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Original Research Article

Gum arabic down-regulate PPAR-γ and SCD mRNA expression in mice

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ABSTRACT

Introduction: Gum arabic is a complex polysaccharide used in the food industry as a thickener and stabilizer. It reduced plasma cholesterol level in animals and humans, and it has prebiotic, anticarcinogenic and anti-oxidant effect with a protective role against hepatic and cardiac toxicities.

Aim: To study the impact of gum arabic on body weight, adipose tissue weight, lipid profiles and expression of some gene control lipid metabolism.

Material and methods: 20 female CD-1 mice at 5 weeks age were divided into two groups, one served as control and the second received 10% of gum arabic in drinking water for 6 weeks. Liver and visceral adipose tissue and serum were collected from all groups. Total cholesterol, triglyceride, HDL-c and LDL-c were assayed using kits, and the expression of lipid metabolic enzyme gene was detected by RT-PCR.

Results and discussion: The results showed that gum arabic significantly decreased body weight ($P < 0.05$) and visceral adipose tissue weight ($P < 0.01$). Gum arabic non-significantly ($P < 0.05$) reduces blood glucose and total cholesterol, and increased HDL-c. The expression of lipid metabolic enzyme gene showed that gum arabic significantly ($P < 0.05$) down-regulated PPAR-γ and SCD expression. However, gum arabic has no significant ($P < 0.05$) effect on HMGR, G6P, CYP17, Sreb, TNF-α, FAS, MGL, ATGL, HSL and ACC gene expression.

Conclusions: The results conclude that gum arabic can reduce body weight and visceral adipose tissue weight, and down-regulated PPAR-γ and SCD gene expression.

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1. Introduction

Gum arabic is an exudative polysaccharide from the African tree *Acacia senegal* and is used widely in the food industry as a thickener and stabilizer.[1,2] It contains high molecular weight (lipoprotein) and low molecular weight (heterogeneous gum polysaccharides) compounds.[3] The use of gum arabic dates back to the second millennium BC when the Egyptians used it as an adhesive and ink. Gum arabic was evaluated for acceptable daily intake levels by man by the Joint FAO/WHO Expert Committee on Food Additives since 1969[4]; however, Sudanese people in Western Sudan have been using it for a longer time without limitations. It is indigestible in both humans and animals, and is not degraded in the intestine but fermented in the colon to give short-chain fatty acids, leading to a large range of possible health benefits.[5] One of these benefits is its prebiotic effect.[6,7] It had been claimed that four week supplementation with gum arabic (10 g/day) led to significant increases in Bifidobacteria, Lactobacteria, and Bacteriodes in humans indicating a prebiotic effect.[7] Other effects include reduction in plasma cholesterol level in animals and humans,[8] anticarcinogenic effect[9] and anti-oxidant effect[10,11] with a protective role against hepatic and cardiac toxicities. Additionally, gum arabic alleviates effects of chronic renal failure in humans; however, further studies are needed for confirmation.[12,13]

Obesity is a well-known risk factor for coronary heart disease, stroke, diabetes and many other abnormalities, including cancer.[14,15] These complications depend not only on absolute amount of fat but also on its distribution. Absolute total body fat and adipose tissue distribution are known to be associated with cardio-metabolic risk in adult females.[16] Processes that determine fat deposition in adipose tissue include the rates of fat uptake, de novo fatty acid synthesis, triacylglycerol synthesis, lipid degradation and transport processes of fatty acids.[17] Several key factors are involved in lipid metabolism in adipose tissues. Lipogenic enzymes include acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and glucose-6-phosphate dehydrogenase (G-6-PDH), and changes in their activities can alter the rates of biosynthesis of fatty acids.[18,19] Furthermore, peroxisome proliferator-activated receptor γ (PPAR-γ) has recently been identified as a key enzyme regulating in lipid metabolism in adipose tissue by regulating targeting gene expression related to lipid metabolism, or by transporting fatty acids.[20] Gum arabic can serve to reduce obesity and therefore prevent associated complications in humans. The current therapeutic options to improve cardiovascular risk and slow progression of renal failure are quite limited. Therefore, our current results together with other authors confirm that gum arabic may be a beneficial dietary addition to this group of patients.

2. Material and methods

2.1. Experimental animals

Twenty female CD-1 mice at 5 weeks age were obtained from the Experimental Animal Center of Nanjing Medical University (Nanjing, China). The mice were housed under controlled lighting (12 h light, 12 h dark), temperature (21 °C–22 °C) and humidity at 65%–70%. The mice were allowed free access to a commercial pellet diet and drinking water throughout the experiment period. The experimental protocol involving mice was approved in accordance with the guide for the care and use of laboratory animals prepared by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

2.2. Experimental design

After an acclimatization period of a week, mice were randomly divided into two equal groups. The first group continued to receive the same diet without treatment until the end of the study (control group). The second group was given normal food and received 0.5% of gum arabic aqueous solution as drinking water for 7 days, and then 10% solution for further 6 weeks. During the treatment period, the mice were weighed weekly. After 6 weeks, the mice were killed. Serum samples and liver and visceral adipose tissue were collected and stored at −80 °C.

2.3. Biochemical measurements

Total cholesterol, triglyceride, high density lipoprotein – cholesterol (HDL-c) and low density lipoprotein – cholesterol (LDL-c) were assayed using kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.4. Lipid metabolic enzyme gene expression

Liver was ground in liquid N_2, and a portion of about 100 mg was used for RNA extraction using TRIzol total RNA kit (Invitrogen, Biotechnology Co, Ltd, Carlsbad, CA, USA) according to the manufacturer's instruction. Two approaches were taken to ensure that all the total RNA preparations are free of genomic DNA contamination. Firstly, total RNAs were treated with 10 U DNase I (RNase Free, D2215, Takara, Japan) for 30 min at 37 °C, and purified according to the manufacturer's protocol. Secondly, the primers for the reference gene (GAPDH) were designed to span an intron, so any genomic DNA contamination can be reported easily with an extra product in the melting curves for real-time PCR. Lipid metabolic enzyme gene expression was carried out by real-time PCR performed in Mx3000P (Stratagene, USA). Mock reverse transcription (RT) and No Template Controls (NTC) were included to monitor the possible contamination of genomic and environmental DNA at both RT and PCR steps. The pooled sample made by mixing equal quantity of RT products (cDNA) from all samples was used for optimizing the PCR condition and tailoring the standard curves for each target gene, and melting curves were performed to insure a single specific PCR product for each gene. The PCR products were sequenced to validate the identity of the amplicons. Primers specific for HMGR, PPAR-γ, CYP17, G6P1, G6P2, ATGL, HSL, ACC, FAS, SCD and MGL were synthesized by Geneary (Shanghai, China) (Table 1). A mouse GAPDH was used as a reference gene for normalization purposes. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time PCR data.[21] The mRNA abundances were presented

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