fish showed ovaries with proper sized oocytes with smooth surfaces at all development stages and mature oocytes had a well uniform distribution of yolk globules (Bhat *et al.*, 2023). Heavy metals can cause greater loss to advance stages of oogenesis, and may reduce the ability of the fish to reproduce (Mehanna, 2005; Raksha and Sharma, 2012). The oocytes of T-2 groups showed irregularly shaped oocytes, with wrinkled, rough, distorted surfaces, oocyte appeared elongated and deformed (Bhat *et al.*, 2023). Development of inter-follicular space in oocytes and dissolution of oocyte wall, vacuolation and broken wall in vitellogenic oocytes, dissolution of yolk globules, clumping of cytoplasm of mature oocytes and

atresia were seen in the maturing ovarian follicle. Inter follicular spaces were larger and vacuolation in developing oocytes were observed in T-3 (Brraich and Jangu, 2015). The fish in the control and T-1 set ups showed normal histological structure of Testis, T-2 group showed disorganised lobule structure, increased interstitial space, in T-3 Severe degenerative and necrotic changes in the cellular elements of the seminiferous tubule, increased interstitial space were observed. (Bhat *et al.*, 2023; Mohamed, 2008; Hanna *et al.*, 2005; Kader and Mourad, 2019).

Arsenic in Tissues

The concentration of Arsenic in various organs (gills, liver, intestine, gonads, and muscle) of the *E. suratensis* species were recorded after terminating the experiments. Mean metal concentration ranged from controls to T1, T2, T3: gills (0.02 ± 0.01 , 1.47 ± 0.38 , 2.20 ± 0.69 , 4.75 ± 1.21); liver (0.05 ± 0.03 , 1.26 ± 0.62 , 2.29 ± 0.31 , 4.47 ± 1.04); intestine (0.03 ± 0.02 , 1.23 ± 0.27 , 0.45 ± 0.37 , 3.33 ± 0.95); Muscle (0.01 ± 0.01 , 0.32 ± 0.35 , 0.52 ± 0.35 , 0.95 ± 0.08); Ovary (0.02 ± 0.01 , 0.11 ± 0.04 , 2.40 ± 0.42 , 2.98 ± 1.07). Fig. 4 and Table 2. The distribution of the Arsenic in the tissues of *E. suratensis* is in the order gills > liver > ovary > intestine > muscle. Because there is no direct interaction of toxicants with muscle tissues, accumulation of As in the muscles was the least in all experiment groups, most of the fish mass and majority of the fish tissues consumed are muscles (Palaniappan and Vijayasundaram, 2009; Kumari *et al.*, 2016). The highest concentration of As were observed in T3 for all the tissues in which gills showed the highest concentration of As. Gills

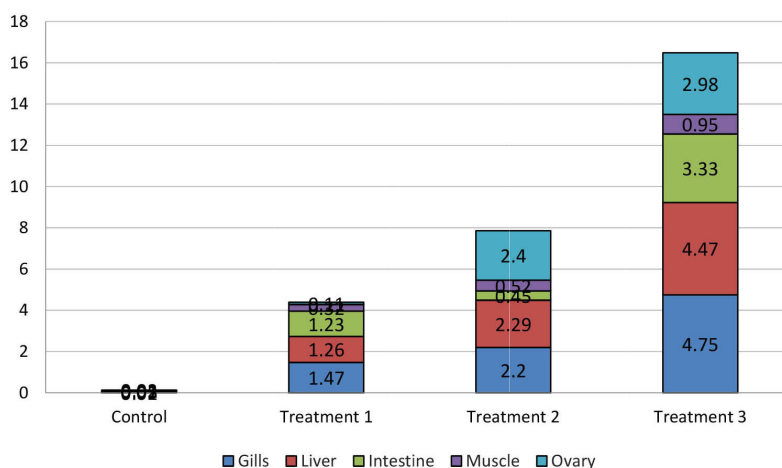


Fig. 4. Concentration of arsenic in various tissues of control and treatments

Table 2. Heavy metal Concentration ($\mu\text{g/g}$) in different tissues (mean $\pm$ SD) of *Etropolis suratensis* exposed to various concentration of Sodium arsenate for 21 days

	Gills	Liver	Intestine	Muscle	Ovary
Control	0.02 $\pm$ 0.01	0.05 $\pm$ 0.03	0.03 $\pm$ 0.02	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01
Treatment 1	1.47 $\pm$ 0.38	1.26 $\pm$ 0.62	1.23 $\pm$ 0.27	0.32 $\pm$ 0.35	0.11 $\pm$ 0.04
Treatment 2	2.20 $\pm$ 0.69	2.29 $\pm$ 0.31	0.45 $\pm$ 0.37	0.52 $\pm$ 0.35	2.40 $\pm$ 0.42
Treatment 3	4.75 $\pm$ 1.21	4.47 $\pm$ 1.04	3.33 $\pm$ 0.95	0.95 $\pm$ 0.08	2.98 $\pm$ 1.07

act as barrier, lowering the uptake of toxic substances by other organs (Kumari *et al.*, 2016). The liver is a key organ in the control of metabolic processes and the majority of the biotransformation of inorganic As substances takes place in the liver, As is actively metabolised in liver and hence tends to accumulate more in the liver. The major route of dietary intake for arsenic is through the gastrointestinal tract. Once absorbed by the intestinal tract, arsenic is distributed to other organs throughout the body via the circulatory system (Rossman, 2003; Kumari *et al.*, 2016). As accumulation in eggs may have a direct effect on the process of oogenesis, Fish exposed to arsenic in the treatment group showed signs of stress, including anxiety in breathing, slimy mucus secretions on the surface of the skin, gulping for oxygen, changes in swimming patterns, and a tendency to settle on the bottom of the tank. These behavioral abnormalities clearly showed the effect of arsenic on these fish. Fish in the control group remained active and healthy throughout the study.

Conclusion

Numerous studies have demonstrated that Humans are exposed to arsenic contamination in various ways including groundwater, the use of Arsenic pesticides in the agricultural sector, contaminated water bodies, and contaminated food produced from contaminated sources, such as vegetables, fruits, and seafood from contaminated sources. There are many biological and environmental factors which aid in arsenic bioaccumulation. Hence it is necessary to explore such possible influencing factors. Since arsenic is classified as a group I carcinogen, extensive research on its effects on histology, haematology, and biochemical processes is conducted to understand its toxic effects on various factors. In this study, *E. suratensis* exposed to sodium arsenate displayed a decreased level of glucose and triglycerides, enhanced protein and cholesterol production, decline in the level of Hb, RBC, MCV

and increased production of WBC, MCH and MCHC. The liver, gonads, and gills all showed extensive necrosis and other abnormalities, As has negative impacts on the gonads of the fish reducing the reproductive capacity of fish. As toxicity increased with its increasing concentration. It is also noted that As rapidly accumulates in all tissues exposed to sodium arsenate. It is undoubtedly clear that As has deleterious effects on biochemical, histopathological, haematology characteristics.

This study is limited to alterations observed in the histopathological, haematological and biochemical parameters that are indicative of effects of Arsenic reflected for a short term exposure. Hence this study opens up the scope for research on the effects of Arsenic for a long term exposure including potential changes to genetic make up and the steroidogenesis. Further study on As risk assessment and its control in marine environment may also be taken up to enhance social sustainability.

Conflict of Interest - None

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