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Hypolipidemic and antioxidant effects of dietary fenugreek (*Trigonella foenum-graecum*) seeds and garlic (*Allium sativum*) in high-fat fed rats

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ABSTRACT

Hypolipidemic and antioxidant influences of dietary fenugreek (*Trigonella foenum-graecum*) seeds (10%) and garlic (*Allium sativum*) (2% powder), individually and in combination for 8 weeks were evaluated in high-fat diet (HFD) fed Wistar rats. Increased serum triglycerides and LDL-cholesterol caused by HFD was countered by these dietary interventions, while HDL-cholesterol was increased. Increase in cholesterol: phospholipid ratio and atherogenicity index caused by HFD was lowered by dietary fenugreek+garlic. Dietary fenugreek or fenugreek+garlic countered the increase in triglycerides and cholesterol: phospholipid ratio in the heart tissue of HFD-fed rats. The increase in liver triglycerides and cholesterol as a result of HFD was countered by dietary fenugreek+garlic. All the 3 dietary interventions lowered dietary fat absorption in HFD-fed situation. The elevated lipid peroxides in serum, cardiac, and liver tissue of HFD-fed rats was countered by dietary fenugreek+garlic. This was accompanied by increased glutathione concentration in serum and liver and of ascorbic acid in heart and liver. Activities of antioxidant enzymes were enhanced by dietary fenugreek+garlic in serum and heart in HFD-fed situation. Thus, dietary fenugreek+garlic produced higher lipid lowering effect and improved the antioxidant status in high-fat fed rats which suggest that these nutraceuticals may have higher cardio protective influence when consumed together.

1. Introduction

Cardiovascular disease (CVD) is a growing health concern both in developed countries and in developing countries resulting from known risk factors such as physical inactivity, overweight, diabetes, hypertension, and hyperlipidemia. Role of food constituents in the prevention of degenerative diseases has received considerable attention in recent years. Effective nutritional intervention strategies for preventing or managing CVD also call for basic information on the nutraceutical potential of non-nutrient phytochemicals/adjuncts, due to their antioxidant property and beneficial modulation of lipid homeostasis (Amani & Sharifi, 2012). Diet choices advocated for the management of CVD include liberal consumption of foods which provide abundant amounts of dietary fiber and antioxidant phytochemicals.

Due to multipronged beneficial physiological influences of spices, *viz*., relieving the oxidative stress, hypocholesterolemic, hypotriglyceridemic, hypotensive, and antithrombotic properties (Srinivasan, 2005), these food adjuncts may offer nutraceutical advantage in the context of prevention of heart diseases. Free radicals and reactive oxygen species have been implicated in cardiac diseases which result from an exposure to chemicals and environmental agents (Srinivasan, 2014). Fenugreek seeds which provide rich amounts of soluble fiber and *Allium* spice – garlic which possess several bioactive sulfur compounds are known to exert beneficial hypocholesterolemic and antioxidant influences (Srinivasan, 2013, 2014). Fenugreek is shown to bring about the hypocholesterolemic effect through increased secretion and excretion of bile acids and neutral sterols (Sharma, 1986) which is accompanied by a depletion of cholesterol stores in the liver. Dietary fenugreek also has the property of stimulating bile acid formation in the liver from cholesterol. Consumption of a high-fat diet may lead to an increase in serum triglycerides, cholesterol and plasma fibrinogen levels which in turn may result in decreased fibrinolytic activity and blood coagulation time. These changes would also increase the risk of atherosclerosis and heart diseases (Srinivasan, 2014). Dietary garlic or its constituents are known to suppress these detrimental factors in animal studies and clinical trials (Srinivasan, 2013).

The mode of action of garlic in exerting the hypolipidemic and antioxidant action is different from that of dietary soluble fiber-rich fenugreek seeds and is mediated through their sulfur compounds (Srinivasan, 2013). Hence, there is a possibility of an additive effect when fenugreek seeds and garlic are consumed in

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combination. Hence, it is desirable to gather more information with regard to hypolipidemic potential of fenugreek seeds in terms of potentiation of this health effect by garlic that works similarly by a different mechanism.

In this investigation, we examined the synergistic/additive effects if any, among dietary fiber-rich fenugreek seeds (*Trigonella foenum-graecum*) and the *Allium* spice garlic (*Allium sativum*) with regard to their hypolipidemic property in the context of the etiology/protection against cardiovascular complications in experimentally induced hyperlipidemia. We have also examined dietary fenugreek and garlic for any beneficial influence on the antioxidant molecules and antioxidant enzymes in blood, heart, and liver tissue in high-fat diet fed situation since oxidant stress is a risk factor for CVD. Since there is a possibility of an additive/synergistic effect when fenugreek seeds and garlic are consumed in combination, the hypolipidemic and antioxidant potential of these two spice ingredients is particularly examined with their combination.

2. Materials and methods

2.1. Materials

Corn starch, sugar powder and refined peanut oil and hydrogenated fat were purchased from local market. Salt mixture (Bernhardt–Tommarelli modified) was purchased from SISCO Research Laboratories (Mumbai, India). Food grade casein was purchased from Nimesh Corporation (Mumbai, India). Fenugreek seeds and garlic were purchased from local market. Fenugreek seeds were powdered, and stored at 4 °C. Garlic was peeled and cloves were freeze-dried, powdered and stored at 4 °C. Cholesterol, dipalmitoyl phosphatidyl choline, triolein, oxidized glutathione, reduced glutathione, nicotinamide adenine dinucleotide phosphate – reduced form (NADPH), glutathione reductase, xanthine oxidase, *t*-butyl hydroxyperoxide, α-tocopherol, cytochrome-C were procured from Sigma-Aldrich Chemical Co., (St. Louis, USA). 1-chloro-2,4-dinitrobenzoic acid, ethylene diamine tetraacetic acid, trichoroacetic acid, *m*-phosphoric acid, sodium citrate were obtained from SISCO Research Laboratory (Mumbai, India). All other reagents and solvents were of analytical grade obtained from SISCO Research Laboratory (Mumbai, India).

2.2. Animal treatment

This animal study was carried out with due clearance from the Institute's Animal Ethics Committee, taking appropriate measures to minimize discomfort or pain taking all precautions regarding the care and use of animals for experimental procedures. Male Wistar rats with initial weights around 70–75 g produced in our Institute's Experimental Animal Production Facility were used for this study. Eight groups of these animals ($n=6$ per group) housed in individual stainless steel cages were maintained at 25 ± 1 °C with humidity 65 ± 5% with 12 h day and night cycle. The animals were maintained on various experimental diets and water *ad libitum* for a duration of 8 weeks. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitaminized starch, 1; Bernhardt–Tommarelli modified NRC salt mixture, 4, fat soluble vitamins at the recommended levels and refined peanut oil, 10. High fat diet (HFD) consisted of (%): casein, 21; cane sugar, 10; corn starch, 34; hydrogenated fat, 25; refined peanut oil, 5; vitaminized starch, 1; and Bernhardt–Tommarelli modified salt mixture, 4 (Pande & Srinivasan, 2012). Dietary interventions with fenugreek, garlic and combination of fenugreek and garlic were made by including 10% fenugreek seed powder, 2% freeze-dried garlic powder, and 10% fenugreek seed powder+2% garlic powder, respectively, at the expense of equivalent amount of corn starch. These dietary doses of fenugreek seeds and garlic were evolved based on earlier studies from this laboratory which have documented hypolipidemic influences in rat model (Mukthamba & Srinivasan, 2015a, 2015b). Thus, the eight animal groups were: (1) Basal Control (C), (2) C+Fenugreek, (3) C+Garlic, (4) C+Fenugreek+Garlic, (5) High-fat diet (HFD), (6) HFD+Fenugreek, (7) HFD+Garlic, and HFD+ Fenugreek+Garlic.

At the end of 8 weeks of diet regimen, after an overnight fast all animals were sacrificed under ether anesthesia. Blood was collected by heart puncture and serum was separated by centrifugation. Liver and heart were quickly excised, weighed and stored frozen until further analyses.

2.3. Dietary fat absorption

Towards the end of the dietary regimen, fecal matter was collected for 72 h. Weight of feces was noted and fecal triglyceride was estimated by the method of Fletcher (1968). Fat absorption was calculated as the difference between fat intake (calculated from food intake data) and excretion through feces.

2.4. Lipid profile

Heart and liver lipid was extracted by the method of Folch, Lees, and Sloane-Stanley (1957). Cholesterol was quantified by the method of Searcy and Bergquist (1960). The HDL and LDL cholesterol in serum was estimated by the protocol given by Warnick and Albers (1978) for the separation of lipoproteins. Phospholipid concentration was estimated by ferrous ammonium thiocyanate method as described by Stewart (1980). Triglycerides was estimated according to the method described by Fletcher (1968). The atherogenecity index was calculated as $[\text{Total cholesterol} - \text{HDL-cholesterol}] \times \text{HDL-cholesterol}^{-1}$.

2.5. Activities of antioxidant enzymes

Serum, liver and cardiac muscle were assayed for the activities of various antioxidant enzymes. Catalase (CAT) activity was assayed according to the method of Aebi (1984) by following the decomposition of hydrogen peroxide, measured by recording the decrease in absorbance at 240 nm for 3 min. The enzyme activity is expressed as the amount of enzyme that decomposes 1 μM hydrogen peroxide per mg of protein. Superoxide dismutase (SOD) activity was measured by quantitating the inhibition of cytochrome-C reduction in the xanthine–xanthine oxidase system as described by Flohe and Otting (1984). Glutathione peroxidase (GPX) was estimated using NADPH oxidation in a coupled reduction system of hydrogen peroxide and oxidized glutathione as described by Flohe and Gunzler (1984). Glutathione reductase (GR) activity was assayed in serum, heart, and liver by measuring the oxidation of NADPH at 340 nm by oxidized glutathione as described by Carlberg and Mannervik (1985). Glutathione-S-transferase (GST) activity was assayed by measuring the chlorodinitrobenzene–glutathione conjugate formed using 1-chloro-2,4-dinitrobenzene as the substrate, as described by Warholm, Guthenberg, Bahr, and Mannervik (1985).

2.6. Antioxidant molecules

Glutathione in serum, liver and heart muscle was determined by using Ellman's reagent according to Beutler, Duron, and Kelly (1963). Ascorbic acid in serum, liver, heart muscle was estimated spectrophotometrically by measuring 2,4-dinitrophenyl-hydrazine derivative of dehydroascorbic acid using the method of Omaye, Turnbull, and Sauberlich (1973). α-Tocopherol in serum, liver and heart was determined by HPLC method described by Zaspel and