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Histopathological Research of the Therapeutic Effects of Grape (*Vitis vinifera* L.) Seeds Extracts on Cadmium-Exposed Carp (*Cyprinus carpio* L. 1758)

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ABSTRACT

This study was designed to investigate the therapeutic effects of *Vitis vinifera* L. seed extract (5 ppm and 10 ppm) on gill and liver tissues histopathology of carp (*Cyprinus carpio* L. 1758) exposed to cadmium chloride ($CdCl_2$=Cd) (20 ppb) acute and subchronic period. A total of 140 fish in 14 groups were used in the experiment. The experiment was designed as in two different periods (acute and chronic) and 7 different dose groups (control, vehicle, *V. vinifera* seed extract 5 ppm, *V. vinifera* seed extract 10 ppm, Cd 20 ppb, *V. vinifera* seed extract 5ppm + Cd 20 ppb and *V. vinifera* seed extract 10 ppm + Cd 20 ppb). At the end of the experiment, the gill and liver tissues dissected from the fish were fixed, dehydrated, cleared, and paraffin impregnated. Appropriately sized blocks were prepared from the tissues, sections of 5-7 µm thickness were taken and covered by staining with hematoxylin-eosin. Stained preparations were examined under a light microscope and photographed. It was determined that all dose groups in the acute period and control groups were not different histopathological. It was determined that the histopathological damage caused by the cadmium group in the subchronic period and tissue damage were reduced in the dose groups with cadmium added with *V. vinifera* seed extract. As a result of the study, it was concluded that *V. vinifera* seed extract could have a therapeutic effect on the gill and liver tissues of carp exposed to cadmium.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 10(2): 182-190, 2022

Üzüm (*Vitis vinifera* L.) Çekirdeği Ekstraktlarının Kadmiyuma Maruz Kalmış Sazan Balıkları (*Cyprinus carpio* L. 1758) Üzerindeki Terapötik Etkilerinin Histopatolojik İncelemesi

MAKALE BİLGİSİ

Araştırma Makalesi

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Anahtar Kelimeler:
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Kadmiyum
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ÖZ

Bu çalışma, *Vitis vinifera* L. çekirdeği ekstraktının (5 ppm ve 10 ppm) kadmiyuma ($CdCl_2$=Cd) (20 ppb) akut ve subkronik süre maruz kalan sazanların (*Cyprinus carpio* L. 1758) solungaç ve karaciğer dokuları histopatolojisi üzerindeki terapötik etkilerini araştırmak için tasarlanmıştır. Deneyde 14 grupta toplam 140 balık kullanılmıştır. Deney iki farklı süre (akut ve subkronik) ve 7 farklı doz grubu (kontrol, vehicle, *V. vinifera* çekirdeği ekstraktı 5 ppm, *V. vinifera* çekirdeği ekstraktı 10 ppm, Cd 20 ppb, *V. vinifera* çekirdeği ekstraktı 5ppm + Cd 20 ppb ve *V. vinifera* çekirdeği ekstraktı 10 ppm + Cd 20 ppb) olarak tasarlanmıştır. Deney sonunda balıklardan alınan solungaç ve karaciğer dokularına fiksasyon, dehidrasyon, saydamlaştırma ve parafin emdirme işlemleri yapılmıştır. Dokulardan uygun büyüklükte bloklar hazırlanarak 5-7 µm kalınlığında kesitler alınıp, hematoksilen-eozin ile boyanarak kapatılmıştır. Boyanmış preparatlar ışık mikroskobunda incelenerek fotoğrafları çekilmiştir. Akut süre tüm doz grupları ile kontrol gruplarının histopatolojik yönden farklı olmadığı tespit edilmiştir. Subkronik sürede kadmiyum grubunun oluşturmuş olduğu histopatolojik hasarın ve *V. vinifera* çekirdeği ekstraktı ilave edilmiş kadmiyumlu doz gruplarında ise doku hasarının kısmen azaldığı belirlenmiştir. Çalışma sonucunda *V. vinifera* çekirdeği ekstraktının kadmiyuma maruz kalmış sazan balıklarının solungaç ve karaciğer dokularında terapötik etki oluşturabildiği kanaatine varılmıştır.

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Introduction

A wide variety of pollutants due to industrialization and agricultural activities enter and pollute freshwater ecosystems (Rajeshkumar and Li, 2018). Anthropogenic activities and pollutants (heavy metals, pesticides, PAH, etc.) adversely affect aquatic ecosystems, thus reducing the quality of the ecosystem and deteriorating its balance (Javed et al., 2016). This significant amount of untreated wastewater from domestic, industry and agriculture, which deteriorates water quality, is discharged into the aquatic environment and has negative effects on freshwater organisms. One of these factors that negatively affect the aquatic environment is heavy metals. As a result of heavy metal pollution, either species or population densities may decrease day by day. At the same time, these pollutants, which cause ecological damage, accumulate in aquatic organisms and pass to humans through the food chain and adversely affect human health (Bonanno and Giudice 2010; Götze et al., 2014; Yong-Bo et al., 2014; Fatima et al., 2015).

Cadmium (Cd) is one of the most toxic heavy metals with toxic effects discharged to the aquatic environment with wastewater. The main sources of Cd are industrial and agrochemical wastes (e.g., nickel cadmium battery industry, lead mining, sewage, Cd-containing fertilizers, plastics, vehicle tires, etc.). Cd mixed with water from these sources is then taken up by aquatic organisms (Abbas et al., 2019).

In recent years, aquaculture has become important with the increase in competition for food. Therefore, a scientific approach is necessary for Cd detoxification and maintaining the health of economically farmed fish. Histopathological studies are one of the necessary parameters today to determine the possible damage that may occur in fish. Natural products such as plants and their extracts have received much attention due to their ability to increase appetite, stimulate growth, enhance immune ability, and have anti-pathogenic properties in fish (Abdel-Tawwab, 2016; Abdel-Tawwab et al., 2018). In addition, it is important to develop natural herbal extracts that can be added to feeds in feed rationing techniques, in the fight against growth and various diseases in a short time. Thus, the application of these extracts can be beneficial both for the protection of fish health and for economic gain.

Plants contain many biologically active compounds (Mohammed et al., 2020a; Pehlivan et al., 2021). These bioactive compounds are responsible for many biological activities in living organisms (Sevindik, 2018; Mohammed et al., 2020b; Mohammed et al., 2020c). It has been reported that phenolics in grape content inhibit human LDL oxidation. The monomeric phenols in its content show antimutagenic and antiviral properties (Saito et al., 1998). It has been reported that black grape seed extract, which has 17 different chemical compounds in its structure, also exhibits antioxidant properties (Meyer et al., 1997). Briefly, antioxidant, anti-carcinogenic, antimicrobial and anti-mutagenic properties of grape *Vitis vinifera* L. seed are reported in the literature.

Carp is one of the most cultivated fish in the world. It is a preferred species in aquaculture because of its high nutritional value and low cost. Carp is frequently consumed by Turkish low-income citizens in the domestic market. In addition, carp is an indicator of the pollution status of inland waters.

Therefore, in our study, different doses (5 ppm and 10 ppm) of ethanol-extracted extract of black grape seed (*V. vinifera*), which is stated to have antioxidant, anti-carcinogen, antimicrobial and anti-mutagenic properties in the literature, for different durations (4 days and 30 days) was applied to cadmium (20 ppb) exposed fish. At the end of the study, it was aimed to determine the changes in gill and liver histopathology in fish.

Material and Method

Animals and Ethics Committee

The fish used in the experiment were obtained from the Ministry of Agriculture and Forestry, 7th Regional Directorate of State Hydraulic Works, Yedikır Fisheries Station. Ethics committee approval of the study was obtained from Selcuk University Faculty of Veterinary Medicine (approval letter dated 25.03.2014 and numbered 2014/14). The fish brought to the laboratory were put into 250 L aquariums and adapted for 20 days. The fish were fed with pellet food during this period. The fish used in the experiment were less than one-year-old, with an average weight of 70-80 g and an average length of 15-17 cm.

The Experimental Design

14 groups were designed in which control, vehicle and 5 doses of extract were administered orally using gavage once a day for 30 days in two different periods (Table 1). 140 fish were randomly distributed, with 10 fish in each group. Samples for histopathological examination were taken on days 4 and 30. The dose of cadmium used in the experiment was arranged according to Drastichová et al. (2004) "Effect of cadmium on hematological indices of common carp (*C. carpio*)" article. The experimental design was renewed daily to keep the dose constant.

Table 1. Experimental design

Acute period (4 days)	N	Subchronic period (30 days)	N
1. Group (A1): Control group	10	1. Group (S1): Control group	10
2. Group (A2): Vehicle (%0.09 ethanol)	10	2. Group (S2): Vehicle (%0.09 ethanol)	10
3. Group (A3): $CdCl_2$ (20 ppb)	10	3. Group (S3): $CdCl_2$ (20 ppb)	10
4. Group (A4): *V. vinifera* seed extract (5ppm)	10	4. Group (S4): *V. vinifera* seed extract (5ppm)	10
5. Group (A5): *V. vinifera*. seed extract (10 ppm)	10	5. Group (S5): *V. vinifera* seed extract (10 ppm)	10
6. Group (A6): *V. vinifera* seed extract (5 ppm) + $CdCl_2$ (20 ppb)	10	6. Group (S6): *V. vinifera* seed extract (5 ppm) + $CdCl_2$ (20 ppb)	10
7. Group (A7): *V. vinifera* seed extract (10 ppm) + $CdCl_2$ (20 ppb)	10	7. Group (S7): *V. vinifera* seed extract (10 ppm) + $CdCl_2$ (20 ppb)	10

Table 2. Quality Criteria of the Water Used in the Experiment

Parameter	Duration (day)	Control	Vehicle	*V. vinifera* seed extract (5ppm)
Water temperature (°C)	4	11.8 ±0.2	12.4 ±0.1	12.4 ±0.3
	30	12.1±0.1	12.4 ±0.3	12.3±0.2
Dissolved O_2	4	7.8±0.1	7.1±0.3	7.9±0.2
	30	7.4±0.1	7.0±0.2	7.8±0.1
pH	4	7.6±0.1	7.9±0.1	7.6±0.2
	30	7.6±0.2	7.8±0.1	7.9±0.2
Dissolved suspended solid (mg/L)	4	27.4±1.2	31.6±1.4	28.0±0.8
	30	28.0±0.8	32.4±0.9	27.6±0.7
Hardness ($CaCO_3$)	4	152.4±1.3	147.6±1.1	153.1±1.2
	30	148.4±1.4	152.3±1.2	152.3±1.2
Ammonium NH_4-N (mg/L)	4	4.6±0.2	5.2±0.4	4.7±0.1
	30	4.7±0.1	5.3±0.2	5.2±0.4
Parameter	***V. vinifera* seed extract (10 ppm)**	**Cd (20 ppb)**	**Cd + *V. vinifera* seed extract (5 ppm)**	**Cd + *V. vinifera* seed extract (10 ppm)**
Water temperature (°C)	13.1±0.4	12.3±0.2	13.2±0.3	12.1±0.1
	12.4 ±0.3	13.1±0.4	12.3±0.2	12.4 ±0.3
Dissolved O_2	7.0±0.3	7.4±0.1	7.0±0.2	7.8±0.1
	7.9±0.2	7.0±0.3	7.4±0.1	9±0.2
pH	7.8±0.1	7.6±0.1	7.9±0.2	7.6±0.1
	7.6±0.1	7.9±0.1	7.6±0.2	7.6±0.2
Dissolved suspended solid (mg/L)	32.4±0.9	27.5±0.6	31.8±0.7	27.6±0.7
	27.4±1.2	28.0±0.8	31.8±0.4	27.4±1.2
Hardness ($CaCO_3$)	148.4±1.4	152.3±1.2	147.5±1.1	152.5±0.9
	147.5±1.1	152.5±0.9	147.6±1.1	153.1±1.2
Ammonium NH_4-N (mg/L)	5.1±0.3	4.7±0.1	5.3±0.2	4.8±0.1
	4.7±0.1	4.6±0.2	4.7±0.1	5.1±0.3

Table 3. Index of gill lesions by groups

GILL	Control		Vehicle (%0.09 ethanol)		*V. vinifera* seed extract application		Cadmium		Cadmium + *V. vinifera* seed extract application	
	4 days	30 days	4days	30 days	4 days	30 days	4 days	30 days	4 days	30 days
Epithelial separation in the secondary lamellar	-	+	-	-	-	-	+	+++	-	++
Shortening of the secondary lamellar	-	-	-	-	-	+	+	+++	-	++
Clubbed in secondary lamellar	-	-	-	-	-	-	-	++	-	+
Falttening in the secondary lamellar	-	-	-	-	-	-	-	+++	-	++
Cartilage damage	-	-	-	-	-	-	-	++	-	+

Table 4. Index of liver lesions by groups

Liver	Control		Vehicle (%0.09 ethanol)		*V. vinifera* seed extract application		Cadmium		Cadmium + *V. vinifera* seed extract application	
	4 days	30 days	4 days	30 days	4 days	30 days	4 days	30 days	4 days	30 days
Hemorrhage	-	-	-	+	-	+	-	+++	-	++
Extracellular degeneration	-	-	-	-	+	+	++	+++	+	++
Mononuclear cell Infiltration	-	-	-	-	-	+	+	+++	-	++

Plant Extract

The seeds of black grape (*V. vinifera*) used in the study were collected from Gaziantep province in June-2013. Healthy seeds were dried in an oven and crushed into small pieces in a mechanical shredder. The crushed seeds were weighed 100 grams and placed in the cartridges of the Soxhlet Device (Gerhardt EV 14). It was extracted for 12 hours at 60-80°C with 500 mL of 96% pure ethyl alcohol (Merck) for each cartridge in a Soxhlet device. After the obtained extracts were filtered with Whatman no:4, they were concentrated under high vacuum in a Rotary Evaporator (Heidolph Hei VAP HB Digit) at 40°C. The obtained extracts were stored at +4°C until the start of the experiment (Goyal et al., 2009; Dasari et al., 2012).

Water Quality Criteria

Water criteria in aquariums are shown in table 2.

Histopathological Examination

Gill and liver tissue pieces taken after dissection were placed in tissue tracking cassettes and labeled. The cassettes were kept in 10% formaldehyde solution for at least 24 hours for fixation. The cassettes removed from the fixative were washed in tap water for 24 hours. The tissue samples in the cassettes placed on the Leica TP1020 model tissue tracking device were dehydrated, cleared and impregnated. After the appropriate paraffin blocks were prepared using the Leica EG1160 model tissue blocking device, 5-6 µ thick sections were attached to the slide using a Leica RM 2125RTS model rotary microtome. After the slides were stained with the procedures in accordance with the Harris Hematoxylin Eosin staining method, they were closed with entellan and examined under a Leica DM3000 model light microscope (Korkmaz and Orun, 2020; Erdoğan et al., 2021).

Results and Discussion

Proper water quality is critical for maintaining healthy aquatic organisms, including fish (Svobodová, 1993). In recent years, heavy metal contamination of aquatic systems due to increased natural and anthropogenic activities has increased public health and ecological concern (Ramachandra et al., 2018). The global concern may be from the tendency of heavy metals to cause toxic damage to living organisms, especially aquatic organisms, and ultimately to humans (Bahnasawy et al., 2011). Fish are frequently exposed to toxic chemicals such as heavy metals, which pollute the aquatic environment and have devastating effects on the ecological balance of the environment and various aquatic organisms (Adebayo, 2017). Cadmium, a toxic heavy metal, is gaining increasing importance as an environmental hazard to both humans and wildlife (Satarug and Moore, 2012).

Fish are key organisms for assessing the ecological status of rivers based on biomarkers (Scardi et al., 2008; Hermoso and Clavero, 2013; Fonseca et al., 2016;). In addition, their histopathology is the standard method for assessing both short term and long term. It is known that exposure to cadmium adversely affects fish morphology and physiology of fish. For example, morphological changes have been reported in the gill (Al-Mansoori, 2006), liver (Ikram and Malik, 2009; Deore and Wagh, 2012) and kidney (Amin et al., 2013) in fish. Fish gills are among the primary target organs of environmental pollutants, as they are in direct contact with water and are characterized by large surface area and absorption rates of chemicals (Pandey et al., 2008). Pathological changes in the gill epithelium are often the result of exposure to pollutants, particularly heavy metals (Fonseca et al., 2016), the severity of which depends on pollutant concentration and duration of exposure (Tchounwou et al., 2012). The liver is the organ most associated with the detoxification and biotransformation process because of its function, position, and blood supply. It is also one of the organs most affected by water pollution (Camargo and Martinez 2007). Pollutants in water can cause histopathological lesions in the liver.

Recently, herbal feed supplements have been used to increase immunity and antioxidant capacity and reduce heavy metal toxicity in fish (Kalavathi and Saradhamani, 2017; Abdel-Tawwab et al., 2018). It has also been reported that herbal extracts have various beneficial properties for fish (Chakraborty and Hancz, 2011; Bulfon et al., 2015; Van Hai, 2015; Reverter et al., 2017).

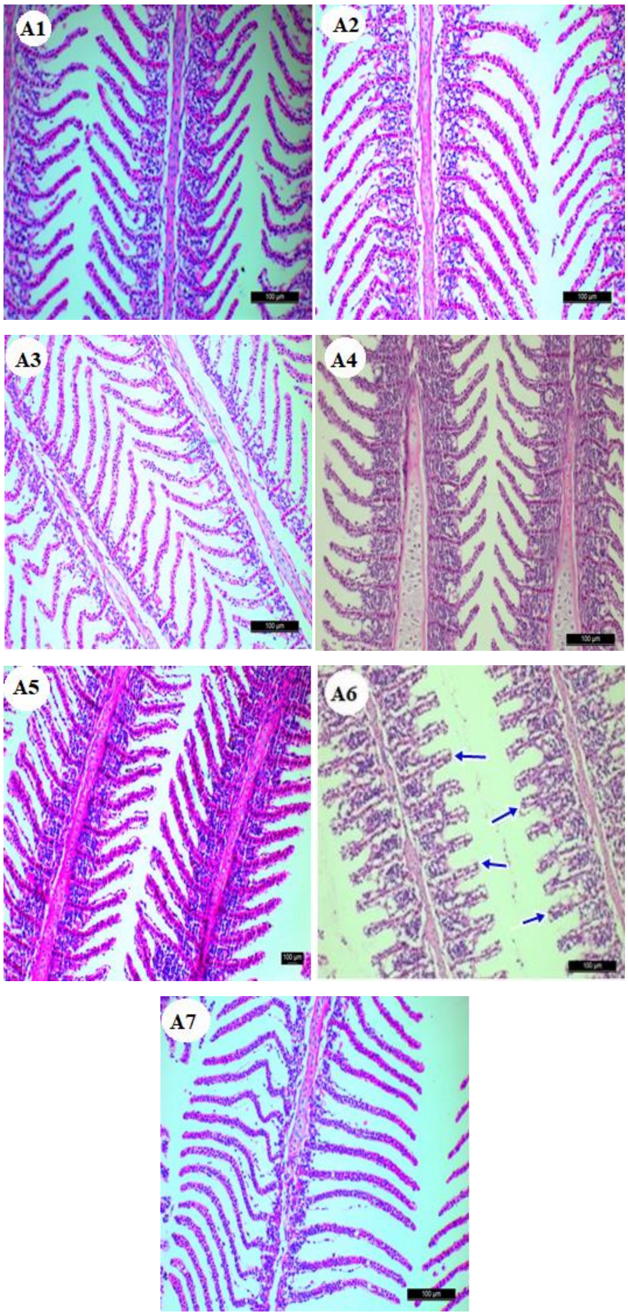

Figure 1. Acute period gill histopathology
A1: Control group gill (HE200X), A2: Vehicle group gill (HE200X), A3: Cadmium exposed group gill (HE 200X), A4: Grape seed (*V. vinifera*) extract (5 ppm) applied group gill (HE 200X), A5: Grape seed (*V. vinifera*) extract (10 ppm) applied group gill (HE 200X), A6: Grape seed (*V. vinifera*) extract (5 ppm) applied to Cd exposed group gill (HE 200X)
Arrows show shortening in the seconder lamellae, A7: Grape seed (*V. vinifera*) extract (10 ppm) applied to Cd exposed gill (HE 200X)

It is stated that black grape and its seeds significantly prevent tumour formation and progression, prevent hepatic cancer, prevent coagulation by inhibiting platelet aggregation, and are beneficial to the cardiovascular system with its pathophysiological effects. It is also known for its antifungal, antiviral, anti-inflammatory, antioxidant, antibacterial and anti-cancer effects.

In this study, the apparent histopathological effect of cadmium in the acute dose and duration was not observed in the gill tissue, but shortening, flattening (unification) and epithelial separation in the secondary lamellae were observed in the subchronic dose and duration. Considering the histopathological damage caused by the cadmium group in the subchronic period, it was determined that *V. vinifera* seed extract partially reduced tissue damage in the plant extract-added cadmium dose groups (Figure 1 and Figure 2). The intensity of some histopathological changes in the gill tissue is shown in Table 3.

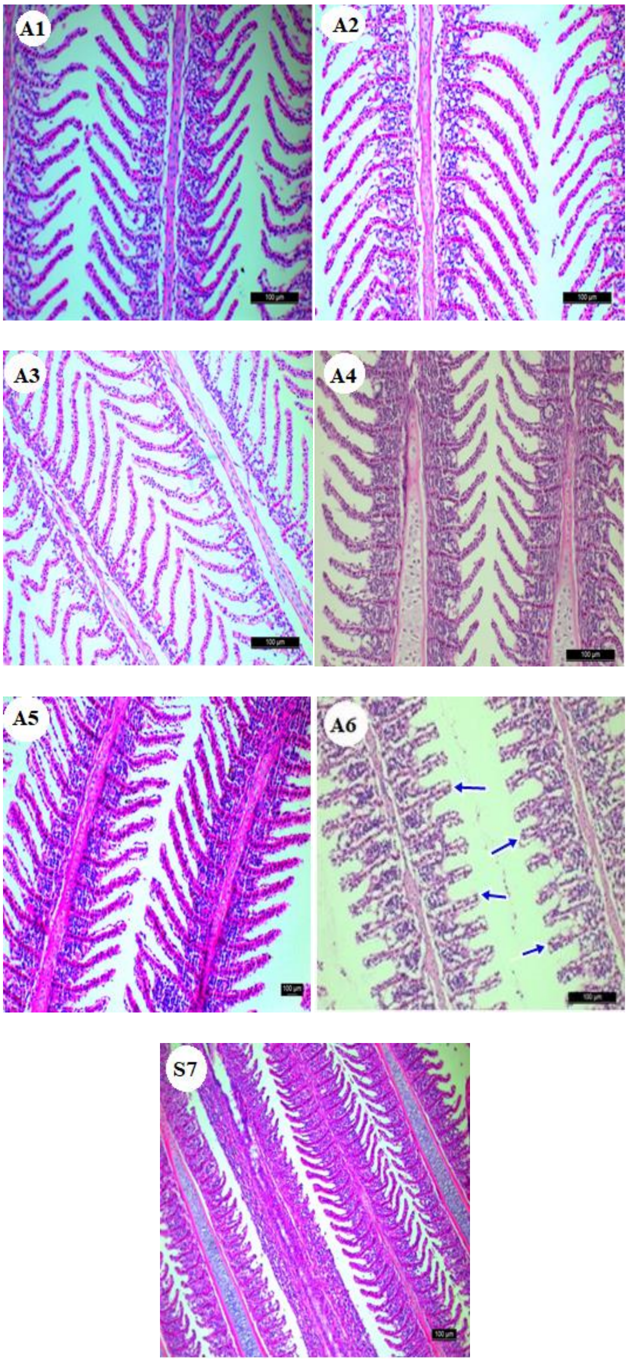

Figure 2. Subchronic period gill histopathology

A1: Control group gill (HE200X) Arrows show separation in the epithelial of the seconder lamellae, A2: Vehicle group gill (HE200X), A3: Cadmium exposed group gill (HE 200X) A- Flattening in the seconder lamellae, B- Cartilage tissue damage, C- Shortening in seconder lamellea, A4: Grape seed (*V. vinifera*) extract (5 ppm) applied group gill (HE 200X) Arrows show shortening in the seconder lamellae, A5: Grape seed (*V. vinifera*) extract (10 ppm) applied group gill (HE 200X), A6: Grape seed (*V. vinifera*) extract (5 ppm) applied to Cd exposed group gill (HE 200X) A- Flattening in the seconder lamellae C- Shortening in seconder lamellea, S7: Grape seed (*V. vinifera*) extract (10 ppm) applied to Cd exposed group gill (HE 200X)

As a result of the study, cadmium did not cause a significant histopathological effect in acute duration and dose groups in liver tissue. Bleeding (hyperemia), mononuclear cell infiltration and degeneration in the extracellular area were observed in the subchronic period and dose. Considering the histopathological damage caused by the cadmium group in the subchronic period, it was determined that *V. vinifera* L. seed extract partially reduced tissue damage in the cadmium dose groups with plant extract added (Figure 3 and Figure 4). The intensity of the histopathological changes in the liver tissue is shown in Table 4.

Many researchers have reported degenerative changes in tissues of animals in response to contamination by various toxic substances (Andhale et al., 2011; Mukke, 2012). Histological changes in fish are a remarkable and promising area for understanding the extent to which structural organization changes in organs due to environmental pollution (Selvanathan et al., 2013).

The primary target of aquatic pollutants is the gills, which are in direct contact with the environment. Therefore, it is stated that contamination of fresh water with metals causes histopathological changes in fish gills depending on metal concentration and exposure duration (Tchounwou et al., 2012; Hermenean et al., 2017). Heavy metals may have effects on filament epithelial proliferation, lamellar fusion and epithelial necrosis in the gills (Fonseca et al., 2017). It has been reported that water quality induces histopathological changes including occlusion of blood vessels and aneurysms in the gills (Santos et al., 2014).

Jayakumar and Subburaj (2017) reported that secondary lamellar erosion, lamellar fusion, blood vessel constriction, secondary lamellar damage, hyperplasia, vacuolation have identified shortening of epithelial secondary lamellae in gill histopathological examination of *Heteropneustes fossilis* exposed to subletal Cd concentrations. Similarly, Thophon et al. (2003) reported gill lesion in sea bass *Lates calcarifer* exposed to Cd. Bais and Lokhande (2012) observed lamellar epithelial hypertrophy, dysfunction of gill lamellae, and blood congestion in *Ophiocephalus striatus* exposed to cadmium chloride. On histopathological examination of *Cirrhunus mrigala* exposed to cadmium, they reported swelling at the tip of the gill lamellae, misalignment of the cells, shrinkage of the epithelial cell of the primary gill lamellae and damage to the blood capillaries, fragmented cells, necrosis of the secondary gill lamellae, and cell atrophy (Prabhaha et al., 2012). Studies on pollution have also reported that water pollution causes histological changes such as hyperplasia, fragmentation of epithelial cells, and edema of epithelial cells (Abalaka, 2015; Mustafa et al., 2020). Rahman et al. (2019) reported that exposure to heavy metals caused hyperplasia and fusion of gill lamellae in the gills of *Oreochromis niloticus*.

Similar findings were reported in the gill examination of *Auchenoglanis occidentalis* fish collected from Tiga Dam, Nigeria by Abalaka (2015). In addition, Mustafa et al. (2019) reported similar observations in gill tissues of *C. carpio* and *Carasobarbus luteus* collected from the Tigris River. According to Pantung et al. (2008) reported histological changes such as hyperplasia of chloride cells, fragmentation of epithelial cells, edema of epithelial cells in hybrid catfish (*Clarias macrocephalus* x *Clarias* *gariepinus*) exposed to Cd in water. In this study, a number of changes were noticed in the gills of fish exposed to Cd. These occurred as hypertrophy, hyperplasia of gill epithelium, mucous cell hypertrophy and proliferation, mucus hypersecretion, proliferation of eosinophilic granule cells, and interlamellar cell hyperplasia, respectively. The first histological changes may be due to the first defence mechanism (Naigaga, 2002).

The toxicity effect of heavy metals on fish liver has been studied by many researchers. Histopathological changes such as hepatocytes degeneration, vacuolization, congestion of hepatic tissues, subcapsular vacuolization, necrosis, indistinct cell borders and pycnotic nuclei were detected in liver of Cd-exposed *C. batrachus* (Selvanathan, 2013).

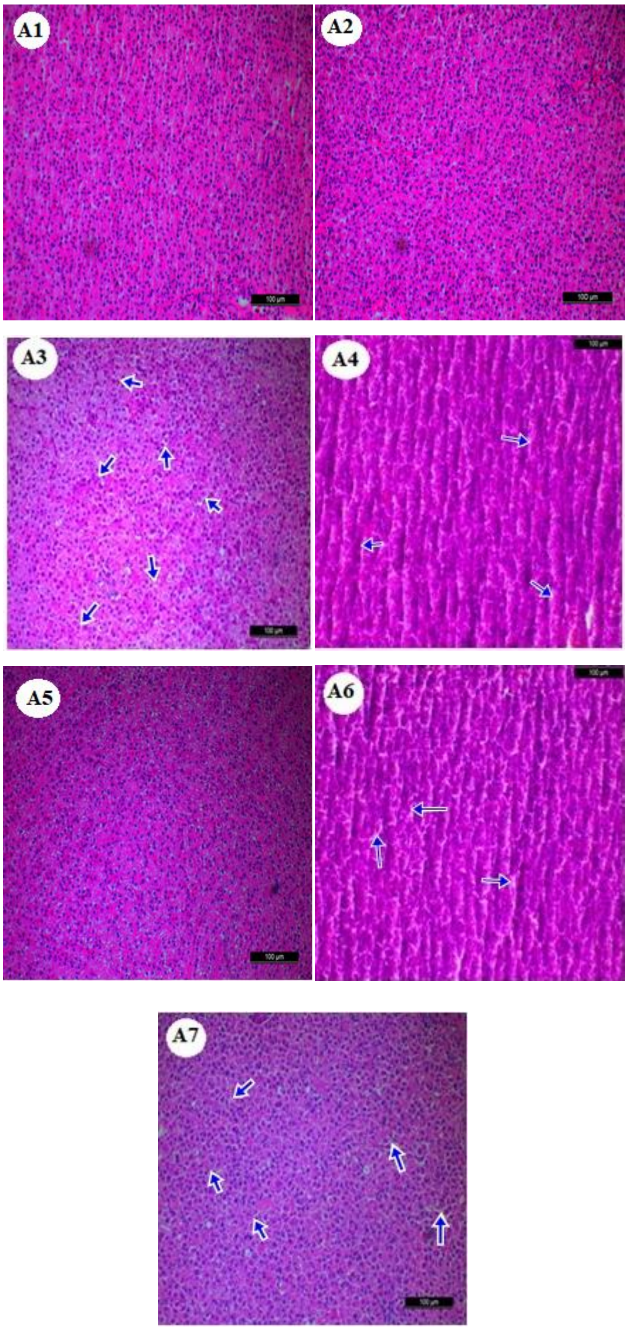

Figure 3. Acute period liver histopathology
A1: Control group liver(HE200X), A2: Vehicle group liver (HE200X), A3: Cadmium exposed group liver (HE 200X) Arrows show extracellular degenerations, A4: Grape seed (*V. vinifera*) extract (5 ppm) applied group liver (HE 200X) Arrows show extracellular degenerations, A5: Grape seed (*V. vinifera*) extract (10 ppm) applied group liver (HE 200X), A6: Grape seed (*V. vinifera*) extract (5 ppm) applied to Cd exposed group liver (HE 200X) Arrows show extracellular degenerations, A7: Grape seed (*V. vinifera*) extract (10 ppm) applied to Cd exposed group liver (HE 200X)
Arrows show extracellular degenerations

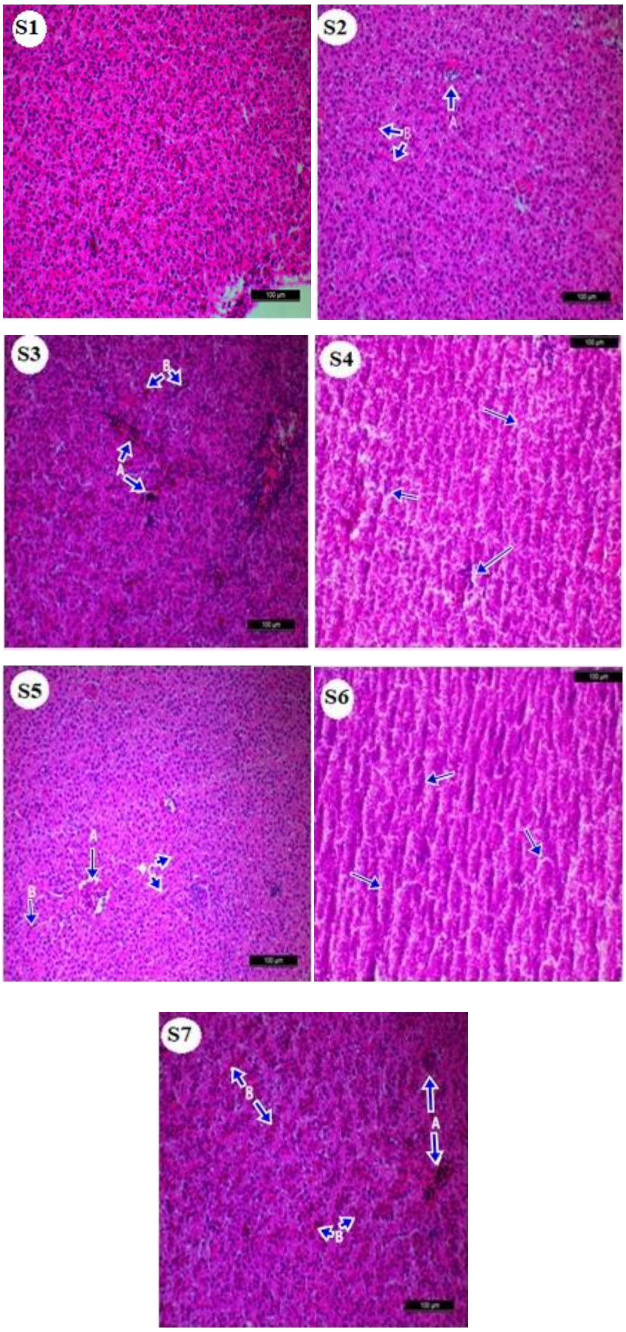

Figure 4. Subchronic period liver histopathology
S1: Control Group liver (HE200X), S2: Vehicle Group liver (HE200X) A- Hemorrhage, B- Mononuclear cell infiltration, S3: Cadmium exposed group liver (HE 200X) A- Hemorrhage, B- Mononuclear cell infiltration, S4: Grape seed (*V. vinifera*) extract (5 ppm) applied group (30 days) liver (HE 200X) Arrows show extracellular degenerations, S5: Grape seed (*V. vinifera*) extract (10 ppm) applied group liver (HE 200X) A- Hemorrhage B- Mononuclear cell infiltration C- Extracellular degeneration, S6: Grape seed (*V. vinifera*) extract (5 ppm) applied to Cd exposed group liver (HE 200X) Arrows show extracellular degenerations, S7: Grape seed (*V. vinifera*) extract (10 ppm) applied to Cd exposed group liver (HE 200X) A- Hemorrhage B- Mononuclear cell infiltration

Degenerative changes such as hepatocellular dissociation, necrosis and hypertrophy were observed in the freshwater fish *O. striatus* exposed to cadmium chloride (Bais and Lokhande, 2012). Rahman et al. (2019) reported hepatopancreatic cell necrosis and degeneration in fish exposed to heavy metals. In the histological examination of liver sections *of H. fossilis* exposed to Cd concentrations, it has been reported that hepatocyte rupture, vacuolization, hemorrhage, cellular necrosis, increased pycnotic nuclei, and melanomacrophages were reported (Jayakumar and Subburaj, 2017).

It has been reported that herbal extracts have various beneficial properties for fish (Chakraborty and Hancz, 2011; Bulfon et al., 2015; Van Hai, 2015; Reverter et al., 2017).

Rahman et al. (2019) reported that Indian lotus ameliorated gill and liver histopathological alterations in heavy metal exposed tilapia. Zahran et al. (2019) reported that fish fed a diet fortified with *Chlorella vulgaris* reduced to some extent the histopathological changes induced by chronic sodium arsenite. Similar results were seen in *O. niloticus* fed with *Thunbergia laurifolia* leaf extract and exposed to lead (II) nitrate (Palipoch et al., 2011).

The histopathological data of the gill and liver in the subchronic period of Cd exposure obtained in this study and the therapeutic results obtained as a result of *V. vinifera* seed extract application are similar to the findings in the literature. These results indicated that dietary supplementation of *V. vinifera* seed extract could have protective effects against the Cd toxic effects of *C. carpio* and therefore serve as a useful feed additive for application in the aquaculture industry.

Conclusion

As a result of the study, it was concluded that *V. vinifera* seed extract has a therapeutic effect on the gill and liver histopathology of carp exposed to cadmium. It can be used in food ration in fish farms to feed fish. Thus, we can have the opportunity to produce quality fish in a short time by preventing histopathological anomalies that may occur in fish due to water quality or environmental conditions. Investigation of the molecular mechanism of action of the protective and curative effect of black grape seed *V. vinifera* extract will make a great contribution to this field.

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Disclosure Statement

The author declares that there is no conflict of interest.

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References

Abalaka SE. 2015. Heavy metals bioaccumulation and histopathological changes in *Auchenoglanis occidentalis* fish from Tiga dam, Nigeria. Journal of Environmental Health Science and Engineering, 13(1): 1-8. https://doi.org/ 10.1186/s40201-015-0222-y

Abbas WT, Authman MMN, Darwish DA, Kenawy AM, Abumourad IMK, Ibrahim TB. 2019. Cadmium toxicity-induced oxidative stress and genotoxic effects on Nile tilapia (*Oreochromis niloticus* L.) fish: the protective role of fenugreek (*Trigonella foenum-graecum*) Seeds. Egyptian Journal of Aquatic Biology and Fisheries, 23(5) (Special Issue): 193-215. https://doi.org/10.21608/EJABF.2019.647 15

Abdel-Tawwab M, Sharafeldin KM, Ismaiel NEM. 2018. Interactive effects of coffee bean supplementation and waterborne zinc toxicity on growth performance, biochemical variables, antioxidant activity and zinc bioaccumulation in whole body of common carp, *Cyprinus carpio* L. Aquaculture Nutrition, 24(1): 123-130. https://doi.org/10.1111/anu.12540

Abdel-Tawwab M. 2016. Feed supplementation to freshwater fish: Experimental approaches. LAP Lambert Academic Publishing.

Adebayo IA. 2017. Determination of heavy metals in water, fish and sediment from Ureje water reservoir. Journal of Environment and Analytical Toxicology, 7(4): 1-4. https://doi.org/ 10.19080/OFOAJ.2017.04.555628

Al-Mansoori AF. 2006. Histological changes induced by cadmium ion in the Gills, Liver and Intestine of juvenile *Carassius carassius* (L.). Basrah Journal of Science (B), 24(1): 32-46.

Amin N, Manohar S, Qureshi TA, Khan S. 2013. Effect of Cadmium Chloride on the Histoarchitecture of Liver of a Freshwater Catfish, *Channa punctatus*. Journal of Chemical, Biological and Physical Sciences (JCBPS), 3(3): 1906.

Andhale AV, Bhosale PA, Zambare SP. 2011. Histopathological study of nickel induced alterations in the fresh water bivalve, *Lammellidens marginalis*. Journal of Experimental Sciences, 2(4): 1-3.

Bahnasawy M, Khidr AA, Dheina N. 2011. Assessment of heavy metal concentrations in water, plankton, and fish of Lake Manzala, Egypt. Turkish Journal of Zoology, 35(2): 271-280. https://doi.org/ 10.3906/zoo-0810-6

Bais UE, Lokhande MV. 2012. Effect of cadmium chloride on histopathological changes in the freshwater fish *Ophiocephalus striatus* (Channa). International Journal of Zoological Research, 8(1): 23.

Bonanno G, Giudice RL. 2010. Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. Ecological indicators, 10(3): 639-645. https://doi.org/ 10.1016/j.ecolind.2009.11.002

Bulfon C, Volpatti D, Galeotti M. 2015. Current research on the use of plant-derived products in farmed fish. Aquaculture Research, 46(3): 513-551. https://doi.org/ 10.1111/are.12238

Camargo MM, Martinez CB. 2007. Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyology, 5(3): 327-336.

Chakraborty SB, Hancz C. 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. Reviews in Aquaculture, 3(3): 103-119. https://doi.org/ 10.1111/j.1753-5131.2011.01048.x

Dasari NP, Rao BG, Rao ES, Rao TM. 2012. Quantification of phytochemical constituents and in vitro antioxidant activity of *Synadium grantii*. Free Radicals and Antioxidants, 2(2): 68. https://doi.org/ 10.5530/ax.2012.2.2.12

Deore SV, Wagh SB. 2012. Heavy metal induced histopathological alterations in liver of *Channa gachua* (Ham). Journal of Experimental Sciences, 3(3): 35-38.

Drastichova J, Svobodova Z, Luskova V, Machova J. 2004. Effect of cadmium on hematological indices of common carp (*Cyprinus carpio* L.). Bulletin of environmental contamination and toxicology, 72(4): 725-732.

Erdoğan K, Kandemir Ş, Doğru MI, Doğru A, Şimşek I, Yılmaz S, Örün G, Altaş L, Yazıcıoğlu O, Korkmaz N, Örün, I. 2021. The effects of seasonal heavy-metal pollution of Lâdik Lake on pike fish (*Esox lucius*). Biological Rhythm Research, 52(6): 821-845. https://doi.org/ 10.1080/09291016.2019. 1607215

Fatima M, Usmani N, Firdaus F, Zafeer MF, Ahmad S, Akhtar K, Dawar Husain SM, Ahmad MH, Anis E, Hossain MM. 2015. In vivo induction of antioxidant response and oxidative stress associated with genotoxicity and histopathological alteration in two commercial fish species due to heavy metals exposure in northern India (Kali) river. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 176: 17-30. https://doi.org/10.1016/j.cbpc.2015.07.004

Fonseca AR, Fernandes LS, Fontainhas-Fernandes A, Monteiro SM, Pacheco FAL. 2016. From catchment to fish: Impact of anthropogenic pressures on gill histopathology. Science of the Total Environment, 550: 972-986. https://doi.org/ 10.1016/j.scitotenv.2016.01.199

Fonseca AR, Fernandes LS, Fontainhas-Fernandes A, Monteiro SM, Pacheco FAL. 2017. The impact of freshwater metal concentrations on the severity of histopathological changes in fish gills: A statistical perspective. Science of the Total Environment, 599: 217-226. https://doi.org/10.1016/ j.scitotenv.2017.04.196

Goyal M, Nagori BP, Sasmal D. 2009. Sedative and anticonvulsant effects of an alcoholic extract of *Capparis decidua*. Journal of natural medicines, 63(4): 375-379. https://doi.org/10.1007/s11418-009-0339-3

Götze S, Matoo OB, Beniash E, Saborowski R, Sokolova IM. 2014. Interactive effects of CO_2 and trace metals on the proteasome activity and cellular stress response of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Aquatic Toxicology, 149: 65-82. https://doi.org/10.1016/ j.aquatox.2014.01.027

Hermenean A, Gheorghiu G, Stan MS, Herman H, Onita B, Ardelean DP, Ardelean A, Braun M, Zsuga M, Kéki S, Costache M, Dinischiotu A. 2017. Biochemical, histopathological and molecular responses in gills of *Leuciscus cephalus* exposed to metals. Archives of environmental contamination and toxicology, 73(4): 607-618. https://doi.org/10.1007/s00244-017-0450-5

Hermoso V, Clavero M. 2013. Revisiting ecological integrity 30 years later: non-native species and the misdiagnosis of freshwater ecosystem health. Fish and Fisheries, 14(3): 416-423. https://doi.org/10.1111/j.1467-2979.2012.00471.x

Ikram SARA, Malik MA. 2009. Histo-pathological profile of organs of immune system (kidney, liver and spleen) on acute cadmium intoxication in *Labeo rohita*. Biologia, 55: 51-58.

Javed M, Ahmad I, Ahmad A, Usmani N, Ahmad M. 2016. Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in *Channa punctatus* (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent. Springer Plus, 5(1): 1-9. https://doi.org/ 10.1186/s40064-016-2478-9

Jayakumar N, Subburaj A. 2017. Sub-lethal cadmium toxicity induced histopathological alterations in the gill, liver and kidney of freshwater catfish (*Heteropneustes fossilis*). Journal of Entomolgy and Zoology Studies, 5(5): 1339-1345.

Kalavathi R, Saradhamani N. 2017. Influence of *Nelumbo nucifera* on the rate of oxygen consumption in *Cirrhinus mrigala* exposed to copper sulphate. European Journal of Biomedical and Pharmaceutical Sciences. 10: 597–600.

Korkmaz N, Orun I. 2020. Research of the hematological, antioxidant and histopathological effects of Neemazal-T/S on common carp fish *Cyprinus carpio* (Linneaus 1758). Fresenius Environmental Bulletin, 29(9): 7246-7256.

Meyer AS, Yi OS, Pearson DA, Waterhouse AL, Frankel EN. 1997. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). Journal of agricultural and Food Chemistry, 45(5): 1638-1643. https://doi.org/10.1021/jf960721a

Mohammed FS, Şabik AE, Sevindik E, Pehlivan M, Sevindik M. 2020a. Determination of Antioxidant and Oxidant Potentials of *Thymbra spicata* Collected from Duhok-Iraq. Turkish Journal of Agriculture-Food Science and Technology, 8(5): 1171-1173.

Mohammed FS, Şabik AE, Doğan M, Selamoğlu Z, Sevindik M. 2020b. Antioxidant potential of *Hypericum spectabile* JAUB. ET SPACH. Bulletin of Biotechnology, 1(2): 43-45.

Mohammed FS, Günal S, Şabik AE, Akgül H, Sevindik M. 2020c. Antioxidant and Antimicrobial activity of *Scorzonera papposa* collected from Iraq and Turkey. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 23(5): 1114-1118.

Mukke VK. 2012. Bioaccumulation study of mercury chloride in selected tissue of fresh water crab, *Barytelphusa guerini* from Aurangabad region. Journal of Chemical and Pharmaceutical Research, 4(1): 398-401.

Mustafa SA, Al-Rudainy AJ, Al-Samawi SM. 2020. Histopathology and level of bioaccumulation of some heavy metals in fish, *Carasobarbus Luteus* and *Cyprinus carpio* tissues caught from Tigris River, Baghdad. The Iraqi Journal of Agricultural Science, 51(2): 698-704. https://doi.org/ 10.1016/j.ejar.2020.01.004

Naigaga I. 2002. Bioaccumulation and histopathology of copper in *Oreochromis mossambicus*. MSc Thesis, Rhodes University,Grahamstown, South Africa, 124.

Palipoch S, Jiraungkoorskul W, Tansatit T, Preyavichyapugdee N, Jaikua W, Kosai P. 2011. Protective efficiency of *Thunbergia laurifolia* leaf extract against lead (II) nitrate-induced toxicity in *Oreochromis niloticus*. Journal of Medicinal Plants Research, 5(5): 719-728. https://doi.org/ 10.5897/JMPR.9001063

Pandey S, Parvez S, Ansari RA, Ali M, Kaur M, Hayat F, Ahmad F, Raisuddin S. 2008. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chemico-biological interactions, 174(3): 183-192. https://doi.org/10.1016/j.cbi.2008.05.014

Pantung N, Helander KG, Helander HF, Cheevaporn V. 2008. Histopathological alterations of hybrid walking catfish (*Clarias macrocephalus* x *Clarias gariepinus*) in acute and subacute cadmium exposure. Environment Asia, 1(1): 22-27.

Pehlivan M, Mohammed FS, Şabik AE, Kına E, Dogan M, Yumrutaş Ö, Sevindik M. 2021. Some Biological activities of ethanol extract of *Marrubium globosum*. Turkish Journal of Agriculture-Food Science and Technology, 9(6): 1129-1132.

Prabhahar C, Saleshrani K, Tharmaraj K, Vellaiyan M. 2012. Effect of cadmium compound on the histological changes of various vital organs of the fresh water fish *Cirrhinus mrigala*. International Journal of Pharmaceutical and Biological Archives, 3(1): 84-8.

Rahman ANA, ElHady M, Hassanin ME, Mohamed AAR. 2019. Alleviative effects of dietary Indian lotus leaves on heavy metals-induced hepato-renal toxicity, oxidative stress, and histopathological alterations in Nile tilapia, *Oreochromis niloticus* (L.). Aquaculture, 509: 198-208. https://doi.org/10.1016/j.aquaculture.2019.05.030

Rajeshkumar S, Li X. 2018. Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. Toxicology reports, 5: 288-295. https://doi.org/ 10.1016/j.toxrep.2018.01.007

Ramachandra TV, Sudarshan PB, Mahesh MK, Vinay S. 2018. Spatial patterns of heavy metal accumulation in sediments and macrophytes of Bellandur wetland, Bangalore. Journal of environmental management, 206: 1204-1210. https://doi.org/ 10.1016/j.jenvman.2017.10.014

Reverter M, Tapissier-Bontemps N, Sasal P, Saulnier D. 2017. Use of medicinal plants in aquaculture. Diagnosis and control of diseases of fish and shellfish, 1: 223-261.

Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaji N. 1998. Antiulcer activity of grape seed extract and procyanidins. Journal of agricultural and Food Chemistry, 46(4): 1460-1464. https://doi.org/ 10.1021/jf9709156

Santos D, Melo MRS, Mendes DCS, Rocha IK, Silva JPL, Cantanhêde SM, Meletti PC. 2014. Histological changes in gills of two fish species as indicators of water quality in Jansen Lagoon (São Luís, Maranhão State, Brazil). International journal of environmental research and public health, 11(12): 12927-12937. https://doi.org/ doi.org/10.3390/ijerph111212927

Satarug S, Moore MR. 2012. Emerging roles of cadmium and heme oxygenase in type-2 diabetes and cancer susceptibility. The Tohoku journal of experimental medicine, 228(4): 267-288. https://doi.org/ 10.1620/tjem.228.267

Scardi M, Cataudella S, Di Dato P, Fresi E, Tancioni L. 2008. An expert system based on fish assemblages for evaluating the ecological quality of streams and rivers. Ecological informatics, 3(1): 55-63. https://doi.org/ 10.1016/j.ecoinf. 2007.10.001

Selvanathan J, Vincent S, Nirmala A. 2013. Histopathology changes in freshwater fish *Clarias batrachus* (Linn.) exposed to mercury and cadmium. International Journal of Life Sciences and Pharma Research, 3(2): 11-21.

Sevindik M. 2018. Pharmacological properties of Mentha species. J Tradit Med Clin Natur, 7(2): 259.

Svobodová Z. 1993. Water quality and fish health (No. 54). Food and Agriculture Organization of the United Nations, European Inland Fisheries Advisory Commission.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. 2012. Heavy metal toxicity and the environment. Molecular, clinical and environmental toxicology, 133-164. https:// doi.org/10.1007/978-3-7643-8340-4_6

Thophon S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, Jaritkhuan S. 2003. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environmental pollution, 121(3): 307-320. https://doi.org/10.1016/S0269-7491(02) 00270-1

Van Hai N. 2015. The use of medicinal plants as immunostimulants in aquaculture: A review. Aquaculture, 446: 88-96. https://doi.org/ 10.1016/j.aquaculture.2015. 03.014

Yong-Bo T, Cheng-Bin L, Hao-Xue Q. 2014. Calculations on polarization properties of alkali metal atoms using Dirac—Fock plus core polarization method. Chinese Physics B, 23(6): 063101. https://doi.org/10.1088/1674-1056/23/6/063101

Zahran E, Awadin W, Risha E, Khaled AA, Wang T. 2019. Dietary supplementation of *Chlorella vulgaris* ameliorates chronic sodium arsenite toxicity in Nile tilapia *Oreochromis niloticus* as revealed by histopathological, biochemical and immune gene expression analysis. Fisheries science, 85(1): 199-215. https://doi.org/10.1007/s12562-018-1274-6