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# Liver alteration and hematological and serum biochemical responses of common carp, *Cyprinus carpio* Linnaeus, 1758, following long-term feeding of pistachio (*Pistacia vera*) green hull extract as a source of natural phenol

By J. Motamedi-Tehrani[1], E. Ebrahimi-Dorcheh[1], P. Malekpouri[2] and S. A. H. Goli[3]

[1]*Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran;* [2]*Young Researchers and Elites Club, Science and Research Branch, Islamic Azad University, Tehran, Iran;* [3]*Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran*

## Summary

This study was conducted to investigate the effect of pistachio green hull extract (PGHE) on hematological and serum biochemical changes in common carp, *Cyprinus carpio*. Three hundred common carp (11.65 ± 1.65 g) were fed one of five different dietary treatments (with three replications) containing 0, 0.5, 1.5, 4.5 or 9 g PGHE $kg^{-1}$diet for ten continuous weeks. Each tank had a 90-L capacity and water flow rate of about 500 ml $min^{-1}$.Total phenolic compounds of the different diets differed significantly ($P < 0.001$) according to the amount of PGHE. At the end of experiment, six fish were removed randomly from each treatment. Blood samples were taken for hematological and serum biochemical analyses at room temperature. Liver tissue samples were processed for histology and stained by H&E. The results indicated that all doses of tested PGHE induced no significant changes in hematocrit, hemoglobin, or erythrocytes, nor alkaline phosphatase, alanine transaminase, lactate dehydrogenase, total protein, albumin, globulin, triglycerides, low-density lipoprotein, high-density lipoprotein, glucose or cholesterol in the serum. Leukocytes were higher ($P < 0.01$) in fish fed a 1.5, 4.5, or 9 g PGHE $kg^{-1}$ diet when compared to the 0.5 g PGHE $kg^{-1}$ diet group or the control. Serum aspartate transaminase in treatments containing a 4.5 or 9 g PGHE $kg^{-1}$ diet was significantly ($P < 0.001$) higher in comparison with the control. Liver histology showed focal necrosis, cytoplasm degeneration, lateral nuclei and an increase in Kupffer cells following PGHE administrations. The results of this trial indicated that although there were no significant changes in most hematological and biochemical parameters, PGHE could induce some adverse pathological effects on liver tissue.

## Introduction

The commonly used synthetic antioxidants are 2,3-tert-butyl-4-methoxyphenol (BHA), 2, 6-di-tert-butyl-4-methylphenol (BHT) and tert-butylhydroquinone (TBHQ) (Mahdavi et al., 1995; Fennema, 1996). However, they have some side effects that might induce liver damage and cancer in laboratory animals (Ito et al., 1983; Burt, 2004; Shahidi et al., 2006). Amongst antioxidants, phenolic compounds are more effective due to the release of hydrogen atoms as well as their radical intermediates, which are stable even following resonance (Fennema, 1996). Because of the potential side effects of synthetic antioxidants, testing for alternatives is increasing. Moreover, plant-based phenolic compounds have various functions such as reducing properties (free radical terminators), metal chelating effects, and singlet oxygen quencher functions (Jadhav et al., 1995; Hosseinzadeh et al., 2012).

Polyphenol-rich foods and plants have some physiological properties, including antioxidant, anti-microbial (Sousa et al., 2006; Pereira et al., 2007; Rajaei et al., 2010), antimutagenic (Duarte et al., 1999; Rocha-Guzmán et al., 2007), anti-allergenic, anti-inflammatory, immunomodulatory, anti-platelet (Pietta et al., 2003), and anti-stress effects (Citarasu et al., 2002, 2003). Total production of pistachios in 2011 was circa 1 005 210 tonnes (t), around half of which were from Iran (472 097 t) (FAOSTAT, 2014). About 40% of the fruit weight is the green hull, which is wasted or used as fertilizer in some local gardens. Goli et al. (2005) reported that pistachio green hull contains large amounts of phenolic compounds, suggesting that considerable phenolic compound resources are produced but wasted annually.

In recent years, studies have examined the effect of plant additives, including green tea (Cho et al., 2007; Abdel-Tawwab et al., 2010), garlic extract (Lee et al., 2012), willow herb, *Epilobium hirsutum* extract (Pakravan et al., 2012), and *Garcinia kola* seed extract (Dada and Ikuerowo, 2009) on some physiological and biochemical parameters. Rajaei et al. (2010) showed that extraction of pistachio green hull causes strong antioxidant activities and inhibits the growth of different $gram^{+}$ pathogenic bacteria. Motamedi-Tehrani et al. (2016) reported that *Cyprinus carpio* fed a diet containing *Pistacia vera* hull extract had no significant effects on growth performance or body composition, and that peroxide values were lower in the *P. vera* hull extract than in the control group.

Based on the previous study that indicated no adverse effects of PGHE on *C. carpio*, our aim was to determine the effects of different levels of dietary PGHE on hematological

and serum biochemical parameters as well as the liver histology of *C. carpio* in order to highlight any possible pathophysiological changes induced by the use of this natural phenolic compound.

## Material and methods

### Preparation of pistachio green hull ethanolic extracts

Pistachio (Ahmadaghaei variety) hulls were obtained from a local garden in Kerman, Iran. The hulls were carefully dried and sieved through a 10-mesh screen to remove dust, sieved through a 40-mesh screen, and the remaining hulls then stored at −20°C until the extraction procedure was commenced. 50 g of the hull powder was then soaked in 200 ml ethyl alcohol (96.6%) for 15 days. The ethanolic solution was then filtered and any extra ethyl alcohol allowed to evaporate under vacuum at 40°C using a Buchi, Suess rotary evaporator to obtain a dark green, nearly odorless, viscous material (Benhammou et al., 2008). This compound was kept at −20°C for preparing dietary treatments (see below).

### Determination of total phenolic content (TPC) in pistachio green hull

The TPC was determined in triplicate in each diet sample by the Folin–Ciocalteu colorimetric method (Waterhouse, 2002). The UV–vis spectrophotometer (Scinco, South Korea) was used to measure absorbance at 765 nm. Calculations were based on a calibration curve, obtained by using gallic acid (Sigma–Aldrich, UK). The TPC was expressed as mg of gallic acid equivalents per g of dry matter.

### Experimental design

Purchased from a local farm in Isfahan (Iran), 300 fish were transferred within one hour in thermal-proof boxes to our laboratory, and reared in a 500-L fiberglass tank prior to the experiment. During acclimation (20 days), fish were fed with a basal diet (3.5% of body mass) daily. Water quality parameters, including temperature, dissolved oxygen and pH, were measured daily.

A commercial basal diet was crushed and mixed with water and a sufficient amount of extract added to obtain the desired amount of supplemented diet, including a 0 (control), 0.5, 1.5, 4.5 and 9 g PGHE $kg^{-1}$ diet. The proximate compositions of the commercial diet (wet basis %) consisted of 8.7% moisture, 32% protein, 10.5% lipid and 11.2% ash (Table 1). In all cases, the diet contained the same volume of ethyl alcohol. The diets were formed as pellets, allowed to dry, then coated with fish oil.

After acclimation, the fish were randomly stocked in 90-L fiberglass tanks with 20 fish per tank. Water flow rate was 500 ml $min^{-1}$. The fish were treated with different amounts of PGHE as mentioned above (three replications for each treatment) and hand-fed at 3.5% of body weight daily at 08:00, 12:00 and 17:00. At each feeding stage, all added foods were consumed and no leftovers observed. Water quality parameters were measured daily during the experiment: temperature (25.15 ± 0.93°C); DO (5.92 ± 0.1 mg $L^{-1}$) using a WTW-OXI 196, Germany; pH (7.55 ± 0.57) with a Metrohm 744 pH meter, Germany; and total ammonia nitrogen (TAN) (<0.03 mg $L^{-1}$) according to APHA (1998).

Table 1
Basal diet formulation applied for carp feeding

| Ingredients | % |
|---|---|
| Fishmeal | 41 |
| Wheat flour | 20 |
| Corn flour | 20 |
| Rice bran | 11 |
| Sugar beet molasses | 5 |
| Vitamin premix | 1.5 |
| Mineral premix | 1.5 |

Vitamin premix (Arasbazar, Iran) (mg/kg), vit. A, 1800 IU; vit. $D_3$, 1200 IU; vit. E, 120 mg; vit. $B_{12}$, 24 mg; riboflavin, 15 mg; niacin, 90 mg; Pantethonic acid, 27; menadion, 3 mg; folic acid, 4.8 mg; pyridoxine, 9 mg; thiamine, 9 mg; biotin, 0.48; choline chloride, 360 mg; cobalamin, 24 mg; ascorbic acid, 156 mg; Nicotinic acid, 90 mg; inositol, 72 mg; antioxidant, 15 mg. Mineral premix (Arasbazar, Iran) (mg/kg), Zn, 18 mg; I, 0.6 mg; Mn, 7.8; Co, 0.5; Se, 0.15; Cu, 1.8 mg; Fe, 12.

### Preparation of food extract

The lyophilized food was then ground and 200 g of the powder extracted three times with 10 volumes of 70% ethyl alcohol at 60°C for 24 h; the ethyl alcohol was removed with a vacuum evaporator (Buchi, Suess) and the extract freeze-dried in a lyophilizer (Oh et al., 2008) and stored at −20°C.

### TPC determination in fish diet

The concentration of phenolic compound in the extract was determined using the method described by Folin and Denis (1915): 5 ml of the extract (0.2 mg in 5 ml) was mixed with 0.5 ml of Foline-Ciocalteu-phenol reagent (Sigma–Aldrich, UK). After 3 min, 1 ml of saturated $Na_2CO_3$ (Merck, Germany) solution was added to the mixture. The reaction was kept in the dark for 1 h. The absorbance was then read at 700 nm using a spectrophotometer (JENWAY 6400, UK).

### Blood sampling

At the end of the trial, the fish were starved for 24 h. No anesthetic was used in order to avoid any probable effect of the anesthesia agent on serum parameters. Blood samples were withdrawn from the caudal vein of six fish selected at random from each replicate. Blood aliquots were added to either heparinized tubes for further hematological investigation or to non-heparinized tubes for serum biochemical analyses.

### Hematology

Hemoglobin concentration (Hb) was measured spectrophotometrically (JENWAY 6400, UK) at 540 nm by the cyanomethemoglobin method. Hematocrit percentage (Hct)

was measured with the microcentrifuge method (Micro-hematocrit centrifuge, 346, UNIPAA, Poland). Red blood cells (RBC) and white blood cells (WBC) were counted manually using a Neubauer hemocytometer (Boeco, Germany) after diluting the blood by adding Daice solution. Blood smears were dried and then fixed by methanol, stained by Giemsa, and examined using a light microscope with 1000 × magnification. The leukocytes cells differentiated based on their morphology, namely lymphocytes, monocytes and heterophils.

#### Serum biochemical parameters

After centrifugation (3000 rpm for 10 min), serum was collected and stored at −20°C for biochemical analysis (n = 6). Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Total Protein (TP), Albumin (Alb), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), Cholesterol, Glucose, and Triglycerides (TG) contents were determined by an automated analyzer system for serum chemistry (Roche Hitachi Cobas Mira Plus CC Analyzer) based on the manual. Serum globulin (Glb) was also calculated with a fraction of serum Alb from TP of each individual fish. All biochemical analyses were done at room temperature.

#### Liver histology

The dissected liver tissues were sampled immediately after blood sampling, then fixed in 10% neutral buffered formalin (pH = 7.2) for further histopathological investigations. All samples were then embedded using paraffin wax; serial sections (5 $\mu$m) were then processed routinely and stained by H&E method.

#### Statistical analysis

All data were subjected to a Shapiro-Wilk test to check their normality, followed by Levene's test for homogeneity of variance; acceptable data were then subjected to one-way analysis of variance (ANOVA). Data comparison was performed with a LSD complementary test. All statistical analyses were carried out using SPSS program version 22.

## Results

Growth parameters and survival test results showed no significant differences between different treatments (further details in Motamedi-Tehrani et al., 2016). The concentration of phenol in the PGHE was about 21.52 mg of gallic acid equivalents per g per sample. The TPC of fish food containing different levels of PGHE is shown in Fig. 1. The TPC of the fish diet was significantly different among treatments ($P < 0.001$). An increasing pattern of TPC as well as an increasing content in the extract can be seen in the diets.

#### Hematology and biochemical alterations

No alterations in serum chemistry parameters or in hematologic indices were observed unless otherwise stated.

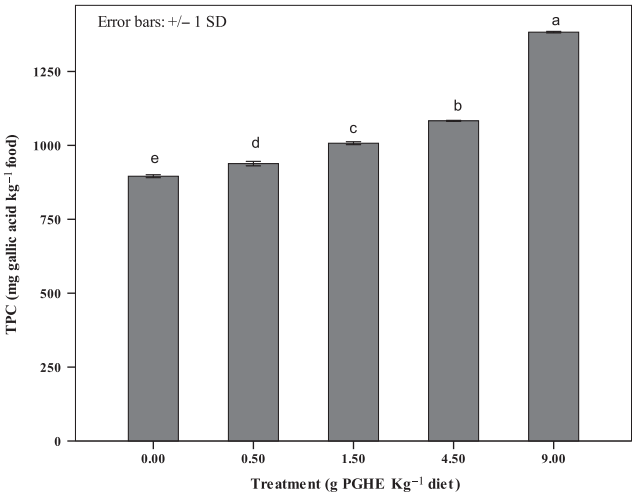


Fig. 1. Total phenolic content of fish food. Each mean is based on three samples. Different letters = significant differences at $P < 0.01$

Hematological parameters of fish fed with different levels of PGHE are given in Table 2. It can be seen that there were no significant differences ($P > 0.05$) in RBC, Hb or Hct among all dietary groups. Different levels of PGHE had a significant influence on WBC ($P < 0.01$). Fish fed with the 1.5, 4.5 or 9 g PGHE $kg^{-1}$ diet had a higher WBC, whereas a lower WBC was observed in fish fed with the control or 0.5 g PGHE $kg^{-1}$ diet. There were no significant differences in lymphocyte, monocyte or heterophil counts in any treatments ($P > 0.05$).

Table 3 shows the serum biochemical parameters of fish fed with diets containing different levels of PGHE. The results indicate no significant difference ($P > 0.05$) in ALP, AST, LDH, TP, Alb, Glb, TG, HDL, LDL, glucose or cholesterol. The sera ALT in all PGHE diet groups were significantly higher compared to the control ($P < 0.001$). Observed was that the ALT concentrations were about 67.96%, 73.96%, 2.36-fold and 3.62-fold higher than in the untreated control fish, respectively, in 0.5, 1.5, 4.5 and 9.0 g in the PGHE $kg^{-1}$ diet.

#### Histological changes in liver

There were no gross pathological changes in carp liver tissue when dissected. The liver of the control group showed a normal appearance (Fig. 2a). On the contrary, pathological changes were observed in all treated groups after 10 weeks of oral administration of PGHE.

After feeding with the 0.5 g PGHE $kg^{-1}$ diet, the liver cells cytoplasm became vacuolated and some nuclei showed changes in shape and size. In some areas of the liver, focal necrosis could be observed. In some slides a dilution in sinusoids was noted (Fig. 2b).

Daily feeding of a 1.5 g PGHE $kg^{-1}$ diet provoked necrosis in some hepatocyte areas. Although vacuolated degeneration was also observed, with some nuclei becoming lateral in the cell, other nuclei still had a regular shape and central position in the cell. Blood congestion could be seen in an irregular pattern in the liver (Fig. 2c).

Table 2
Mean ± SD of hematological parameters (n = 6) of *Cyprinus carpio* following 70 days feeding with different pistachio green hull extract

| Parameters | Treatments (g PGHE $kg^{-1}$ diet) | | | | | P value |
|---|---|---|---|---|---|---|
| | Control | 0.5 | 1.5 | 4.5 | 9 | |
| Hb (g $dl^{-1}$) | 6.5 ± 1.32 | 6.26 ± 0.66 | 5.93 ± 1.1 | 6.7 ± 0.88 | 6.66 ± 0.66 | 0.886 |
| Hct (%)[a] | 18.66 ± 6.5 | 20.33 ± 2.51 | 19 ± 3 | 18.5 ± 0.5 | 21.66 ± 4.5 | 0.84 |
| RBC (cell $mm^{-3}$) | 1.18 × $10^6$ ± 76 376 | 1.76 × $10^6$ ± 11 500 | 1.85 × $10^6$ ± 86 602 | 1.78 × $10^6$ ± 76 376 | 1.96 × $10^6$ ± 28 867 | 0.082 |
| WBC (cell $mm^{-3}$) | 15 000 ± 500[b] | 16 333 ± 577[b] | 20 000 ± 1732[a] | 19 000 ± 1000[a] | 20 677 ± 333[a] | <0.01 |
| Lymphocyte (%) | 82.5 ± 3.27 | 80.5 ± 3.01 | 81.16 ± 1.16 | 81 ± 2.78 | 80.83 ± 2.13 | 0.724 |
| Monocyte (%) | 12.5 ± 1.87 | 16.16 ± 3.25 | 14.33 ± 1.63 | 14.5 ± 2.5 | 15.16 ± 1.47 | 0.104 |
| Heterophil (%) | 5.0 ± 2.0 | 3.33 ± 1.63 | 4.5 ± 1.51 | 4.33 ± 1.21 | 3.83 ± 1.16 | 0.4 |

Means with the different letter is significantly different ($P < 0.05$).
All measurements were done at room temperature.
[a]Samples for Hct were dilated by adding heparins anticoagulant.
Hb, hemoglobin; Hct, hematocrit; RBC, red blood cells; WBC, white blood cells.

Table 3
Changes in the serum biochemical parameters of *Cyprinus carpio* fish following 70 days feeding with different levels of PGHE.

| Biochemical parameters | Treatment (g PGHE $kg^{-1}$ diet) | | | | | P value |
|---|---|---|---|---|---|---|
| | Control (0) | 0.5 | 1.5 | 4.5 | 9 | |
| AST (IU $L^{-1}$) | 108.7 ± 7.57 | 117 ± 6.11 | 115 ± 5 | 116 ± 4.35 | 118.34 ± 5.5 | 0.341 |
| ALT (IU $L^{-1}$) | 16.67 ± 7.63[a] | 28 ± 4.16[b] | 29 ± 3.05[bc] | 39.33 ± 3.21[c] | 60.33 ± 7.76[d] | <0.001 |
| ALP (IU $L^{-1}$) | 160.66 ± 6.42 | 169.33 ± 16.04 | 182.66 ± 10.69 | 178.3 ± 9.01 | 184 ± 4.93 | 0.08 |
| LDH (IU $L^{-1}$) | 823 ± 59.9 | 801 ± 24.54 | 754.33 ± 30.53 | 752 ± 15.71 | 772 ± 23 | 0.116 |
| TP (g $dl^{-1}$) | 3.26 ± 0.76 | 3.76 ± 0.28 | 2.8 ± 0.51 | 3.96 ± 0.32 | 3.4 ± 0.14 | 0.103 |
| Albumin (g $dl^{-1}$) | 1.18 ± 0.3 | 1.34 ± 0.21 | 0.96 ± 0.23 | 1.51 ± 0.24 | 1.4 ± 0.28 | 0.17 |
| Globulin (g $dl^{-1}$)[a] | 2.08 ± 0.46 | 2.42 ± 0.23 | 1.83 ± 0.28 | 2.45 ± 0.3 | 2 ± 0.14 | 0.294 |
| TG (mg $dl^{-1}$) | 236.6 ± 7.03 | 234 ± 6.42 | 242 ± 7.3 | 237.6 ± 9.86 | 234 ± 4.5 | 0.601 |
| HDL (mg $dl^{-1}$) | 69.0 ± 7.54 | 79.0 ± 2.64 | 67.6 ± 9.6 | 70.33 ± 8.05 | 68.0 ± 4.58 | 0.325 |
| LDL (mg $dl^{-1}$) | 28.0 ± 2.64 | 35.0 ± 2.5 | 34.0 ± 3 | 29.0 ± 3.6 | 33.0 ± 4.93 | 0.098 |
| Glucose (mg $dl^{-1}$) | 74.66 ± 3.5 | 82.33 ± 6.65 | 83.0 ± 4.9 | 78.3 ± 9 | 89.0 ± 9.07 | 0.148 |
| Cholesterol (mg $dl^{-1}$) | 130.0 ± 8.02 | 146.0 ± 5.19 | 131.6 ± 5.05 | 130 ± 7 | 129.6 ± 9.5 | 0.085 |

Means with the different letter is significantly different ($P < 0.05$).
Means and SD was represented and calculated from six samples.
[a]Globulin content was determined by subtracting albumin from the total protein.
AST, Aspartate transaminase; ALT, Alanine transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TP, total protein; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

When fish were administrated a 4.5 g PGHE $kg^{-1}$ diet, more focal necrosis was observed. There was a degeneration in the hepatocyte cytoplasm, with a smaller nuclei size and variation in shape (Fig. 2d).

PGHE (9.0 g PGHE $kg^{-1}$ of diet) caused a significant increase in the number of Kupffer cells with vacuole degeneration. Changes in the position as well as in size of the nuclei were also observed (Fig. 2e).

## Discussion

Recently shown is that different parts of the *P. vera* extract can provide some antioxidant properties *in vitro* (Hosseinzadeh et al., 2012). This research investigated the effects of *P. vera* hull extract (as a resource of phenol) on liver tissue, hematology and serum biochemical parameters of common carp as an important species in aquaculture.

Hematological and biochemical tests are important tools to assess the health status of fish (Abdel-Tawwab et al., 2010). It has been suggested that hematological indices can reflect the effects of dietary adequacy (Řehulka, 2002; Ewuola et al., 2008). There were no significant changes in RBC, Hb, or Hct levels among any treatments, and fish fed with PGHE diets showed the normal ranges for common carp blood chemistry (Weiss and Wardrop, 2011). Pakravan et al. (2012) obtained similar results by feeding *C. carpio* with diets containing extracts of willow herb, *Epilobium hirsutum*. However, Goda (2008) reported that there were significant increases in RBC, Hct, and Hb for Nile tilapia, *Oreochromis niloticus* fed diets containing Ginsana G115 at levels ranging from 50 to 250 mg $kg^{-1}$. These might be due to a difference in the antioxidant capacity.

WBC counts increased significantly ($P < 0.05$) in dietary PGHE treatments compared to the control (Table 1). Abdel-

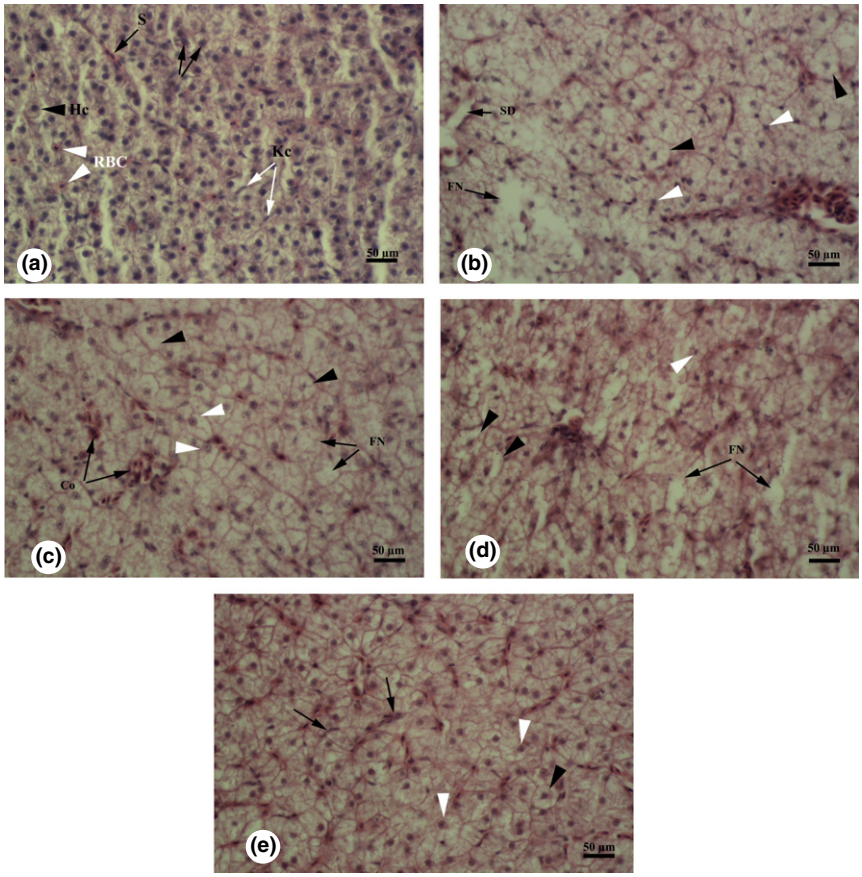


Fig. 2. (a–e) Photomicrgraph of *C. carpio* liver tissue following dietary PGHE administration, stained with H& E. (a) normal liver tissue. Arrows show nuclei with nucleuli included. (b) hepatocyte from 0.5 g PGHE $Kg^{-1}$. (c) 1.5 g PGHE $Kg^{-1}$ diet. White arrowhead, smaller nuclei; Black arrowhead, cytoplasm degeneration. (d) liver tissue from 4.5 g PGHE $Kg^{-1}$ diet. White arrowhead, lateral nuclei; Black arrowhead, cytoplasm degeneration. (e) liver tissue from 9.0 g PGHE $Kg^{-1}$ diet. White arrowhead, smallsized nuclei; Black arrowhead, cytoplasm degeneration. diet. Black arrow, increase in kupffer cells; White arrowhead, smaller nuclei, Black arrowhead, cytoplasm degeneration. Hc, hepatocyte; RBC, red blood cells; S, sinusoid; Kc, Kupffer cells; FN, focal necrosis; SD, sinusoid dilation; Co, blood congestion.

Tawwab et al. (2010) found that RBC and WBC counts were significantly different in Nile tilapia, *O. niloticus,* following dietary administration of 0.5, 1.0, or 2.0 g green tea, *Camellia sinensis* $kg^{-1}$ diets. The authors suggested that green tea enhanced the hematological function of the fish due to its natural antioxidant capacity. Pistachio hulls contain phenolic compounds as well as an antioxidant activity (Goli et al., 2005; Rajaei et al., 2010). The results of the present study indicate that PGHE is able to increase the WBC count in common carp, which might be the result of antioxidant compounds in *P. vera* extract. This suggests that the addition of PGHE to diets can either stimulate the immune system or be the result of a defense reaction (Abdel-Tawwab et al., 2010). High doses of phenolic compounds can probably induce activities in the anterior part of the kidney. However, histological studies on the hematopoietic systems are needed to confirm this hypothesis.

No significant differences were observed among treatments in differential counts of leukocytes. These results agree with the findings of Pakravan et al. (2012), who found no increases in the lymphocytes, monocytes or granulocytes counts after the feeding of common carp with different levels of willow herb, *Epilobium hirsutum*.

AST and ALT are considered to be important in assessing the state of the liver tissue (Coz-Rakovac et al., 2005). The liver is rich in AST and ALT (Vaglio and Landriscina, 1999), and injury to this tissue results in the release of these substances into the bloodstream, leading to high serum AST and ALT activities.

Abdel-Tawwab et al. (2010) showed that glucose, TP, and albumin were higher in Nile tilapia *O. niloticus* fed a diet containing 0.5, 1, or 2 g green tea $kg^{-1}$. Significant differences in TP, glucose, TG, and cholesterol were shown in *O. mossambicus* when fed diets containing a variety of herbal plants (Immanuel et al., 2009). The present study indicated that ALT was elevated in carp sera by increasing the PGHE dosage. An increase in ALT might be the result of a toxic effect of phenolic compounds of PGHE in the liver (Fig. 2). However, an ALT elevation does not necessarily indicate a liver disorder, but may be related to other physiological effects because the liver acts as a detoxifying system, reflecting any sort of hepatoxicity following a drug administration or dietary supplement. Liver cell necrosis, observed frequently in this study, might be a possible cause of increased serum ALT activity (Giboney, 2005). Some phenol compounds can elevate hepatic transaminases, which might be related to tissue damage (Tiedge et al., 1986; Amacher, 1998; Barse et al., 2006; Abdel-Hameid, 2007). Therefore, it could be interpreted that phenolic substances, existing markedly in PGHE, induced some pathological changes in the liver, including focal necrosis, cytoplasm degeneration, lateral nuclei, and an increase in kupffer cells. Natural phenols are able to stimulate Kupffer cells to regulate some cytokines (Kawada et al., 1998). This response was also observed here

following administration of PGHE as a natural source of phenol. Aside from the significant effect of herbal plants (Kikuyu grass and Moringa leaves) on fish growth, this might engender some hepatocyte necrosis as well as have a degrading effect on the liver (Hlophe Samkelisiwe and Moyo Ngonidzashe, 2014).

Based on the previous study and data of the present investigation, we therefore surmise that the only beneficial effect of PGHE in this species is a lower peroxide value of the fish meat. Poor growth performance found in the two studies might be a result of anti-nutritional factors such as phenolic compounds. It is important to determine whether this compound is an immunostimultant. This cannot, however, be completely concluded from the increase in WBC under the present conditions, but additional immunity parameters should and could be measured following the dietary administration of PGHE.

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**Author's address:** Javad Motamedi-Tehrani, Department of Natural Resources, Isfahan University of Technology, Isfahan, 8415683111 Iran.
E-mail address: jmotamedi124@gmail.com