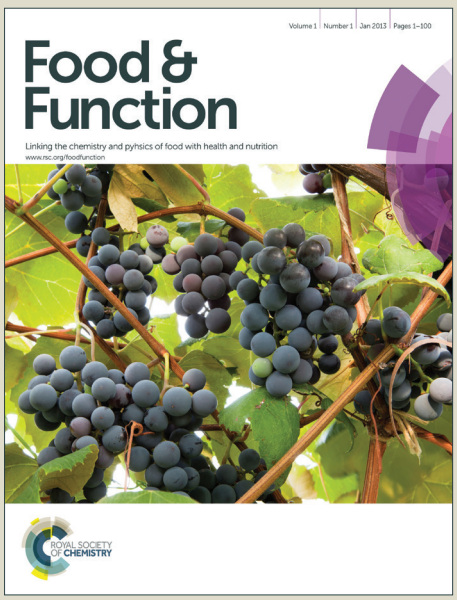
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Evaluation of hypocholesterolemic effect and antioxidant activity of *Boops boops* proteins in cholesterol fed rats.

Imen Lassoued[1], Mariem Trigui[1], Zohra Ghlissi[2], Rim Nasri[1], Kamel Jamoussi[2], Mondher Kessis[2], Zouheir Sahnoun[2], Tarek Rebai[2], Ahmed Boualga[3], Myriem Lamri-Senhadji[3], Moncef Nasri[1], Ahmed Barkia[1*]

1. Laboratoire de Génie Enzymatique et de Microbiologie, Université de Sfax, Ecole Nationale d'Ingenieurs de Sfax, B.P 1173-30387, Sfax-Tunisie.

2. Faculte de Medecine, Université de Sfax, Tunisia

3. Laboratoire de Nutrition Clinique et Métabolique, Département de Biologie, Faculté des Sciences, Université d'Oran. B.P 16 Es-Sénia, Oran, Algérie.

* Corresponding author: E-Mail: ahmedbarkia_lgem@yahoo.fr

Abstract

Dietary proteins affect blood cholesterol concentrations and antioxidant status which are in relation with several diseases such cardiovascular disease. The present study attempts to investigate the potential of *Boops boops* proteins (Bb-NHP) and its hydrolysate (Bb-HP) in the prevention of hypercholesterolemia and oxidative stress in rats fed a high cholesterol diet (HCD). After four weeks treatment, serum lipid profiles (total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol), activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the level of malonaldehyde (MDA) and the activities of antioxidant enzymes [Catalase (CAT) and glutathione peroxidase (GPx)] in liver were determined. Compared with fed standard diet, high cholesterol diet induced dyslipidemia, oxidative stress and aorta structure alterations. Interestingly, supplementing the HCD with *Boops boops* proteins attenuated these abnomalies in a dose-dependent manner.

These observations suggested that *B. boops proteins* might provide health benefits by helping to reduce the deleterious effects of increased intake of cholesterol that characterize modern diets.

Keywords: *Boops boops*, Hypocholesterolemic, Antioxidant, Atherosclerosis, Protein hydrolysate

1. Introduction

Elevated plasma cholesterol and oxidative stress (higher pro-oxidant level than antioxidant level) promote the development of several serious pathologies such cardiovascular diseases.[1,2] Proatherogenic effect of hypercholesterolemia could be attributed, at least partially, to its induction of unfavourable changes of serum lipoproteins profile as the increase of the atherogenecity index [low-density-lipoproteins-cholesterol (LDL-ch)/ high-density lipoproteins cholesterol (HDL-ch) ratio]. For its part, oxidative stress promotes the oxidation of various substances and, consequently, causes the alteration of their functional properties. In particular, oxidation increases the atherogenecity of LDL giving them the ability to interact with the scavenger receptors of macrophages which are more actively converted into foam cells. Also, the oxidation reduces the atheroprotectivity of HDL which may even become atherogenic.

Atherosclerosis is a disease that develops over a long period. Consequently, its prevention is possible, notably, by the use of substances which are able to prevent and/ or attenuate the atherogenic abnormalities. For a long time, synthetic compounds have been used as antioxidants and lipid-lowering agents, but today they are subject to serious suspicion because many of them have adverse effects[3,4]. So, others lipids lowering and antioxidants agents are actively sought.

Numerous studies have demonstrated the beneficial effects of various proteins on lipid metabolism and antioxidant status.[5-7] Such effect could be due to the amino acids composition[7], but it was attributed mainly to peptides that are generated following incubating of proteins with a protease preparation and during the physiological digestion.[8] As it was reported in numerous reports, the effects of these peptides may depend on their characteristics

including their physico-chemical properties, their amino acid composition and sequence, and their size.[6,8,9]

In recent years, research has investigated cholesterol lowering and/or the potential antioxidant of many marine proteins such as muscles proteins of Alaska pollock (*Theragra chalcogramma*)[10], freshwater clam[11], sardine[12], zebra blenny[13], and Loach (*Misgurnus anguillicaudatus*)[14], sardine by-product proteins[15] and skin gelatine of jumbo squid (*Dosidicus gigas*)[16] and Hoki (*Johnius belengerii*) skin[17]. Obtained results showed that fish proteins have real antioxidant and hypocholesterolemiant potential. Indeed, the antioxidant properties (radical scavenging, metal ion chelation properties, lipid peroxidation inhibition)[11,16] of hydrolysates and/or peptides from different marine proteins and their ability to inhibit the cholesterol micellar solubility and to interact with bile acids[10,11] have clearly demonstrated in vitro studies. Further, in vivo studies, realized in rats, showed that marine protein hydrolysates or peptides improve defence mechanisms to eliminate reactive oxygen species, such as superoxyde dismutase (SOD) GPx and CAT[12,14], and serum lipids profile. In particular, the consumption of such hydrolysates by hyperlipemic animals led to a significant reduction of serum cholesterol (TC), triglycerides (TG) and LDL-ch[12,13,16] and/or an increase in HDL-ch[12,13] and HDL_2-ch[17].

The *Boops boops* (*Linnaeus*, 1758) (Sparidae teleost fish) is a responded fish (especially in the Atlantic and the Mediterranean) with a large biomass. It has a fusiform and elongated body. In Tunisia, it is one of the main fish caught in Tunisian waters, with an exploitable biomass estimated at about 8000-10000 tons annually[18].

The objective of the present paper was to investigate the cholesterol-lowering and antioxidant effects, in diet-induced hypercholesterolemic male *wistar* rats, of *Boops boops* undigested proteins and its hydrolyzate obtained by the action of crude alkaline protease extract from the intestine of smooth hound (*Mustellus mustellus*). The profile of plasma lipids

and the hepatic antioxidant enzymes (GPx, EC 1.11.1.9 and CAT, EC 1.11.1.6), and cytotoxic parameters (ALT and AST) were determined. In addition, we have investigated, by microscopy**,** the effects of fish proteins and its hydrolysate on the aorta structural change which are induced by hypercholesterolemia.

2. Materials and methods

2. 1. Material

Boops boops, with an average length of 16±5 cm and a weight of 80±13 g, were captured in the Gulf of Gabes and purchased from the fish market in the city of Sfax, Tunisia. They were packed in polyethylene bags, placed in ice and transported to the research laboratory within 30 min.

2. 2. Preparation of crude alkaline protease from smooth hound

Viscera (500 g) from smooth hound were rinsed with distilled water and then homogenized for 1 min with 300 ml of extraction buffer (10 mM Tris -HCl , pH 8.0). The homogenate was centrifuged at 8500 rpm for 30 min at 4°C. The pellet was discarded and the supernatant was collected and used as crude alkaline protease extract. The alkaline protease activity in crude extract was measured by the method of Kembhavi et al.[19] using casein as a substrate.

2. 3. Preparation of *B. boops* proteins hydrolysate (Bb-HP)

Fish fillets (100 g) were collected, rinsed three times with cold distilled water to remove salts and other undesirable elements. They were cooked for 20 min at 90°C in 200 ml of distilled water (90 °C, 20 min) and then homogenised. The homogenate was incubated for 4

hours with smooth hound alkaline crude protease extract at a 3:1 (U/mg) enzyme/ substrate ratio. During the digestion, temperature and pH were maintained at their respective values for maximum activity (pH 8, 50 °C). The control of pH was carried out by continuous addition of 4 N NaOH solution. The reaction was stopped by thermal inactivation of enzyme at 80 °C for 20 min. After centrifugation at 5000 g for 20 min, the soluble phase, which was regarded as hydrolysate, was collected, freeze dried and then stored at -20°C for further use.

The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds cleaved (*h*) to the total number of peptide bonds in the studied substrate (*htot*), was calculated from the amount of base (NaOH) added to keep the pH constant during the hydrolysis as according to Adeler-Nissen[20].

2.4. Chemical composition of Bb-HP

The proximate contents of *B. boops* hydrolysate in moisture, fat and ash were determined following the AOAC methods (1995). Fat was determined by Soxhlet extraction method. Total nitrogen content was analyzed by the Kjeldahl (AOAC, 1995), and the conversion factor of N x 6.25 was used to quantify the crude protein content.

2.5. Amino-acids composition

The dried samples were hydrolyzed with 0.5 ml of 6 N hydrogen chloride at 112 °C for 24 h on a heating block, and then filtered through a 0.45 μm membrane filter prior to analysis. Ten μl of the treated sample was derivatized using a Waters AccQ.Fluor Reagent Kit (according to the waters AccQ.Tag Chemistry Package Instruction Manual). The HPLC analyses were performed with a waters 2996 Separation Module equipped with a waters 2475 multi-wavelentgth fluorescence detector and the amino acids were separated on a Waters

ACCQ.Tag amino acid analyzing column (Nova-Pak C18, 150 x 3.9 mm). The amount of amino acid was calculated, based on the peak area in comparison with that of a standard.

2.6. Experimental procedure

In this study, we followed the guidelines for Care and Use of Laboratory Animals as approved by the local Institute Ethical Committee. Male *wistar* rats weighing 200-250 g were purchased from the breeding center of the Central Pharmacy of Tunis (Tunisia). The animals were housed in individual cages in controlled breeding room (temperature: 22±2 °C, humidity: 60±5%, 12 h light/dark cycle) and allowed free access to water and alimentation.

Rats were divided into seven experimental groups of five each receiving different diets during one month. The experimental groups were as follows:

Group I (G1): Control rats fed a standard diet. The characteristic of the standard diet, provided by the Society of Animal Nutrition (SAN), Sfax, Tunisia is reported in Table 1.

Group II (G2): rats fed a cholesterol-enriched diet (HCD), prepared by supplementing 1% cholesterol and 0.1% cholic acid to the standard diet.

Groups III (G3), IV (G4) and V (G5): rats fed a HCD gavaged daily by 0.1, 0.5 and 2 g of hydrolysed *B. boops* proteins (Bb-HP)/Kg of animal body weight (BW), respectively.

Groups VI (G6) and VII (G7): rats fed a HCD gavaged daily by 0.1 and 0.5 g of non hydrolysed *B. boops* proteins (Bb-NHP)/Kg of animal, respectively.

2.7. Collection of blood, liver and aorta

At the end of the experimental period, animals were fasted for 12 hours and then sacrificed under diethyl ether anesthesia. Blood samples were collected by cardiac puncture in EDTA-containing tubes and plasma was prepared by centrifugation (3000 tours/min for 15 min and at 4 °C) and stored at -80 °C until use. Liver and aorta were excised from each

animal. Aorta was immediately placed in the formal, while liver was frozen at – 80°C until further use.

2.8. Blood analysis

The levels of total cholesterol (TC), total triglycerides (TG) and low density lipoprotein-cholesterol (LDL-ch), high density lipoprotein-cholesterol (HDL-ch), and the activities of ALT and AST were determined in fasting blood using commercially available kits (Bekman Counter, Galway, Ireland). The LDL-ch was calculated according to Friedewald equation:

$$\text{LDL-ch} = [\text{TC} - \text{HDL-ch}] - [\text{TG}/5]$$

2.9. Evaluation of liver antioxidant status

Sample from liver tissue (1 g) was homogenized in 10 ml of cold TBS buffer (50 mM Tris, 150 mM NaCl, pH 7.4) at 4°C using Ultra-turax homogenizer. The cytosolic fraction (supernatant) was prepared by centrifugation (8000 trs/min, 4 °C, 10 min) and its level of malonaldehyde (MDA) and its activities of GPx and CAT were determined according to Draper and Hadley[21], Flohe and Gunzler[22] and Aebi[23], respectively.

2.10. Histological evaluation

The aorta is a privileged site for the development of atherosclerotic plaque. So, a histological comparison of aortas of rats from different groups was realized. Each piece of aorta was immediately fixed in formalin solution, embedded in paraffin, thin sections of about 5 µm thick were made, stained and, then, examined in the light microscope.

2.11. Statistical analysis

Values were given in means ± SD of five rats per group. High cholesterol diet fed rats group was compared to standard diet fed rats group and to different groups of Bb-HP or Bb-NHP supplemented rats. Differences were analyzed by Student t-test. The relation between assayed lipids parameters and MDA, CAT, GPx, AST and ALT was examined by the test of linear correlation. $P<0.05$ was considered statistically significant.

3. Results

3.1. Preparation and characterization of *B. boops* proteins hydrolysate

B. boops protein hydrolysate was prepared by the action of crude alkaline protease extract from *M. mustellus* intestine. The hydrolysis degree (DH) of the obtained hydrolysate was 20%. Proteins and fats are, respectively, 63% and 4.3% of the dry powder of intact *B. boops* proteins, and 67.65% and 3.6% of the dry powder of hydrolysate.

The amino acids composition of *B. boops* proteins and its hydrolysate, expressed as residues /1000 total amino acid residues, are summarized in Table 2. It was noted that there is a slight difference in amino acid composition between undigested and digested proteins. High levels of Gly, Thr, Arg, Glu, His, Ser and Leu amino acids were observed in both Bb-NHP and Bb-HP. Gly and Thr are the most dominant amino acids. The Gly content in Bb-NHP and Bb-HP were 150.6‰ and 157.6‰, and those of Thr were 108.3‰ and 110.3‰, respectively. The hydrophobic amino acids *in* Bb-NHP and Bb-HP were 453.0‰ and 481.0‰ of total amino acids, respectively. Outside the Trp that could be destroyed by acid hydrolysis, aromatic acids were 84.7‰ and 93.4‰ of the total amino acids in Bb-NHP and Bb-HP, respectively.

3.2. Anti-hyperlipidemic effect of Bb-HP and Bb-NHP

Plasma cholesterol, triglycerides, LDL-ch and HDL-ch levels obtained in different experimental rat groups at the end of the experiment are shown in Table 3. As expected and compared to standard diet fed rats, those fed HCD showed an important increase in plasma TC (+52.34 %, $p<0.01$), TG (+66.1 %, $p<0.01$) and LDL-ch (+236.11 %, $p<0.001$), whereas, their plasma HDL-ch was significantly decreased (-22.62 %, $p<0.05$). Consequently, the LDL-ch/HDL-ch ratio, which is unanimously considered as atherogenecity index, was increased at an important manner (4.4-fold higher, $p<0.001$). It is interesting to note that supplementation of HCD fed rats with *B. boops* proteins was found to attenuate lipid disorders at a dose-dependent manner. Indeed, all plasma lipids were significantly reduced as compared to HCD group, and values obtained with higher concentrations were similar to those of the control group.

3.3. Antioxidant activity and hepatoprotective effects

The MDA levels and the activities of CAT and GPx enzymes in the liver of the different groups of rats are shown in Fig 1. The activities of serum ALT and AST enzymes are presented in Fig 2. Compared to control group, HCD fed rats showed an increase of MDA (+84.14 %, $p<0.001$), ALT (+ 31.93 %, $p<0.01$) and AST (+ 94.46 %, $p<0.01$), but a decrease of CAT (-27.54 %, $p<0.05$) and of GPx (-69.34 %, $p<0.01$). The administration of *B. boops* proteins to HCD fed rats allowed preventing these deleterious changes at a dose dependant manner.

3.4. Histological observation

Aortas coming from rats under different diet (standard, HCD, HCD + Bb-HP, and HCD + Bb-NHP) were analyzed by microscopy sections. The obtained results reported in Fig 3 showed that high cholesterol diet induces a structural alteration predisposing to the

development of the atherosclerotic plaque. These alterations such as the formation of foam cells were virtually absent when rats were fed HCD supplemented with Bb-NHP/ kg BW or Bb-HP/kg BW.

4. Discussion

Literature reported that hydrolysates of marine proteins are composed by less than 5% fat and 60-90% protein, and that are characterized by a large presence of hydrophobic amino acids especially Gly, Ala, Leu and Val[10,24]. In consistence with these dada, Bb-HP and Bb-NHP had 63% and 68% of proteins, and 4.3% and 3.6% of lipids, and 453.0 ‰ and 480.8 ‰ of their amino acids were hydrophobic. Nevertheless, while glutamine-glutamic acid and asparagine-aspartic acid predominate in protein hydrolysates of several fish[10,24], Gly is the major amino acid in *B. boops* proteins and its hydrolysate.

Previous studies showed that proteins from different origins including fish proteins possess a variety of biological activities such as anti-hypercholesterolemic and antioxidant activities. However, the concomitant study of these two activities has been carried out only in a few previous studies[12].

Using *Wistar* rats as animal model, we have examined whether the treatment of rats by *B. boops* proteins or its hydrolysate might improve the lipid profile and oxidative damage resulting from a high-cholesterol diet. As expected, rats treated with the hypercholesterolemic diet showed a significant increase in serum lipids (TC, TG and LDL-ch). However, supplementing the HCD with Bb-NHP and Bb-HP promote a significant decrease in TC, TG and LDL-ch in all treated groups (Table 3). This effect was dose-dependent with both non-hydrolysed and hydrolyzed fish proteins. The daily supplementation of HCD rats by 2 g of Bb-HP/Kg BW, completely prevents the deleterious effect of HCD on the profile of serum lipids.

The obtained results were in agreement with several other findings previously reported in literature with regards to the hypocholesterolemic effects of protein hydrolysates[10,12,25]. For example, Ben Khaled et al.[12] reported a significant derease of TC, TG and LDL-ch in high cholesterol diet fed rats treated with sardinelle protein hydrolysates.

determinent However, different effect on serum levels of cholesterol and other lipids was observed with some hydrolysates. For example, salmon proteins did not show any effect except when they were administered in combination with fish lipids[26], while saithe proteins hydrolysate was found to reduce TG levels without affecting plasma total cholesterol and HDL-ch[16]. In another study, Louala et al.[17] have reported that and sardine proteins are without effect on LDL concentration. The differences recorted between fish protein hydrolysates in the prevention of dyslipidemia could be essentially due to the nature of the protein substrate and the enzyme used for the hydrolysis of fish proteins. Indeed, the amino acids composition and sequence of proteins, and specificity of the used proteases determine peptide composition and, therefore, the biological properties of the hydrolysate.

According to the literature, oxidative stress is hypercholesterolemia subjacent phenomenon[27]. In accordance with this data, our results showed that hypercholesterolemia was associated with deterioration of antioxidant status, resulting from increasing of MDA level and decreasing of CAT and GPx enzymes activities, and conversely, that the amelioration of cholesterolemia was accompanied by an improvement in this status, and the observed effects were more prominent with higher protein or peptide concentration.

The biological consequences of oxidative stress and hypercholesterolemia include cell lysis and the development of atherosclerotic plate. The hepatocyte is particularly vulnerable to oxidative stress which can cause deterioration of the membrane integrity resulting in the escape of several components from its cytosol such as transaminases. Serum activities of alanine aminotransferase and aspartate aminotransferase enzymes were tested in the different

diet groups of rats. Hypercholesterolemic diet significantly increased the activity of both serum AST and ALT activities which reflects a hepatocyte lysis. Interestingly, when the HCD was supplemented with *B. boops* proteins these two enzyme activities decreased and become comparable to control group at higher doses.

The observed antioxidant activity of *B. boops* proteins could simply be a consequence of the reduction of plasma cholesterol and / or an expression of the presence of antioxidant agents that may possess or not an hypocholesterolemic activity. Anyway, a relationship seems to exist between anti-hypercholesterolemia and antioxidant activities. Indeed, the results presented in Table 4 show that the increase of TC, LDL-ch and TG was associated with worsening of oxidative stress and alteration of the hepatocyte membrane structure, while the increase of HDL-ch was associated with an attenuation of these two abnormalities as shown by the significant negative correlation of these cholesterol fraction with MDA and AST. Such protective effect of HDL is not surprising since they possess antioxidant properties.

By their induction of CAT and GPx activities, the Bb-NHP and Bb-HP may be particularly effective in the prevention of lipid peroxidation. Indeed, CAT and GPx catalyze the detoxification of hydrogen peroxide (H_2O_2) and/or organic hydroperoxide (ROOH) which are involved in the initiation of peroxidation.

The contribution of individual amino acid residues or peptides in the biological activities of food protein hydrolysates has been investigated in some studies. Obtained results argue for the importance of hydrophobic amino acids in the power antioxidant[28] and hypocholesterolemic[29]. Also, these results associate the high intake of methionine and lysine levels and the low intake of arginine and glycine with increased cholesterolemia.[30,31] Consequently, the effects exhibited by the *B. boops* proteins or its hydrolysate can be explained, at least in part, by their high content in hydrophobic amino acids and the low

values of Lys/Arg (0.23 and 0.32, respectively) and Met/Gly (0.26 and 0.22, respectively) ratios.

Based on literature data, different mechanisms of action may be considered to explain the observed effects. In particular, because the *B. boops* proteins are hydrophobic amino acids rich, their hydrolysis probably generates hydrophobic peptides which act as radical scavengers or chelating transition metals, which justify, at least partially, the antioxidant activity of Bb-HP and Bb-NHP. In addition, hydrophobic peptides can act as anti-hypercholesterolemic, in particular by their ability to reduce the cholesterol micellar solubility which leads to reduce cholesterol absorption. Such peptides may also interact with bile acids causing their greater fecal excretion which results in the activation of their formation from cholesterol[32]. Furthermore, this lipid lowering effect could be explained also by the fact that Bb-NHP and Bb-HP can reduced by 3-hydroxyl-3-methyl-glutaryl-Coenzyme A (HMG-CoA) reductase activity involved in cholesterol biosynthesis[10].

Many previous findings support the absorption from the intestinal lumen of small peptides in intact form and without losing their biological activity[33]**.** Bb-HP and Bb-NHP could contain such peptides that can exert their effects in the circulation and/or in some target tissues. In particular, peptides might possess antioxidant properties and/or induce genes expression involved in antioxidant status. These peptides might also affect different lipoprotein fractions levels by acting on factors that influence serum and cellular lipid metabolism. For example, they could help in the observed cholesterol-lowering effect by inducing the activity of the LDL receptor. Indeed, it has been reported that this receptor activity which is depressed in hypercholesterolemia or in response to administration of high dietary cholesterol level, was positively influenced by food proteins both in the hepatic[34] and extrahepatic[34] cells. Additionally, absorbed peptides could affect the metabolism of triglycerides rich lipoproteins (TRL) through several possible mechanisms. Those include the

increase in hepatic clearance of these lipoproteins as well as the activation of their degradation by lipoprotein lipase (LPL) which may follow an induction of the expression of this enzyme' activator (apolipoprotein (apo) CII)[36]. Also, a decrease in the formation of TRL may occur due to decreased synthesis of TG[37], apo B and microsomal triglyceride transfer protein (MTP), or inhibition of the activity of MTP.

Furthermore, the decrease of triglyceridemia in response to the Bb-HP or Bb-NHP treatment could be partially due to the reduction of LDL. Indeed, rat LDL are characterized by a high TG content[38].

Considering the biochemical analysis, *B. boops* proteins could exert a beneficial action against a deleterious effect of high cholesterol diet. Such potential is comforted by the results of our histological analysis of the aorta (Fig 3). Indeed, the structural changes predisposing to the development of atherosclerotic plaque induced by high cholesterol diet have been prevented by the animal supplementation with *B. boops* proteins.

5. Conclusion

The data obtained in the present study suggest that both hydrolyzed and intact *B. boops* proteins exert preventive effects on atherosclerosis through their antioxidative properties and and their improving of the blood lipids profile.

Acknowledgements

Funding for this research was provided by the Ministry of Higher Education and Scientific Research, Tunisia.

References

1. K. M. Anderson, W. P. Castelli and D. Levy, *JAMA*, 1987, **257**, 2176-2180.
2. M. N. Diaz, B. Frei, J. A. Vita and J. F. Keaney, *N. Engl. J. Med*., 1997, **337**, 408-416.
3. G. Bjelakovic, D. Nikolova, L. Gluud, R. G. Simonetti, C. Gluud, *JAMA*. 2007, **297**, 842-857.
4. I. Agalliu, C. A. Salinas, P. D. Hansten, E. A. Ostrander and J. L. Stanford, *Am. J. Epidemiol.*, 2008, **168**, 250-260.
5. C. Brandsch, A. Shukla, F. Hirche, G. Stangl and K. Eder, *Nutrition*, 2006, **22**, 1162-1170.
6. C. F. Ajibola, J. B. Fashakin, T. N. Fagbemi and R. E. Aluko, *Int. J. Mol. Sci.,* 2011, **12**, 6685-6702.
7. M. W. Huff and K. K. Carroll, *J. Nutr.,* 1980, **110**, 1676-1685.
8. B. Walther and R. Sieber, *Int. J. Vitam. Nutr. Res.*, 2011, **81**, 181-92.
9. A. Iwaniak and J. Dziuba, *Food Technol. Biotechnol*., 2011, **49**, 118-127.
10. S. Hosomi, O. Motoki, K. Fukunaga, M. Okuno, R. Yagita, S. Kanda, T. Nishiyama, and M. Yoshida, *Trace-Nutr. Res*., 2010, **27**, 21-27
11. Y-H. Lin, J-S. Tsai, L-B. Hung and B. Sun Pan, *Food Chem*., 2010, **123**, 395-399.
12. H. Ben Khaled, Z. Ghlissi, Y. Chtourou, A. Hakim, N. Ktari, F. Ayadi, A. Barkia, Z. Sahnoun and M. Nasri, *Food Res. Int.,* 2012, **45**, 60-68.
13. N. Ktari, K. Mnafgui, R. Nasri, K. Hamden, I. Bkhairia, A. Ben Hadj, T. Boudaoura, A. Elfeki and M. Nasri, *Food funct.,* 2013, **4**, 1691-1699.
14. L. You, M. Zhao, J. M. Regenstein and J. Ren, *Food chem*., 2011, **124**, 188-194.
15. A. Barkia, A. Bougatef, H. Ben Khaled and M Nasri, *J. Food Biochem.*, 2010, **34**, 303-320.

16. B. Liaset, L. Madsen, Q. Hao, G. Criales, G. Mellgren, H. U. Marshall, P. Hallenborg, M. Espe, L. Froyland and K. Kristiansen, *Acta,* 2009, **1791**, 254-262.

17. S. Louala, S. Hamza-Rguig, A. Benyahia-Mostefaoui, A. Boualga and M. Y. Lamri-Senhadji, *J. Funct. Foods,* 2011, **3**, 321-328.

18. A. Hattour, L. Ben Abdallah, and Y. Guennegan, *Bull. Instit. Nat. Sci. Technol. Mer,* 2004, **31**, 5-16.

19. A. A. Kembhavi, A. Kulkarni and A. Pant, *Appl. Biochem. Biotechnol*., 1993, **38**, 83-92.

20. Adler-Nissen, J. (1986). In J. Adler-Nissen(Ed.), Enzymic hydrolysis of food proteins (pp. 57–109). Copenhagen, Denmark: Elsevier Applied Science Publishers.

21. H. H. Draper and M. Hadley, *Methods in Enzymol.,* 1990, **186**, 421-431.

22. L. Flohé and W. A. Gunzler, *Methods in Enzymol.*, 1984, **105**, 114-121.

23. H. Aebi, *Methods in Enzymol.,* 1984, **105**, 121-126.

24. C. B. Ahn, J. L. Je and Y. S. Cho, *Food Res. Int.,* 2012, **49**, 92-98.

25. H. Wergedahl, B. Liaset, O. A. Gudbrandsen, E. Lied, M. Espe, Z. Muna, S. Mork and R. Berge, *J. Nutr.,* 2004, **134**, 1320-1327.

26. H. Wergedahl, O. A. Gudbrandsen, T. H. Rost and R. K. Berge, *Nutrition*, 2009, **25**, 98-104.

27. A. R. Schultz-Moreira, J. Benedí, L. González-Torres, R. Olivero-David, S. Bastida, M. I. Sánchez-Reus, M. J. González-Muñoz and F. J. Sánchez-Muniz, *Food Chem.,* 2011, **129**, 1623-1630.

28. C. C. Udeniqwe, R. E. Aluko, *Int. J. Mol. Sci.,* 2011, **12**, 3148-3161.

29. F. Zhong, X. Zhang, J. Ma, C. F. Shoemaker, *Food Res. Int*., 2007, **40**, 756-762.

30. T. Rajamohan, A. Kurup, J. Biosci., 1990, **15**, 305-311.

31. O.A. Gudbrandsen, H. Wergedahl, B. Liaset, M. Espe and K. Berge, Brit. J. Nutr., 2005, **94**, 321-330.

32. S. Nagaoka, Y. Futamura, K Miwa, T. Awano, K. Yamauchi, Y. Kanamaru, K. Tadashi, T. Kuwata, *Biochem. Biophys. Res. Comm*., 2001, **281**, 11-17.

33. E. Ziv, M. Bendayan, *Microsc. Res. Tech*., 2000, **49**, 346-352.

34. S. J. Cho, M. A. Juillerat and C. H. Lee, *J. Agric. Food Chem*., 2008, **56**, 4372-4376.

35. M. R. Lovati, C Manzoni, A. Canavesi, M. Sirtori, V. Vaccarino, M. Marchi, G. Gaddi and C. R. Sirtori, *J. Clin. Invest*., 1987, **80**, 1498-1502.

36. H. R. Kast, C. M. Nguyen, C. J. Sinal, S. A. Jones, B. A. Laffitte, K. Reue, F. J. Gonzalez, T. M. Willson and P. A. Edwards, *Mol. Endocrinol*., 2001, **15**, 1720-1728.

37. I. Demonty, Y. Deshaies, B. Lamarche and H. Jacques, *J. Nutr.,* 2003, **133**, 1398-1402.

38. Y. Oschry and S. Eisenberg, *J. Lipid Res.,* 1982, **23**, 1099-1106.

Figure legends

Fig 1: Effects of different diets on liver malonaldehyde level, glutathione peroxydase and catalase activities. Data are expressed as the mean ± SD (n = 5). Significant differences of means were indicated by asterisks *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

G1, Control group; G2, HCD fed rats; G3, G4 and G5 are HCD fed rats gavaged by 0.1, 0.5 and 2 g of Bb-HP/ kg of animal BW daily, respectively. G6 and G7 are HCD fed rats gavaged by 0.1 and 0.5 g of Bb-NHP/ kg of animal BW daily, respectively.

Fig 2: Serum activities of alanine aminotransferase and aspartate aminotransferase in different rat groups. Values represented means ± SD, for 5 rats per group. Significant differences of means were indicated by asterisks *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

G1, Control group; G2, HCD fed rats; G3, G4 and G5 are HCD fed rats gavaged by 0.1, 0.5 and 2 g of Bb-HP/ kg of animal BW daily, respectively. G6 and G7 are HCD fed rats gavaged by 0.1 and 0.5 g of Bb-NHP/ kg of animal BW daily, respectively.

Fig 3: Aorta histological analysis (x 400) in rats receiving different experimental diets.
G1, Control group; G2, HCD fed rats; G5, HCD fed rats gavaged by 2 g of Bb-HP/ kg of animal BW daily; G7, HCD fed rats gavaged by 0.5 g of Bb-NHP/ kg of animal BW daily.

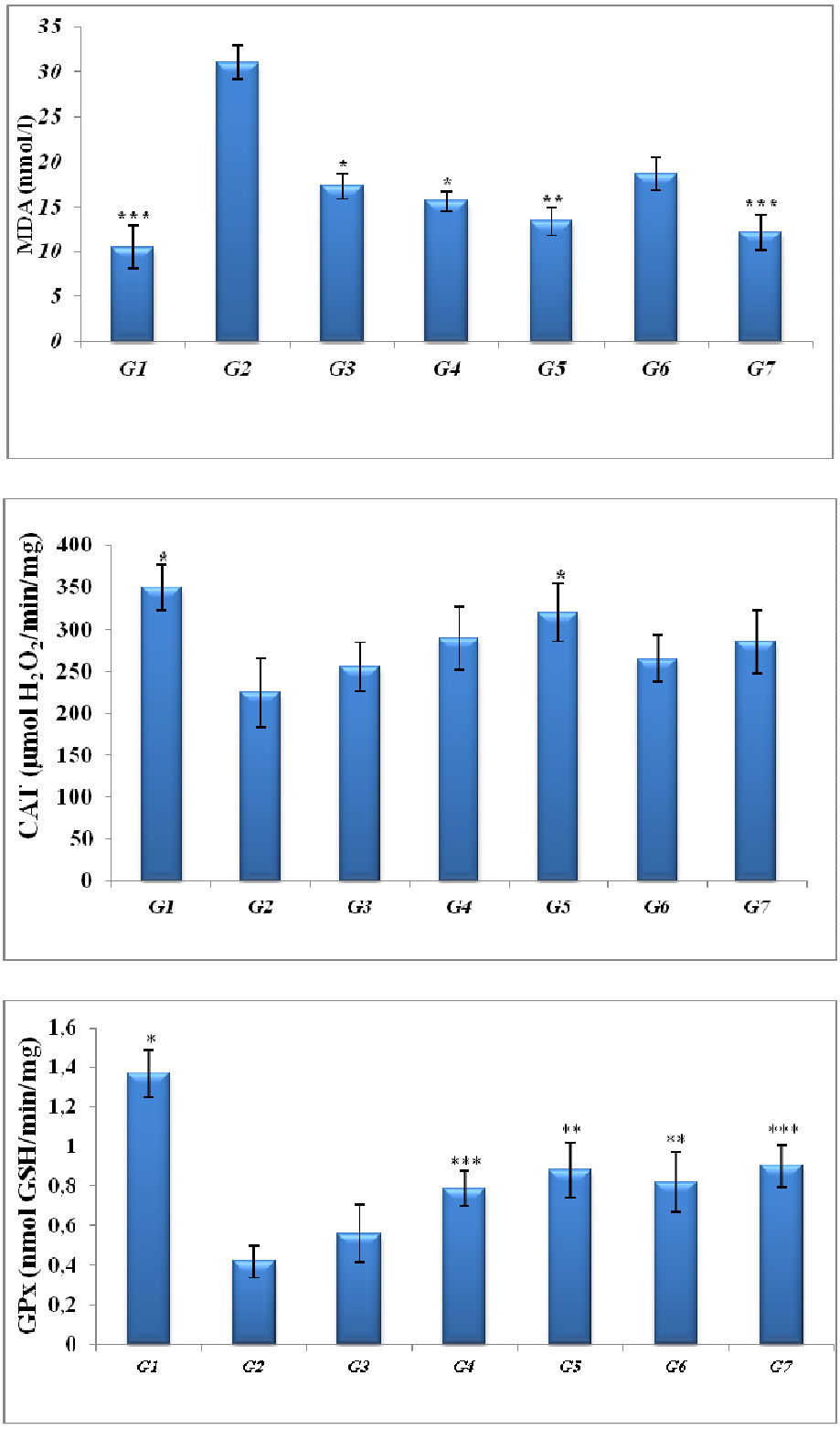
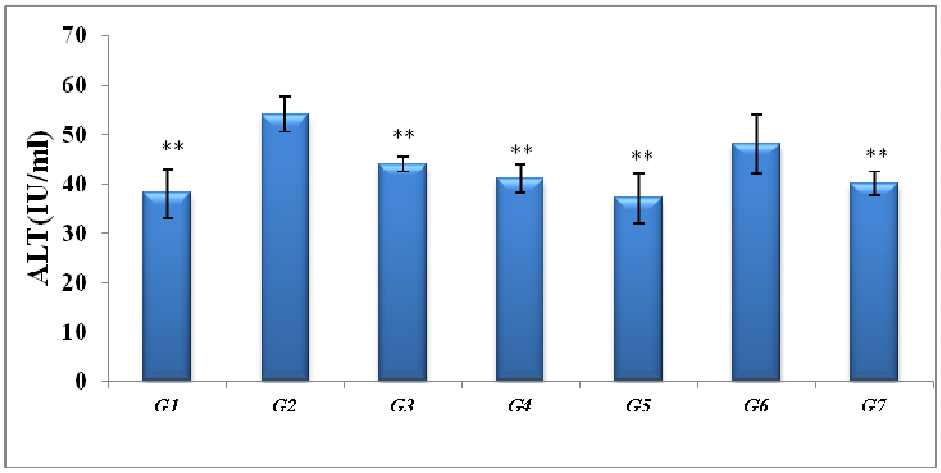


Fig 1



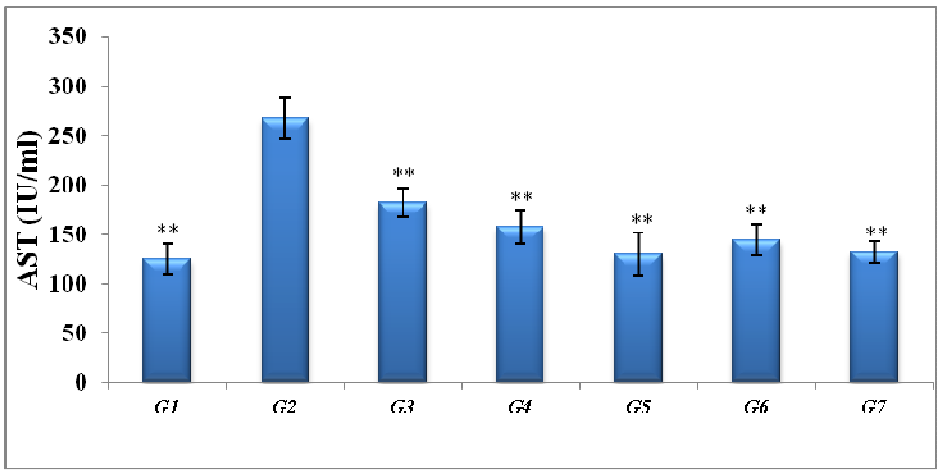
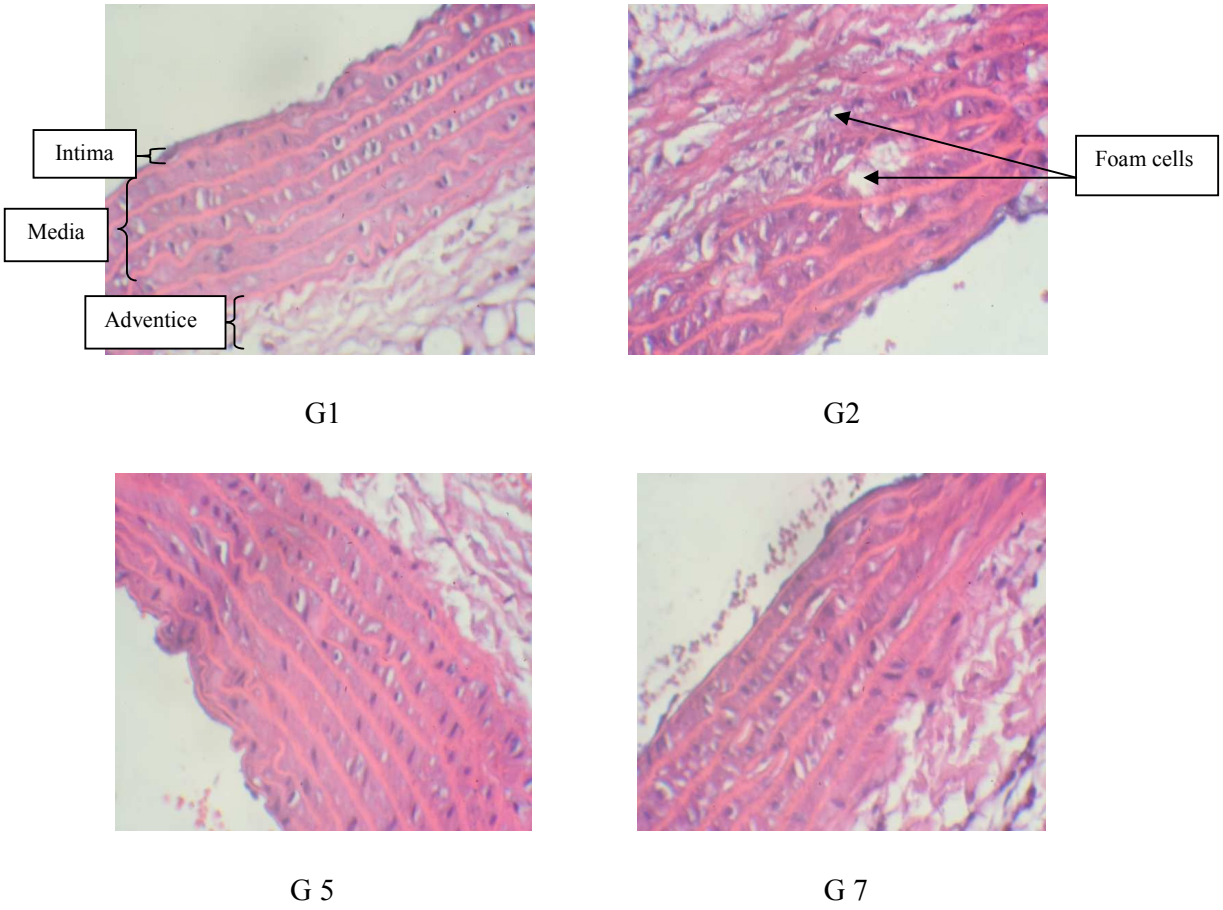


Fig 2



.

Fig 3

Table 1: Composition of basic food (Society of Animal Nutrition « SNA» Sfax Tunisia). This food consists of corn, soya VMC (Vitamins minerals compound) with the following characteristics.

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 acids (%)	
Methionine	0.36
Cysteine	0.26
Threonine	0.62
Tryptophane	0.2
Mineral mix (mg/kg)	
Manganese	80
Iron	48
Copper	18
Zinc	64
Selenium	0.28
Cobalt	0.2
Iodine	2
Vitamin and antioxidant (mg/kg)	
Vitamin A	11.200
Vitamin D3	2800
Vitamin H	25
Antioxydant (BHA – BHT)	100

Table 2: Amino acid composition (‰) of intact *Boops boops* proteins and of Boops boops proteins hydrolysates

Amino acids	Hydrolysate	Proteins
Asp	3.45	3.6
Ser	6.83	7.9
Glu	7.30	6.8
Gly	15.76	15.06
His	6.95	6.85
Arg	7.59	9.75
Thr	11.03	10.83
Ala	5.43	5.15
Pro	3.92	3.67
Cys	2.23	2.95
Tyr	4.04	3.70
Val	4.65	4.40
Met	3.42	3.90
Lys	2.47	2.22
Ile	3.07	2.70
Leu	6.53	5.65
Phe	5.30	4.77

Table 3: Plasma lipids in different rat groups

	G1	G2	G3	G4	G5	G6	G7
TC	1.28±0.09 **	1.95±0.28	1.64±0.15	1.52±0.15 *	1.31±0.07 **	1.58±0.10 *	1.27±0.28 **
TG	0.65±0.17 **	1.08±0.19	0.92±0.14	0.83±0.14 *	0.62±0.10 **	0.83±0.03	0.86±0.18
LDL-ch	0.36±0.11 ***	1,21±0.21	0.71±0.2 *	0.59±0.06 *	0.34±0.08 ***	0.68±0.17 **	0.45±0.13 *
HDL-ch	0.84±0.08 *	0.65±0.11	0.76±0.11	0.76±0.11	0.79±0.09	0.74±0.15	0.74±0.11
L/H	0.44±0.17 ***	1.98±0.34	0.96±0.42 **	0.79±0.10 ***	0.42±0.12 ***	0.98±0.41 **	0.62±0.11 ***

L/H : LDL-ch/HDL-ch

Concentration lipids (mmol/ l) are given as mean ± SD for 5 rats. All groups were compared to group 2. Statistical significance: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

G1, Control group; G2, HCD fed rats; G3, G4 and G5 are HCD fed rats gavaged by 0.1, 0.5 and 2 g of Bb-HP/ kg of animal BW, daily, respectively. G6 and G7 are HCD fed rats gavaged by 0.1 and 0.5 g of Bb-NHP/ kg of animal BW, daily, respectively.

Table 4: Correlation of lipids parameters with indicators parameters of liver antioxidant status and damage

	MDA	CAT	GPx	AST	ALT
TC	0.45 *	- 0.47 *	- 0.58 *	0.45 **	0.34 *
TG	0.30	- 0.27	-0.46 **	0.56 **	0.25
HDL-ch	- 0.32 *	0.22	0.24	-0.47 **	-0.29
LDL-ch	0.47 *	- 0.59 **	-0.6 **	0.58 **	0.53 **
L/H	0.49 **	- 0.42 **	-0.57 **	0.77 ***	0.51 **

Correlation was investigated using the test of linear correlation. L/H, LDL-ch/ HDL-ch; MDA, malonaldehyde; GPx, glutathione peroxidase; CAT, catalase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Statistical significance: $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.