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# Toxicological impact of silica nanoparticles on the liver of freshwater spotted snakehead *Channa punctatus* (Bloch, 1793): Light microscopic study

**Gyanendra B. Chand* & Aparna Singh**
Aquatic Toxicology Laboratory, P.G. Department of Zoology, Patna University, Patna, Bihar, India

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**Abstract-** The present study investigates the toxicological effects of silicon dioxide nanoparticles ($SiO_2$NPs) on the liver of the freshwater fish *Channa punctatus,* a species widely used as a bioindicator of aquatic pollution due to its ecological and economic importance. Pre-characterized $SiO_2$NPs (<100 nm, 99.9% purity) were administered at varying concentrations of 1.2 ppm and 2.3 ppm for 7 days and 14 days respectively to explore acute and sub-acute toxicity in fish. Histological examinations revealed progressive liver damage including fibrosis, hepatocellular necrosis, vascular congestion, cytoplasmic vacuolization, infiltration of inflammatory cells, and signs of hepatocellular Carcinoma etc. All these changes are indicative of severe hepatic stress and immune response. Observed histopathological alterations intensified with both concentration and exposure duration. The findings align with existing literature on nanoparticle-induced hepatic toxicity in aquatic organisms and raise concerns about the environmental and biological risks of $SiO_2$NPs, especially considering their increasing industrial application. Given the potential for bioaccumulation and trophic transfer, this study underscores the urgent need for regulated nanoparticle use and conservation strategies to protect aquatic ecosystems and public health.

**Keywords: Acute toxicity, Behavioural responses, *Channa punctatus*, Conservation, Environment, Histopathology, Hepatic tissue, $LC_{50}$, Silica nanoparticles**

## INTRODUCTION

Nanotechnology is a rapidly evolving scientific field that integrates principles of engineering and technology to create, manipulate, and utilize particles ranging in size from 1 to 100 nanometers. It is inherently interdisciplinary, merging knowledge from physics, chemistry, biology, mathematics, materials science, and engineering to form a collaborative framework for nanoscale innovation.[1,2] As per the definition of International Organization for Standardization (ISO) and the European Committee for Standardization, which was later adopted by the Organization for Economic Co-operation and Development (OECD), nanomaterials are substances with at least one dimension in the nanoscale range (1–100 nm) and display novel properties distinct from those of the same materials in larger forms.[3] Nanomaterials exhibit unique features compared to their larger counterparts, such as increased surface area, altered solubility, and varied aggregation behaviour. These changes make them advantageous for a range of scientific and industrial applications. Nanomaterials may be natural, incidental, engineered, and anthropogenic.

*Corresponding author :
Phone : 9431406660
E-mail : gbchand@patnauniversity.ac.in
singhanna001@gmail.com

Silica nanoparticles ($SiO_2$NPs) were chosen for this study due to their extensive application across various sectors, including biomedicine, agriculture, and industry, which raises concerns about their potential environmental and biological impacts. Their unique physicochemical properties-such as high surface area, stability, and modifiable surfaces, bio-compatibility, low toxicity, tuneable properties, surface functionalization, stability, porosity-make them effective tools in various biotechnological applications.[4] The global market for silica has experienced notable expansion in recent years, driven by its extensive use across multiple industries. In terms of market size, the global silica market was valued at approximately USD 48.2 billion in 2023. It is projected to increase to USD 51.28 billion by 2024 and reach around USD 84.10 billion by 2032, registering a compound annual growth rate (CAGR) of 6.38% over the forecast period from 2025 to 2032.

Silica occurs in two primary structural forms: crystalline and amorphous. Crystalline silica has a highly ordered atomic structure, while amorphous silica features a more disorganized molecular arrangement.[5,6] The toxicity and environmental impact of $SiO_2$NPs in living organisms depend on multiple factors, including their chemical stability, tendency to persist in the environment, and potential for biological accumulation.[7-9] Silica nanoparticles have been reported to cross the blood-brain barrier in animal models.[10] *In vitro* studies using human cell lines have demonstrated that $SiO_2$NPs can cause mitochondrial damage, oxidative stress, and cell death.[11] Reduced cell survival and increased cytotoxicity have been reported in endothelial cell lines (EAHY926), following exposure to $SiO_2$ nanoparticles.[12] Intranasal administration of silica nanoparticles caused reduced antioxidant activity and disrupted hepatocellular architecture.[13] Similarly, fullerene C60-a different type of nanomaterial-was found to trigger oxidative stress in liver cells of freshwater fish *Pseudetroplus maculatus* (Bloch, 1795)[14].

*Channa punctatus*, commonly referred to as the spotted snakehead, is a notable freshwater species belonging to the family *Channidae.*[15] The fish is recognized for classifying it as a lean species throughout the year. Due to its low lipid content, nutritional value and market demand, it holds significant economic importance.[16-19] This species is widely distributed across South Asian countries, including Afghanistan, Pakistan, India, Nepal, Bangladesh, Sri Lanka, Myanmar, and parts of southern China such as Yunnan.[20] Although commonly found in freshwater habitats such as rivers and ponds[21,22] the species is also known to inhabit brackish waters and wetlands including beels and ditches.[23-25]

The liver plays a crucial role in the fish's metabolism and serves as the primary organ for accumulating, transforming, and eliminating contaminants.[26] It is involved in producing and secreting important substances such as albumin, prothrombin, fibrinogen, lipoproteins, bile, and cholesterol. Additionally, it supports lipid metabolism, detoxification of harmful substances, nitrogen breakdown, fat digestion, and immune defense, while receiving arterial blood through the hepatic artery. Though the liver has the capacity to neutralize toxins, continuous exposure can lead to damage over time. Due to its sensitivity to various toxins, the liver is often used as a biological indicator of water pollution.[27,28] The present study has been designed to study the systematic deleterious impact of silicon dioxide nanoparticles ($SiO_2$NPs) on the liver of the freshwater fish *Channa punctatus.*

## MATERIALS & METHODS

### Experimental Animal

Healthy *Channa punctatus* specimens were procured from the local fish market at Bazar Samiti, Patna and brought to the Aquatic Toxicology Laboratory, Department of Zoology, Patna University, disinfected with 0.1% KMnO solution for 30 minutes. The fish were then acclimated for a period of 15 days under ideal laboratory conditions, in accordance with APHA (2017) guidelines. Water was largely replaced daily using a pump to maintain cleanliness. Throughout the acclimation period, the fish were fed to satiation each day with Tokyu and Ultima micro-pellets.

### Procurement, Characterization and Preparation of Stock Solution of $SiO_2$NPs

$SiO_2$NPs was purchased from Nano Research Lab (NRL), Jharkhand, India. $SiO_2$ nanoparticles with a size less than 100nm were used in this study with a purity of 99.9% and a molecular weight of 60.08g/mol. The morphology was determined to be spherical with a bulk density of 0.10 g/cm$^3$. $SiO_2$NP stock solutions were prepared by dispersing NPs in double -distilled water by sonication. Ultrasonic treatment was performed for 45 minutes in a bath type sonicator (40kHz) and stored as a stock. During experimental period, the test solution in each

experimental group was replenished daily to keep the concentration and distribution of $SiO_2$NP relatively constant.

**Experimental Design**

Pre-characterized silicon nanoparticles (SiNPs) were used to assess toxicity in fish. The $LC_{50}$ of silica nanoparticles for the fish was determined by probit regression analysis.[29] Two doses of 1.2 ppm and 2.3 ppm was selected for its administration to the fish for 7 days and 14 days respectively to explore acute and sub-acute toxicity in fish. The acclimated fish were grouped into **Control** Group (C) and Treated Groups: **$T_1$** - 1.2 ppm SiNPs treated for 7 days; **T2**- 1.2 ppm SiNPs treated for 14 days; **T3-** 2.3 ppm of SiNPs treated for 7 days and **T4**-2.3 ppm of SiNPs treated for 14 days respectively.

Throughout the experimental period, fish were monitored daily to record mortality, clinical signs, and behavioural changes through direct observation. Notable behavioural alterations included increased air gulping, heightened aggressiveness, and rapid pectoral fin movements during the initial stages. As exposure progressed, fish exhibited reduced pectoral and caudal fin movements, decreased air gulping, and a tendency to remain stationary in one corner of the tank.

## HISTOLOGICAL SAMPLE PREPARATION & MICROPHOTOGRAPHY

After the schedule exposure, the fishes were decapitated and the liver tissue were removed and cleaned in cold normal saline. The hepatic tissues were fixed for 5 days in buffered Neutral Formalin and washed overnight under running tap water. They were dehydrated in graded series of alcohol for 2 hours in each grade with changes in between, cleaned in absolute alcohol and xylene (1:1) and then taken in pure xylene for 1 hour with a change in between. Tissues were embedded in molten paraffin wax with ceresin (Melting point 58°C-68°C) after passing through xylene and molten wax (1:1). Paraffin spread sections were double stained using hematoxylin and eosin, cleared in xylene and mounted in DPX. The microphotography has been done on the Trinocular Olympus 2000 Light microscope fitted with camera and laptop. The micro-photographic interpretations have been done using standard histological Atlas of Teleost.

## RESULTS & DISCUSSION

The health status of fish in all experimental groups was consistently monitored throughout the study. The behavioural alterations included weak (+) to very severe (++++) increased air gulping, heightened aggressiveness, erratic swimming and rapid pectoral fin movements during the initial stages. As exposure progressed, fish exhibited reduced pectoral and caudal fin movements, decreased air gulping, and a tendency to remain stationary in one corner of the tank (Table-1).

**Table-1: Showing behaviour score of *C. punctatus* on exposure to SiNPs**

| Concentration (mg/L) | Air gulping | Swimming | Aggression | Resting at the bottom |
|---|---|---|---|---|
| **0** | - | - | - | - |
| **600** | + | + | + | + |
| **700** | ++ | ++ | + | ++ |
| **800** | ++ | +++ | ++ | +++ |
| **900** | ++ | ++++ | ++ | ++++ |

**Note**: The symptoms scores were recorded as follows:
(-) No, (+) weak, (++) moderate, (+++) severe, (++++) very severe.

## HISTOLOGICAL OBSERVATION OF THE LIVER IN CONTROL FISH:

In teleost fishes, the liver is a large organ with two primary lobes-left and right-which are further subdivided into anterior and posterior sections. Each of these is again divided into multiple smaller lobes. The fundamental structural and functional unit of the liver, the hepatic lobule contains two or sometimes three types of tissues: hepatic, parenchymal tissue and the bile excretory system. Hepatic cells are arranged in hepatic chords, well separated by sinusoids, draining into the central vein. Besides bile artery, hepatic portal vein showed the normal cytoarchitecture (Plate- 1, Fig. 1, 2, 3 & 4; Plate 2, Fig.1, 2, 3 & 4).

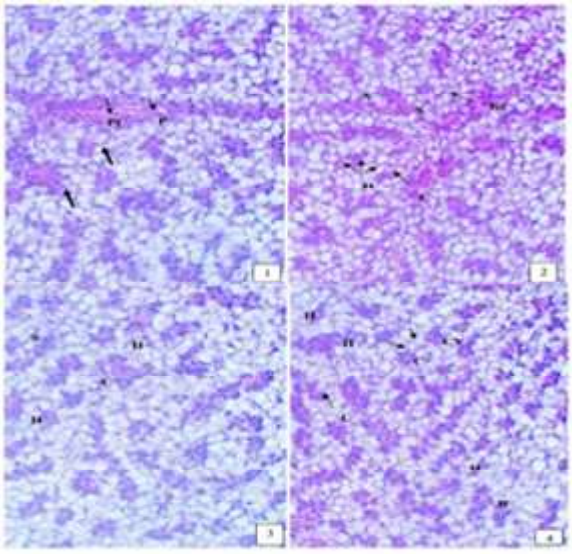

**Plate-1**

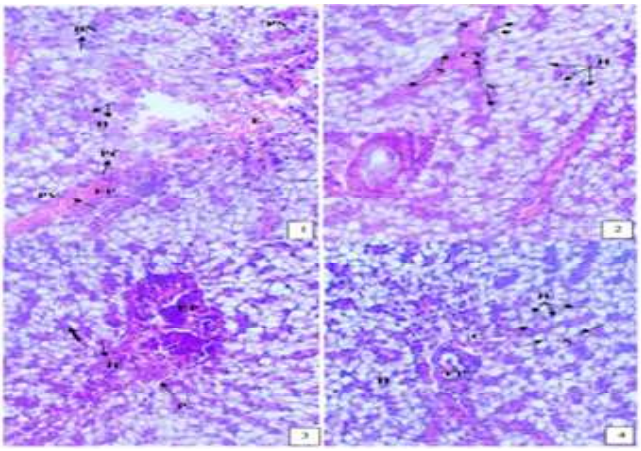

**Plate-II**

**Fig Control liver of fish: Hepatic cells (H) are arranged in hepatic chords, well separated by sinusoids (S), draining into the central vein (CV). Besides bile artery (BA), hepatic portal vein (PV) showed the normal cytoarchitecture X400**

### 1.2 ppm $SiO_2$NPS treated liver of fish

Transverse section of liver of $SiO_2$NPs (1.2 ppm for 7days) treated fish showed deposition of fibrous tissue around the central vein which was itself filled with eosinophilic inclusion. Besides, dilated sinusoids and enucleated hepatocytes, abundant connective tissue and blood vessels were marked at this exposure level. Besides, congested central vein, necrotic clump of degenerating hepatocytes, bridging necrosis of hepatocytes, degeneration in bile duct and appearance of clumps of eosinophils were prominent anomalies at this exposure level (Plate III. Fig. 1, 2, 3 & 4).

Transverse section of liver of $SiO_2$NPs (1.2 ppm for 14 days) treated fish showed fibrosis in the hepatic artery, appearance of cytoplasmic lipid vacuoles and complete degeneration in the cytoarchitecture of hepatic tissues. However, enucleated hepatocytes, presence of bridging necrosis, appearance of wide and disorganized hepatocytes were prominently marked. The ballooning of hepatocytes was seen at few places. Portal vein gets filled with eosinophilic inclusions, basophils, eosinophils and marked with a massive thickening of the parenchymal cell surrounding the portal vein. The sinusoids showed congestion and filled with eosinophilic inclusions and few pus cells. Kupffer cells showed increase in number marking induced phagocytic activities. The clumps of pyknotic nuclei, congested central vein, excessive vacuolations were prominently marked indicating severe liver damage and inflammatory responses following nanoparticle exposure (Plate-IV, Fig. 1, 2, 3 & 4).

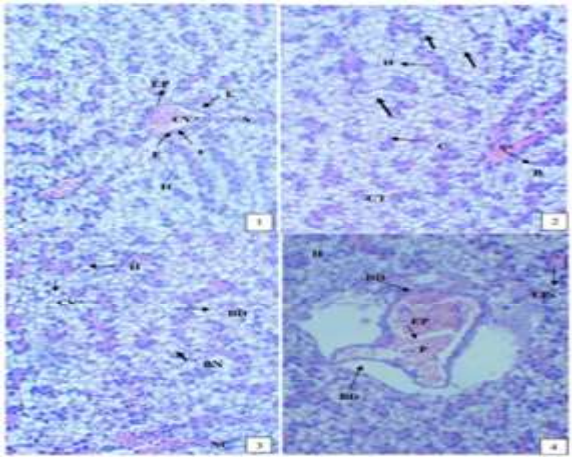

**Plate-III, Fig. 1,2,3 & 4: 1.2 ppm $SiO_2$ NPs treated liver of fish for 7 days showing deposition of fibrous tissue around the congested central vein with eosinophilic inclusions, necrotic clump of degenerating enucleated hepatocytes, bridging necrosis of hepatocytes, degeneration in bile duct and appearance of clumps of eosinophils X400**

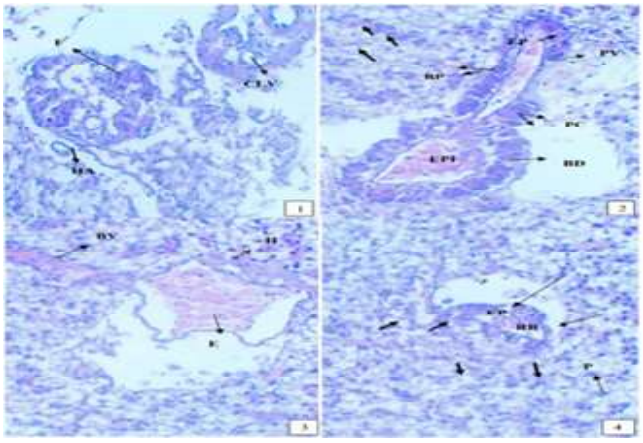

**Plate-IV, Fig. 1, 2, 3 & 4: 1.2 ppm $SiO_2$NPs treated liver of fish for 14 days showing clumps of pyknotic nuclei, congested central vein, excessive vacuolation, fibrosis in the hepatic artery, enucleated hepatocytes with bridging necrosis and ballooning of hepatocytes, sinusoids congested with eosinophilic inclusions and few pus cells; Portal vein filled with eosinophilic inclusions, basophils, and marked with a massive thickening of the parenchymal cell around it. X 400**

### 2.3 ppm $SiO_2$NPS treated liver of fish

The transverse section of liver of 2.3 ppm $SiO_2$NPs treated fish for 7 days showed increased number of swollen hepatocytes, few binucleated hepatocytes. At few places, the sign of hyperplasia of hepatocytes were prominently marked, indicating both degenerative and proliferative responses to the nanoparticle exposure. Besides dilation

of sinusoids, appearance of prominent clumps of pycnotic nuclei, haemorrhagic clot within bile duct were prominent anomalies incurred at this exposure level. However, dilated lumen of portal vein was reported to be filled with eosinophilic inclusions, erythrocytes, plasma cells and even few degenerating hepatocytes (Plate- V, Fig. 1, 2, 3 & 4). The exhibited pronounced histopathological changes were considered as indicative of cellular stress and immune response initiated by the liver of the fish. These findings collectively suggest significant liver damage and immune modulation due to the administered $SiO_2$ nanoparticles.

The transverse section of 2.3 ppm $SiO_2$NPs treated liver of fish for 14 days showed fibrosis of chronic inflammatory cells suggesting backgrounds of cirrhosis. Slightly enlarged nuclei and occasional prominent nucleoli with HCC (Hepatocellular Carcinoma), and congested sinusoids were prominently marked (Plate VI, Fig.1).

Besides, massive necrosis in hepatocytes, infiltration of macrophages & lymphocytes, clumps of pyknotic nuclei, enucleation of hepatocytes with hyalinized cytoplasm, increased vacuolization, appearance of increased number of Kupffer cells, widespread hepatocellular necrosis, dissociation of hepatocyte chords, massive hepatocellular dissociation and necrotizing hepatitis were prominent anomalies incurred at this exposure level. Besides, swollen and disintegrated parenchyma cells with thick deposition of fibrous tissue around the portal vein was prominently marked. Infiltration of numerous macrophages and lymphocytes in the portal vein was marked. The central vein showed congestion. Both portal vein & hepatic artery was congested with eosinophilic inclusions, plasma cell, and pus cells (Plate –VI Fig. 2, 3 & 4). The presence of plasma cells and eosinophilic inclusions are indicative of an immune response. The deposition of fibrous tissue around the bile duct suggests fibrosis. The presence of pus cells within the sinusoidal Spaces, further points toward an inflammatory or possibly infectious process within the hepatic parenchyma. The heavy depositions of silica nanoparticles in the hepatic parenchyma advocates the tendency of silica nanoparticles to be deposited in the fatty tissues of the aquatic organism (Plate-VII, Fig. 1). Congested sinusoidal spaces are also evident, which may reflect dilation or disruption of normal hepatic architecture due to stress or damage with heavy deposition of silica nanoparticles. The appearance of densely packed inflammatory cells and erythrocytes, eosinophilic inclusions, haemorrhagic clots within bile duct and portal vein marks a characteristic of portal inflammation or cholangitis and chronic vascular congestion (Plate VII, Fig.2, 3 & 4; Plate VIII, Fig. 1, 2, 3 & 4). These observations collectively indicate that prolonged exposure to $SiO_2$NPs at this concentration induces severe liver damage, chronic inflammation, and potentially carcinogenic changes.

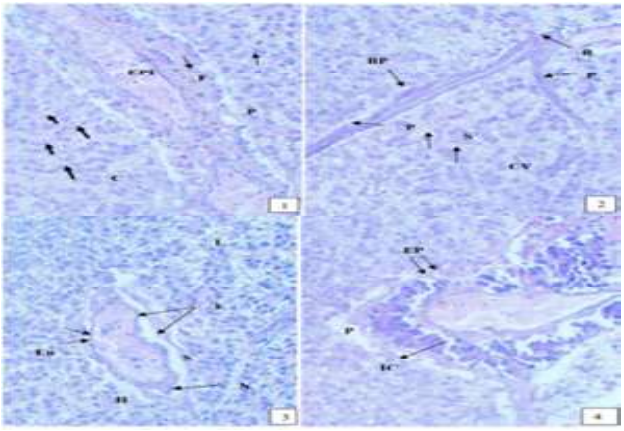

**Plate-V Fig. 1, 2, 3 & 4: 2.3 ppm $SiO_2$NPs treated fish for 7 days showing sign of hyperplasia of swollen binucleated hepatocytes, dilation of sinusoids, prominent clumps of pycnotic nuclei, bile duct congested with haemorrhagic clot, dilated lumen of portal vein filled with eosinophilic inclusions, erythrocytes, plasma cells and few degenerating hepatocytes X 400**

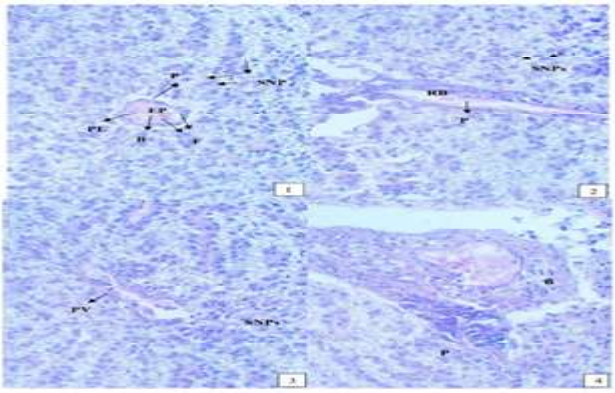

**Plate-VI Fig. 1, 2, 3 & 4: 2.3 ppm $SiO_2$NPs treated fish for 14 days showing solid compact mass of polygonal cells and fibrosis of chronic inflammatory cells, enlarged nuclei and occasional prominent nucleoli with HCC (Hepatocellular Carcinoma), and congested sinusoids. massive necrosis in hepatocytes, infiltration of macrophages & lymphocytes, clumps of pyknotic nuclei, enucleation of hepatocytes with hyalinized cytoplasm, increased vacuolization, appearance of increased number of Kupffer cells, widespread hepatocellular necrosis, dissociation of hepatocyte chords, massive hepatocellular dissociation and necrotizing hepatitis X 400**

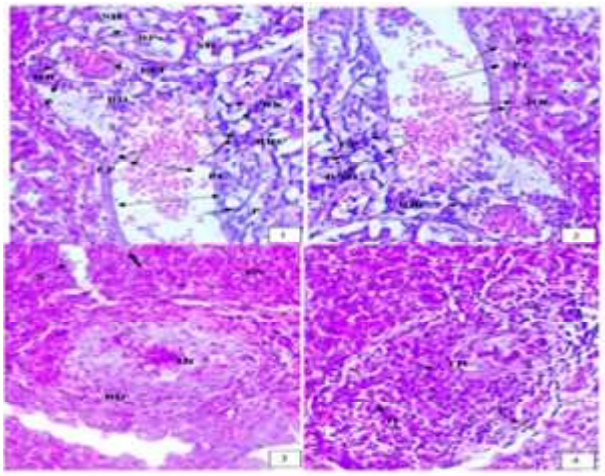

**Plate-VII Fig. 1, 2, 3 & 4: 2.3 ppm $SiO_2$NPs treated fish for 14 days showing plasma cells and eosinophilic inclusions in the portal vein, deposition of fibrous tissue around the bile duct, pus cells within the sinusoidal spaces, heavy depositions of silica nanoparticles in the hepatic parenchyma, densely packed inflammatory cells and erythrocytes, haemorrhagic clots within bile duct and portal vein. X 400**

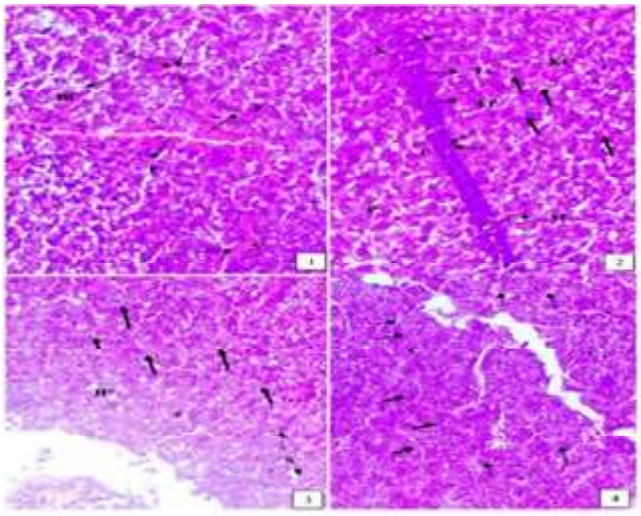

**Plate-VIII Fig. 1, 2, 3 & 4: 2.3 ppm $SiO_2$NPs treated fish for 14 days showing the appearance of densely packed inflammatory cells and erythrocytes, eosinophilic inclusions, haemorrhagic clots within bile duct and portal vein marking a characteristic of portal inflammation or cholangitis and chronic vascular congestion X 400**

Duan *et al*. (2018)[30] investigated the effects of silica nanoparticles ($SiO_2$NPs) on liver lipid metabolism both *in vivo* and *in vitro*. Their histopathological analysis revealed that, by day 15 (end of the exposure period), granulomas had formed in the livers of SiNP-treated mice, consisting primarily of macrophages, necrotic hepatocytes, and infiltrating lymphocytes. However, by day 60 (following a 45-day recovery), these granulomas showed reduced cellularity and the presence of pink-stained collagen fibres. Additionally, mild hepatic steatosis was evident at this later time point, characterized by round lipid droplets in the hepatocyte cytoplasm, with some vacuoles merging into larger ones. $SiO_2$NPs were also detected in the peri-sinusoidal space alongside collagen fibres, suggesting progression from inflammation to lipid accumulation in liver tissue following repeated $SiO_2$NPs exposure.

Our findings are in agreement with the findings of Vidya *et al*. (2019)[31] who examined the hepatic responses of *Oreochromis mossambicus* to silica nanoparticle toxicity. In untreated fish, liver tissues displayed normal architecture with well-defined parenchymal hepatocytes possessing clear cytoplasm and round nuclei. However, exposure to $SiO_2$NPs at 5 mg/L resulted in disrupted liver structure. After 24 hours, mild vacuolization was noted, which progressed to nuclear disintegration and more pronounced vacuole formation by 48 hours. After 96 hours, hepatocytes exhibited severe cytoplasmic vacuolization and infiltration of leukocytes.

These hepatic changes, such as disorganized parenchyma and altered nuclear morphology, reflect acute toxicity effects. The increased vacuolization indicated a disruption in the balance between the synthesis and release of cellular lipids and glycogen. This vacuole formation likely serves as a defense mechanism, attempting to isolate harmful substances and protect hepatocyte function. The continued exposure led to cytoplasmic vacuoles filled with lipids and glycogen-key metabolic components-indicating cellular degeneration. Such histological damage can impair liver function and potentially impact the survival and ecological fitness of affected fish populations.

Similar kind of $SiO_2$NPs toxicity was assessed in gill, liver, and brain tissues in *Oreochromis mossambicus.*[32] They reported that after 96 hours of exposure, segmented hepatocytes and spindle-shaped nuclei were prominent. Prolonged exposure (60 days) caused extensive tissue damage, including severe vacuolization, nuclear loss, and complete disruption of hepatocyte structure. Appearance of vacuoles in hepatocyte cytoplasm, typically containing glycogen and lipids essential for normal liver function, indicated a metabolic imbalance caused by the nanoparticles. This vacuolization, often considered the liver, being central to metabolism and detoxification, highly

susceptible to damage from environmental pollutants, with $SiO_2$NPs accumulating in hepatic tissue at levels far higher than those in surrounding environments.

Furthermore, short-term exposure led to minor neuronal damage, while long-term exposure resulted in significant neurodegeneration, including vacuole formation, cerebral swelling, neuro-fibrillary necrosis, and damage to the choroid plexus. Comparable neurotoxic responses have been observed in *Pseudetroplus maculatus* exposed to other environmental pollutants like chlordecone.[32]

Somani and Sharma (2020)[33] investigated the impact of $SiO_2$NPs on freshwater fish *Clarias batrachus*. Their findings revealed several histopathological alterations in the liver tissues of exposed fish. These included increased hepatocyte proliferation, infiltration by inflammatory cells, nuclear pyknosis, cytoplasmic vacuolation, aggregation of melano-macrophages, blood vessel dilation, liver cell necrosis, central vein wall rupture, and the presence of apoptotic cells. Additionally, a marked reduction in liver glycogen content was noted, indicated by a faint magenta coloration. The study also reported a statistically significant increase in both the size and number of melano-macrophage centres (MMCs) in the liver tissues of all exposed groups when compared to the control group.

Duan *et al.* (2018)[30] while conducteing a study on $SiO_2$NPs induced hepatic lipid metabolism disorder *in vivo* and *in vitro*. revealed that the Toll-like receptor 5 (TLR5) signalling pathway plays a central role in the disruption of hepatic lipid metabolism caused by SiNP exposure. The nanoparticles were found to induce hyper-lipidemia and interfere with normal lipid regulation in the liver. Specifically, SiNPs promoted lipid synthesis by upregulating key enzymes such as acetyl-CoA carboxylase 1 (ACC1) and fatty acid synthase (FAS), while simultaneously reducing fatty acid β-oxidation through suppression of carnitine palmitoyl transferase 1A (CPT1A) protein levels. This disruption in the balance between lipid production and breakdown can result in metabolic disturbances, which are major contributing factors to the development of fatty liver disease and atherosclerosis. The findings suggest that prolonged or repeated exposure to SiNPs may increase the risk of liver steatosis and cardiovascular disease.

Kawade (2020)[34] examined the similar histo pathological changes in the liver of the freshwater fish *Channa gachua* following acute exposure to nickel. The study identified several structural alterations, including the shrinkage of the central vein, accumulation of blood cells within it, sinusoidal rupture, and degeneration and necrosis of both hepatocytes and connective tissue. Our findings align with the liver tissue damage observed in *Oreochromis niloticus* (Nile tilapia) subjected to nickel and lead exposure, as reported by Shahid *et al.* (2020)[35]; cellular shrinkage and nuclear degeneration in the liver of *Channa punctatus* exposed to lead reported by Sastry and Gupta (1978)[36]. These pathological alterations are generally attributed to interactions between metal ions and liver tissues, leading to cellular degradation and impaired metabolic processes, as described by Hinton and Lauren (1990)[37] and Nikalje *et al.* (2012)[38]. According to Sorenson *et al.* (1980)[39], the build-up of nickel in liver tissues disrupts essential physiological functions, including digestion, protein synthesis, and detoxification. Such disruptions compromise the internal stability of fish, as also emphasized by Jalaludeen *et al.* (2012)[40].

Abdel-Latif *et al.* (2021)[41] investigated hazardous effects of $SiO_2$NPs on liver and kidney functions, histopathology Characteristics, and transcriptomic responses in Nile Tilapia (*Oreochromis niloticus*) juveniles.

Mengqi Sun *et al.* (2024)[42] explored on silica nanoparticles induced liver lipid metabolism disorder via ACSL4-mediated ferroptosis. Lipid metabolism imbalance in the liver is recognized as a crucial factor in the development of non-alcoholic fatty liver disease (NAFLD). However, the specific molecular pathways by which SiNPs contribute to such disturbances and NAFLD progression remain unclear. Acyl-CoA synthetase long-chain family member 4 (ACSL4) functions as a key regulatory enzyme in the oxidation of long-chain polyunsaturated fatty acids (PUFAs), playing a direct role in lipid metabolism within liver cells. Ferroptosis, a distinct type of regulated cell death, is uniquely linked to ACSL4 activity and not associated with other enzymes in the same family.[42] While reports of SiNP-induced ferroptosis are limited, recent findings suggest that SiNP exposure may trigger this form of cell death in human umbilical vein endothelial cells UVECs).[42]

Kaur and Mishra (2019)[43] examined the liver histopathology of *Channa punctatus* following exposure to sub-lethal levels of a hybrid pesticide. Their observations revealed severe cellular degeneration in the liver,

hypertrophy of hepatocytes, and damage to the hepatic portal vein. These findings point to a direct toxic impact of hybrid pesticide exposure on liver tissues. Similar liver damage has been observed in *Siluriform corydoras* exposed to organophosphate pesticides, where abnormalities like irregularly shaped nuclei in hepatocytes and cytoplasmic vacuoles were noted.[43] Srivastava and Prakash (2019)[44] suggested that the significant hepatocyte destruction and the appearance of intercellular gaps might result from the rapid breakdown and use of glycogen reserves to meet the energy demands induced by toxic stress. The current study also reported the appearance of intracellular vacuoles, necrosis, and pyknotic nuclei in *Channa punctatus* exposed to pesticides. Vacuoles within the hepatocyte cytoplasm are often associated with stored lipids and glycogen, essential for normal liver metabolic functions. Under stress, a decline in hepatic glycogen is commonly observed, as glycogen is converted into glucose to meet elevated energy requirements.[44] According to Pacheco and Santos (2002)[45], increased vacuolization in hepatocytes can be an indicator of degenerative changes, pointing to possible metabolic dysfunctions due to exposure to pollutants. These histological alterations not only impair liver function but may also lead to bile accumulation, a sign of disrupted hepatic metabolism.[45] Supporting this, Olarinmoye *et al.* (2009)[46] reported extensive liver damage, including vacuole degeneration of hepatocytes, necrosis, pancreatic tissue damage, and overall structural disorganization.

Abdel Rahman *et al.* (2022)[47] have studied on Silica nanoparticles acute toxicity alters ethology, neuro-stress indices, and physiological status of African catfish (*Clarias gariepinus*). $SiO_2$NPs have been shown to exert harmful cellular effects primarily through triggering inflammation and oxidative stress.[48] ROS generation is considered as one of the primary mechanisms behind $SiO_2$NPs-induced cellular damage[48], as these reactive species initiate oxidative modifications within cells and can lead to apoptosis via intrinsic pathways.[49,50] In a related study on *Oreochromis mossambicus*, Vidya and Chitra (2015)[51] reported that $SiO_2$NPs exposure increased intracellular ROS levels and depleted antioxidant reserves in the liver. Their follow-up study further noted a reduction in key antioxidant enzymes (SOD and GPX) and an increase in hydrogen peroxide and lipid peroxidation after exposure to SiO, nanoparticles.[52] Similarly, Gopi *et al.* (2019)[53] observed that *O. niloticus* exposed to sub-lethal concentrations of copper experienced oxidative stress and significant changes in antioxidant enzyme activity.

## CONCLUSION

The present study focused on the toxicological impact of $SiO_2$NPs on the liver of the freshwater fish *Channa punctatus.* Histological examination of the liver in $SiO_2$NPs treated fish showed significant hepatic damage, particularly affecting the central vein, sinusoids, hepatocytes, and various vascular structures. The scientific output of the present study may be considered for making conservation strategies for the aquatic community against non- judicious use of $SiO_2$NPs for the welfare of the human kind, especially for developing countries like India. Since in the present Global scenario, India and China are the two major countries, who are leading global economy related to engineered nanoparticles. Any xenobiotics finds its way to the aquatic bodies through agricultural run-off and other anthropogenic interventions. Fishes get easily victimized to such toxicants. Even the exposure of these fishes to $SiO_2$NPs at environmentally relevant concentration led to such an extensive alteration in the hepatic tissues. In future, if we add the use of $SiO_2$NPs in such a pace, it will definitely be going to have some detrimental effect. Through food chain, it may enter in the human beings especially in piscivore community and would have serious implications. From the bio-conservation of the aquatic organism point of view, the present study holds substantive scientific importance.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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