Int. J. Aquat. Biol. (2019) 7(5): 315-321
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

The protective effect of Damask rose, *Rosa damascena* extract on the liver of *Cyprinus carpio* following zinc exposure

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Abstract: This study aimed to examine two concentrations of *Rosa damascene* extract on toxic effect of Zn on the liver of common carp. For this purpose, four treatments with three replicates were designed as following: treatment one as control, treatment 2 exposed to 3 mg/l $ZnSO_4$, and treatments 3 and 4 supplemented with 2.5 and 5 g of *R. damascene* extract along with exposure to 3 mg/L $ZnSO_4$. After 45 days, the bioaccumulation of Zn and histopathological alternation in the liver, as well as aspartate aminotransferase (AST), alanine transaminase (ALT), triglyceride, cholesterol and albumin were measured. The results showed the highest concentration of Zn in treatment, and its decreasing trend in treatments 3 and 4. The histopathological examinations showed the most alternations in treatment 2, but lower changes in treatment 3 and 4 due to application of *R. damascene* extract. In addition, those measured biochemical parameters had increased in treatment 2 following exposure to $ZnSO_4$, whereas they had decrease in treatments 3 and 4. These results revealed positive effect of *R. damascene* extract to decrease toxicity of Zn on the liver of common carp.

Article history:
Received 25 February 2019
Accepted 21 October 2019
Available online 25 October 2019

Keywords:
Common carp
Bioaccumulation
Histopathology
Biochemical parameters

Introduction

Damask rose, *Rosa damascene*, is one of important species of the Rosaceae family (Kaul et al., 2000; Cai et al., 2005) and its main components are anthocyanin, cyanidin, 3-5 diglucoside, kaempferol, quercetin, galactoside, arabinoside, citronellol, linalool, geraniol and terpenes (Velioglu and Mazza, 1991), having antibacterial and antioxidant activities (Ozkan et al., 2004). Among these components, quercetin can prevent many types of cancers e.g. intestine cancer (Shoskes et al., 1999) and reduce blood pressure (Edwards et al., 2007). In addition, Kaempferol extracted from damask rose reported to prevent the activity of the HIV virus's proteases (Mahmood et al., 1996).

Zinc is an essential and second trace element in vertebrates' body (Pagiannisa et al., 2004), necessary for physiological processes such as cell division, metabolism, wounds healing, defense and reproduction systems, and taste and sight performances (Aggett and Comerford, 1995). Its concentrations in aquatic environments vary due to anthropological activities, and natural geological events (Janssen et al., 2000; Luoma and Rainbow, 2008; Wood et al., 2012). Total Zn concentrations has been reported ranging 0.02 µg/L in rivers to more than 1000 µg/L in freshwater water bodies near mining and metal industrial activities (Luoma and Rainbow, 2008; Wood et al., 2012). Despite the essentiality of Zn, it can be toxic and considered as a serious threat for health, if organisms exposed to a high concentrations (Mazrouh and Mahmoud 2009; Murakami and Hirano, 2008). Zinc can be toxic for fish, causing disturbances in ionoregulation and hypoxia (Murugan et al., 2008), and histological damages e.g. edema, epithelial cell destruction, hyperemia, inflammatory cell infiltration, leydig cell hyperplasia, severe fibrosis, loss of natural structure, and reduction of spermatids and spermatozoa in the lumen of the seminiferous tubules have been reported following

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DOI: https://doi.org/10.22034/ijab.v7i5.749

zinc poisoning in *Carassius auratus* (Hoseinifard et al., 2018).

The liver is an important organ for Zn storage in fishes, and increasing the levels of Zn will lead different degrees of abnormalities effecting fish performance (Gregorović et al., 2008), therefore liver is a proper biomarker for evaluation of environmental pollution (Chavan and Muley, 2014). Hence, this study aimed to examine two concentrations of *R. damascene* extract on toxic effect of Zn on the liver of common carp (*Cyprinus carpio*).

Materials and Methods

A total of 120 healthy fish with an average weight and length of 50±2.14 g and 13±0.25 cm, respectively, were purchased from a local fish farm and transferred to the laboratory. Fish were acclimated in an aerated freshwater 1000L tank (24±1°C; pH, 7.2±0.2) for two weeks. During the acclimatization period, fish were fed with commercial pelleted feed including 41% crude protein, 6% crude fat, 5% crude fiber and 12% moisture (Chineh Co.). Fish were fed as 1% of body weight twice a day during the acclimatization period. They were also maintained at 24±1°C with continuously aeration, 6.5 mg/L DO, and pH 7.2±0.2.

For experiment, fish were randomly distributed into 12 circular 100 L tanks as 4 treatments with three replications. Treatments were fed with commercial pelleted feed. Treatment 1 was control one, treatment 2 exposed to 3 mg/L $ZnSO_4$ (Merck, Germany) without adding *R. damascene* extract, and treatments 3 and 4 were exposed to 3 mg/L $ZnSO_4$ along with 2.5 and 5 g *R. damascene* extract supplementation in 100 gr commercial feed. Each supplemented diet was mixed in a mixer for 30 min and then homogenized into a paste by adding fish oil and distilled water into the food mixer. The amount of distilled water required for pelleting (40% of feed weight) was then added to the mixture and further homogenized. This mixture was passed through a meat grinder, producing string shapes, dried and then broken to produce pellets. The control diet was prepared by the same process, although no supplement was added. The fish were subjected to artificial light of 12h D: 12h L. After 45 days, fishes were taken out randomly, anesthetized with clove oil and sacrificed for further analysis after blood sampling. The water conditions were similar to those of the acclimatization period.

Damask rose extract analysis: The extract component of *R. damascena* was analyzed based on Yassa et al. (2015). Determination of total amount of phenol was measured using folin-ciocaltue colorimetric method (Slinkard and Singleton, 1977) and total flavonoid by aluminum chloride colorimetric method (Kim et al., 2002).

Histopathological analysis: The liver samples were removed and fixed into 10% buffered formalin. Histological sections were prepared based on Eagderi et al. (2013) using tissue processor device of DID SABZ2080/H (Iran), and microtome of Leitez 1512 (Germany) with a diameter of 5 µm. The tissue sections were stained with Haematoxylin and Eosin (H&E) (Bancroft and Gamble, 2008; Moëzzi et al., 2013). The histological sections were examined with light microscope (Olympus CX31, Japan) equipped to a digital camera (Tucsen TrueChrome Metrics). The histopathological alternations were graded as - , +, ++ and +++ showing no change, mild, moderate, and severe lesions, respectively (Thophon et al., 2003; Nasrolah Pourmoghadam et al., 2014).

Biochemical parameters analysis: The blood samples were taken and their serum were separated by 3000 r/m centrifuging for 10 min (Ahmadivand et al., 2014, 2015). The biochemical parameters, including aspartate aminotransferase (AST), alanine transaminase (ALT), triglyceride, cholesterol and albumin were measured by Pars Azmoon kit (Iran) using autoanalyzer (Hitachi -911, Japan).

Bioaccumulation analysis: The samples of liver tissue were removed from fish and dried in the oven (105°C) for 96 hours and powdered, then 1 g of this powder was mixed with 5 ml of nitric acid (Merck, Germany) in the polyethylene tubes and then 2 ml of perchloric acid (Merck, Germany) were added. The samples were kept overnight at room temperature, and afterward transferred to the water bath for 2 hours at 100°C. After digestion process, they filtered using filter paper, and their volume increased to 25 ml by adding

Table 1. Histopathological alternations of the liver of *Cyprinus carpio* in different treatments (-, +, ++ and +++ indicate no change, mild, moderate, and severe lesions, respectively).

	Hyperaemia	Hepatocytes necrosis	Bile duct hyperplasia	Inflammatory cells infiltration	Vacuolar degeneration
Treatment 1	-	-	-	-	-
Treatment 2	++	++	++	++	+++
Treatment 3	+	++	+	+	++
Treatment 4	-	+	-	-	+

Table 2. Biochemical parameters (Mean±SE) of *Cyprinus carpio* in different treatments (letters above the column indicate significant differences; $P<0.05$).

	AST (U/Lit)	ALT (U/Lit)	Triglyceride (mg/dl)	Albumin (g/dl)	Cholesterol (mg/dl)
Treatment 1	85 ± 3.16^{b}	8.50 ± 0.67^{c}	$174\pm4.60^{a,b}$	$1.05\pm.02^{a}$	70 ± 1.78^{b}
Treatment 2	130 ± 9.48^{a}	20.25 ± 1.77^{a}	225.50 ± 27.05^{a}	$1.25\pm.11^{a}$	125.5 ± 18.64^{a}
Treatment 3	89.50 ± 2.50^{b}	12 ± 0.89^{b}	166 ± 16.09^{b}	$1.15\pm.08^{a}$	125 ± 9.83^{a}
Treatment 4	86.50 ± 4.94^{b}	$9\pm0.70^{b,c}$	130 ± 14.75^{b}	$1.02\pm.05^{a}$	$98\pm3.13^{a,b}$

1% nitric acid (Al-Weher, 2008). Zn concentration measured using Atomic Absorption Spectrophotometer (Analytic jenaAA-400, Germany), and the following formula was used to calculate the concentration (Al-Weher, 2008):

Zn concentration in sample (µg/g) = AAS reading of digest (µg/ml) × volume of digest used (ml)/weight of sample digested (g)

Statistical analysis: Normality of the data were checked using Kolmogorov-Smirnov. One-way analysis of variance (ANOVA) was used for analysis of significant difference among treatments. Means were compared by Duncan's test and a $P<0.05$ was considered statistically significant. Statistical analysis was performed using software SPSS ver. 22. Data are presented as mean±SE.

Results

Damask rose extract components: The total phenol and flavonoid in *R. damascena* extract were 18.6 and 1.54 mg/g, respectively.

Histopathology finding: In treatment 1, the prepared histological sections showed normal conditions. Those of treatment 2 had altered as moderate hyperaemia, hepatocytes necrosis, bile duct hyperplasia and inflammatory cells infiltration and sever vacuolar degeneration. In treatment 3, mild hyperaemia, inflammatory cells infiltration and bile duct hyperplasia, moderate vacuolar degeneration and hepatocytes necrosis were observed. In treatment 4, the histopathological alternations significantly were healed, and only mild necrosis and vacuolar degeneration were observed (Table 1, Fig. 1).

Bioaccumulation of Zn in the liver tissue: The highest Zn of the liver was measured in treatment 2, significant than the others ($P<0.05$). In those treated with *R. damascene*, Zn concentration in the liver were lower, and no significant difference found between treatments 4 and control one ($P>0.05$) (Fig. 2).

Biochemical parameters: The measured biochemical parameters showed an increase in treatment 2, whereas, those of 3 and 4 had decreased due to application of using *R. damascene* extract. The results are shown in Table 2.

Discussions

The present study examined the toxic effect of Zn and therapeutic properties of *R. damascene* in liver tissue of common carp. Liver is a biological indictor for determination of environmental pollution (Sadauskas et al., 2007; Chavan and Muley, 2014) and the main organ for heavy metals bioaccumulation (Wood et al., 2012; Chavan and Muley, 2014; Georgieva et al., 2016). Histopathological changes such as hepatocyte necrosis, degenerative vacuolation and hyperemia in liver were reported subsequent Zn poisoning in common carp (Georgieva et al., 2016).

The highest Zn concentration was observed in

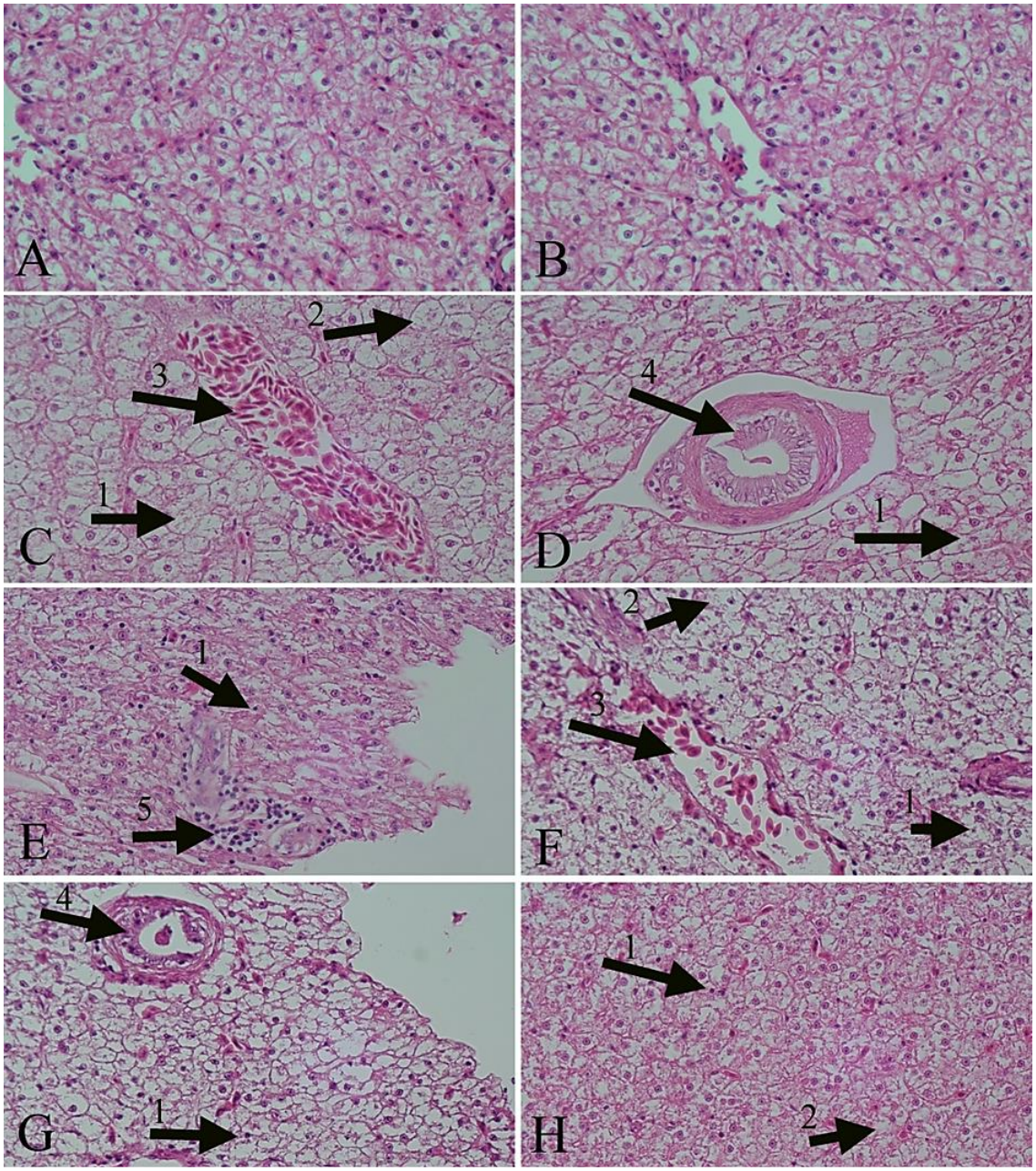

Figure 1. (A, B) Treatment 1, normal liver tissue, (C, D, E) Treatment 2, (1) necrosis, (2) vacuolar degeneration, (3) congestion, (4) bile duct hyperplasia, (5) inflammatory cells infiltration, (F, G) Treatment 3, (1) necrosis, (2) vacuolar degeneration, (3) congestion, (4) bile duct hyperplasia, and (H) Treatment 4, necrosis (1), vacuolar degeneration (2) (X40 H&E).

treatment 2 i.e. the group only exposed to Zn, whereas this metal was low in treatments 3 and 4 due to application of *R. damascene* extract. The results also revealed that Zn has been caused sever histopathological lesions in treatment 2, whereas in the supplemented treatments with *R. damascene* i.e. groups 3 and 4, the histopathological alternation were less. These results revealed the positive effect of *R. damascene* extract to decrease toxicity of Zn on the liver of common carp. Damask rose possesses the flavonoids, phenol and phenolic compounds with valuable antioxidant and anti-aging effects (Kalim et al., 2010). The other works revealed higher antioxidant effect of *R. damascene* extract in compared to usual antioxidants such as ascorbic acid (Alam et al., 2008) and in this regard, quercetin, an important flavonoids, is a main compounds of Damask rose extract (Velioglu et al., 1991; Arts et al., 2004). Previous studies indicated that quercetin can reduce necrosis and apoptosis in the injured liver of rats

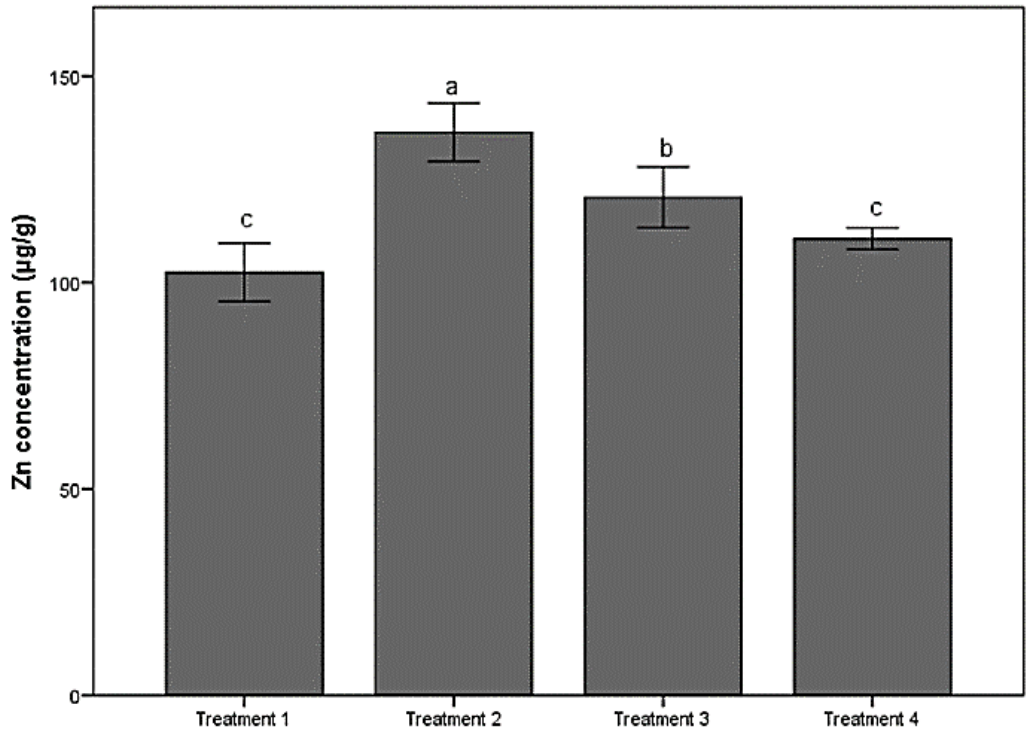

Figure 2. Zn concentrations (Mean±SE) of *Cyprinus carpio* liver in different treatments (the letters above the column indicate significant

(Velioglu et al., 1991; Khaki, 2010). In addition, quercetin has shown protective effect on liver damage induced by biliary obstruction in rats (Kanter, 2010).

Antioxidant and hepatoprotective effect of the ethanolic extract of the *R. damascene* have been studied by Alam et al. (2008), showing that this ethanolic extract reduces some hepatic injuries such as hepatocyte necrosis, degenerative vacuolation, inflammatory cells infiltration and congestion in central vein and sinusoids. Moreover a dose dependent decrease of AST, ALT, ALP enzymes in rat liver that damaged by paracetamol was reported (Alam et al., 2008). In line with the above-mentioned work, in the current study, an increase of the biochemical parameters was observed in treatment 2, whereas in the treatments 3 and 4 they were lower. Shalaby (2000) showe the increase of ALT and AST in liver of common carp following exposure to ZnO. In addition, application of quercetin have improved the biochemical parameters, including activities of cholinesterase, AST, ALT, ALP, bilirubin, total lipids, cholesterol and triglycerides in the rat liver, damaged due to exposure to ammonium fluoride (Czerny et al., 2000). As conclusion, the result of the present study showed *R. damascene* can decrease the toxic effect of Zn in liver tissue of common carp.

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