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Ameliorating effects of goby fish protein hydrolysates on high-fat-high-fructose diet-induced hyperglycemia, oxidative stress and deterioration of kidney function in rats

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ABSTRACT

This study investigated the therapeutic potential of undigested goby fish (*Zosterisessor ophiocephalus*) muscle proteins (UGP) and their hydrolysates on high-fat-high-fructose diet (HFFD)-fed rats.

HFFD induced hyperglycemia, manifested by a significant increase in the levels of glucose and glycogen as well as α-amylase activity when compared to normal rats. The administration of GPHs to HFFD-fed rats significantly decreased α-amylase activity and the contents of blood glucose and hepatic glycogen. By contrast, the UGP increased the glucose metabolic disorders in HFFD-fed rats.

Furthermore, HFFD-fed rats showed oxidative stress, as evidenced by decreased antioxidant enzyme activities and glutathione (GSH) levels and increased concentration of the lipid peroxidation product malondialdehyde in liver and kidney. Interestingly, the daily gavage of UGP and GPHs improved the redox status in liver and kidney of HFFD-rats by ameliorating or reversing the above-mentioned changes. Moreover, GPHs exhibited a renal protective role by reversing the HFFD-induced decease of uric acid and increase of creatinine levels in serum and preventing some HFFD-induced changes in kidney architecture. The results demonstrate that GPHs contain bioactive peptides that possess significant hypoglycemic and antioxidant properties, and ameliorate renal damage in rats fed hypercaloric diet.

1. Introduction

It is well known that cardiovascular diseases (CVD) represent the major cause of human death all over the word. In fact, the National Cholesterol Education Program's Adult Treatment Panel III defined CVD as the primary clinical outcome of metabolic syndrome (MS) [1]. Most individuals, who suffer from cardiac problems, develop multiple diseases, which are commonly associated with different risk factors including abdominal obesity, atherogenic dyslipidemia, hypertension, prothrombic state, type 2 diabetes mellitus T2DM (non-insulin-dependent diabetes mellitus NIDDM) [1]. The interaction between all these changes promotes the development of MS syndrome. Commonly, MS is accompanied by insulin resistance syndrome and impaired glucose tolerance [2].

Universally, T2DM, characterized by a persistent hyperglycemia, is associated with several metabolic abnormalities in protein and lipid regulation, and results in severe micro- and macrovascular problems, including failing of organ functions, especially in eyes, kidneys, nerves, heart and blood vessels [3]. In fact, under the obesity and insulin resistance conditions, hepatic and/or renal failure associated with the perturbation of lipid metabolism and atherosclerosis status are widely detected [4].

The major organs involved in the excretion of xenobiotics are liver and kidneys. In fact, considered as the primary site of metabolism and detoxification, liver is vulnerable to infections [5]. The metabolism of toxic chemicals, drugs, and virus

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infiltration leads to the generation of reactive oxygen species (ROS) and hepatic cell death causing hepatotoxicity or liver damage [6]. Kidneys are considered as the principal organs of xenobiotics excretion [7]. They are very sensitive to oxidative stress damage because of their high content of long-chain polyunsaturated fatty acids [8]. For these reasons, liver and kidneys' protection remains the major clinical challenge, involving the development of new substances to reduce in these vital organs from serious risks of toxicity. Available pharmacotherapeutic options for liver diseases are very limited and there is a great demand for the development of new effective substances [9]. Actually, in the modern system of medicine, there is a huge lack of reliable liver protective treatments and they are still restricted, principally, to the treatment with steroids, vaccines, and antiviral drug; all of which have shown poor therapeutic success and are generally associated with toxicity risks. Clearly, there exists a critical need for exploring novel alternatives for the prevention and treatment of diseases of vital organs. Thus, recent scientific studies are focusing on producing bioactive compounds derived from natural products without toxic side effects.

Regardless of their origins, biological substances have shown abundant therapeutic benefits and are considered as a natural gift for promoting human health. Indeed, it has been reported that certain plant extracts may provide a preventive effect against insulin resistance and oxidative stress in rats [10]. Also, protein hydrolysates offer a huge pool of bioactive peptides characterized by different biological activities, including antioxidation, hepatoprotection [11,12], hypoglycemia [13], anticoagulant [14] and antihypertension [15] effects. Fish muscles are widely studied thanks to their richness in proteins, and their hydrolysates have shown several biological effects such as antihyperlipidemic, antioxidative, ACE-inhibitory [16,17], antiproliferative [18], anti-inflammatory [19] activities. We have previously shown the potential bioactivities of protein hydrolysates produced from goby fish (*Zosterisessor ophiocephalus*) muscle [14,20,21].

In the present study, we investigated the hypoglycemic and antioxidant properties of undigested goby fish muscle proteins (UGP) and their hydrolysates (GPHs) on high-fat-high-fructose diet (HFFD)-induced hyperglycemia and oxidative stress in rats.

2. Materials and methods

2.1. Biological material

Marine fish Goby fish (*Z. ophiocephalus*), a marine fish, was freshly purchased from the fish market of Sfax City, Tunisia. The samples were packed in polyethylene bags, placed in ice with a sample/ice ratio of approximately (1:3) (w/w) and transported to the research laboratory within 30 min. Upon arrival, muscles were separated, rinsed with cold tap water and then with cold distilled water in order to remove salts and other contaminants. They were used immediately or stored in sealed plastic bags at −20 °C until they were used for protein hydrolysates production less than one week later.

2.2. Preparation of proteolytic enzymes

Enzyme preparation from *Bacillus mojavensis* A21 was prepared in our laboratory as reported by Mhamdi et al. [22] Regarding the grey triggerfish digestive enzymes, 150 g of intestines were thoroughly washed 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 10,000 g for 30 min at 4 °C. The pellet was discarded and the supernatant, referred to as the protease extract, was collected. The alkaline protease activity was determined by using the method of Kembhavi et al. [23] using casein as a substrate. One unit of protease activity was defined as the amount of enzyme required to liberate 1 μg of tyrosine per minute under the experimental conditions used.

Casein zymography was performed on native-PAGE according to the method of Garcia-Carreno et al. [24] to estimate the number of proteases in each enzyme preparation.

2.3. Preparation of undigested goby fish protein and its hydrolysates

For the preparation of UGP, raw muscle from goby fish (500 g) in 1000 ml distilled water was cooked for 20 min at 100 °C. The bones were removed from cooked fish and muscles were collected and dried in an oven at 80 °C for 18 h. The dried muscle preparation was minced to obtain fine powder used as UGP.

Regarding protein hydrolysates, goby fish muscle (500 g), in 500 ml distilled water, was first minced using a grinder (Moulinex Charlotte HV3, France) then cooked at 90 °C for 20 min to inactivate endogenous enzymes. The cooked muscle sample was then homogenized in a Moulinex® blender for about 5 min. The samples were adjusted to optimal pH and temperature for both enzyme fractions (pH 10.0, 50 °C). Then, the goby fish proteins were digested with enzymes at a 1:3 (U/mg) enzyme/protein ratio. The proteolytic enzyme fractions were used at the same activity levels to compare degree of hydrolysis (DH). During the reaction, the pH of the mixture was maintained at the desired value by continuous addition of 4 N NaOH solution. After incubation, the reactions were stopped by heating the solutions for 20 min at 80 °C to inactivate enzymes. Protein hydrolysates were then centrifuged at 10 000 g for 20 min. Finally, the supernatants, referred to as protein hydrolysates (GPHs), were freeze-dried using freeze-dryer at a temperature of −50 °C and a pressure of about 121 mbar through a lyophilizer (Moduloyd-230, Thermo-Fisher Scientific, USA) and then stored at −20 °C for further use. Hydrolysates obtained with the *B. mojavensis* A21 protease fraction and the triggerfish protease fraction are referred to as GPH-A and GPH-TF, respectively.

The DH, defined as the percent ratio of the number of peptide bonds cleaved to the total number of peptide bonds in the substrate studied, was calculated from the amount of base (NaOH) added to keep the pH constant during the hydrolysis as described by Adler-Nissen [25].

The approximate composition of GPHs was determined according to the AOAC methods. Moisture content was determined keeping in a dry oven at 105 °C for 24 h. Crude ash content was determined by calcinations in furnace at 550 °C and crude protein content was determined by the Kjeldahl method. Crude lipid content was determined by the Soxhlet method.

The GPH samples were hydrolyzed by addition of hydrochloric acid 12 M (30%), and the amino acid composition was determined as previously reported [26].

2.4. Animals, diets and experimental study design

Healthy adult male Wistar rats weighing about 110–150 g were purchased from the Central Pharmacy of Tunisia (SIPHAT, Tunis City, Tunisia). Animals were housed in an environmentally controlled room (temperature, 25 ± 1 °C; humidity 60 ± 5% and a 12 h light/dark cycle) in the laboratory of Animal Ecophysiology of Sfax City, Tunisia. Animals were allowed free access to tap water and alimentation during the experimental period. Laboratory animal handling and experimental procedures were performed according to the guidelines of the Tunisian Ethical Committee for the care and use of laboratory animals.