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Pomegranate Peel Extract Powder: Mitigating Cadmium Accumulation and Oxidative Damage in Common Carp Fillets

Hayder Al-Iessa[1], Mohammadreza Rezaeigolestani[1*], Davar Shahsavani[1*], Hasan Baghishani[2]

1. Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Equally corresponding authors

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ABSTRACT

Introduction: Cadmium (Cd) contamination in aquatic environments threatens fish health by inducing oxidative stress and bioaccumulation. Natural antioxidants like pomegranate peel extract powder (PPEP) may help mitigate these effects through metal chelation and oxidative defense. This study evaluates PPEP's potential to reduce Cd accumulation and oxidative damage in common carp.

Methods: The protective effects of PPEP, as a natural dietary supplement, against Cd accumulation was evaluated by an inductively coupled plasma mass spectrometer, and the effect of PPEP on the oxidative effects of Cd in common carp was assessed via the determination of 2-thiobarbituric acid reactive substances (TBARs) and carbonyl content. Moreover, the bioaccumulation of some minerals (copper (Cu), magnesium (Mg), and zinc (Zn)) was also evaluated in the studied carps.

Results: The feeding with different concentrations of PPEP (1, 2 and 4% wt) could significantly reduce the level of Cd in the fillet samples ($P<0.05$). Moreover, this supplementation also resulted in lower concentrations of Cu and Mg, while Zn was unaffected. Based on TBARs analysis, the levels of oxidation in Cd-supplemented samples were reduced (27-38%) by the PPEP treatments ($P<0.05$). However, no correlation was detected between protein oxidation and lipid peroxidation markers in the fish samples ($P>0.05$).

Conclusion: This study demonstrates that PPEP effectively reduces cadmium accumulation and oxidative stress in common carp fillets. While PPEP mitigates lipid peroxidation, its impact on essential minerals like Cu and Mg requires further investigation. These findings support PPEP as a natural dietary strategy to improve fish health in aquaculture.

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Introduction

Food security, both quantitatively and qualitatively, is a top priority for sustainable global development. In recent decades, the unexpected adverse effects of contaminants on food quality have threatened food security and human health. Among these contaminants, heavy metals (HMs) are chemicals that can disrupt metabolism and enter food chains via the environment, contributing to disease and even death (1). Heavy metals are generally classified into two categories: essential heavy metals, like copper and zinc, which are necessary for vital processes such as metabolism and the growth and development of various organs, and non-essential heavy metals, like cadmium and Lead, which are not required by organisms for any metabolic processes (2). Due to human activities, such as mining, improper waste disposal, and fuel combustion, levels of heavy metals in the environment have increased, particularly in aquatic ecosystems adjacent to industrial areas (3). Among the most important of these are fish, aquatic organisms that live in these polluted ecosystems. As a widely consumed nutritious food group, fish can contain significant amounts of various types of heavy metals, such as cadmium (Cd) and lead (4). This pollution is a serious problem that may outweigh the benefits of consuming seafood. Of all heavy metals, cadmium is one of the most biologically and kinetically toxic, and its toxicity against all life

* *Corresponding author(s):* Mohammadreza Rezaeigolestani, PhD, Assistant Professor, Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. Phone: +98 5138805646, Email: mr.rezaee@um.ac.ir.
Davar Shahsavani, PhD, Professor, Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. Phone: +98 38805624, Email: shahsavani@um.ac.ir.

forms, including mammals, fish, and plants, has been sufficiently demonstrated. High intake of cadmium in the human diet can lead to various chronic toxicities, such as impaired kidney function, and the International Agency for Research on Cancer has classified it as a Group 1 human carcinogen (5). Furthermore, cadmium causes various systemic toxic effects in fish, including changes in behavior, gill morphology, energy balance, endogenous antioxidant status, increased micronuclei, and death (6). It is known that tissue damage caused by cadmium is mainly attributed to oxidative stress caused by toxicants (7). Cadmium stimulates the formation of reactive oxygen species (ROS) and increases lipid peroxidation, causing oxidative damage to various tissues and leading to loss of membrane function (8).

Researchers are currently working on several strategies to mitigate the harmful oxidative effects of HMs in animal tissues. In this regard, chelating HMs and enhancing endogenous antioxidant defense mechanisms through herbal supplements containing antioxidant compounds is one innovative approach that has been introduced and applied in various animal models to combat oxidative stress induced by HM toxicity. These compounds can destroy free radicals and are responsible for initiating or propagating the reaction. This is done through a chain-breaking mechanism by donating free electrons to active oxygen radicals and lipids present in biological systems, converting them into stable molecules. This prevents or delays the oxidation process. Key components include flavonoids and ascorbic acid (9–11).

The most important of these herbal supplements is pomegranate (Punica granatum), which belongs to the Punicaceae family and is widely cultivated in Central Asian countries such as Iran and Afghanistan (12). This fruit is a valuable source of natural phenolic compounds such as catechins, anthocyanins, tannins, gallic acid, and ellagic acid, known for their beneficial health-promoting properties (9). The protective effects of pomegranate extracts, especially its juice, against oxidative stress resulting from chronic exposure to various high-impact chemicals such as aluminum and lead have previously been reported in animal models such as mice (13,14). Indeed, these effects have been linked to the presence of certain bioactive compounds, particularly polyphenols, in pomegranate. Although almost all parts of the pomegranate, including fruit juice, peel, and leaf extracts, have been shown to possess potent antioxidant activity (15), the majority of studies have focused on fruit juice or evaluated in vivo animal models (16), while information on the use of the peel in various meat products is scarce. Pomegranate peel (PP, approximately 40–50% of the total fruit weight), the main by-product of industrial processing of pomegranate fruit, is usually discarded as waste. However, numerous nutritious and health-promoting compounds, such as phenolics, proteins, bioactive peptides, and polysaccharides, can be found in PP (17). Therefore, it has great potential for use as a natural, antioxidant-rich, and low-cost substance for various purposes.

This study aimed to evaluate the protective effects of polyphenol extract powder (PPEP), as a natural food supplement, against cadmium accumulation and the oxidative effects of this toxicant in farmed carp. Furthermore, the effect of PPEP supplementation on the bioaccumulation of some metals, including copper (Cu), magnesium (Mg), and zinc (Zn), was evaluated. Common carp was chosen due to its high consumption rate among various seafood species in Iran and its susceptibility to HM-induced tissue damage (18). The use of herbal supplements from food industry waste, such as pomegranate peels, to eliminate the bioaccumulation of heavy metals such as cadmium in common carp meat, which ranks first in global consumption, and to improve its quality in terms of food safety and health, as well as the elimination of the most important elements that cause cancer in humans, represents a new approach to preserving human health, food safety, and improving the environment.

Materials and Methods

Pomegranate Peel Extraction

PPEP was prepared following the method of Abdel Moneim et al. (2012) with some modifications (1). In brief, 3 kg of sweet-type whole Iranian pomegranates grown in Kashmar, Iran, was purchased from a local store in Mashhad and transported to the laboratory. Then, they were peeled, cut into small pieces, washed with distilled water and dried in an oven at 40 °C for 48 hours. After drying, the peels were ground to obtain a fine powder. The pomegranate peel powder was mixed with

ethanol (96% v/v) in a 1:10 ratio with continuous stirring for 2 min, and after 48 hours it was filtered with a vacuum pump. The obtained extract was concentrated using a rotary evaporator (Model N-100, Eyela, Tokyo, Japan) at 40 °C. Finally, the concentrated extract was poured into a Petri dish and held in an incubator at 40 °C for 24 h to completely dry.

Experimental Design and Sampling

A total of 100 common carp (*Cyprinus carpio*) at the age of or fingerlings (20.3 ± 0.8 g) were obtained from a local fish farm or hatchery (Saft Khalid, Khorasan Province, Iran), transported to the lab, and acclimated to the new environment for 10 days, during which the subjects were fed on a basal commercial diet (EX-TG2, Beyza Feed Mill, Iran) containing 43-46% crude protein and 11-15% crude fat. The fish were then randomly divided into five groups, each containing 20 fish, and stocked in five 120-liter aquariums equipped with air pumps for continuous aeration. The level of Cd in the water was measured before fish stocking and reported as 1.1 ± 0.82 µg/L. The chemical standards for drinking water issued by the Environmental Protection Agency (EPA) and the World Health Organization (WHO). The optimal intake for cadmium set by the WHO is 0.002 and 0.005 mg per liter. The amount of cadmium found in fish flesh is 15.3 parts per billion. Cadmium concentrations in environmental water can be significant, ranging from 0.01 to 1.16 mg per liter. Therefore, the cadmium concentration (0.5 mg/L) was adopted. The five treatments were subjected to the following conditions: the first group (control) stocked in plain water; the second group (Cd) exposed to $CdCl_2$ (0.5 mg/L); third (Cd/1%PPEP), fourth (Cd/2%PPEP) and fifth (Cd/4%PPEP) groups were exposed to $CdCl_2$ (0.5 mg/L) and fed PPEP at levels of 1, 2, and 4% (w/w) of the daily diet, respectively, according to a previous study (2).

Different concentrations of PPEP were dispersed in 2 mL of distilled water (at 30 °C) and then sprayed on the applied diets. The fish were fed three times per day (7, 1, 17) at a rate of 2.5% body weight day^{-1}. The length of the experiment was 30 days and the health status of the fish was evaluated by visual examination during this period. Throughout the experiment, to maintain clear healthy water, the water of the aquaria was replaced with fresh well-aerated water twice a week to remove uneaten food and feces. The temperature, pH and oxygen concentration of the water were maintained at 20±1°C, ~7.5 and 5.6-6 ppm respectively during the acclimation time and afterward. These values are acceptable for fish farming (3).

At the end of the experiment, eight fish from each group were randomly selected and anesthetized with clove powder (0.5 g/L). Studies have shown that cadmium accumulates in fish tissues within days of exposure, following the order: gills > liver > muscle > brain. Fish primarily eliminate metals through bile, urine, gill excretion, and mucus. Among these, the gills exhibited the highest rate of demineralization, while the liver showed a significant but lower degree of metal removal. In contrast, the brain and muscles displayed minimal demineralization even after 30 days. Consequently, muscles were selected for measuring cadmium accumulation. The fish were then headed and cleaned, and appropriate muscle samples were manually excised using a sterile scalp. Subsequently, the fillets were washed with physiological serum and stored at −70 °C until analysis.

Heavy Metals Analysis

Each sample (0.5 g) was digested in concentrated nitric acid (98%, 5 mL) and hydrogen peroxide (3 mL) in a beaker. Then, the samples were diluted to 50 ml with ultra-pure water. Afterward, the prepared samples were determined for Cu, Mg, Zn and Cd using an inductively coupled plasma mass spectrometer (ICP-MS, model 7700 series, Agilent Technologies, Tokyo, Japan). Metals assayed in the present study were: copper (Cu), magnesium (Mg), zinc (Zn) and cadmium (Cd). Standard solution of the element (Perkin Elmer) was prepared by diluting stock solutions of 100 mg/mL of each HM based on Taweel et al. (2013) (4). The concentrations of HMs in the fish samples were reported in mg/kg tissue.

Oxidative Status Tests

In order to use the frozen samples for oxidative status tests, they were quickly thawed and homogenized (Heidolph homogenizer, Germany) for five min in 10 volumes (w/v) of ice-cold 0.05 M phosphate buffer (pH 7.4). Finally, the resultant mixture was centrifuge (Eppendorf 5417c centrifuge, Germany) at 5000 rpm for 10 min, and the clear supernatant fraction was collected and used for oxidative assays.

TBARs (2-thiobarbituric acid reactive substances) method was carried out to measure

the lipid oxidation in the samples according to the spectrophotometric (Optizen 2120 UV Plus spectrophotometer, Korea) procedure described by Maraschiello et al. (1999) (5). The TBARs value was expressed as mg malonaldehyde (MDA) equivalents/kg tissue.

Protein oxidation in the tissue samples as measured by the protein carbonyl content was assessed using the 2,4-dinitrophenylhydrazine (DNPH) method as described by Levine et al. (1990) (6). The carbonyl content was estimated by a spectrophotometric assay at 370 nm and expressed as nmol/mg tissue.

Statistical Analysis

The data were expressed as mean values with their standard deviation indicated (mean ± SD). All data were statistically analyzed using SPSS 16 software by one-way analysis of variance (ANOVA), and multiple comparisons were done by Tukey's tests. A level of probability $P<0.05$ were considered statistically significant.

Results

Heavy Metal Trace in Fish Samples

Cadmium

The concentration of Cd in the muscle tissue of common carp was drastically increased in the fish groups exposed to 0.5 mg/L of Cd, compared to the control ($P<0.05$) (Table 1). Among those groups, maximum and minimum Cd contents were recorded for the Cd group (0.02 mg/kg) and Cd/1%PPEP group (0.011 mg/kg) respectively. Feeding with different concentrations of PPEP could efficiently reduce the level of Cd in the carp fillets ($P<0.05$). Based on the result, the changes in Cd content of cd/PPEP-exposed samples were not dependent on the concentration of PPEP ($P<0.05$).

Table 1. Metal accumulation (mg/kg tissue) in carp fillets exposed to cadmium (Cd: 0.5 mg/L) and pomegranate peel extract powder[1].

	Treatment				
Heavy metals	Control	Cd	Cd/1%PPEP	Cd/2%PPEP	Cd/4%PPEP
Cd	0.0012 ± 0.0005^{a}	0.02 ± 0.0023^{b}	0.011 ± 0.0015^{c}	0.013 ± 0.0021^{c}	0.013 ± 0.0028^{c}
Cu	1.27 ± 0.2^{a}	0.84 ± 0.38^{ab}	0.66 ± 0.21^{b}	0.68 ± 0.28^{b}	0.91 ± 0.51^{ab}
Mg	256 ± 30^{a}	247.5 ± 33^{a}	216.7 ± 11^{ab}	222.2 ± 17^{ab}	196.4 ± 17^{b}
Zn	45 ± 14.2^{a}	32.6 ± 16.2^{a}	32.1 ± 17.3^{a}	36.3 ± 5.9^{a}	25.7 ± 3.4^{a}

[1]The mean ± standard deviations are presented.

$^{a-c}$ Different lowercase letters within a row indicate significant differences ($P<0.05$)

Copper, Magnesium and Zinc

Table 1 shows the level of metal accumulation in the fish fillets induced by dietary cadmium and PPEP. The results of the analysis of copper concentration in muscle tissue of common carp showed that although all of the Cd-exposed samples exhibited lower Cu concentrations (0.66-0.91 mg/kg) compared to the control group (1.27 mg/kg), only the Cu content of Cd/1%PPEP and Cd/2%PPEP carp fillets were significantly lower than the relevant value in control ($P<0.05$) (Table 1).

The concentrations of Mg ranged between 196.4 and 256 mg/kg (Table 1). The supplementation of PPEP resulted in lower content of Mg in the fillet samples, particularly in the Cd/4%PPEP group ($P<0.05$), compared to the control.

The analysis of Zn concentration revealed that the control group and the fifth group of carps contained maximum (45 mg/kg) and minimum (25.7 mg/kg) concentrations of Zn respectively (Table 1). However, the differences recorded between the treated samples were not significant, mainly due to the variations among the Zn values in each group of fish fillets. Moreover, it seems that the addition of Cd to the experimental diets reduced the concentrations of the metal in the fillets.

Oxidative Markers in Tissue Samples

Lipid Oxidation

The TBARs values of the fish muscle samples from different experimental groups are presented in Fig. 1A The minimum and maximum lipid oxidation were recorded for the control group and group 2 with the TBARs values of 0.13 and 0.22 mg MDA equivalents/kg muscle tissue respectively. With the addition of Cd, the level of lipid oxidation was significantly increased in the group 2 samples, while the differences in TBARs values among the actual treatments and also the control group were not significant ($P<0.05$). However, some slight differences can be observed between the fish fed with different levels of PPEP, and they exhibited slightly higher lipid oxidation compared to the control.

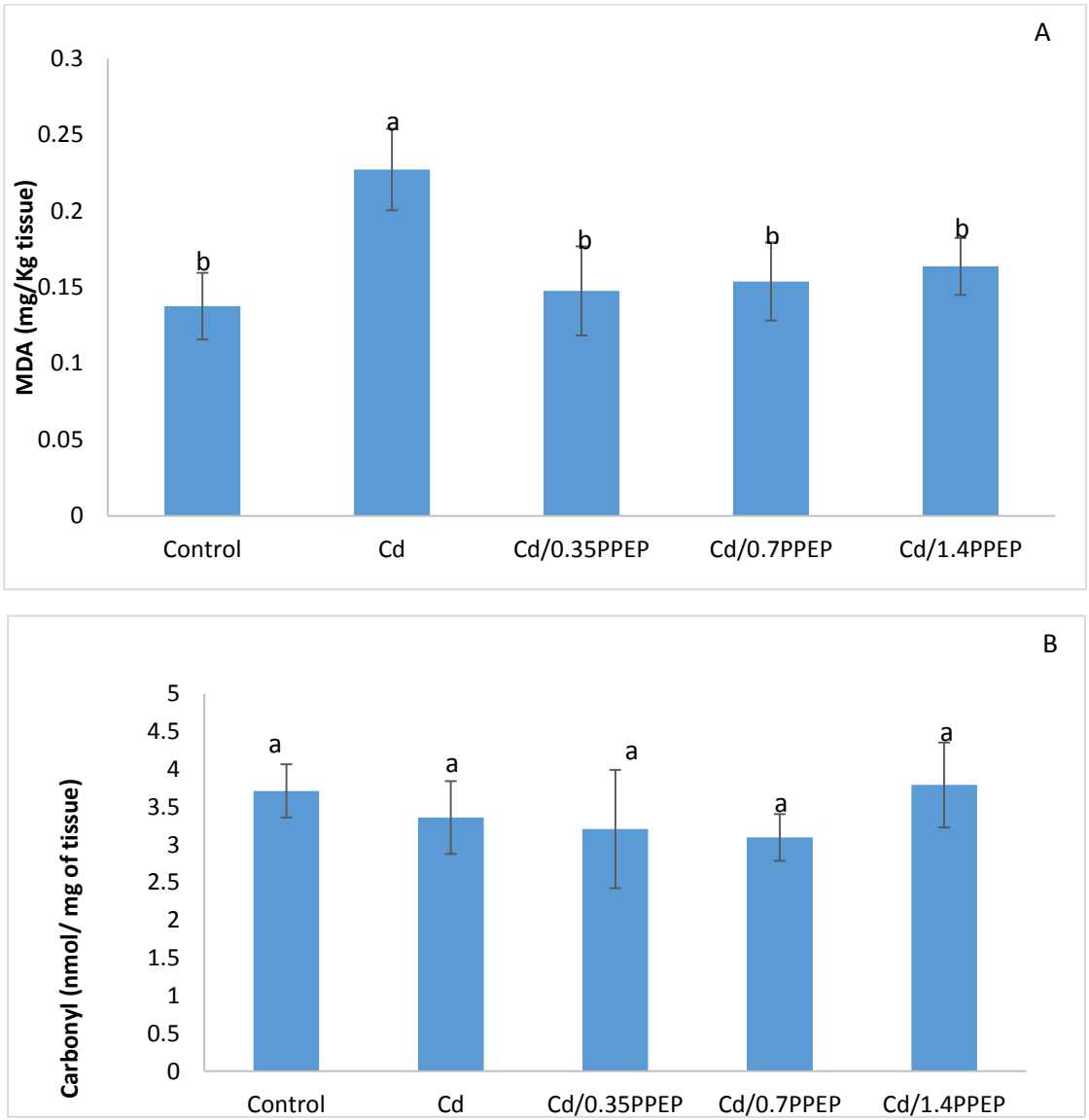

Figure 1. Effect of cadmium and different concentrations of pomegranate peel extract powder (PPEP) on TBARS (A) and carbonyl content (B) of carp fillets. Data are presented as means of three replicates and the error bars show the standard deviation. Different letters represent a significant difference (P<0.05).

Protein Oxidation

The measurement of protein carbonyl content is the prevalent method used for the determination of the extent of protein oxidation in food systems (7). As shown in figure 1B, carbonyl content in the fish samples ranged between 3.1 and 3.8 (nmol/mg tissue), and minimum and maximum values were recorded for Cd/2%PPEP and Cd/4%PPEP respectively. These values are in accordance with the values reported in the literature for carp muscles (8). Neither the addition of Cd to the aquarium water nor the feeding with PPEP significantly changed the carbonyl content of muscle proteins compared to the control (P>0.05). However, among the tested groups, the fish fed with 1% or 2% PPEP showed lower values. No correlation was detected between protein oxidation and lipid peroxidation markers in the fish samples.

Discussion

The elevation of Cd content in the fish samples exposed to Cd was predictable as it has been frequently stated that higher levels of heavy

metals, like cadmium and lead, in the aquatic ecosystems finally lead to higher levels of the elements in the tissues of the resident aquatic organisms (9,10). In general, muscles accumulate fewer HMs than the metabolically active tissues, like liver, kidneys, or gills, and this bioaccumulation occurs in a species-specific manner (9). The previous study by Vinodhini et al. (2008) showed that HMs, particularly Cd, could significantly accumulate in the tissues of common carp exposed to sublethal concentrations of the metals for periods of 32 days (10).

Based on the result, PPEP had a decreasing effect on the Cd content of the carp fillets. As it has been widely demonstrated in the literature, some synthetic and natural compounds can chelate metals and limit their reactivity (11). Moreover, metal chelators can be used for the detoxification of HMs-contaminated foods or feeds. Among natural metal chelators, flavonoids and vitamin C have shown promising chelating properties (11). The chemical composition of PPE was evaluated in several studies, which indicated that PPE is a good source of flavonoids (12). Furthermore, vitamin C was also detected in PPEs obtained from different sources (13,14). Consequently, the lower Cd concentrations in the carp groups fed with different levels of PPEP might be related to the presence of active agents like flavonoids and vitamin C that probably chelate the metal and decreased its level in the flesh of tested fish. The levels of cadmium reduction in the fourth and fifth groups were lower than in the third group. This may be due to the fact that pomegranate peels also contain some important minerals like manganese, copper, iron, zinc, lead, and cadmium at different concentrations (15). It has been demonstrated that due to the competition and interaction between the ions, the rate of heavy metals removal using pomegranate peel is relatively low when applied to mixed ions (16).

Similar effects were observed by Aksu et al. (2017) in the rats exposed to lead and fed with pomegranate juice (17). They reported that the levels of lead in all tested rats' tissues including kidney, liver, heart and testis, significantly declined with the addition of pomegranate juice to their diets ($P<0.05$).

Regarding the impacts of dietary bioactive herbal materials on mineral bioaccumulation in animal tissues various studies have been published. Some reports mentioned that tissue metal uptake was not affected by herbal supplementation, while others demonstrated different impacts of dietary herbs on the accumulation of minerals in animal tissues (18). Stef and Gergen (2012) reported a poor or moderate correlation between total phenols and the bioaccumulation of some minerals, like Zn and Cu in the muscles of chickens supplemented with medicinal herbs rich in polyphenols (19). On the other hand, the four-week inclusion of polyphenol-rich herbal products in the diet of piglets had a marginal effect on the levels of Zn, Cu and Fe (20). Kalay and Canli (2000) indicated that Cu and Zn are both non-chelatable, but due to the metabolism of copper and the lack of zinc metabolism in the body, the level of Cu and Zn in the tissues decreased and remained almost unchanged during the study, respectively (21). In another study, similar to the results of the current study, antioxidant agents caused the negative removal of some beneficial mineral elements such as calcium or magnesium (4). Čobanová et al. (2020) mentioned that the uptake of Zn and Cu by lamb tissues was not affected by herbal supplementation (18). Therefore, great variability in the effects of the supplementation of plant materials on minerals uptake is evident, but it seems that the extent of chelation is depend on the chemical constituents of the herbs particularly their polyphenols (19). In the present study, the concentrations of Cu and Mg in the fillets were mainly affected by supplementation of some levels of PPEP, probably due to the chelating ability of PPEP constituents, while Zn contents were less influenced. The results of analysing the concentration of magnesium in the muscle tissue of common carp showed a significant decrease in the concentration of magnesium in the fifth group compared to the control group. In another study similar to our findings, antioxidant agents, in addition to the removal of heavy metals, caused the loss of some beneficial minerals such as calcium or magnesium from the body in an inappropriate manner through the chelation of those elements (22).

It is noted that toxic levels of cadmium can impede zinc absorption (23). Then, the lower levels of zinc in the cadmium-induced samples may be justified by this fact.

MDA is a highly reactive dialdehyde that is produced through the oxidation of unsaturated fatty acids with toxic nature. This substance is a

common marker widely used for tracing the progress of lipid oxidation in different matrices like foodstuffs and animal tissues (24). Reactive oxygen species (ROS) like superoxide radicals, hydroxyl radicals, and hydrogen peroxide are active compounds that can trigger the peroxidation of lipids, resulting in the formation of MDA. Among different agents that can stimulate the formation of ROS, HMs, like Cd, have shown strong potential to develop those active species (25). Therefore, as the level of Cd was higher in the Cd-exposed fish samples, then the increase of lipid peroxidation in those samples was probably attributed to the stimulating effects of the deposited Cd on the formation of ROS. In accordance with our findings, the induction of oxidative stress by several HMs like Cd and Pb were similarly reported in animal models like rat, common carp and several aquatic organisms (25–27).

The lower MDA values in the PPEP-fed groups compared to group 2 indicate the protective effects of the extract against the oxidative effects of Cd. Regarding the effects of feeding with herbal substances on the MDA content of different animal tissues, several reports have been published. The analysis of TBARs values in different animal tissues including liver, kidney, heart and testis showed that pomegranate juice and PPE both provided a protection against the oxidative stresses induced by HMs (28,29). The effect of piper betle leaf extract (PBE) against Cd-induced oxidative hepatic dysfunction in rats was examined by Milton Prabu et al. (2012) (30). Besides, the elevation of TBARs values in the liver samples exposed to Cd, the authors observed that the supplementation of PBE (200 mg/kg BW) significantly reduced the level of TBARs in the Cd-induced samples ($P<0.05$). This antioxidant effect has similarly attributed to the presence of polyphenols in the PBE. The supplementation of grape pomace in the diets of sheep was also effective in bringing down the level of lipid oxidation (31).

The antioxidant activity of dietary PPEP in fish samples implies that the active antioxidant agents of PPEP, like polyphenols, can be absorbed through the gastrointestinal tract and transferred to the muscle tissue. This phenomenon was previously demonstrated by several authors for other natural dietary compounds (31). In this regard, Nardoia et al. (2018) showed that polyphenols present in wine by-products can be absorbed, distributed, and remain their active antioxidant activity in chicken breast meat (32).

Although the aforementioned studies have suggested that the inclusion of dietary phenolic compounds favors the antioxidant stability of flesh food products during storage, the exact mechanisms of action have not been fully established. For a better understanding of the mechanism that PPEP confront the lipid oxidation progress, the chemical composition of the extract must be considered. Pomegranate peel contains various active compounds with strong antioxidant activity like polyphenols, flavonoids, and anthocyanins that can destroy ROS (33). In fact, high levels of phenols were previously reported for pomegranate peel obtained from different geographic areas (12). It has been demonstrated that phenolic compounds possess a cell membrane stabilizing activity by inhibiting the generation of ROS induced by Cd (30). However, it seems that the Cd chelating activity of PPEP was the leading factor that inhibited lipid peroxidation and limited the production of MDA in the carp samples as the Cd analysis showed lower content of the metal in the PPEP-supplemented samples.

To explain the lack of correlation between protein oxidation and lipid peroxidation markers, it should be mentioned that protein carbonyl groups are formed by the oxidation of certain amino acids like lysine, threonine, arginine, proline, and histidine, while other amino acids might be oxidized without any alteration in carbonyl content (34). Moreover, it has been stated that the onset of muscle protein oxidation takes place slower than the oxidative degradation of lipids in meat systems (35). So, although TBARs analysis showed significant differences between different groups of carps, the inclusion of Cd or PPEP in the diets did not significantly change the carbonyl content of the samples.

The effects of dietary herbal materials and heavy metals on the carbonyl content of different animal tissues have been assessed in various studies. Milton Prabu et al. (2012) stated that the oxidizing effects of Cd in the liver of rats were significantly hampered by the pre-oral supplementation of piper betle leaf extract (200 mg/kg BW) (30). Moreover, a positive correlation between lipid oxidation and carbonyl content of the liver tissue was also reported.

Ortuño et al. (2016) observed a similar phenomenon in chilled meat obtained from lambs fed on a diet supplemented with rosemary extract (35). In fact, both TBRAs values and carbonyl content of the lamb meat supplemented with rosemary declined compared with the control (fed only with basal diet). However, it is worth mentioning that the differences between the carbonyl content of the reinforced lamb samples and the control were significant since day 11 of storage, while the effects of the inclusion of rosemary extract on the lipid oxidative degradation were observable from day 7 ($P<0.05$). So, as the fish samples in the present study were immediately frozen and used for antioxidant assays, further storage of the samples might be needed for detecting the differences in protein oxidation among the treatments

Mechanism of Action of PPEP against Cadmium Toxicity

The protective effects of PPEP against cadmium toxicity are primarily attributed to its metal-chelating properties, antioxidant activity, and regulation of metal metabolism. The polyphenols and flavonoids in PPEP can bind to cadmium ions, reducing their bioavailability and limiting their accumulation in fish tissues. Additionally, PPEP acts as a potent antioxidant by scavenging reactive oxygen species (ROS), thereby mitigating oxidative stress-induced lipid and protein oxidation (36). This study demonstrated a significant reduction in thiobarbituric acid reactive substances (TBARs) in PPEP-fed groups, supporting its protective role against lipid peroxidation. Furthermore, PPEP appears to influence the uptake of essential minerals, such as copper and magnesium, which may be due to competitive interactions between cadmium and these elements. While these findings suggest PPEP as a promising dietary strategy to reduce heavy metal toxicity in aquaculture, further research is needed to clarify its long-term effects and optimize its application.

Conclusion

This study demonstrated that PPEP supplementation effectively reduced cadmium accumulation and oxidative stress in common carp fillets, likely due to its known antioxidant and metal-chelating properties. These findings suggest that PPEP could be a valuable dietary strategy for mitigating heavy metal toxicity in aquaculture. However, this study has some limitations. The exact molecular mechanisms underlying PPEP's protective effects remain unclear and require further investigation. Additionally, the potential impact of PPEP on the sensory properties and overall quality of fish fillets was not assessed. Future studies should explore the long-term effects of PPEP supplementation, optimize its dosage for different exposure levels of heavy metals, and evaluate its influence on fish health and product quality in real aquaculture conditions. Investigating the synergistic effects of PPEP with other natural antioxidants could also provide valuable insights into enhancing its efficacy.

Declarations

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hayder Al-Iessa, Davar Shahsavani, Hasan Baghishani and Mohammadreza Rezaeigolestani. The manuscript was written by Hayder Al-Iessa and Mohammadreza Rezaeigolestani, and revised by Hasan Baghishani. All authors read and approved the final manuscript.

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