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ORIGINAL ARTICLE

Histological Effects of Sodium Metabisulfite Exposure on the Gills, Kidneys, and Liver of the *Labeo rohita*

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ABSTRACT

Background: Sodium metabisulfite ($Na_2S_2O_5$) is a disinfectant and preservative agent that is commonly used in the food industry and polluted water bodies, and spread through industrial wastes.

Objective: Current study was conducted to observe the impact of widely used sodium metabisulfite ($Na_2S_2O_5$) on the gills, kidneys, and liver of the *Labeo rohita*.

Methods: During this experimental research, fish were divided into three groups. One group was treated as control. Two groups were exposed to sodium metabisulfite in different concentrations ranging from 29 mg/L to 68 mg/L for 28 days.

Results: The 96h LC_{50} value for sodium metabisulfite ($Na_2S_2O_5$) was 130 mg/L. Histological changes revealed moderate to severe damage in time and concentration-dependent manners. Photomicrographs of the gills of exposed fish showed different histological alterations such as hyperplasia, marginal gill lamellae, blood congestion, gills lamellae aneurysm, and distortion of the gill cells. Results indicated that exposure to sodium metabisulfite caused severe progressive alterations such as cytoplasmic vacuolization, melanomacrophagy, and expression of hepatocytes and cluster nuclei in the liver tissues of the exposed fish. Kidney tissues of the treated *L. rohita* revealed different histological changes including the wide hypertrophied nucleus, sinusoidal spaces, cloudy swelling, glomerular expansion, and tubules starting the degeneration process compared to control fish.

Conclusion: Our results represent the pioneering report demonstrating that sodium metabisulfite can act as a potent toxic agent for *Labeo rohita*.

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Sodium Metabisulfite, Nephrotoxicity, Hepatotoxicity, Fish, Histology.	*dr.khurram@uo.edu.pk*	*Received: December 27, 2023* *Accepted: February 14, 2024*

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INTRODUCTION

Aquatic toxicants such as salts, metals, pesticides, and other industrial pollutants are causing serious health risks to many aquatic organisms if they are present in high concentrations. These pollutants were toxic to human health and the environment. Water plays a significant role in nutrient recycling and is a crucial natural source used for drinking and different developmental purposes. Due to the rise in contaminants and the overuse of water resources for a variety of developmental activities, i.e., agriculture, manufacturing activities, industrial processes, and also thermal power plants to meet the requirements of a large-scale population, this significantly reduces their assimilation capacity. Thus, the double pressure exerted on the water bodies is ultimately faced by the biological communities[1].

Sodium metabisulfite ($Na_2S_2O_5$) is a disinfectant and preservative agent that is commonly used in the food industry for things like cheese, sweets, drinks, beef, margarine, drugs, fruit, sausages, pastries and fish. It has been considered an antimicrobial food additive[2]. It is a commercial reducer highly used in the leather tanning industry, water dichlorination, rubber industry, and chemical synthesis[3].

Many sources are responsible for the drainage of sodium metabisulfite into water bodies, such as industrial effluents, domestic sewage, and rainwater overflow. These pollutants, when released into the water bodies without suitable treatment, become very harmful to any type of aquatic animal, especially fish[4]. Sodium metabisulfite can be toxic to aquatic life if it is present in high concentrations in the water. It can cause a range of negative effects on aquatic organisms, including fish, shellfish, and other aquatic organisms. These effects can reduce growth, reproduction, changes in behavior, and even death. In addition, sodium metabisulfite also affects the health of predators that consume organisms that have been exposed to the chemical. Numerous studies are related to the effect of sodium metabisulfite on rats, resulting in an increase in biochemical parameters and a reduced immunoglobulin concentration[5]. Also, the effect of SMB on shrimp melanosis development was assayed by sensory analyses and bacterial counts were reported[6].

Fish are used for this experiment because they are sensitive to changes in their environment and can be used as health indicators for aquatic ecosystems. Fish are an important part of the aquatic food chain, which means they can accumulate toxicants in their bodies[7]. The *L. rohita* is an extensively used fish in Pakistan and a commercially important fish due to its high nutritive value[8]. Hence, the impact of sodium metabisulfite on different organs of this species was chosen for the study.

The current study was directed at observing the effect of sodium metabisulfite on the histological changes in major organs such as the gills, kidneys, and liver of *L. rohita* throughout both short and long experimental periods.

MATERIALS AND METHODS

Chemicals and Reagents

Sodium metabisulfite ($Na_2S_2O_5$) was purchased from Merck Company and has 99.9% purity. The appropriate amount of salt was mixed with distilled water to make a stock solution (1 g/L).

Ethical Approval

The study was conducted domesticating *Labeo rohita*. The statement of responsible use and conduct of the experiment was submitted to the institutional bioethics and biosafety committee (IBBC) with full-length research proposal. The committee recommended the study and submitted the proposal to Institutional Research Board and they granted the approval of the study. No animal was harmed nor stressed by conscious or unconscious means.

Experimental Organism

Labeo rohita (average length 33.4 ± 4.30 cm and weight 14.67 ± 4.22g) were purchased from Head Balloki fish hatchery, District Kasur, Pakistan, in a plastic bag with aerated water. There was no mortality during the transportation of fish from the hatchery to the zoology lab at the University of Okara where further study was completed. The fish were kept in a large aquarium made up of glass with height, length, and width (*44″* × *91″* × *30″*) for 7 days and fed with commercial feed containing 22% protein regularly. The mechanically aerated water was changed daily to remove the feces of fish to maintain the level of Biological Oxygen Demand in ideal conditions.

Experimental Design

After acclimatization, fish were divided into three groups and each group contained 20 fish. It was preferred that fish of similar size and weight be placed in the same group. Group 1 was assigned as a control group or chemical-free group and groups 2 and 3 were given 29 mg/L and 68 mg/L sodium metabisulfite, respectively. The experimental design had short (14 days) and long-term (28 days) phases. Temperature 24.5 ± 2.7ºC, oxygen concentration 7.25±0.23 mg/L, and pH 7.46±0.28 of water were upheld throughout the experimental duration. After one day, 90% of the water was changed.

Histological Study

At day 14 (short-term experimental conditions), fish (n = 5) from each group were removed and dissected humanely. Vital organs like gills, kidneys, and liver were removed for histological study. Tissue samples were fixed in a 10% formalin solution and dehydrated in 70–100% alcohol. An automatic microtome machine was used to cut tissues into small sections (4-5µm) and stained with hematoxylin and eosin stains following the method of

Afzal *et al*.[9]. Prepared slides were photographed at 40X with the help of an optical microscope (Lieca DM 750). Similarly, after 28 days (long-term experimental conditions), fish (n = 5) from each group were removed and the above-mentioned process was performed again.

RESULTS

Histological Observations of Gill Tissues

In histological study of gill tissues, short and long-term exposure was done by using Hematoxylin and Eosin staining and examined histological changes as shown in Figure **1** (a-e). Alterations in the gill tissues were examined in the exposed fish which were found to be rising with the exposure period while in the control fish, a normal gill lamellar pattern was examined (Figure **1a**). In gills, after short-term and long-term exposure, histological changes, including hyperplasia of epithelial cells of gills which gives rise in the quantity of cells in an organ or tissue, marginal gill lamellae with marginal gill channels dilated, blood congestion swelling bodily tissues, gills lamellae aneurysm cause irregular blood filled enlargement of blood vessels which may affect the blood movement in the gills and alterations of the bone cell in gills and curved secondary lamellae were observed.

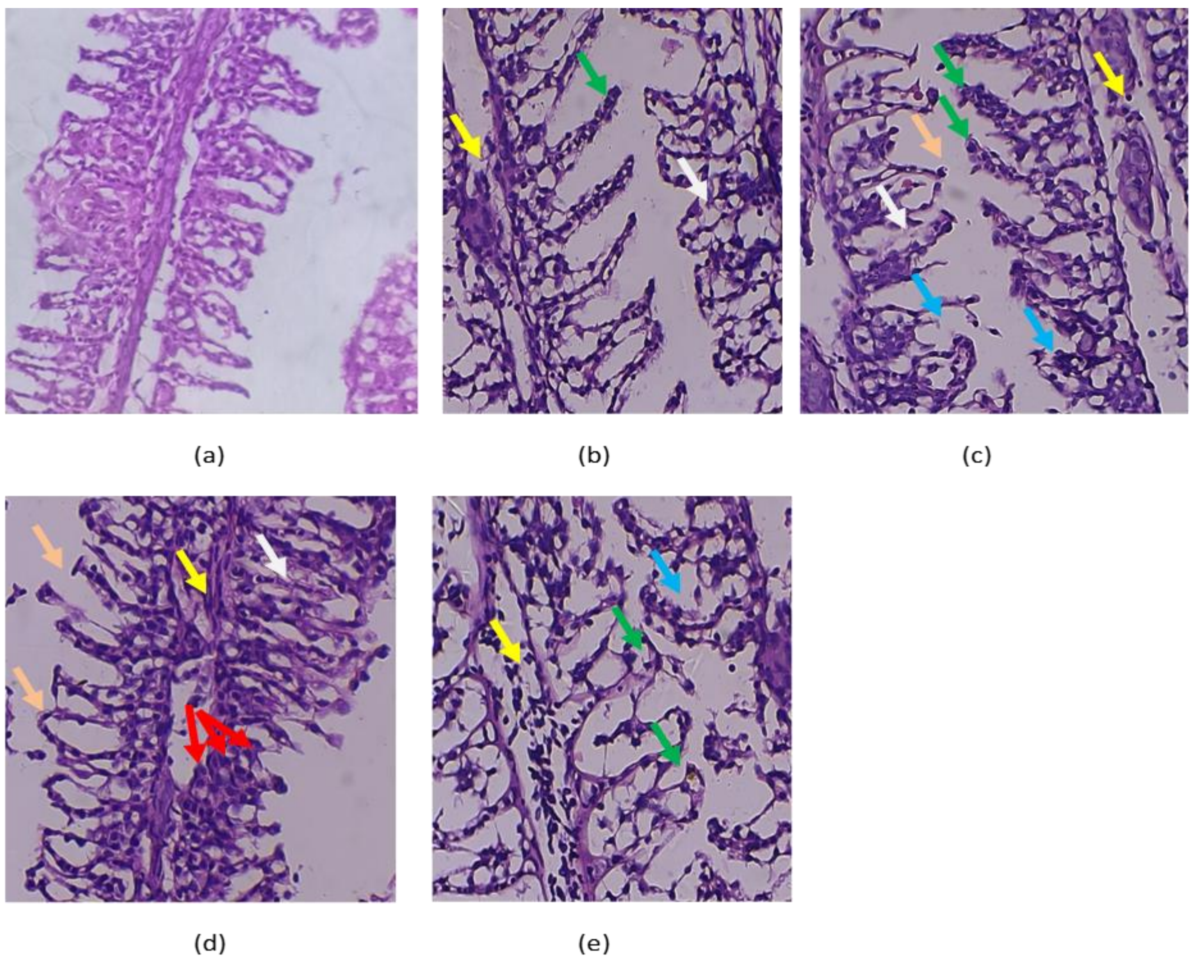

Figure 1. 1(a) Shows the microscopic structure of normal gill tissues of *L. rohita*. **1(b)** and **1(c)** shows a high dose effect of 14 and 28 days. **1(d)** and **1(e)** shows a low dose effect of 14 and 28 days. The white arrow shows the effects such as hyperplasia of epithelial cells of gills which gives rise to the number of cells in an organ or tissue. The green arrow shows marginal gill lamellae with marginal gill channels dilated. The blue arrow shows the effect of blood congestion swelling bad tissues. The red arrow shows gills lamellae aneurysm causes irregular blood-filled enlargement of blood vessels which may affect blood flow in the gills. The yellow arrow shows distortion of the bone cell in the gills and the orange arrow shows curved secondary lamellae.

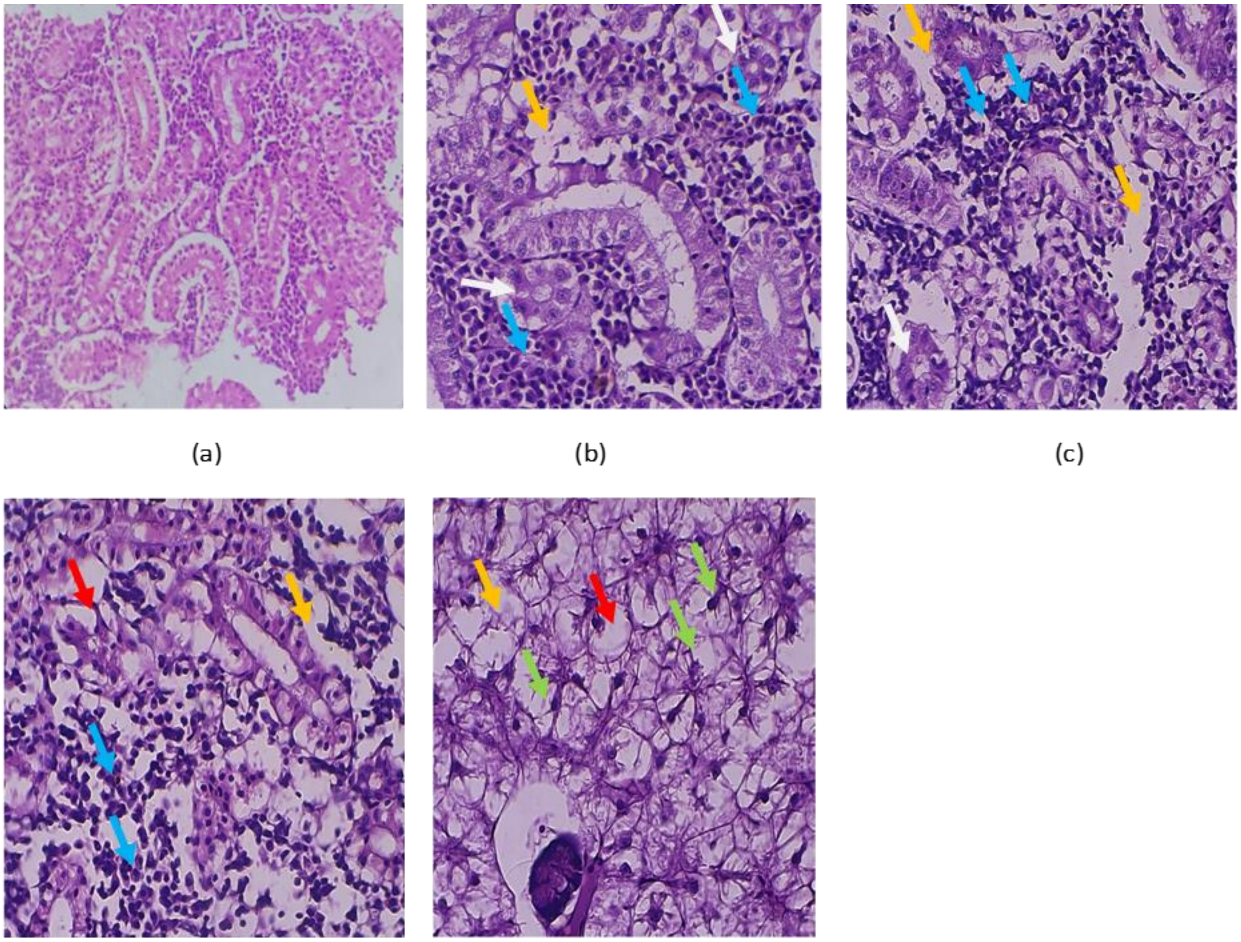

Figure 2. Shows the result of the histological examination of the kidney in the control and experimental fish after each of the different treatments. Figure **2(a)** shows the microscopic structure of normal kidney tissues of *L. rohita.* **2(b)** and **2(c)** shows high dose effects of 14 and 28 days. **2(d)** and **2(e)** shows low dose effect of 14 and 28 days. White arrow shows the tubule cells hypertrophied nucleus which causes a decline in renal function. The orange arrow shows sinusoidal spaces. The red arrow shows cloudy swelling degeneration. Blue arrow shows the tubules starting the degeneration process. The green arrow shows the effect of glomerular expansion or dilated renal tubules which may cause the development of fibrosis within the kidney and it is a significant structural lesion that is characterized by the abnormal explosion of mesangial cells and large production of matrix protein.

Histological Observations of Kidney Tissues

Histological examination of kidney tissues of the short-term and long-term exposed and control fish was done, by using Hematoxylin and Eosin staining, and reported histological changes are shown in Figure **2** (a-e). Changes in the kidney tissues were examined in the exposed fish which were found to be rising with the exposure period as compared to control fish, normal kidney tissues were reported in Figure **1a**. In the kidney, after short-term and long-term expsure, histological changes, including tubules cells hypertrophied nucleus which cause a decline in the renal function, sinusoidal spaces, cloudy swelling degeneration, glomerular expansion or dilated renal tubules which may cause the development of fibrosis within in the kidney and it is significant physical lesions that are characterized by the abnormal explosion of mesangial cells and large production of matrix protein, tubules starting the degeneration process were observed.

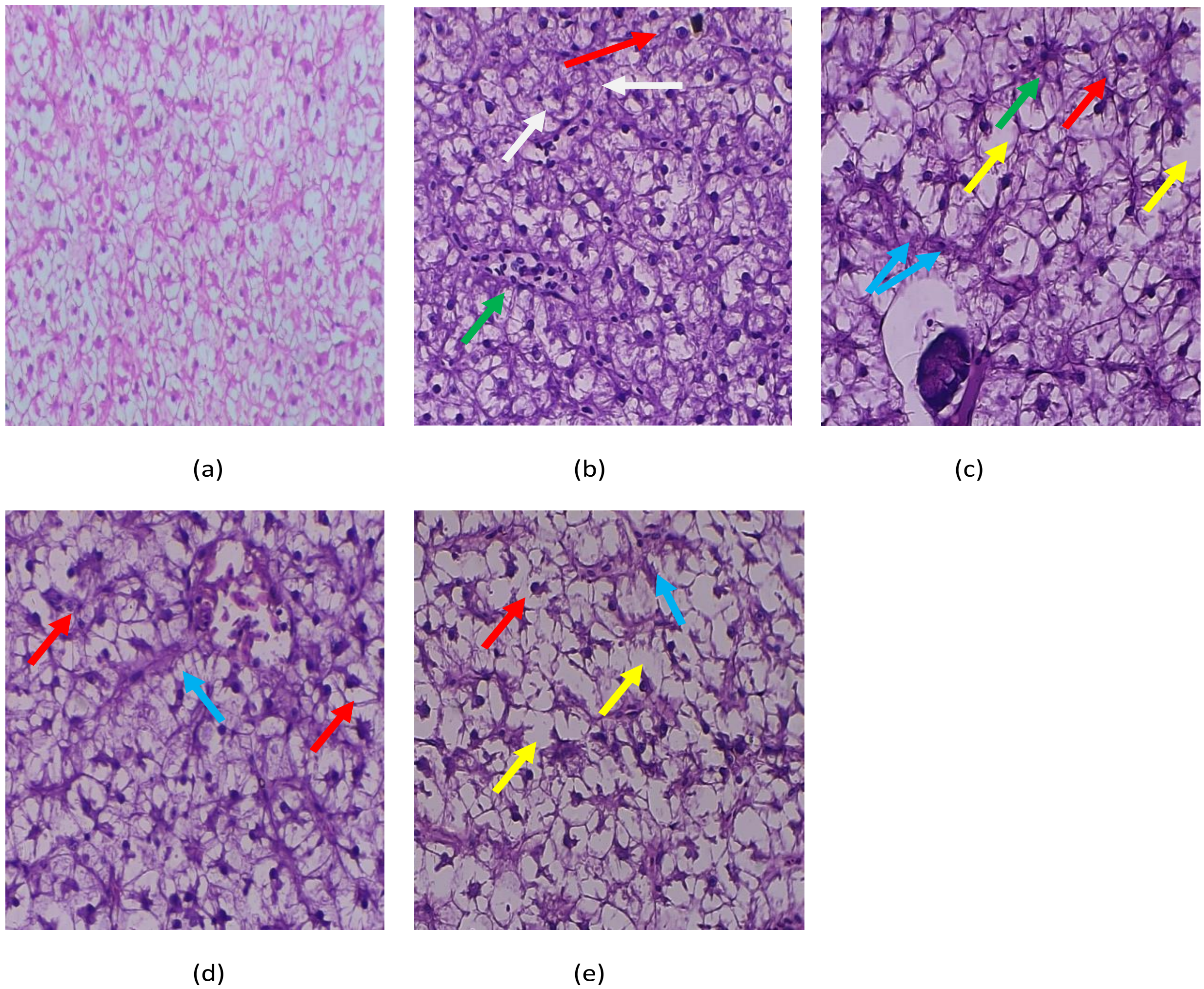

Figure 3. 3(a) Shows the microscopic structure of normal liver tissues of *L. rohita* at 40X. **3(b)** and **3(c)** shows a high dose effect of 14 and 28 days. **3(d)** and **3(e)** shows a low dose effect of 14 and 28 days. The white arrow shows the melanomacrophage which are phagocytes that synthesize melanin. The yellow arrow shows cytoplasmic vacuolization. The red arrow shows hepatocytes with irregular shaped nuclei. The green arrow shows cluster nuclei and the blue arrow shows cytoplasmic degeneration which shows pathological conditions that cause cells to change in structure and functions. Injury is mainly in the cytoplasm of the cell these lesions are reversible when pathological stimuli are reduced or eliminated.

Histological Observations of Liver Tissues

In the histological study, examining the liver tissues of fish *L. rohita* throughout both short-term and long-term exposure and control fish was done; by using Hematoxylin and Eosin staining, and the reported histological lesions are shown in Figure **3** (a-e). Significant alterations in the liver tissues were observed in the treated fish which were found to be rising exposure time however, in the control fish, normal hepatic cells were observed (Figure **1a**). In the liver, after short-term and long-term exposure, histological changes, including melanomacrophage that are phagocytes that synthesize melanin, cytoplasmic vacuolization, hepatocytes with irregular shapes nucleus, cluster nuclei and cytoplasmic degeneration which shows pathological conditions that cause cells to change in the structure and functions were observed.

DISCUSSION

The natural freshwater impurities with salt ruminants have become a major issue throughout the world and have harmful effects on aquatic life [10, 11]. Biological study is not sufficient to ensure the extreme position of various organs under biological stress. Therefore, it is significant to understand histological examinations and they proceed as natural indicators to evaluate the toxicity [12, 13]. Hence, this research was conducted to estimate the effects of sodium metabisulfite on soft tissues such as the gills, kidneys and liver of freshwater fish *L. rohita.*

Histological changes can be used as a marker to determine the effects of various toxicants on organisms[14]. Previous studies reported that various toxicants (agricultural and industrial effluents) caused several histological changes in different tissues of the fish [15]. Histology is considered a definitive tool to examine the effect of toxicants such as sodium metabisulfite on fish organs because these salts affect the regular functioning of the organism[16].

Gills were the main target organ affected by sodium metabisulfite ($Na_2S_2O_5$). Gills are commonly considered high-quality indicators of water [17]. Hence the gills are the principal way for the entrance of salts. In fishes, gills are important organs for their osmoregulatory, excretory, and respiratory functions. Several histological alterations in the gills of different fish species exposed to salts were observed[18]. Histological alterations examined the hyperplasia of epithelial cells which gives rise to the number of cells in an organ or tissue. Blood congestion swelling bodily tissues produced by high blood vascular flow and limited increase in blood pressure, Marginal gill lamellae with marginal gill channels dilated which might increase surface area in contact with the ecosystem to increase gas exchange between blood and water, alterations of the cell in gills. Curved secondary lamellae cause an offshoot of the primary filaments. Gills lamellae aneurysms which cause irregular blood-filled enlargement of blood vessels which cause disruptions in the moment of blood in the gills were observed in *L. rohita* exposed to sodium metabisulfite.

The kidneys perform a significant role associated with water balance, and electrolytes, and maintain homeostasis by eliminating nitrogenous compounds like creatinine, ammonia and urea[12, 19]. In a recent study, in both treatment groups, the kidney of *L. rohita* showed various histological alterations such as sinusoidal spaces, tubules with hypertrophied nucleus in renal tubular cells which cause a decline in renal function, cloudy swelling degeneration means vacuoles are absent in pretentious cells, instead, the cytoplasm is weak and organelles are usually detached within the complex cytoplasm and inflammation resulted in blocking of the tubular lumen[20]. Glomerular expansion or dilated renal tubules may cause the development of fibrosis within the kidney and it is a significant physical lesion that is categorized by the abnormal proliferation of mesangial cells and extra production of matrix protein, a fusion of individual cell margins, condensation of epithelial cells of tubules was represented the toxicity of sodium metabisulfite. Similar progressive variations like distention of tubules and necrosis of tubular epithelium of the kidney were observed by Majumder and Kaviraj[21].

The liver is an essential organ that is damaged by the pollutants in the water bodies owing to its role in decontamination and biological conversion methods[22-24]. In recent research examined that the liver of *L. rohita* was exposed to sodium metabisulfite ($Na_2S_2O_5$) showed progressive alterations like cloudy swelling of hepatocytes, degeneration of hepatocytes with enlargement of nucleus, cluster nuclei, melanomacrophage that are phagocytes that synthesize melanin, cytoplasmic vacuolization is a recognized process observed in mammalian cells after exposed to viral pathogens and bacteria as well as to several natural and artificial lower molecular weight compounds, hepatocytes (major parenchyma cells in the liver) with irregular nucleus in both long and short periods. Sarkar, Chatterjee[25] and Waris, Hayat[26] described hepatocyte disruption, vacuolation, and cytoplasmic degeneration which shows pathological conditions that cause cells to change in structure and functions. Injury is mainly in the cytoplasm of the cell these lesions are reversible when pathological stimuli are reduced or eliminated, melanomacrophage, cluster nuclei in *L. rohita* upon sodium metabisulfite exposure. Irregularities such as hepatocytes and access amount of blood in arteries were observed in the liver of *L. rohita* upon exposure to sodium metabisulfite.

CONCLUSION

It was concluded that sodium metabisulfite caused degenerative effects in vital organs such as gills, liver, and kidneys of the exposed fish *Labeo rohita*. It affected the morphology and physiology of the gills, kidneys, and liver of the exposed fish. Since it adversely affects the normal organs of the fish, it may have an indirect impact on the consumers within the food chain. So, there is a dire need for adequate checks and balances on the industrial effluents that contaminate freshwater bodies like rivers and lakes. There is a need to avoid the introduction of such toxicants into the aquatic life and water bodies.

CONFLICT OF INTEREST

All authors have no potential conflict of interest.

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