Effect of humic acid on the brain tissue of brown trout treated with cadmium

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ABSTRACT
The present study was aimed to investigate histopathological changes in brain tissue following cadmium (Cd) treatment and to observe whether humic acid (HA) has an effect against to brain damage caused by cadmium. For this aim, brown trout were randomly separated into four groups: (I) control group, (II) cadmium group (2 ppm), (III) humic acid group (5 ppm), (IV) humic acid +cadmium group. The brown trout were exposed to heavy metal stress cadmium at 2 ppm dosage and humic acid at 5 ppm dosage. After the fish were sacrificed, brain tissues were examined histopathologically by light microscopy. The histopathological results indicated damage of the fish brain tissues in the Cd and Cd+HA groups. However, HA had no effect when compared to the other groups.

Keywords: Humic acid, cadmium, brain, histopathology, brown trout.

1. Introduction
Heavy metal contamination occurring in the aquatic ecosystem has drawn increasing attention and it may cause toxic effects in aquatic organisms, especially the fish and disruption of the ecological balance [1, 2]. Cadmium is a heavy metal which have no biological function and may lead to physiological changes in aquatic organisms [3, 4]. It was reported that the acute toxicity of cadmium for fish is different and is related to free cadmium ion concentration [5]. Cadmium used in ecotoxicological studies rises in the aquatic environment because of domestic wastes and industrial [6, 7]. It is important to note that cadmium is toxic to fish and mammals and cause ecological problems due to the accumulation in tissues. By contrast, the excretion of cadmium from living organisms is a slow process [8]. In fish, cadmium has been shown to alter the structure and to cause morphological changes of varying severity in various organs [9].

Humic substances are organic molecules that constitute the major part (up to 95%) of the dissolved/natural organic matter (DOM/NOM) in freshwater [10]. There are natural organic matters (NOM) such as humic acid (HA) in the aquatic environment [11]. Humic substances can react with various chemicals in water or in aquatic organisms and bioconcentration of humic substances has been reported in freshwater organisms [12]. Humic substances are found in the environment and have been shown to affect physiological functions of aquatic organisms [10]. Therefore, the present study was aimed to investigate whether HA has an effect against cadmium-induced neurotoxicity in the brain tissues after Cd and HA exposure.

2. Materials and method
Fish Maintenance, Treatment Unit, Water and Toxicant
A group of 40 brown trout fish (with an average weight of 145±12 g) was obtained Atatürk University, Faculty of Fisheries Inland water fish breeding and Research Center. Fish were acclimated to conditions of the research unit for three weeks before the treatment. The research platforms were 400 lt fiberglass circular tanks with a constant under natural light conditions. The fish had an average 203.31±8.09 g weight and 22.21±0.49 cm length. Water quality parameters of tank water are: temperature (10-12 °C); pH (7.0±0.3); dissolved oxygen (7.52±0.50 mg/L); water hardness (164.1±4.17 mg/L). The stock solutions of cadmium chloride (Sigma) and humic acid (Farmavet Medicine) were used and the final concentration was...
achieved [13, 14]. Ten fish were placed into each of the tanks. Fish were divided into four groups, as the group I was designed as a control group, group II as cadmium group, group III as only humic acid group, group IV as cadmium + humic acid group. Fish in group II were given a single dose of 2 ppm concentration of cadmium chloride (CdCl₂). This dose was selected as it has been previously reported to induce toxicity in rainbow trout [13]. Fish in group III were exposed to a single dose of 5 ppm concentration of humic acid. Fish in group IV were exposed to a mixture dose of 2 ppm cadmium chloride and 5 ppm humic acid. These doses were administered to fish for seven days.

2.1 Histopathological analysis

The tissue samples for light microscopic examination were fixed in 10% formaldehyde, dehydrated in a graded alcohol series, and cleared in xylol. After dehydration, specimens were embedded in fresh paraffin. Sections were cut using a microtome (Leica, Germany). Each paraffin block was serially cut into 5 μm-thick sections. The sections were stained with hematoxylin-eosin (H-E) for light microscopic examination. (Olympus BX52 with DP72 camera system). All histopathological alterations were estimated with an image processing system (Olympus, DP2-BSW). The scores were derived semi-quantitatively using light microscopy on the preparations and were reported as follows: none: −, mild: +, moderate: ++, and severe: +++.

3. Results

Histopathological results

There were no histopathological changes in brain tissues (Fig 1) of the control group. Similarly, no histopathological changes except for a limited edema and limited neuronal degeneration were observed in humic acid group (Fig 2). Prominent changes were observed in the experimental cadmium group. Neuronal necrosis and edema in a purkinje layer of cerebellar sections were marked (Fig 3). Similar histopathological changes were observed in cadmium + humic acid group (Fig 4). Neuronal changes were similar level with cadmium group. The intensity and severity of histopathological changes were displayed in table 1.

Table 1: The intensity and severity of histopathological changes in brain tissues.

<table>
<thead>
<tr>
<th>Histopathological lesion</th>
<th>Control</th>
<th>HA</th>
<th>Cd</th>
<th>Cd+HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperemia</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Edema</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Neuronal degeneration and necrosis</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
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Fig 1: Normal histologic layers of cerebellum and Purkinje cells with nuclei (arrows) in control group. H-E.

4. Discussion

Histological changes occurred in brain tissues of fish exposed to different pollutants is lacking in the literature. In fish, cadmium may cause different changes in organ structural [9]. Brain has important functions in governing the whole body function [15]. Evaluation of neurotoxicity of environmental pollutants is important because of excessive of these compounds [16]. Many pollutants in aquatic environment cause neurotoxicity on central nervous system [17]. Neurotoxic injury can result in behavioral changes that may impair the subsequent survival or reproduction of exposed organisms [16].

Cadmium is a more persistent neurotoxic contaminant [17]. Histopathology is the manifestation of cellular injury [18]. The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs [19]. In the present study, histopathological results showed that the brain of the control group showed normal histological features, but the Cd-treated group revealed edema and severe neuronal degeneration and necrosis (Fig 3). It may be suggested that neuronal degeneration may be indicative of neurotoxic effects of cadmium. It had been previously reported to induce toxicity in *Cyprinus carpio communes* of Cd [2].
**Fig 2:** Some degenerative neurons and edema (arrows) in HA group. H-E.

**Fig 3:** Edema and severe neuronal degeneration and necrosis in Cd group (arrows). H-E.

**Fig 4:** Severe neuronal changes (arrows) similar with Cd group in Cd-HA group. H-E
Our research results in this respect were similar to the findings of Patnaik et al (2011). We can say that cadmium may have a direct effect on brain tissue. Probably, this process may be related to the occurrence of oxidative products in the brain. However, an increase in the rate of ROS production causes mitochondrial DNA, nuclear DNA and protein and membrane damage [20]. These composed lesions of ROS may cause the necrosis [28, 21, 22]. Additionally, there is no documentation in the literature on whether HA has an effect on brain neurotoxicity induced by Cd. Our results showed that humic acid did not improve cadmium-induced histopathological damage. Humic substances are found in the environment and have been shown to affect physiological functions of aquatic organisms [19]. It has been detected that humic acid consistently reduced Cd accumulation in all the tissues/organs [14]. In conclusion, Cd treatment induces brain damage as indicated by the elevation of histopathological alterations. HA did not have much effect against damage induced by cadmium.

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6. Reference
13. Talas ZS, Orun I, Ozdemir I, Erdogan K, Alkan A, Yilmaz I. Antioxidative role of selenium against the toxic effect of heavy metals (Cd^{2+}, Cr^{3+}) on liver of rainbow trout (Oncorhynchus mykiss Walbaum 1792). Fish Physiol Biochem 2008; 34:217–222.