RESEARCH ARTICLE

Antihyperlipidemic and antioxidant effects of feather protein hydrolysate in high-fat diet-fed mice

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The hyperlipidemia is a serious health problem that increases the risk of many complications including cardiovascular disease. This study aims to evaluate the possible antihyperlipidemic effects of the feather protein hydrolysate (FPH) in a mice fed with a high-fat diet (HFD)-fed mice during 5 weeks. The FPH administration improved dose-dependent lipid profile, as well as the liver and renal dysfunction indices in hyperlipidemic mice. The FPH also restored the antioxidant status in liver, kidney, and heart by lowering the lipid peroxidation and enhancing the antioxidant enzymes (catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase [SOD]). Moreover, the histological studies proved that FPH administration prevents hepatic steatosis, glomerular hyperfiltration risk, and cardiac muscle hypertrophy. Accordingly, the FPH is a promising novel medicinal ingredient for possible use in the hyperlipidemic treatment and related complications.

KEYWORDS

antihyperlipidemic effect, antioxidant enzymes, feather protein hydrolysate, high fat-fed mice

1 | INTRODUCTION

Diet is a key factor in the human health control. In fact, high-fat diet (HFD) is the major cause of metabolic syndrome risks development including hyperlipidemia, abdominal obesity, etc. Indeed, the hyperlipidemia is characterized by high levels of serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C). It is an important risk factor for atherosclerosis, the underlying cause of coronary heart disease leading to mortality in the world [1]. The hyperlipidemia state represents one of the crucial factors of oxidative stress development caused by the increased free radical production and the decreased antioxidant enzyme activities [2].

Lipid-lowering drugs have markedly reduced the risk of heart disease, but they are associated with adverse effects [3]. Thus, natural lipid-lowering agents are sought such as protein hydrolysate, biosurfactants, polyphenolic, etc. Several works

have reported the beneficial effects of protein and protein hydrolysates in the lipid metabolism regulation [4–8]. Indeed, the antioxidant activities of protein hydrolysate and their abilities to inhibit cholesterol micellar solubility and interact with bile acids are demonstrated in vitro studies [5]. Further, in vivo studies showed that the consumption of some protein hydrolysates by hyperlipidemic rats leads to a reduction in serum TC, TG, and LDL-C and an increase in high-density lipoprotein cholesterol (HDL-C) and improves defense mechanisms [9–11].

Feathers represent the major by-product generated from poultry processing plants with 10% of the total weight of the poultry, and they constitute an important source of proteins (85%) [12]. The antioxidant activity of feather protein hydrolysates (FPH) was previously investigated [13]. The present study was undertaken to test the FPH potential to lower serum cholesterol and TG levels in HFD-fed mice and their beneficial role on antioxidant enzymes (CAT, GPx, and SOD).

2 | MATERIALS AND METHODS

2.1 | Production of feather protein hydrolysate

The feathers were freshly collected from a poultry processing unit in Sfax (Tunisia), washed fully with distilled water and dried at 90°C for 5 h. The FPH was prepared by the method of Ben Hamad Bouhamed et al. [12], with some modifications. It was conducted in jacketed glass reactor with aqueous H₂SO₄ (0.5 N) and feather: solvent ratio (w/v) of 1:20 at 90°C for 100 min. The reaction was stopped by adjusting the pH to 7.0 with NaOH (4 N) and the solution was centrifuged at 5000 rpm for 30 min. The supernatant was dialyzed (MWCO 3000) against distilled water for 3 days to remove inorganic salts. The FPH obtained after drying was stored at −20°C until further use.

2.2 | Animals

Forty adults' male albino mice, weighing 27–32 g were purchased from the Pasteur Institute of Tunis (Tunisia) and kept in a controlled breeding laboratory (12 h light/dark cycle, at 25 ± 2°C and 60 ± 5% relative humidity). They were acclimatized to laboratory conditions for a week prior to the experimentation. The animals had access to standard laboratory diet (Table 1) and tap water. This study was conducted with the Tunisian Ethical Committee Guidelines for the Care and Use of Laboratory Animals.

2.3 | Experimental design

The animals were randomly divided into four groups of ten, each receiving different diets during 5 weeks.

Group 1 (G1): mice were fed with control diet.

Group 2 (G2): mice were fed with a HFD. The HFD was prepared by adding 10% sheep fat and 0.1% cholic acid to the standard diet. Indeed, the cholic acid is a bile acid involved to facilitate the micelles formation, which promotes processing of dietary fat.

Group 3 (G3): mice were fed with HFD, gavaged daily by 100 mg FPH/kg body weight (BW).

Group 4 (G4): mice were fed with HFD, gavaged daily by 200 mg FPH/kg BW.

The BW of the mice was monitored twice a week throughout the treatment.

2.4 | Collection of blood, liver, kidney, and heart

At the end of the experiment period (5 weeks), the mice were weighed and then sacrificed by decapitation, after an overnight fasting, to avoid stress. The blood was collected and plasma was immediately separated by centrifugation at 1500 g at 4°C for 15 min, and then stored at −20°C until analysis.

PRACTICAL APPLICATION

High-fat diet (HFD) induced hyperlipidemia associated with oxidative stress in the liver, kidney, and heart of mice. The feather protein hydrolysate (FPH) orally administered were found to attenuate hyperlipidemia and oxidative stress in HFD-fed mice. Thus, the FPH is a promising novel medicinal ingredient for possible use in the hyperlipidemic treatment and related complications.

The liver, kidney, and heart were neatly removed, weighed and stored at −80°C for further studies. Some pieces of the liver, kidney, and heart were fixed immediately in formalin solution (10%) for histological examination.

2.5 | Biochemical analysis

The plasma urea and the creatinine levels were measured using commercially diagnostic kits (Biomagreb, Tunisia). The

TABLE 1 Composition of control diet used in this study (obtained from the Society of Animals Nutrition (SNA), Sfax, Tunisia)

Nutritional properties (%)	
Moisture (maximal)	14
Fibers (maximal)	5
Proteins (minimal)	18
Fat (maximal)	3
Ash (maximal)	13.5
Carbohydrate	46.5
Calorific value (kcal/kg)	2846
Amino acid (%)	
Methionine	0.36
Cysteine	0.26
Threonine	0.62
Tryptophan	0.2
Mineral mix (mg/kg)	
Manganese	80
Fer	48
Cuivre	18
Zinc	64
Selenium	0.28
Cobalt	0.2
Iode	2
Vitamin and antioxidant (mg/kg)	
Vitamin A	11 200 UI
Vitamin D3	2800 UI
Vitamin H	25
Antioxidant (BHA–BHT)	100

TABLE 2 Effects of different treatments on body weight gain and relative organ weights

Parameters	G1	G2	G3	G4
Body weight gain (g/mice/5 weeks)	4.81 ± 0.11 ^b	14.80 ± 0.21 ^c	6.10 ± 0.10 ^d	4.95 ± 0.09 ^b
Liver relative weight (g/100 g BW)	4.77 ± 0.64 ^b	6.05 ± 1.22 ^c	5.08 ± 0.125 ^d	4.93 ± 0.65 ^c
Kidney relative weight (g/100 g BW)	1.33 ± 0.15 ^a	1.36 ± 0.09 ^a	1.35 ± 0.10 ^a	1.32 ± 0.15 ^a
Heart relative weight (g/100 g BW)	0.50 ± 0.03 ^a	0.55 ± 0.09 ^a	0.55 ± 0.06 ^a	0.52 ± 0.05 ^a

Body weight gain (g) = Final body weight – Initial body weight

G1 = normal mice; G2 = hyperlipidemic mice; G3 = hyperlipidemic mice given FPH (100 mg/kg BW); G4 = hyperlipidemic mice given FPH (200 mg/kg BW).

The data are represented as mean ± SE for 10 mice in each group. Means with the different superscript letters within the same line are significantly different ($p < 0.05$).

TC, TG, LDL-C, HDL-C levels, as well as the AST, ALT, ALP, LDH, and T-Bilirubins were estimated in frozen plasma using standardized enzymatic procedures by commercial kits (Abbot Laboratories, Germany). The atherogenic index (AI) was obtained referring to the Friedewald equation [14] given below:

$$AI = \frac{LDL - C}{HDL - C}$$

2.6 | Evaluation of liver, kidney, and heart antioxidant status

Some livers, kidneys, and hearts were washed and homogenized in 2 mL of cold TBS buffer (50 mM Tris, 150 mM NaCl, pH 7.4) at 4°C using an Ultra-Turrax homogenizer and then centrifuged at 9000 g at 4°C for 20 min to remove cellular debris. The supernatants were stored at –80°C for further analysis. The protein contents in supernatants were measured by the Lowry's method [15]. Their level of malondialdehyde (MDA), as well as the antioxidant activities of CAT, GPx, and SOD, were determined according to Berton's method [16], Aebi's method [17], Flohé and Günzel [18], and Asada et al. [19], respectively.

2.7 | Histopathological analysis

Some pieces of liver, kidney, and heart were fixed in a 10% formalin solution, dehydrated in alcohol, embedded in paraffin, and finally sectioned at 4 µm thick. The sections were then stained with hematoxylin–eosin and the slides were examined under an Evos XL light microscope.

2.8 | Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Version 10.0, SPSS Inc., Chicago, IL) was used for the statistical analysis. The statistical differences were calculated using the one-way analysis of variance (ANOVA) for comparison between mice groups. Data are given as means ± SE and the differences were considered significant at $p < 0.05$.

3 | RESULTS

3.1 | FPH effects on the body weight gain and the relative organs weights

Table 2 shows the growth parameters of the different mice groups during 5 weeks' treatment. The BW gain of mice fed with a HFD were significantly ($p < 0.05$) higher than those of the control group. In fact, the BW gain of the HFD group increased by 207.69% as compared to the control group. However, comparatively to untreated HFD-fed mice, the treated groups with FPH (G3 and G4) recorded a significant ($p < 0.05$) decrease in BW gain by 58.87 and 66.55%, respectively.

The relative weights of kidney and heart of all groups were not affected by the HFD ($p > 0.05$). However, the relative liver weight of HFD group was increased by approximately 27% as compared to the control group ($p < 0.05$). The oral administration of the FPH (100 mg/kg) and FPH (200 mg/kg) reduces the liver weight by 16 and 18%, respectively, in comparison with HFD mice.

3.2 | FPH Effects on lipid profile

The plasma lipid levels of the experimental groups are presented in Table 3. After the experimental period, the TC, TG, and LDL-C levels of mice fed with a HFD showed a significant increase as compared to the control group. There was also a significant decrease in the HDL-C level by 44.13%.

The hyperlipidemic mice orally administrated with FPH had lower cholesterol level than mice fed with a HFD. Furthermore, in G3 and G4, the TC, TG, and LDL-C levels reduced by 19.05, 25.51, 19.16% and 26.66, 40.32, 53.33%, respectively, as compared to the HFD group. Therefore, the present study shows that G3 and G4 significantly re-established their HDL-C level ($p < 0.05$).

It is interesting to note that the treatment with FPH at 100 and 200 mg/kg BW reduced the atherogenic index (AI) by 49.32 and 68.91%, respectively, as compared to those HFD group.

TABLE 3 Lipid profiles in different mice groups^a

Parameters	G1	G2	G3	G4
TC (mmol/L)	2.11 ± 0.08 ^a	3.15 ± 0.07 ^b	2.55 ± 0.05 ^d	2.31 ± 0.09 ^{c,d}
TG (mmol/L)	1.51 ± 0.03 ^a	2.43 ± 0.04 ^c	1.81 ± 0.06 ^b	1.45 ± 0.03 ^a
HDL-C (mmol/L)	1.45 ± 0.09 ^d	0.81 ± 0.11 ^a	1.05 ± 0.08 ^{a,b}	1.21 ± 0.09 ^{b,c}
LDL-C (mmol/L)	0.47 ± 0.06 ^a	1.20 ± 0.08 ^b	0.79 ± 0.06 ^a	0.56 ± 0.11 ^a
AI = LDL/HDL	0.32 ± 0.05 ^a	1.48 ± 0.07 ^c	0.75 ± 0.11 ^b	0.46 ± 0.09 ^{a,b}

^aG1 = normal mice; G2 = hyperlipidemic mice; G3 = hyperlipidemic mice given FPH (100 mg/kg BW); G4 = hyperlipidemic mice given FPH (200 mg/kg BW).

The data are represented as mean ± SE for 10 mice in each group. Means with the different superscript letters within the same line are significantly different ($p < 0.05$).

TABLE 4 Liver profiles indices (AST, ALT, ALP, LDH, and T-Bilirubin) and kidney parameters (creatinine and urea) of different mice groups^a

Parameters	G1	G2	G3	G4
Liver dysfunction indices				
AST (U/L)	222.45 ± 5.22 ^a	346.71 ± 6.11 ^b	235.11 ± 4.87 ^a	225.54 ± 6.61 ^a
ALT (U/L)	32.9 ± 2.45 ^a	64.45 ± 2.87 ^b	34.91 ± 1.98 ^a	32.91 ± 2.45 ^a
ALP (U/L)	60.12 ± 4.21 ^a	124.45 ± 3.87 ^c	72.52 ± 4.05 ^b	66.19 ± 3.77 ^{a,b}
LDH (U/L)	2081.14 ± 10.45 ^a	3612.11 ± 11.45 ^c	2784.22 ± 11.31 ^b	2055.22 ± 14.17 ^a
T-Bilirubin (μmol/L)	2.50 ± 0.12 ^a	4.11 ± 0.25 ^c	3.21 ± 0.09 ^b	2.74 ± 0.20 ^a
Kidney toxicity indices				
Creatinine (μmol/L)	21.22 ± 1.25 ^a	40.45 ± 1.14 ^d	31.22 ± 2.05 ^c	24.12 ± 1.89 ^b
Urea (mmol/L)	4.40 ± 0.33 ^a	6.71 ± 0.25 ^b	4.97 ± 0.19 ^a	4.52 ± 0.55 ^a

^aG1 = normal mice; G2 = hyperlipidemic mice; G3 = hyperlipidemic mice given FPH (100 mg/kg BW); G4 = hyperlipidemic mice given FPH (200 mg/kg BW).

The data are represented as mean ± SE for 10 mice in each group. Means with the different superscript letters within the same line are significantly different ($p < 0.05$).

3.3 | Liver–kidney dysfunction indices

The plasma levels of AST, ALT, ALP, LDH, and T-bilirubins are shown in Table 4. Compared to the control group, the HFD-fed mice showed a significant ($p < 0.05$) increase in the AST, ALT, ALP, LDH, and T-bilirubin levels by 55.58, 95.89, 107.00, 73.56, and 64.40%, respectively.

Interestingly, the AST, ALT, ALP, LDH, and T-bilirubin levels decreased significantly in G3 and G4 by 32.18, 45.83, 41.72, 22.92% and 21.98, 34.95, 48.93, 46.81, 43.10, and 33.33%, respectively.

In order to examine the FPH role on kidney function, the plasma creatinine and urea levels were measured, and the results are presented in Table 4. The HFD treatment induced a significant ($p < 0.05$) increase in the creatinine and urea levels by 90.71 and 52.38%, respectively, as compared to the control group. Five weeks' treatment with FPH significantly reversed this increase to normal values.

3.4 | Lipid peroxidation

The MDA levels in the liver, kidney, and heart are shown in Figure 1. An increase in the MDA levels by 250.00, 114.43, and 62.50% was observed in the liver, kidney, and heart, respectively, of HFD-fed mice as compared with the control group. By contrast, the FPH administration to mice fed with a HFD inhibited the MDA formation. In fact, the FPH

diminished the liver, kidney, and heart MDA concentrations, compared to the HFD group.

3.5 | FPH effect on antioxidant status

As reported in Figure 2, the mice fed with a HFD exhibited a marked decrease in the CAT, GPx, and SOD activities in liver, kidney, and heart as compared to the control group.

This decrease was significantly restored in the HFD mice supplemented with the FPH. In the liver tissue (Figure 2A), the 100 mg/kg FPH administration improved ($p < 0.05$) the CAT (31.81%), GPx (28.25%), and SOD (16.25%) activities, respectively, as compared to the HFD group, whereas the FPH (200 mg/kg) induced a significant increase in the CAT, GPx, and SOD activities by 56.15, 82.80, and 32.97%, respectively, as compared to the HFD group. In the kidney tissue (Figure 2B), the FPH (100 mg/kg) treatment enhanced the CAT, GPx, and SOD activities by 47.91, 28.55, and 28.08%, respectively, when compared with the HFD group. The FPH (200 mg/kg) showed a remarkable improvement of the CAT, GPx, and SOD activities by 77.15, 42.85, and 60.04%, respectively, compared to the HFD group. In the heart tissue (Figure 2C), the FPH (100 mg/kg) treatment increased the CAT, GPx, and SOD activities by 37.45, 37.51, and 40.83%, respectively, when compared with the HFD group. The FPH (200 mg/kg) improved the CAT, GPx, and SOD activities by

FIGURE 1 MDA level in liver (A), kidney (B), and heart (C) of the experimental mice groups. G1 = normal mice; G2 = hyperlipidemic mice; G3 = hyperlipidemic mice given FPH (100 mg/kg BW); G4 = hyperlipidemic mice given FPH (200 mg/kg BW). Each bar represents mean $\pm$ SE from 10 mice. Bars with different letters differ significantly at $p < 0.05$

43.97, 61.17, and 60.85%, respectively, compared to the HFD group.

In this study, the hyperlipidemic mice showed a marked decline in the hepatic, renal, and cardiac CAT, SOD, and GPx activities as compared to the control mice, and the treatment with FPH has significantly restored the enzymes activities in a dose-dependent manner when compared with the HFD group.

3.6 | Histological examination

The biochemical modifications were further supported by the histopathological study (Figure 3). As shown in Figure 3A (G2), in HFD group, lipids droplets accumulated in the hepatocytes were observed as spherical vacuoles which have a clear appearance (indicated by the arrows). The light microscopic findings showed that the FPH administration shows a marked improvement in the liver. The liver sections of G4 (Figure 3A [G4]) showed decreased hepatic steatosis with a prominent nucleus and a preserved cytoplasm.

The histological studies of the renal tissues have been examined to evaluate the HFD treatment effect on kidney function (Figure 3B). As shown in Figure 3B (G1), the renal tissue architecture of the control mice showed normal glomeruli and tubules. However, by feeding HFD, a glomerular atrophy and an increase in Bowman's space were detected as shown in Figure 3B (G2). Moreover, the HFD caused a significant increase in degenerative vacuolated renal tubules, resulting from lipid droplet deposited nephropathies.

The histological examination of the heart tissues in HFD group disclosed remarkable hypertrophy of cells (Figure 3C [G2]). The Figure 3C (G3, G4) depicts a more or less normal cellular architecture of the heart muscle cells, with notable alleviation of cardiac muscle hypertrophy in mice fed with a HFD supplemented with FPH.

4 | DISCUSSION

The previous study reported that various animal protein hydrolysate possesses different biological activities such as antihyperlipidemic, antidiabetic, and antioxidant activities [9, 20,21]. However, there are no reports on the FPH for the treatment against hyperlipidemia.

The present study examined whether the treatment of mice by FPH might improve the lipid profile and mitigate oxidative damage resulting from a HFD. As expected, mice treated with hyperlipidemic diet showed a significant increase in TC, TG, and LDL-C levels and a decrease in HDL-C levels. In the human body, high TC and TGs in the blood were related to atherosclerosis, thus, affect the risk of coronary heart disease. The administration of FPH at 100 and 200 mg/kg BW promoted a reduction in TC, TG, and LDL-C levels and an increase in HDL-C level. It is widely accepted that the HDL-C plasma reduction presented a risk factor for atherosclerosis development. The HDL-C facilitates the cholesterol transports from the tissues to the liver, where it is eliminated from the body after conversion to bile acids in the process known as reverse cholesterol transport [22]. The atherosclerosis process may slow down by the increase of HDL-C. In fact, peptides can act as antihyperlipidemics, particularly,

FIGURE 2 FPH effect on the CAT (A), GPX (B), and SOD (C) activities in liver, kidney, and heart of the experimental mice groups. G1 = normal mice; G2 = hyperlipidemic mice; G3 = hyperlipidemic mice given FPH (100 mg/kg BW); G4 = hyperlipidemic mice given FPH (200 mg/kg BW). Each bar represents mean $\pm$ SE from 10 mice. Bars with different letters differ significantly at $p < 0.05$

through their ability to suppress the cholesterol absorption in the intestinal epithelia, caused by the inhibition of the cholesterol micellar solubility [23]. The bile acid reabsorption in the ileum could be inhibited by the high bile acid binding capacity of the peptide, caused an important cholesterol-lowering effect [24]. Indeed, the FPH effect in lipid-lowering could be explained by the fact that FPH can reduce the 3-hydroxyl-3-methyl-glutaryl-Coenzyme A (HMG-CoA) reductase activity involved in the cholesterol biosynthesis [25].

The obtained results are in agreement with several other studies that reported that protein hydrolysates have a similar antihyperlipidemic effects pattern [8,9,26]. For example, Ben Khaled et al. [9] reported that the administration of sardinelle protein hydrolysate in hypercholesterolemic rat decreased the TG, TC, and LDL-C levels significantly and increased the HDL-C level.

The hyperlipidemic and the oxidative stress consequences include cells lysis, atherosclerotic plaques development, and liver disorders. The hepatocyte is sensitive to oxidative stress, which can cause the escape of several components from its

cytosol such as the transaminases, as a result of membrane integrity deterioration [20]. The increased transaminases levels in the plasma served as biomarkers for liver injury in the clinical examination [27]. The serum activities of AST, ALT, ALP, LDH, and T-bilirubin were tested in different groups. The hyperlipidemic diet significantly increased these activities, which reflects the hepatocyte lysis. Interestingly, when hyperlipidemic mice were supplemented with FPH, these activities decreased and became comparable to the control group. Similar findings were observed in previous studies showing the hepatoprotective effects of protein hydrolysates on hypercholesteromic rats [20].

The creatinine and urea are considered as significant markers of kidney function. The creatinine is a metabolic waste product that is naturally removed from the blood by kidneys via the glomerular filtration. Thus, the creatinine level increase in the blood is exclusively caused by a renal failure problem. In fact, our investigation showed an increase of serum creatinine and urea in HFD-fed mice. However, the protective action on the renal function was achieved owing to the supplementation of FPH, which was manifested by decreased

FIGURE 3 Histological observation of liver (A), kidney (B), and heart (C) tissues of different groups. G1 = normal mice; G2 = hyperlipidemic mice; G3 = hyperlipidemic mice given FPH (100 mg/kg BW); G4 = hyperlipidemic mice given FPH (200 mg/kg

BW). Lipid vacuoles →; glomeruli ▲; Bowman's space ▴; tubular dilatation ●

serum indices of renal toxicities. This indicated that FPH could avoid renal damage caused by hyperlipidemia.

The imbalance between reactive oxygen species and their scavenging by the natural antioxidant system is a causal factor of oxidative stress. It has been proved that the oxidative

stress is a phenomenon underlying the hyperlipidemia and the atherosclerosis pathogenesis [28]. It is well-established that HFD leads to an increasing effect on lipid peroxidation in tissues [10]. The present study appears to validate the view that the hyperlipidemic mice have shown a marked increase in MDA levels in the liver, kidney, and heart tissues. Treatment with FPH at 100 and 200 mg/kg BW prevented the lipid peroxidation in the liver, kidney, and heart tissues in hyperlipidemic mice. This normalization might be accomplished by the antioxidant activities of peptides presented in FPH [13]. This power may be due to their ability to decompose free radicals by quenching reactive oxygen species and by trapping radicals before reaching their cellular targets.

Previous studies suggested that hyperlipidemia depresses the antioxidant defense system due to the increase of free radicals formation [9,29]. In accordance with this data, our results showed that hyperlipidemia was associated with the deterioration of antioxidant status, resulting from decreased SOD, CAT, and GPx activities. Conversely, the treatment with FPH has significantly restored these enzymes in a dose-dependent manner when compared with the hyperlipidemic group. This increase may have been due to the activation of enzymes by the hydrolysate. The higher SOD, CAT, and GPx activities could lead to the reduction of reactive oxygen species level in the mice fed with a HFD supplemented with FPH. This normalization is seen as an adaptation process, to face the oxidative stress by preventing the free radical production, so inhibiting the atherosclerosis development. Our results are in agreement with Kim et al. [11] that showed that protein hydrolysate reduces the oxidative stress in vivo and increase the antioxidant activities.

Considering the biochemical analysis, the FPH could exert a beneficial action against the deleterious effect of HFD. Such potential is supported by the histological findings in liver, kidney, and heart. In addition, the increase in the liver weight due to the hepatocyte hypertrophy was confirmed by the lipids accumulation in the liver induced by the HFD. Indeed, there was a statistical correlation between atrophic tubules, scarred glomeruli, and elevated creatinine level [30]. The positive effect of FPH was confirmed by the histological analysis, which showed that FPH prevented the fat appearance in the liver, the glomerular atrophy in the kidney, and the hypertrophy of cells in the heart.

5 | CONCLUDING REMARKS

This study suggests that the HFD-fed mice exhibited a noticeable increase in the BW, TC, TG, LDL-C, AST, ALT, ALP, LDH, T-Bilirubin, creatinine, and blood urea levels. However, they exhibited a decrease in HDL-C levels. In addition, the hepatic, renal, and cardiac MDA levels were increased and their antioxidant enzyme activities (CAT, GPx, and SOD)

were reduced. Moreover, the histological findings of this study revealed that hyperlipidemia induced fat cysts accumulation in the liver, lipid droplet deposition-related renal tubular vacuolization degenerative lesions, and cardiac muscle hypertrophy. However, all the above-mentioned hyperlipidemia-related complications were dose-dependent and significantly inhibited after 5 weeks of the consecutive oral administration of 100 and 200 mg/kg FPH. In addition, FPH dose-dependent improved the liver, kidney, and heart antioxidant defense system and inhibited lipid peroxidation. These results indicate that FPH is a promising new potent medicinal ingredient that can be used in the hyperlipidemic treatment.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

- Ross, R., Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* 1999, 340, 115–126.
- Jiangwei, M., Zengyong, Q., Xia, X., Aqueous extract of *Astragalus mongholicus* ameliorates high cholesterol diet induced oxidative injury in experimental rats models. *J. Med. Plants Res.* 2011, 5, 855–858.
- Smilde, T. et al., Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001, 357, 577–581.
- Brandsch, C. et al., Effect of proteins from beef, pork, and turkey meat on plasma and liver lipids of rats compared with casein and soy protein. *Nutrition* 2006, 22, 1162–1170.
- Hosomi, R. et al., Fish protein decreases serum cholesterol in rats by inhibition of cholesterol and bile acid absorption. *J. Food Sci.* 2011, 76, H116–H121.
- Nagaoka, S. et al., Identification of novel hypocholesterolemic peptides derived from bovine milk β -lactoglobulin. *Biochem. Biophys. Res. Commun.* 2001, 281, 11–17.
- Wang, M.-F., Antihypercholesterolemic effect of undigested fraction of soybean protein in young female volunteers. *J. Nutr. Sci. Vitaminol. (Tokyo)* 1995, 41, 187–195.
- Yamamoto, S. et al., Favorable effects of egg white protein on lipid metabolism in rats and mice. *Nutr. Res.* 1993, 13, 1453–1457.
- Ben Khaled, H. et al., Effect of protein hydrolysates from sardinelle (*Sardinella aurita*) on the oxidative status and blood lipid profile of cholesterol-fed rats. *Food Res. Int.* 2012, 45, 60–68.
- Hosomi, R. et al., Effects of dietary fish protein on serum and liver lipid concentrations in rats and the expression of hepatic genes involved in lipid metabolism. *J. Agric. Food Chem.* 2009, 57, 9256–9262.
- Kim, M.-R. et al., Anti-obesity effects of yellow catfish protein hydrolysate on mice fed a 45% kcal high-fat diet. *Int. J. Mol. Med.* 2017, 40, 784–800.
- Ben Hamad Bouhamed, S., Kechaou, N., Kinetic study of sulphuric acid hydrolysis of protein feathers. *Bioprocess Biosyst. Eng.* 2017, 40, 715–721.
- Ben Hamad Bouhamed, S., Krichen, F., Kechaou, N., Feather protein hydrolysates: a study of physicochemical, functional properties and antioxidant activity. *Waste Biomass Valor.* 2018. <https://doi.org/10.1002/s12649-018-0451-2>. (In Press).
- Friedewald, W. T., Levy, R. I., Fredrickson, D. S., Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* 1972, 18, 499–502.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951, 193, 265–275.
- Berton, T. R. et al., The effect of vitamin E acetate on ultraviolet-induced mouse skin carcinogenesis. *Mol. Carcinog.* 1998, 23, 175–184.
- Aebi, H., [13]Catalase in vitro. In: *Methods in Enzymology*. Cambridge, MA: Academic Press; 1984. p. 121–126.
- Flohé, L., Günzler, W. A., [12]Assays of glutathione peroxidase. In: *Methods in Enzymology*. Cambridge, MA: Academic Press; 1984. p. 114–120.
- Asada, K. et al., Superoxide dismutases from a blue-green alga, *Plectonema boryanum*. *J. Biol. Chem.* 1975, 250, 2801–2807.
- Lassoued, I. et al., Evaluation of hypocholesterolemic effect and antioxidant activity of Boops boops proteins in cholesterol-fed rats. *Food Funct.* 2014, 5, 1224–1231.
- Harnedy, P. A. et al., Atlantic salmon (*Salmo salar*) co-product-derived protein hydrolysates: a source of antidiabetic peptides. *Food Res. Int.* 2018, 106, 598–606.
- Peter A. Mayes, Kathleen M. Botham, Cholesterol synthesis, transport, & excretion. New York, NY: *Harper's Illustrated Biochemistry*. 2003, 26, 219–230.
- Nagaoka, S. et al., Serum cholesterol reduction and cholesterol absorption inhibition in CaCo-2 cells by a soyprotein peptic hydrolyzate. *Biosci. Biotechnol. Biochem.* 1997, 61, 354–356.
- Nagaoka, S. et al., Soy protein peptic hydrolysate with bound phospholipids decreases micellar solubility and cholesterol absorption in rats and Caco-2 cells. *J. Nutr.* 1999, 129, 1725–1730.
- Wergedahl, H. et al., Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol, and lowers Acyl-CoA:cholesterol acyltransferase activity in liver of Zucker rats. *J. Nutr.* 2004, 134, 1320–1327.
- Ktari, N. et al., Cholesterol regulatory effects and antioxidant activities of protein hydrolysates from zebra blenny (*Salaria basilisca*) in cholesterol-fed rats. *Food Funct.* 2015, 6, 2273–2282.
- Fortson, W. C., Tedesco, F. J., Starnes, E. C., Shaw, C. T., Marked elevation of serum transaminase activity associated with extrahepatic biliary tract disease. *J. Clin. Gastroenterol.* 1985, 7, 502–505.

28. Young, I. S., McEneny, J., Lipoprotein oxidation and atherosclerosis. *Biochem. Soc. Trans.* 2001, 29, 358–362.
29. Belguith-Hadriche, O. et al., Comparative study on hypocholesterolemic and antioxidant activities of various extracts of fenugreek seeds. *Food Chem.* 2013, 138, 1448–1453.
30. Schelling, J. R., Tubular atrophy in the pathogenesis of chronic kidney disease progression. *Pediatr. Nephrol. Berl. Ger.* 2016, 31, 693–706.

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