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Antioxidant effect of nondigestible levan and its impact on cardiovascular disease and atherosclerosis

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ABSTRACT

Levan polysaccharide, a type of fructan, has been shown to favorably affect diabetes type 2 and hypercholesterolemia. Recent reports have indicated that excessive oxidative stress contributes to the development of atherosclerosis linked metabolic syndrome. The objective of this current study was to investigate the possible protection against oxidative stress linked atherosclerosis. A group of twenty four male rats was divided into four subgroups; a normal diet group (Control), normal rats received levan (L), a high-cholesterol diet group (Chol) and a high-cholesterol diet with 5% (w/w) levan group. After the treatment period, the plasma antioxidant enzymes and lipid profiles were determined. Our results show that treatment with levan positively changed plasma antioxidant enzyme activities by increasing superoxide dismutase (SOD) and catalase (CAT) by 40% and 28%, respectively, in heart. Similarly, the treatment of Chol fed groups with levan positively changed lipid profiles by decreasing total cholesterol, triglycerides and LDL-cholesterol by 50%, 38.33% and 64%, respectively. Thus may have potential antioxidant effects and could protect against oxidative stress linked atherosclerosis.

1. Introduction

Atherosclerotic disease causes over 19 million deaths annually [1,2]. The etiology of atherosclerosis appears to be a multi-factorial series of events, but the oxidation of lipoprotein is believed to be a primary event in the pathogenesis of atherosclerosis [3]. Recent studies have pointed out oxidative damage as an important etiologic factor in atherosclerosis. Especially, according to the oxidative stress theory, oxidative modification of low-density lipoprotein (LDL) is thought to play a key role in the development of atherosclerosis [4,5]. Hypercholesterolemia or more specifically elevated plasma low density lipoprotein cholesterol (LDL-C) is an important risk factor for development and progression of atherosclerosis. In fact, several studies have shown that an increased dietary intake of cholesterol results in hypercholesterolemia, which is known to eventually generate atherosclerosis and enhance the risk of coronary heart disease (CHD), fatty liver disease and cancer associated with hydroxyl radical formation [6,7]. Atherosclerosis is accompanied with the production of free radicals by endothelial and vascular smooth muscles. Thus, recent interest has been focused on strategies to enhance the removal of reactive oxygen species (ROS) by using antioxidants to enhance endogenous antioxidant responses. Antioxidative substances are believed to suppress the onset and development of atherosclerosis. After decades of clinical and epidemiological research, there is a general consensus about the overall dietary pattern which is best associated with good cardiovascular health. The evidence for links between dietary fiber and atherosclerotic cardiovascular disease (ASCVD) is very strong; it arises from animal studies [8], epidemiologic observations [9] and a limited number of clinical trials [10].

Dietary fiber, made up of indigestible carbohydrates, is one of the most frequently studied dietary components because of its cardiovascular protective effects. The beneficial properties of fiber for cardiovascular health in general were reported [11]. Its effects on digestive physiology, lipid metabolism, glucose homeostasis and general health have raised considerable interest since 1974, when Burkitt et al. [12] formulated the hypothesis that a low consumption of fiber was one cause underlying the increased incidence of a variety of diseases linked to progress. Among these are obesity, diabetes, coronary heart disease (CHD) and colorectal cancer. Moreover, Trowell [13] proposed that a deficient fiber intake might contribute to the high prevalence of coronary heart disease (CHD) among Western people and that a generous fiber intake in other areas of the world was protective from CHD. Recently, Rimm et al.

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Table 1
Composition of the control diet (g/kg).

Ingredient	Concentration
Casein	200
L-Methionine	3
Corn starch	393
Sucrose	154
Cellulose	50
Mineral mix[a]	35
Vitamin mix[b]	10

[a] Mineral mixture contained (mg/kg of diet) the following: $CaHPO_4$, 17.200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4{\cdot}7H_2O$, 200; trace elements, 400; $MnSO_4{\cdot}H_2O$, 98; $CuSO_4{\cdot}5H_2O$, 20; $ZnSO_4{\cdot}7H_2O$, 80; $CoSO_4{\cdot}7H_2O$, 0.16; Kl, 0.32; sufficient starch to bring to 40 g (per kg of diet).

[b] Vitamin mixture contained (mg/kg of diet) the following: retinol, 12; cholecalciferol, 0.125; thiamine, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid,10; p-aminobenzoic acid, 100; biotin, 0.6; sufficient starch to bring to 20 g (per kg of diet).

[9] reported that higher levels of fiber intake were associated with lower rates of myocardial infarctions and death from CHD.

Levan, a β-(2→6) fructan biopolymer with occasional β-(2→1) branching, was found in many plants and microbial products [14]. Microbial levans are produced from sucrose-based substrate by transfructosylation reaction of levansucrase (β-2,6-fructan: D-glucose-fructosyl transferase, EC 2.4.1.10) by a variety of microorganisms [15,16]. Levan has also potential applications in the cosmetic and pharmaceutical industries due to its biological functions, particularly those pertaining to the dermatological field [17], as an antidiabetic [18], hypo-cholesterolemic [19] and an antitumor agent [20]. The present study deals with the evaluation of the potential of in vivo protection against oxidative stress linked atherosclerosis.

2. Materials and methods

2.1. Preparation of levan

The crude enzyme solution (levansucrase of *Bacillus licheniformis*) was used for the production of levan from the sucrose. The enzyme solution was incubated with 20% sucrose in a 20 Mm acetate buffer; pH 5.6 at 40 °C for 24 h. Ethanol (96%) was added to cell free culture liquid (2.5:1.0) mixing at room temperature. The precipitated polymer was washed twice with ethanol, dissolved in water and dialyzed against deionized water. After centrifuging, the resulting precipitate was collected and vacuum-dried at 40 °C, giving a white powder [21].

2.2. Animals

Adult male wistar rats weighting about 180–200 g, and obtained from the Central Pharmacy, Tunisia, were used in this study. All the animals were fed with a pellet diet (Sico, Sfax, Tunisia) and water was allowed ad libitum under strict hygienic conditions. Before initiation of experiment, the rats were acclimatized for a period of 7 days standard environmental conditions such as temperature (26 + 2 °C), relative humidity (45–55%) and 12 h dark/light cycle were maintained in the quarantine. The handling of the animals was approved by the Tunisian Ethical Committee for the care and use of laboratory animals.

2.3. Experimental procedure

The rats were randomly divided into four experimental groups ($n = 6$). Group I: (Control) normal rats which were fed a standard laboratory diet (Table 1). Group II: (Chol) rats which were fed a cholesterol-rich diet (normal diet supplemented with 1% cholesterol and 0.25% bile salts). Group III: (L) normal rats received levan at 5% (w/w). Group IV: (Chol + L) rats which received a cholesterol-rich diet with levan at dose of 5% (w/w) dissolved in drinking water. After 2 months of treatment, the animals were sacrificed by decapitation in order to minimize the handling stress, and blood samples were collected to determine the plasma lipid profile. The hearts and aorta tissues were removed and rinsed with physiological saline solution. All samples were stored at −80 °C until analyzed.

2.4. Lipid profiles

Concentrations of total cholesterol (TC), total triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein (LDL-C) in serum were determined by enzymatic colorimetric methods using commercial kits from Biomaghreb (Ariana Tunis, Tunisia).

2.5. Antioxidant enzyme assay

2.5.1. Superoxide dismutase activity

Superoxide-dismutase (SOD) activity was assayed by measuring its ability to inhibit the photoreduction of nitroblue terazolium (NBT). In this assay, one unit of SOD is defined as the amount required inhibiting the photo reduction of NBT by 50%. Riboflavin (0.26 mM final concentration) was added to start the reaction and absorbance was recorded at 560 nm for 20 min. The activity was expressed as units/mg protein, at 25 °C. Proteins content was estimated by the method of Lowry et al. [22] using the bovine serum albumin as standard.

2.5.2. Catalase activity

Catalase (CAT) activity was measured according to Aebi [23]. 20 μl liver homogenate (about 1.5 mg proteins) were added to 1 ml phosphate buffer (0.1 M, pH 7) containing 100 mM H_2O_2. Rate of H_2O_2 decomposition was followed by measuring the decrease in absorbance at 240 nm for 1 min. The enzyme activity was calculated using an extinction coefficient of 0.043 $mM^{-1}\ cm^{-1}$ and expressed in international units (I.U.), i.e., in μmoles H_2O_2 destroyed/min/mg protein, at 25 °C.

2.6. TBARS assay

As a marker of lipid peroxidation, the TBARS (thiobarbituric acid-reactive substances) concentrations were measured in heart using the method of Buege and Aust [24]. Briefly, 200 μl of a 10% (w/v) tissue homogenate solution was mixed with 600 μl of distilled H_2O and 200 μl of 8.1% (w/v) SDS, vortexed, and incubated for 5 min at room temperature. The reaction mixture was heated at 95 °C for 1 h after the addition of 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) TBA. After cooling the reaction, 1.0 ml of distilled water and 5.0 ml of butanol:pyridine (15:1) solution were added and vortexed. The mixture was centrifuged at 1935 × *g* for 15 min and the resulting colored layer was measured at 532 nm using malondialdehyde (MDA) made by the hydrolysis of 1,1,3,3-tetramethoxypropane as standard.

2.7. Histopathological analysis

At the time of sacrifice, aorta tissues were removed and fixed in 10% formaldehyde solution. The washed tissues were dehydrated in increasing gradient of ethanol and finally cleared in toluene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 μm thickness and stained with hematoxylin and eosin.