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Histopathological Effects of Silicon Dioxide Nanoparticles on Some Tissues of *Labidochromis caeruleus* in the Larval Period

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Key words: Silicondioxide, nanoparticle, *Labidochromis caeruleus,* histopathology, gill, liver, eye and brain

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Abstract: Silicon dioxide is widely used today for different purposes in engineering, biotechnology, medicine and in many daily life products. Large-scale production and use have increased the risk of exposure to silicon dioxide nanoparticles (SiO_2NPs). The present study has focused on the evaluation of this issue by using a nanoparticle, SiO_2NP, in an aquatic model organism, *Labidochromis caeruleus* larvae. Fish were exposed to SiO_2NPs in three different doses and general histological methods were applied. At the same time, histopathological changes were categorized by semiquantitative scoring. Histological sections of gill, liver, eye and brain tissues were examined under a light microscope after staining with hematoxylin and eosin. Distortion of lamellae shapes in gill tissue, rupture and detachment of primary lamellae, shortening and rupture of secondary lamellae and separation of epithelium were observed. Oedema under the capsule in the liver, pycnotic nucleus in hepatocytes, necrosis in different parts of the parenchyma and large vacuoles were observed. Thinning of the corneal layer in the eye tissue, separation of the lens epithelium and fragmentation of the lens fibres were noted. In the brain tissue, astrocytes were intensely observed in the forebrain, medulla oblongata and spinal cord only in the high dose group. These structural changes are advanced in gill and liver tissues, moderate in the eye, and mild in the brain. These findings reveal that even short-term exposure of fish to SiO_2NPs may pose a potential risk.

INTRODUCTION

With the increase in nano technological applications, nano materials have started to be used for many consumer products and have led them to enter our daily lives at a higher rate[1]. The functions and usage areas of these materials differ according to the size and composition of the nanoparticles[1-4]. Nano particles are important due to their high surface-to-volume ratio and are used in many areas including household appliances, the pharmaceutical

industry, optical materials, sensors studies, industry and biomedical applications[2].

In recent years, research on the toxicologically relevant properties of engineered nano particles has increased tremendously. Their widespread use causes potential exposure to engineered nano particles throughout the entire life cycle of living things. When looking at the possible exposure routes for the produced nano particles, it is clear that inhalation, dermal and oral exposure are the most dependent on the product type in which the nano particles are used.

SiO_2 is the basic component of materials such as glass and cement. It is used in furnace construction due to its high melting temperature in many materials such as concrete, tiles and porcelain. SiO_2NPs are used in drug applications, cancer therapy, enzyme immobilization, biosensor and biomarker in engineering, biotechnology and medicine[5-14]. In addition, it is widely used as a food additive in toothpaste and skin care in cosmetics, as a flavour and consistency enhancer in salt, spice and sugar products and as an anti-foaming food additive in some beverages[15-19].

In recent years, studies on nano-sized materials have shown great progress towards becoming an important field on its own. As with every new technology, the effects of this technology and its products on the environment and health are also of interest. Rapid developments in nano technology have focused attention on nano particles, which form the essence of this technology and although the first experimental observations have reached the dimensions to justify this suspicion, it is still controversial.

Information on the interaction of nano particles with biotic and abiotic components is limited and there is currently no reliable method to assess nano material toxicity. It is seen that most of these studies conducted with histological, biochemical and molecular methods focus on the effects of various metal nanoparticles at different doses on different tissues of experimental animals such as mice and rats[20-24].

It has been reported that especially as a result of metal NPs causing water pollution and accumulating in fish, their bio availability poses a significant risk to human health and the environment[25-26]. For example; it has been reported that Cu-NPs have toxic effects on kidney, liver and gill tissues in three-month-old *Cypnus carpio* species[27].

Toxicological studies of toxic substances discharged into aquatic environments are of great importance in terms of the existence of biological life and the protection of nature. There is a lack of data on the bio accumulation, biotoxicity and bio degradation of NP in environmentally-related species. Therefore, comprehensive statements about the toxicity of nano-sized materials cannot be made at present. This study was planned and thought to be useful In order to contribute to the completion of this deficiency in the future.

Silicon dioxide (silica) is among the widely used nano particles today. Large-scale production and use have increased the risk of exposure to SiO_2NPs. Although there are a few studies conducted in recent years, the ecoto xicological effects on fish are not fully understood[29-30]. In this study, the possible toxic effects of SiO_2, which is used for different purposes in many areas, were histologically evaluated on the gill, liver, eye and brain tissues of *L. caeruleus* larvae, which are thought to be an example of the aquatic environment.

MATERIAL METHODS

Fish care and work plan: All fish care and experimental processes were conducted with the permission of the Local Animal Experimentation Ethics Committee of Adnan Menderes University (Approval number: FEF-2017/046) and performed in accordance with Adnan Menderes University Animal Ethics Rules. The number of fish was limited to three runs for each experimental procedure. For the study, rootstock fish were obtained from ADU/Agriculture Faculty, Department of Fisheries Engineering. They were transferred to 30×80×40 cm glass aquariums containing sterile water. Fish were housed at a temperature of 26±1°C with 14 h light: 10 h dark cycle in aquariums with continuously aerated and filtered water for two weeks. All these housing conditions were maintained for two weeks until the beginning of the experiments. Fish were fed twice a day with a commercial fish food containing protein selected according to the species and promoting their nutrition and egg production. *L. caeruleus* rootstock and larvae are shown in Fig. 1a,b.

L. caeruleus fish have swelling in their lower jaws after fertilization of the egg. They carry their fertilized eggs in their mouths during the incubation period (21 days), at the end of this period, they remove the larvae from their mouths. For this reason, the rootstock fish were monitored daily and the fish with swollen under the jaw was intra orally checked. Fish with fertilized eggs in their mouths were transferred to a glass aquarium (35×25×20 cm) where they were awaited for the development of larvae. Fish were housed at a temperature of 26±1°C with 14 h light: 10 h dark cycle in aquariums with continuously aerated and filtered water until the end of the incubation period. At the end of the incubation period, when the larvae that had become prominent and reached the required maturity were seen, the lower jaw of the brood fish was gently stroked/squeezed, thus vomiting/milking was performed and the larvae were collected and then transferred to the aquarium. All these

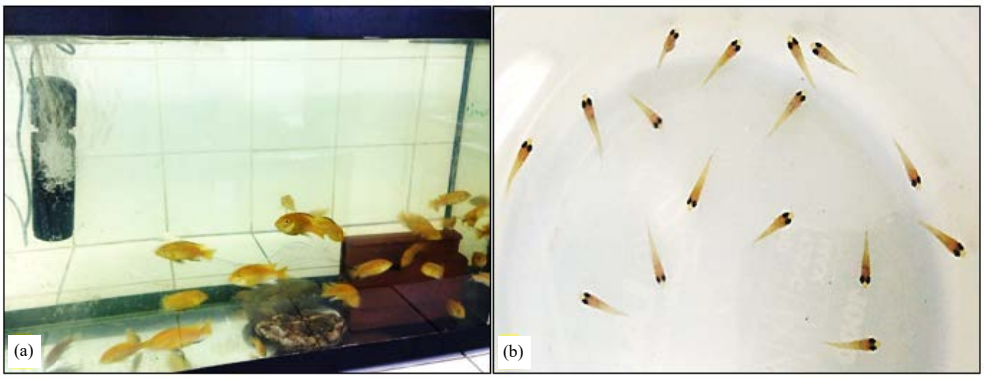

Fig. 1(a-b): (a) *L. caeruleus* fishes in aquarium and (b) *L. caeruleus* larvae

housing conditions were maintained for two weeks until the beginning of the experiments.

SiO_2NP (High purity powder-99%, particle diameter 20-30 nm, amorphous, Cas: 7631-86-9, Nanoshel-India) was purchased from a commercial company (Triogen Biyoteknoloji Company-İstanbul, Turkey). The nanodispersions were prepared just before exposure by ultra-sonication at 100 kHz for 10 min using double distilled water and maintained as stock. Doses were determined according to Vidya and Chitra's (2017, 2018) reports[28-29]. The water of the experimental groups was renewed for 24 h periods, taking care not to change SiO_2NP concentrations[30]. One day old larvae were divided into control and experimental groups. No treatment was applied to the control group. Ninety-six hours after the application of the SiO_2NP doses, the larvae belonging to the experiment and control groups were turned into a permanent preparation by histological methods.

Randomly selected fish (larvae) were separated into 4 groups each was included 10 fish as follows (totally n = 10×4 groups ×3 repeats):

- **Control group:** Fish not exposed to any chemicals (n = 10 for each repeat)
- **Group 1 (G1):** Fish exposed to 50 mL L^{-1} of SiO_2NP in aquarium water (n = 10 for each repeat)
- **Group 2 (G2):** Fish exposed to 100 mL L^{-1} of SiO_2NP in aquarium water (n = 10 for each repeat)
- **Group 3 (G3):** Fish exposed to 200 mL L^{-1} of SiO_2NP in aquarium water (n = s 10 for each repeat)

Semiquantitative scoring: Semiquantitative scoring was performed as described[31]. Fivelarvae were randomly selected from each group and ten slides were investigated per larva. All the slides were examined by the same observer who was blind to the tissue sections between the control and experimental groups. The histopathological changes were categorized as none (-), mild (+) (25% of sections), moderate (++) (25-50% of sections) and severe (+++) (>50% of sections).

Histopathological method: The larvae of the experimental and control groups were fixed as a whole with Bouin's solution for 24 h. After the fixation at +4°C for 24 h, larvae were dehydrated with graded ethanol series and cleared with xylene prior to paraffin embedding. Paraffin blocks were sectioned at 5-7 mm by using a rotary microtome (Leica RM 2145) and mounted on slides for Mayer's hematoxylin and eosin (H and E) staining procedures[32]. The stained sections were evaluated and captured using an Olympus BX51 brightfield microscope equipped with Olympus E-330 digital camera (Shinjuku City, Japan).

RESULTS

Semiquantitative scoring: The results of this study indicate that SiO_2NP causes histopathological changes in the gill, liver, eye and brain of *L. caeruleus*. The histopathological lesions observed according to the exposure concentrations are given in Table 1 and are described by semiquantitative scoring as mild, moderate and severe.

Histopathological results: It was observed that the total larval sections of all experimental groups were similar to the control group (Fig. 2a,b and 3a,b). Microscopic changes in gill, liver, eye and brain tissues are given belowan organ basis.

Gill: When the preparations obtained from the fish belonging to the control group were examined, it was observed that the gill arches, filaments, lamellae and gill epithelial cells were normal in appearance and arranged. It was observed that the secondary lamellae separated

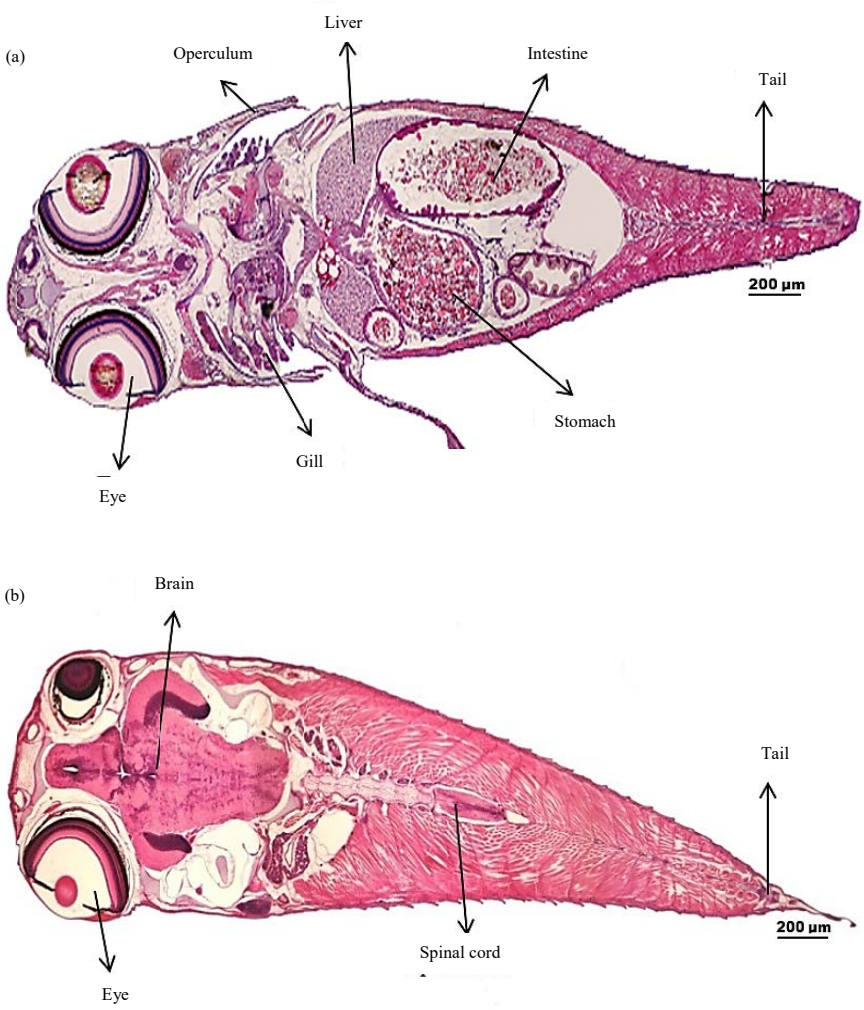

Fig. 2(a-b): Control group *L. caeruleus* larvae, (a) Total coronal dorsal section and (b) Total coronal ventral section. Staining, H and E

radially from the primary lamella supported by the connective and cartilage tissue were in the known normal structure. The central vena sinusoid is located in the middle of the primary lamellae. The outer surface of the secondary lamellae is covered with epithelium and secretory cells located between them (Fig. 4a). In the gill tissues of the fish belonging to all experimental groups to which silicon dioxide nano particles were applied, some structural changes that increased in a dose-dependent manner were detected, the most prominent being the deterioration in the lamella shapes (Fig. 4b-f). In the examinations, rupture and separation of the primary lamellae Fig. 4b,c deformation of the secondary lamellae and separation of the epithelium were observed in all experimental groups Fig. 4c-e. It was noted that some secondary lamellas shortened or even spilt Fig. 4c-f in some secondary lamellas the capillaries expanded (ballooning) Fig. 4f.

Liver: In histological sections, the liver of the control group *L. caeruleus* was seen to have two lobes, anterior and posterior. The hepatocytes forming the parenchyma, which is observed to show normal structure, are in the form of irregularly arranged cell groups rather than regular cords. Hepatocytes contain a prominent nucleus and abundant oil droplets in their cytoplasm. Depending on the amount of fat the hepatocytes contain, the nuclei are located in different parts of the cell, usually in the peripheral cytoplasm. Oil droplets were observed in the cytoplasm as numerous large and small vacuoles. In the

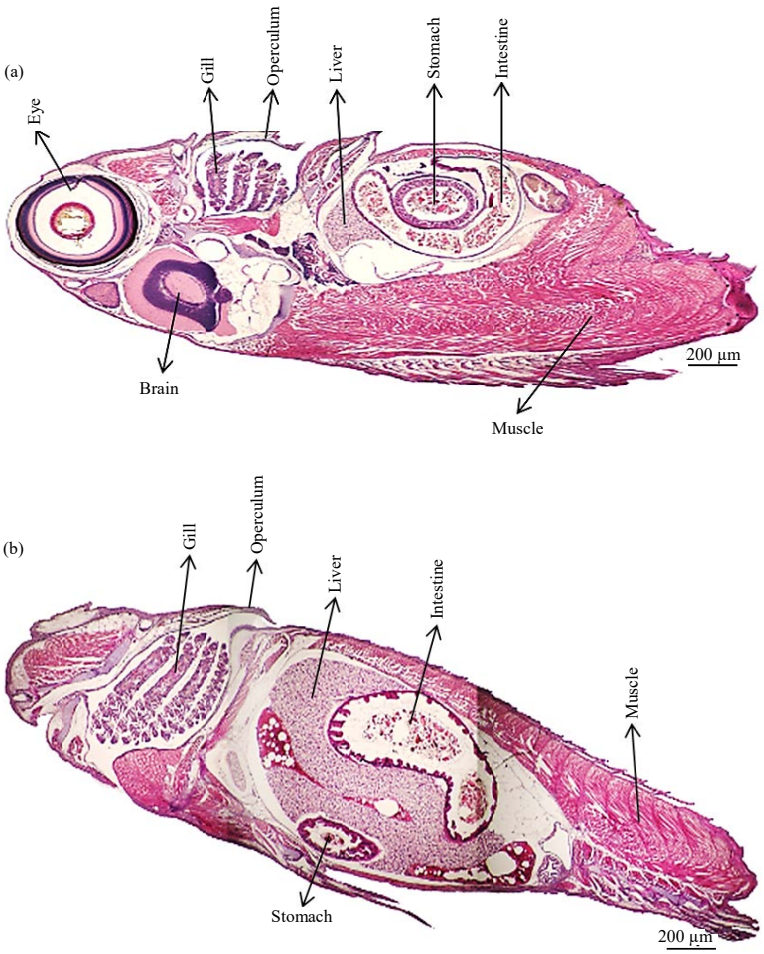

Fig. 3(a-b): Control group *L. caeruleus* larvae. (a) Total sagittal right lateral section and (b) Total sagittal left lateral section. Staining, H and E

Table 1: Semiquantitative scoring of organs of *L. caeruleus* exposed to 50, 100 and 200 mg L^{-1} of SiO_2NPs

Organ	Histopathological lesions	Control	50 (mg L^{-1})	100 (mg L^{-1})	200 (mg L^{-1})
Gill	Rupture and separation of the primary lamellae	-	+	+	+
	Shortening in secondary lamellae	-	+++	+++	+++
	Loss in secondary lamellae	-	++	+++	+++
	Separation in respiratory epithelium	-	++	+++	+++
	Balloning dilatation in capillaries	-	-	+++	++
Liver	Hepatocellular necrosis	-	+	+++	+++
	Pycnotic nuclei in hypatocyte	-	+	+++	+++
	Alteration in staining of hepatocyte nuclei	-	+	++	+++
	Sparation in the capsule	-	-	++	++
	Parenchymal vacuol	-	++	++	++
Eye	Thinning of the corneal layer	-	++	++	++
	Thinning and loss of the lens capsule	-	+++	+++	+++
	Dissolution in primary lens fibers	-	+	++	+++
	Change in lens position	-	++	++	++
	Change in the retina and optic nerve	-	-	-	-
Brain	Degeneration in part of the cerebrum	-	-	-	-
	Degeneration in cerebrum	-	-	-	-
	Increase in astrocytes	-	-	+	+++

Histopathological lesions were scored to the to their severity, -: None, +: Mild, ++: Moderate, +++: Severe

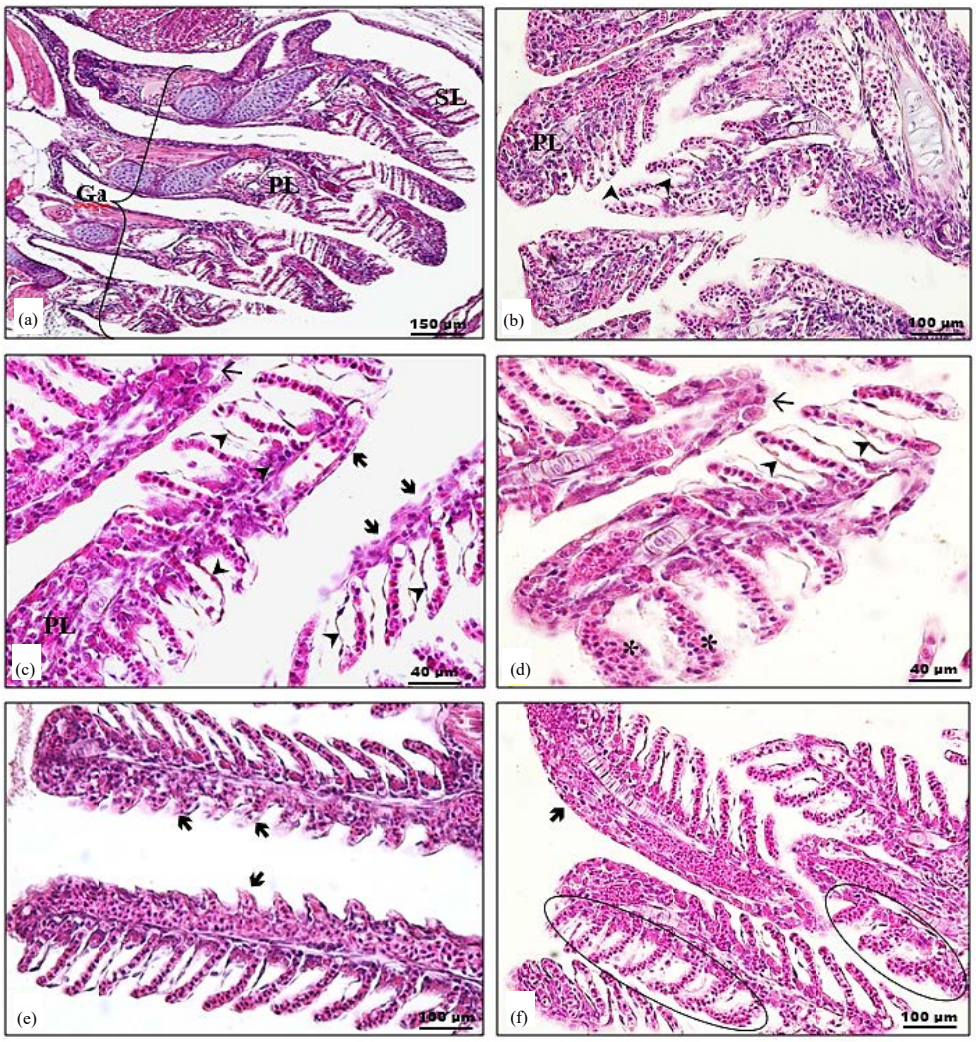

Fig. 4(a-f): (a) Control and (b-f) Experimental group gill sections. Separation in the epithelium (➤), shortening and loss of secondary lamellae (➜), impaired circulation in secondary lamellar vessels (*), deformity of secondary lamellae (oval rings) are observed in the experimental groups. Ga; Gill arch, PL; Primary lamellae, SL; Secondary lamellae. Doses; (a) Control, (b) 50 mg, (c-d) 100 mg, (e-f) 200 mg. Staining, H and E

parenchyma, the sinusoids formed by the portal vein by dividing into many branches are in normal structure (Fig. 5a and b). In the examinations made in the larvae of the experimental group, it was observed that the liver parenchyma and hepatocytes were similar to the control group in the group administered 50 mg L^{-1} SiO_2NPs. The hepatocyte cytoplasm contains a large number of fat vacuoles and the nuclei are eccentrically located (Fig. 5c). In the group administered 100 mg L^{-1} SiO_2NP, wide vacuole-like openings in different parts of the liver parenchyma were remarkable (Fig. 5d). As seen in Fig. 5e,f, hepatocellular necrosis and separation in the capsule were detected in the parenchyma. In hepatocytes located close to the sub capsule, pycnotic nuclei were distinguished (Fig. 5f). In the group administered 200 mg L^{-1}, alterations in staining were observed in the nuclei of hepatocytes located in the peripheral parenchyma (Fig. 5g,h).

Eye: Lenses, lens suspending ligaments (iris), lens fibrils and epithelial cells forming these fibrils were observed in normal structure in the eye tissue sections of the control group. The retina, the choroid covering the retina, the sclera covering the choroid and the choroidal cavity were observed normal structure and well-arranged. The optic nerves and iris were normal-looking. The optic nerves and

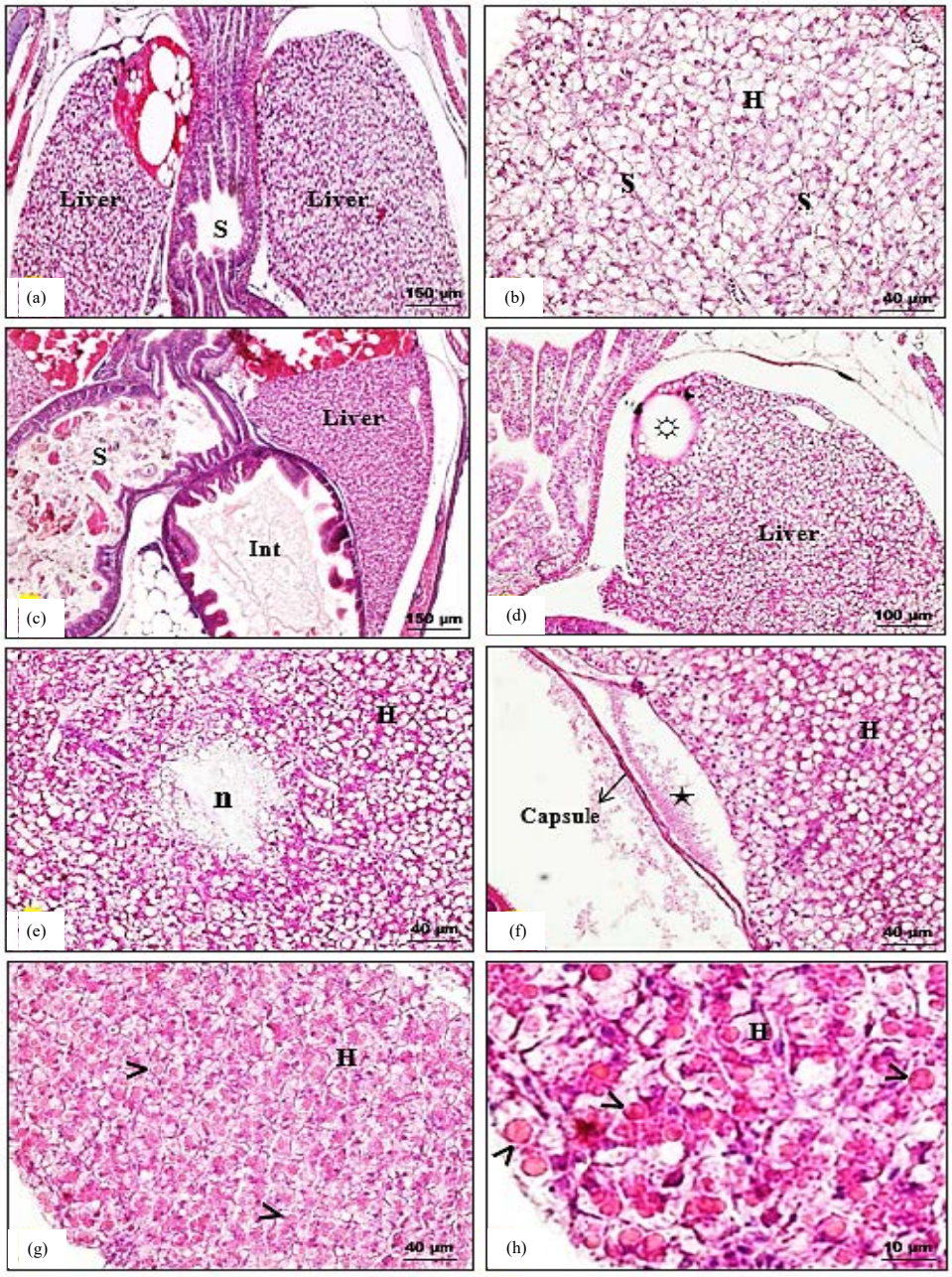

Fig. 5(a-h): (a-b) Control and (c-h) Experimental group liver sections. H: Hepatocyte, S: Stomach, Int: Intestine, s: Sinusoid, n: Necrosis, ☼: Parenchymal space. Separation in the liver capsule (★),difference in staining in nuclei (>) were detected in the sections of the experimental groups. Doses; (a-b) Control, (c) 50 mg, (d-f): 100 mg, (g-h) 200 mg. Staining, H and E

iris were normal-looking. Retina and pigment epithelium, rods and cone optic cells, outer limiting membrane, outer nuclear layer, outer retinal layer, inner nuclear layer, inner retinal layer, ganglion cell layer, nerve fibrils layer and inner limiting membrane layers were normal structures (Fig. 6a and b). In the group administered 50 mg L^{-1} SiO_2, loss of the lens capsule and mild dissolution of the primary lens fibres were observed (Fig. 6c). In the group administered 100 mg L^{-1} SiO_2, thinning and loss of the lens capsule and mild dissolution of the primary lens fibres were detected (Fig. 6d-f). In the group treated with 200 mg L^{-1} SiO_2, some samples were seen thinning of the

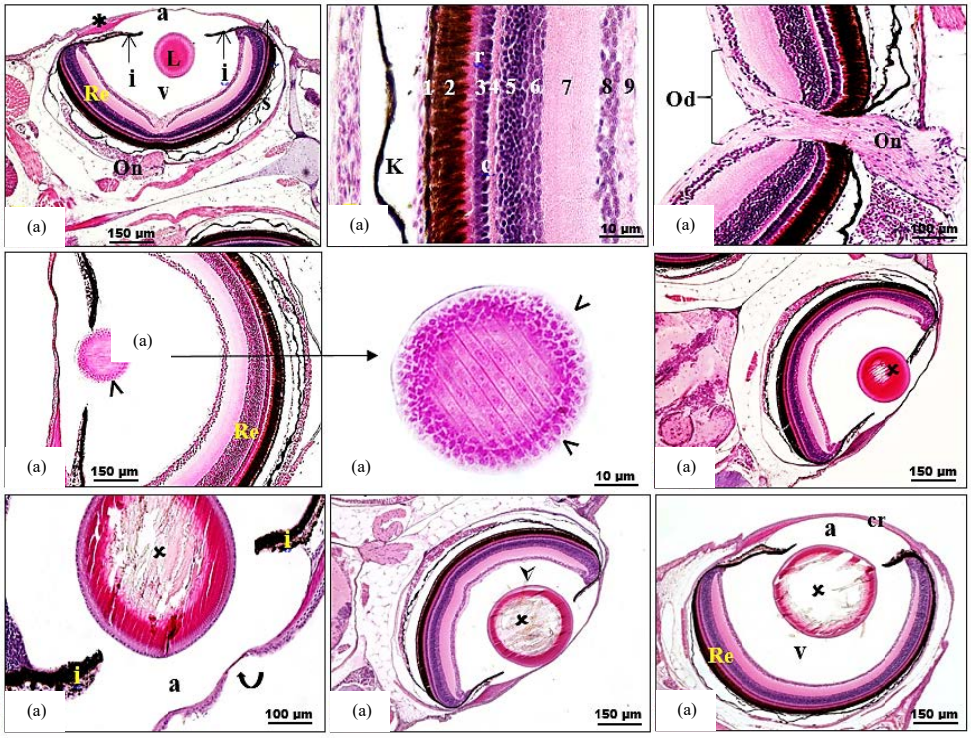

Fig. 6(a-h): (a-c) Eye sections of *L. caeruleus* larvae belonging to control and (d-h) Experimental groups. Loss in lens capsule (>), thinning of the corneal layer (∪), dissolution and disintegration in lens fibers (✕) and change in lens position are seen in the experimental groups. cr: Cornea, a: Anterior space, i; iris, lens (L), v: Vitreous humor, Re: Retina, ✱: Choroid body, k: Choroid layer, s: Sclera, Od: Optic disk, Os: Optic nerve fibers. 1: Retinal pigment epithelium, 2: The outer portion of the photoreceptors, 3: The interior of the photoreceptors, 4: Outer limiting membrane, 5: Outer nuclear layer, 6: Inner nuclear layer, 7: Müller cells, 8: Ganglion cell layer, 9: Optic nerve fibers, k: Choroid, c: Cone cells, r: Rod cells. Doses, (a-c) Control, (d-f) 50 mg, (g-h) 100 mg, (i) 200 mg. Staining, H and E

corneal layer (Fig. 6g), separation of the lens epithelium from the lens fibres and advanced fragmentation of the lens fibres in many sections (Fig. 6g-i). It was noted that there were no structural changes in the retina and optic nerve in all experimental groups (Fig. 6c,f,h,i).

Brain: *L. caeruleus* brain is composed of five main parts: telencephalon, diencephalon, mesencephalan, metencephalon and mylencephalon located in the cranial cavity. The brain is composed of olfactory lobes, brain and cerebellum lobes, the medulla oblangata that forms the myelencephalon, followed by the spinal cord, interconnected neurons and their supporting axon, dendrites and glial cells (Fig. 7a). In the brain tissue sections of all experimental groups, it was determined that the telencephalon, consisting mainly of olfactory lobes, bulbs and telencephalic lobes, had a similar structure to the controls. The mesencephalon, which is associated with the integration of visual information and motor functions, and the metencephalon, which includes the part of the cerebellum, were structurally normal. It was observed that the medulla oblongata and spinal cord were similar to the control group (Fig. 7b-e). It was noted that astrocytes were densely located in different sections such as forebrain, medulla oblongata and spinal cord in high dose (200 mg) group sections (Fig. 7d and e).

DISCUSSION

With the increase in nanomaterials used in commercial products, it is a question of whether the benefits or harms outweigh when considering the environmental and social costs of nanotechnology. Considering the increased interest in biomedical applications recently, it is important to evaluate the potential side effects of SiO_2NPs. It is necessary to understand the biological response to toxicity following SiO_2NP applications. Fish, which form the lowest vertebrate group in the aquatic environment and are an important source of food/protein for humans, can be

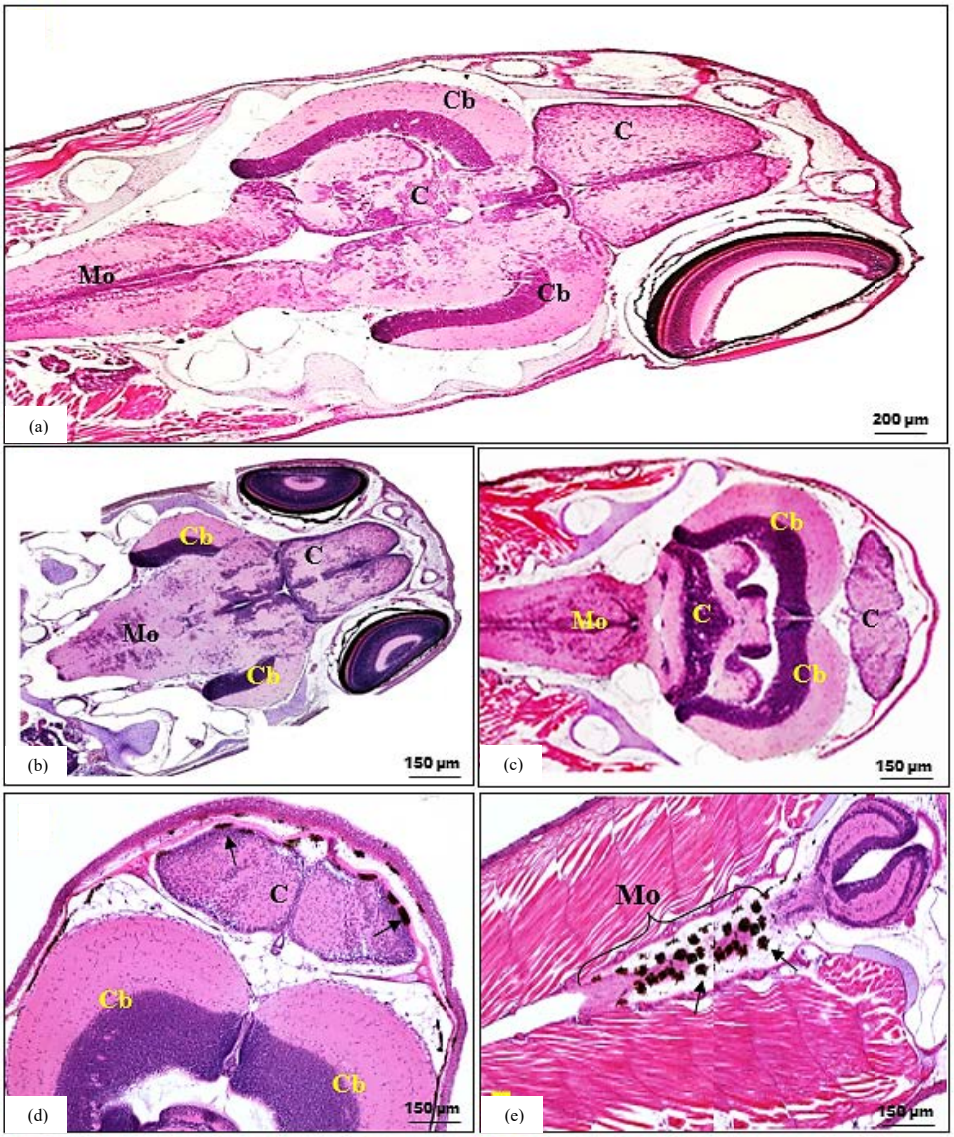

Fig. 7(a-e): (a) Brain frontal section view of control group and (b-e) Experimental groups of *L. caeruleus* larvae. Astrocytes (arrow) found in the forebrain and medulla oblongata. C: Brain, Cb: Cerebellum, Mo: Medeulla oblangata. Doses, (a) Control, (b) 50 mg, (c) 100 mg, (d-e) 200 mg, Staining, H and E

considered as an early warning system for many pollutants in the water[34]. Histopathological studies have an important place in the determination of tissue disorders that these pollutants may cause.

From the examinations and observations made in this study, it is possible to say that the most affected organ by SiO_2NP is the gills. This situation can be explained by the fact that it is the primary target of all kinds of substances in water due to their role in osmoregulation, regulation of acid-base balance and discharge of nitrogenous wastes. Because gills are sensitive to changes in water quality and are in close contact with the external environment.

Changes such as epithelial separation, hyperplasia and hypertrophy, as well as the partial fusion of some secondary lamellae, are examples of defence mechanisms. Because these generally cause an increase in the distance between the external environment and the blood, thus creating a barrier to the entry of pollutants[33-36]. We can say that the changes such as shortening and rupture of the gill secondary lamellae observed in this study occur in order to reduce the surface area. Thus, the organ will be exposed to less surface and less substance in the water. As the distance between water and blood increases due to epithelial separation deterioration of oxygen uptake is inevitable[33]. However fish have the capacity to increase aeration rates to compensate for their low oxygen in take. Similar changes have also been reported in the gills of fish exposed to metals[37-39] and organic pollutants[40-41].

According to Mallatt[42], these changes are not specific and can be induced by different types of substances.

The liver, which is the main organ for biochemical conversion and detoxification of pollutants, is used as a reliable biomarker for histopathology of hepatocytes in toxicity studies. In one study, the freshwater fish Oreochromis mossambicus was exposed to 12 mg L^{-1} SiO_2NP for 96 h and histological changes such as segmentation and spindle-shaped nuclei were observed in hepatocytes. In the group exposed for 60 days, it is stated that the severity of morphological tissue damage such as severe vacuolization, absence of nuclei and completely irregular hepatocytes is more prominent[29].

Vacuoles in the hepatocyte cytoplasm contain lipid and glycogen, which is related to the normal metabolic function of the liver[43]. Therefore, the vacuolization in hepatocytes indicates the imbalance between the synthesis rate of substances and the rate of release into the systemic circulation[44]. We agree with the comments of these researchers. Based on cytoplasmic vacuolization, pycnotic nucleus and nuclear staining difference in hepatocytes, it is possible to say that SiO_2NPs interact with proteins and enzymes in liver tissue, affect antioxidant defence mechanisms and cause necrosis by leading to the formation of reactive oxygen species that promote atrophy. Similarly, in the studies of Alarifi *et al*.[45] examining the effects of TiO_2 nanoparticles on hepatic tissue, it is mentioned that nuclear and cytoplasmic damage in hepatocytes may result in apoptosis and necrosis as a result of disruptions in intracellular mechanisms.

It has been reported that the chromatographic and electrophoretic profiles of the lens proteins did not change in cultured eye cells exposed to cerium oxide nanoparticles (nanoceria) and that nanoceria did not have a detrimental effect on the eye lens proteins[46]. Jo *et al*. Stated that they did not observe direct SiNP toxicity in retinal tissue neuronal and endothelial cells in their study that aimed to demonstrate the antiangiogenic effect of silicate nanoparticles on retinal neovascularization[47]. In oxygen-dependent retinopathy mice, intravitreal injection of SiNPs has been found to effectively reduce abnormal retinal angiogenesis. It has been stated that SiNPs effectively inhibit vascular endothelial growth factor (VEGF)-induced angiogenesis in vitro. Besides, it has been stated that SiNPs block ERK 1/2 activation upon suppression of VEGF receptor-2 phosphorylation caused by VEGF. In conclusion, they concluded that SiNPs could be used as inhibitors of VEGF-mediated retinal neovascularization and may be suggested in the future for the treatment of angiogenesis-related blindness.

In one study, SiNP-induced cytotoxicity (50, 100 and 150 nm size) in cultured Human Corneal Epithelial Cells (HCEC) was evaluated and it was determined that these nanoparticles did not induce significant cytotoxicity in HCECs. After 48 h of treatment, it has been stated that SiNPs was taken up by HCECs and the formation of cellular reactive oxygen species was slightly increased in a dose-dependent manner. However, no significant reduction in cellular viability has been reported up to a concentration of 100 μg mL^{-1} for the three different sizes of SiNPs[48].

According to the report of Kim *et al*.[49], human corneal endothelial cells exposed to SiNP (50, 100, 150 nm size) for 48-72 h engulfed the nanoparticles, but mitochondrial damage did not occur. It is said that cell viability and LDH level remained unchanged in 100 μg mL^{-1} dose, autophagy showed significant dose-dependent activation, but m TOR (rapamycin) activation remained unchanged. In the same study, it is stated that human corneal tissue culture at 100 μg mL^{-1} SiNP concentration for 72 h did not show significant endothelial toxicity. In addition, it is emphasized that it does not induce acute significant cytotoxicity at concentrations up to 100 μg mL^{-1}, however, the long-term toxicity of SiNPs is unknown. It has been determined that in primary human corneal epithelial cells exposed to ultra-thin (30 nm and 40 nm) SiO_2NPs in-vitro for 24 h cause changes in cell membrane damage, reduction of cell life span, cell death and mitochondrial dysfunction. Observable corneal damage has also been detected in the case of *in-vivo* exposure to the same nanoparticles. It has been reported that these effects are more severe than those of ultra-thin (100 and 150 nm) SiO_2NPs and that commonly used antioxidant compounds do not protect the cornea from damage caused by SiO_2NP[50]. As in the above study, it was seen in this study that SiO_2NPNs caused damage to the eye tissue. These were loss of the lens capsule in the 50 mg experimental group, thinning of the corneal layer, separation of the lens epithelium, dissolution of the lens primary fibres in the 100 mg L^{-1} group, and advanced fragmentation of the lens fibres in the 200 mg L^{-1} application group.

In a study conducted at the electron microscope level, it was shown that SiO_2NPs administered to rats at a size of 6, 20, 50 nm and at a dose of 150 mg mL^{-1} cause oxidative stress and structural damage in the liver, kidney, and brain. It has been emphasized that the effects of SiO_2NPs should be thoroughly investigated before they are used in humans, especially since they cause structural changes in the brain compatible with neurotoxicity[51]. In the study Vidya and Chitra[29], while mild degeneration was observed in the brain tissue of fish treated with SiO_2NP for a short time, severe neurodegeneration, vacuolization formation, brain edema, necrosis of the neurofibrillary region and lesion have detected in the choroid plexus in long-term exposure. In the same study, it is stated that the choroid plexus lesion caused by nanoparticles shows pathological brain damage that may cause sublethal neurological disorders and that these lesions are preserved even after the exposure is removed. In our study, it was observed that the telencephalon,

mesencephalon, metencephalon, medulla oblongata and spinal cord were structurally similar to the controls in all experimental groups. Only in the high dose group, astrocytes were found to be dense in the medulla oblongata and spinal cord. Astrocytes, as is known, are the glial cells of the nervous system that protect neurons. In this sense, we can say that SiO_2NPs can cross the blood-brain barrier, as these cells are frequently encountered in the high dose group.

After 90 days of oral administration of colloidal silica nanoparticles in different sizes (20 and 100 nm) and different doses (550-1000-2000 mg kg^{-1}) to rats, clinical changes and histopathological findings have not been detected in any of the experimental groups. In addition, it has been stated that there was no animal death and there is a statistically significant increase in the weight of the rats[52]. Therefore it has been said that the NOAEL value should be greater than 2.000 mg kg^{-1}. Similar to the studies of Kim *et al*., the macroscopic increase in fish growth due to dose increase in our study compared to controls made us think that SiO_2NPs may have a positive effect on the growth and development[52].

CONCLUSION

As can be understood from the above studies, the positive or negative effects of nanoparticles on living systems are not only related to the physical and chemical properties of the particle but also to the properties of the living system and the dose, route and duration of exposure. In this study, it was determined that SiO_2NPs caused some histopathological changes at different levels in different tissues of *L. caeruleus* larvae. In addition to the findings of this study, which can be considered as preliminary information, toxicological and ecotoxicological studies should be carried out with different species and with different methods in both natural and laboratory environments.

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