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Dietary supplementation with Ethiopian pepper (*Xylopia aethiopica*) modulates angiotensin-I converting enzyme activity, antioxidant status and extenuates hypercholesterolemia in high cholesterol fed Wistar rats

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ABSTRACT

The lipid lowering, antihypertensive, hepatoprotective and antioxidant activities of Ethiopian pepper (*Xylopia aethiopica*) supplemented diet in hypercholesterolemic rats were investigated in this study. Animals were divided into five groups (n = 7); normal control rats (NC); untreated hypercholeterolemic rats (Hyper-C diet); hypercholesterolemic rats treated with oral dose of 1 mg/kg of simvastatin (Hyper-C diet + STATIN); hypercholesterolemic rats treated with 2% (Hyper-C diet + 2%ETP) or 4% (Hyper-C diet + 4%ETP) supplementation of ETP. ETP-supplemented diets (2% and 4%ETP) reduced total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and concomitantly increased high-density lipoprotein (HDL). There was no significant difference between the TG and LDL levels of groups fed with Hyper-C diet + 4%ETP and Hyper-C diet + STATIN. Furthermore, Hyper-C diet + 4%ETP significantly reduced plasma angiotensin-I converting enzyme (ACE) levels compared to Hyper-C diet + 2%ETP and Hyper-C diet + STATIN. Furthermore, elevated serum levels of ALT, AST and ALP induced by the high cholesterol diet were reversed by ETP and STATIN supplemented diet. Rutin, quercetin and ellagic acid were the dominant phenolic compounds in ETP as revealed by high performance liquid chromatography analysis. The observed hypolipidemic, antihypertensive and hepatoprotective effects exhibited by ETP suggest its potentials to prevent lipid abnormalities and associated risk factors, and this could be attributed to its phenolic constituents.

1. Introduction

Hyperlipidemia is the most common form of dyslipidemia and is characterized by elevated plasma levels of total cholesterol (TC), triglyceride (TG), very low-density lipoproteins (VLDL), low density lipoproteins (LDL), free fatty acids (FFA) and apolipoprotein B (APO B), as well as low levels of high density lipoprotein (HDL) [1]. Lipid abnormalities are triggered by high fat diet, lifestyle habits, oxidative stress and metabolic disorders such as diabetes [2]. Moreover, there is a strong correlation between alteration of lipid biomarkers and the risk of hypertension [3], ischemia, [4] and cardiovascular diseases [5]. Hyperlipidemia and oxidative stress have also been linked to incidences of atherosclerosis and cardiovascular disease which is one of the leading causes of death in developing countries [6–8]. Strategies used for the treatment of atherosclerosis and prevention of cardiovascular events involve the use of antioxidants and hypolipidemic agents. Statins are synthetic drugs which possess hypolipidemic and anti-atherosclerotic activity. The continuous use of these drugs has been reported to cause several undesirable side effects such muscle fatigue, pain, muscle cramp, soreness and muscle break down which can lead to death [9]. However, complementary and alternative therapies which involve the use of plants as food supplements have gained interests recently.

Xylopia aethiopica (Dunal) A. Rich. (Annonaceae) is a shrub that is locally referred to as Ethiopian pepper, Negro pepper, Guinean pepper, Senegal pepper, Kili pepper and spice tree in the savanna zone and coastal regions of Africa [10]. The fruit of Ethiopian pepper (ETP) is popularly used as a condiment in many local dishes by ethnic groups and communities in Africa. The seeds have musky flavor and is used as a pepper substitute in Nigeria. The fruits are used in folklore for the treatment of dysentery and diabetes [11]. Some researchers have reported the antihelmintic [12], anti-inflammatory, antimicrobial [13] and antiproliferative [14] activities of ETP. The hypolipidemic, antioxidant and antidiabetic activities of ethanolic and acetone extracts from *Xylopia aethiopica* seeds have also been reported [15,16]. Previous

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work from our laboratory have revealed the safety profile of ETP-supplemented diet in normal rats [17]. To the best of our knowledge, there are no reports on the effect of dietary inclusion or supplementation of ETP in hypercholesterolemic rat model. The objective of this study was to investigate the effect of varying levels of ETP-supplemented diet on changes in serum lipid biomarkers, atherogenic index, plasma ACE, oxidative stress and liver function enzymes in hypercholesterolemic rats. In addition, *in vitro* antihypertensive, radical scavenging and metal chelating activities of phenolic extract from ETP were also investigated.

2. Materials and methods

2.1. Chemicals

Reduced glutathione (GSH), thiobarbituric acid (TBA), were sourced from Sigma −Aldrich Chemical Co. (St. Louis, MO), Chemie GmbH (Steinheim, Germany) and BDH Chemicals Ltd., (Poole, England). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and glyceride (TG) kits were purchased from Randox laboratories Ltd. (Admore, Crumlin, Co-Antrim, UK). All chemicals and reagents used were of analytical grade while the water was glass distilled.

2.2. Plant materials and extraction of free phenolics

Fruits of Ethiopian pepper [*Xylopia aethiopica* (Dun.) A. Rich (Annonaceae)] were purchased from Akure main market, Akure Nigeria. The taxonomic classification of the sample was authenticated at the Department of Biology, Federal University of Technology Akure, Ondo State Nigeria and a voucher specimen was deposited at the herbarium. The fruits were air dried and ground into fine powder using a laboratory mill until they could pass through a 1.0 mm screen. Ten grams of ETP powder was soaked in 200 mL of the extraction solvent (methanol and HCl (1N); 1:1 v/v) for 24 h. The mixture was filtered through a filter paper (Whatman [Sigma Aldrich]). The filtrate was concentrated under pressure until about 90% of the solvent had been evaporated. The residue obtained was lyophilized and was used for *in vitro* enzyme inhibition and antioxidant studies.

2.3. Handling of experimental animals

Animal experiment was carried out under standard laboratory conditions in natural photo period of 12-h light–dark cycle at the Functional foods and Nutraceuticals Laboratory, Biochemistry Department, Federal University of Technology, Akure. The Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA) [18] was strictly followed in this study.

2.4. Experimental design

All the rats were provided with commercially available rat normal pellet diet (NPD) and water *ad libitum*, prior to the dietary manipulation. After two weeks of acclimatization, rats were placed on two dietary regimens namely: normal control (NC) or basal diet [(skimmed milk (50%), corn starch (36%), groundnut (10%), and mineral and vitamin premix (4%)] and high-cholesterol (HC) diet (basal diet plus cholesterol) as a percentage of total weight of diet for a period of 30 days. The rats were randomly divided into five (5) groups of seven (7) animals each. The experiment lasted for 30 days after which the animals were decapitated after an overnight-fast by cervical dislocation. The blood was rapidly collected by direct heart puncture and the plasma was prepared and used for biochemical analysis

2.5. Feed formulation and treatment groups

The diets were freshly formulated according to the modified method of and were kept in air tight containers and stored at 4 °C until needed for use.

- Group I (NC-diet)–normal control rats, fed with basal diet (50% skimmed milk, 36% corn starch, 4% mineral & vitamin premix and 10% groundnut oil).
- Group II (Hyper-C diet)–rats fed with basal diet plus 2% cholesterol.
- Group III (Hyper-C diet +STATIN)–rats fed with basal diet plus 2% cholesterol and given 1 mg/kg simvastatin dissolved in 0.9% normal saline.
- Group IV (Hyper-C diet +2%ETP)–rats fed with diet supplemented with 2% Ethiopian pepper plus 2% cholesterol.
- Group V (Hyper-C diet +4%ETP)–rats fed with diet supplemented with 4% Ethiopian pepper plus 2% cholesterol.

Note: Skimmed milk = 36% protein; 1 g of the mineral and vitamin premix contains; 3200 i.u vitamin A, 600 i.u vitamin D_3, 2.8 mg vitamin E, 0.6 mg vitamin K_3, 0.8 mg vitamin B_1, 1 mg vitamin B_2, 6 mg niacin, 2.2 mg pantothenic acid, 0.8 mg vitamin B_6, 0.004 mg vitamin B_{12}, 0.2 mg folic acid, 0.1 mg biotin H_2, 70 mg choline chloride, 0.08 mg cobalt, 1.2 mg copper, 0.4 mg iodine, 8.4 mg iron, 16 mg manganese, 0.08 mg selenium, 12.4 mg zinc, 0.5 mg antioxidant. Inclusion of 2% cholesterol and Ethiopian pepper (2 and 4%) were in equi-weight basis.

2.6. Determination of hypolipidemic activities

Cholesterol levels were measured according to the method described by Allain et al. [19] Triglyceride concentration was determined according to the principle described by Tietz et al. [20]. HDL concentration was measured according to the method of Lopes-Virella et al. [21]. The LDL-cholesterol concentration of the plasma samples was determined according to the equation of Friedewald et al. [22] as described in the kit's manufacturer (Randox Laboratories Ltd) manual. Atherogenic index was calculated as described by Takasaki, [23] using the ratio of TC/HDL-C and LDL-C/HDL-C

2.7. Determination liver function enzymes

The following biochemical assays were determined using commercial kits according to manufacturer's instructions. Activities of aspartate aminotransferase (AST) and alanine

aminotransferase (ALT) were measured according to Reitman and Frankel [24]; alkaline phosphatase (ALP) was measured according to the method of Klen et al. [25].

2.8. Determination of plasma ACE activity

Plasma ACE activity was determined according to the method of Cushman and Cheung [26] and was expressed as mmol/L

2.9. Determination of oxidative stress biomarkers

Reduced glutathione (GSH) content of the rats' liver was determined according to the method of Ellman [27]. Liver malondialdehyde (MDA) levels were also determined according to the method of Ohkawa et al. [28]

2.10. *in vitro* angiotensin-I converting enzyme (ACE) activity

In vitro ACE activity assay was measured according to the method of Cushman and Cheung [26]. For the *in vitro* assay, different concentrations (0.10-2.4 mg/mL) of the extract or standard (captopril) and 50 μL of rabbit lungs ACE (EC 3.4.15.1) solution (4 mU/mL) were