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RESEARCH ARTICLE

Study the Ascorbic Acid Levels, Lipoprotein Ratio and Hypocholesterolemia Action of Dry Okra Extract on Experimental Model of Locally Male Rabbits

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Abstract

Okra (Abelmoschus esculentus) is a flowering plant in the mallow family. Okra is important for its edible green and dry seed pods. The plant is cultivated in tropical, subtropical and warm temperate regions around the world. The geographical source of okra now is very available in Iraq. The rabbit rapidly develops severe hypercholesterolemia and decrease in levels of ascorbic acid and increase in ratio of bad cholesterol to good cholesterol in response to dietary manipulation. The results of this study showed that, there was a significant decrease in serum total cholesterol and lipoprotein ratio for groups of rabbits that administrated with dry okra aqueous extract with its seeds (0.5g/kg) (DOE+ cholesterol oil-fed) as compared to control group(not administrated) at the end of six week of experiment (398±4.0) vs. (175± 5) ($P < 0.05$). On the other hand, a significant difference in the level of serum total cholesterol ($P<0.05$) starts in control group treated with cholesterol-oil fed at the end of six week of experiment as compared to cholesterol+ DOE group (398±4) vs. (543±4). The aqueous extract of dry okra may be enhancing the conversion of cholesterol to bile acids in the liver by activation of an enzyme α-hydroxylase. DOE may be having inhibition action on HMG-COA synthase that responsible on endogenous synthesis of cholesterol in liver.

Keywords: *Dry okra extract, Induce cholesterol. Lipoprotein ratio, Ascorbic acid.*

Introduction

Okra (Abelmoschus esculentus) is a flowering plant in the mallow family. It is important for its edible green seed pods. The geographical source of okra is disputed, with supporters of West African, Ethiopian, and South Asian origins [1]. Dry okra extract (DOE) composition is nil water, protein, carbohydrates and negligible in fat. In a 100 gram amount, DOE is rich in dietary fiber, vitamin C and vitamin K, with moderate contents of thiamin, folate and magnesium [2].

The importance of plasma lipoprotein and lipid metabolism abnormalities characterized by hyperlipidemia and/or hypercholesterolemia as the cause of coronary heart diseases and potential atherosclerosis is increasingly being supported by a considerable number of population-based and epidemiological studies today. Hypercholesterolemia-inducing diets in rabbits have been largely used as a model to study the development of human atherosclerosis [3]. In order to induce hypercholesterolemia in animals, cholesterol-containing diets have been used, and these vary from commercial chow supplemented with substantially different levels of cholesterol, to changes in the amount of lipids, carbohydrates and the different fat sources and contents [4].

Since rabbits have been widely used to study the development of atherosclerosis in humans, rapid development of lesions has been achieved by supplementing their diet with cholesterol (<0.5%), thus reaching moderate hypercholesterolemia, with plasma cholesterol levels in the range of 200 to 800

mg/ dl [5]. Additionally, long-term experiments in rabbits fed diets containing large amounts of cholesterol are discouraging due to hepatotoxicity and failure of the animal to thrive [6]. The cholesterol-fed rabbit model is remarkable because of the rapid development of aortic lesions and low maintenance cost, and the typical diet to induce atherosclerosis involves supplementation of 0.5% to 4% cholesterol per weight for approximately 8 to 16 weeks.

Under these conditions, rabbits rapidly become hypercholesterolemic and the resulting lesions primarily consist of macrophage-derived foam cells [7]. Some researchers support the suggestion that the formation of advanced lesions depends on the age of the animal [8].

Aged rabbits, with 3-4.5 years of age, show fibrotic plaques, whereas young animals (4 months of age) do not show advanced lesions [9]. Rabbits are an animal species that have several aspects similar to those of humans as regards the lipoprotein metabolism, except for hepatic lípase deficiency [10].

Several characteristics of rabbits make them an excellent model for the assessment of effects of human trans genes on lipoprotein metabolism and susceptibility to atherosclerosis: a) apoB-containing lipoproteins are similar to those seen in humans [11] b) rabbit liver produces apoB-100-containing VLDL, like in humans [12] ; c) abundance of ester-transfer protein in rabbit plasma [13].

The present study aims to illuminate the antioxidant activity of feeding with DOE on decreasing cholesterol and lipoproteins ratio and increasing levels of ascorbic acid in response to fed-induce cholesterol with locally rabbits model.

Materials and Methods

Cholesterol, 94%, and ascorbic acid were purchased from Sigma Aldrich (USA). Dry okra was purchased from the city market.

Animals and Study Design

This study was performed at the laboratory of physiology, chemistry, and pharmaceutical Department, College of veterinary medicine, Al-Qasim green University. The collection of samples was conducted during the period from 1st of March 2018 till 1st of July 2018.

Twenty rabbits (950-1500gm) were divided into four groups, each group was five animals.

The first group was control (G1), the second was (cholesterol-oil fed induced) (G2), the third group was (cholesterol-induced+ DOE) (G3) and the fourth group was (only DOE) (G4). The diet of rabbits was natural food and the ethical issue of deals with animals is consideration.

Cholesterol Administration

Rabbits were made hypercholesterolemia by oral administration of cholesterol (100mg/kg body weight/day) suspended in corn by gastric intubation. Cholesterol was given to rabbits for six week (daily) and the degree of hypercholesterolemia required and duration of study of hypocholesterolemia and lipoproteins ratio was investigate by measuring of total serum TCH, VLDL, HDL, TG and LDL.

Sample Preparation

Three kilograms of dry okra pods were using in this experiment. The dry okra was extracted with 2L boiling water for 5 times (each time for 30 min.). Then the filtered liquid was combined and concentrated under vacuum, to yield dry okra extract (near about 15gm). The content of total poly-phenols in dry okra was 30%, which was determined using previous methods. Extract was stored at -4C for chemical analysis and mixed with distilled water to locally rabbit administration experiment.

Preparation of Standard Solution

A volume of 25 µL of ascorbic acid (AA) was dissolved in the methanol to the desired concentrations and measured by HPLC.

Measurement of Serum Total Cholesterol

Cholesterol concentration was determined enzymatically according to the method described by kit manufacture.

Measurement of Ascorbic Acid

Dry okra with seeds (50 mg) was dissolved in methanol (5mL), and the solution was filtered before analysis by HPLC and compared with standard curve of ascorbic acid, the mobile face was: acetic acid and methanol.

Statistical Analysis

Statistical analysis of data in this study was performed utilizing student's t-test. Multiple groups' comparisons were made using P values less than 0.05 and 0.01 was considered significant for all data showed in the results.

Results

The results of the present study showed that, there was a significant decrease in serum total cholesterol for group of rabbits that administrated with DOE (0.5g/kg) and (DOE) + cholesterol oil-fed as compared to G2 group (only cholesterol administration) at the end of six week of experiment (322±4) vs. (534±6) ($P < 0.05$) and highly significant differences ($p<0.01$) between G2 and G4 as shown in Table 1.

Table1: levels of lipid profile in four groups during time of experiment

Groups / Parameters	G1	G2	G3	G4
TCH(mg/dl)	198±5	534±6	322±4 *	179±6 **
HDL (mmol/l)	1.8±0.7	0.7±0.1**	1.5±0.4*	1.9±0.2**
VLDL(mmol/l)	0.6±0.01	1.2±0.02**	0.7±0.02*	0.4±0.01**
LDL(mmol/l)	1.9±0.03	2.8±0.7**	1.7±0.04*	1.6±0.03**
TG(mg/dl)	120±3	290±5**	190±4*	118±2**

TCH: Total Cholesterol, G1: Control, G2: Cholesterol-fed, G3: DOE+ Ch, G4: DOE

Figures (1 and 2) showing the differences in four groups in the levels of lipid profile with and without administration of DOE:

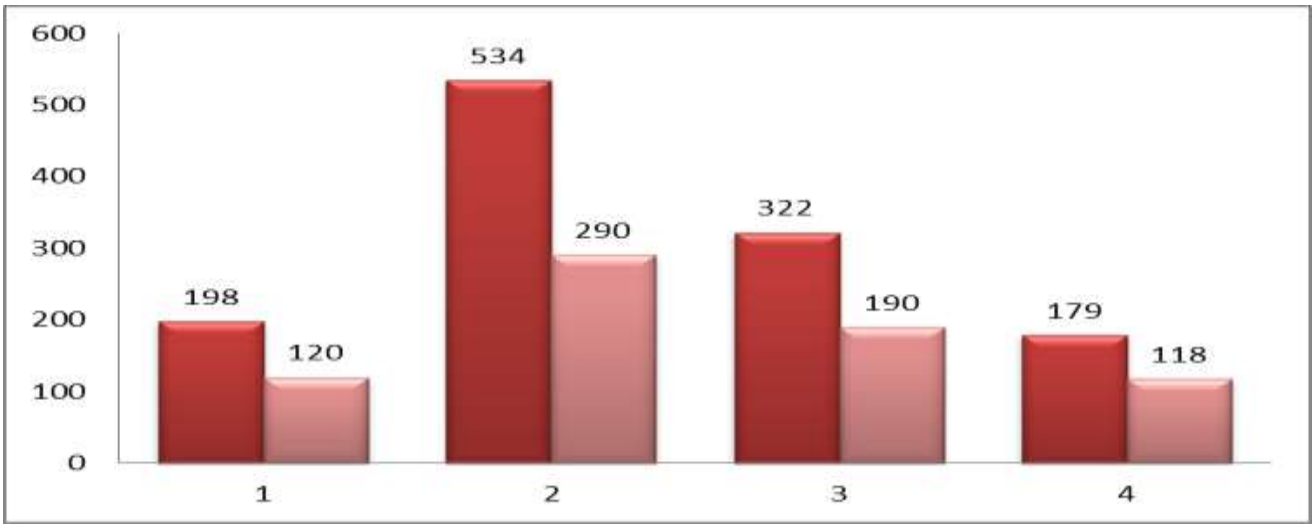

Fig.1: The differences between means of TG and TCH in four groups

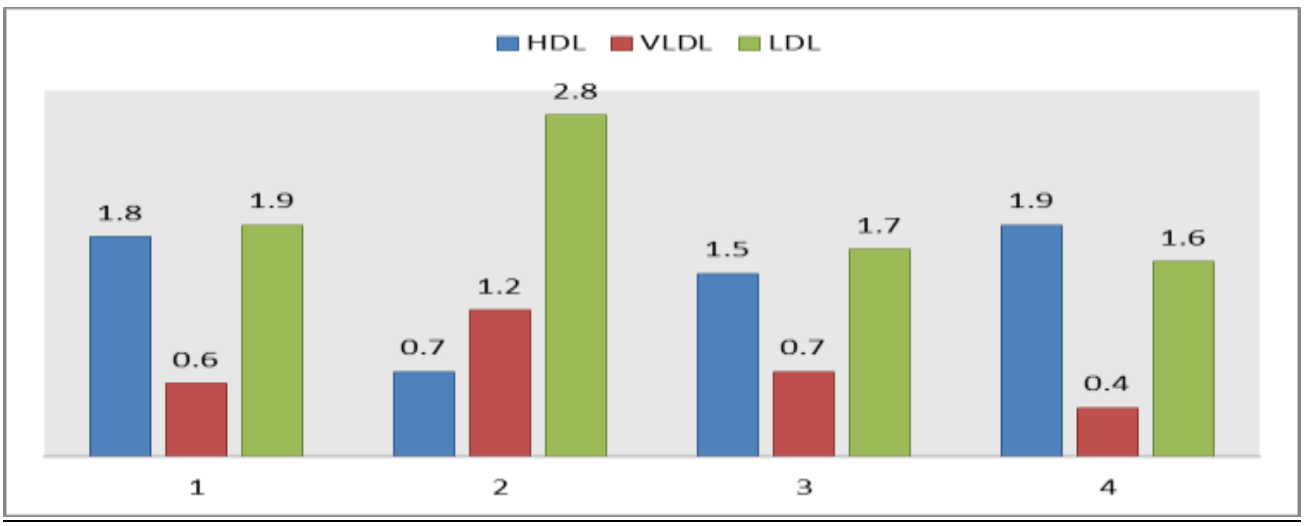

Fig.2: The differences between means of HDL, VLDL and LDL in four groups

The results of current study suggested the ratio of lipoproteins as shown in Figure3.

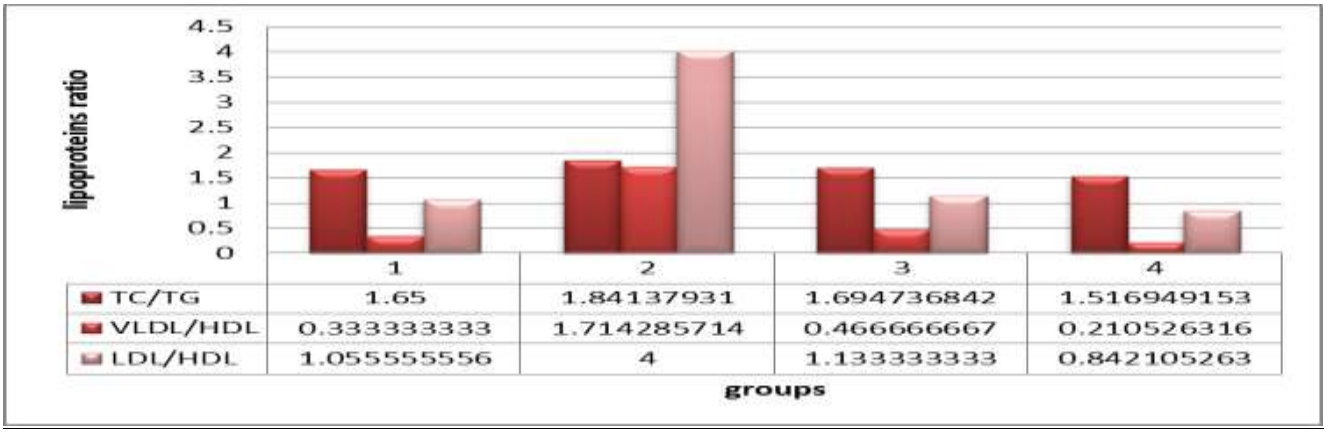

Fig.3: The differences between means of lipoproteins ratio in four groups

Conditions for Ascorbic Acid Measurement by HPLC (Shimadzu)

Mobile phase: A= (50 Mm acetic acid), B= (50 Mm acetic acid and 20% Me OH)

Type of Column: C18 – ODS (25cm x 4.6 mm X 10µL)

Volume injection sample: 100 µl

Detector UV Spectrophotometer: 254 nm

Flow Rate: 0.8 ml / min

Temperature: 25ºC

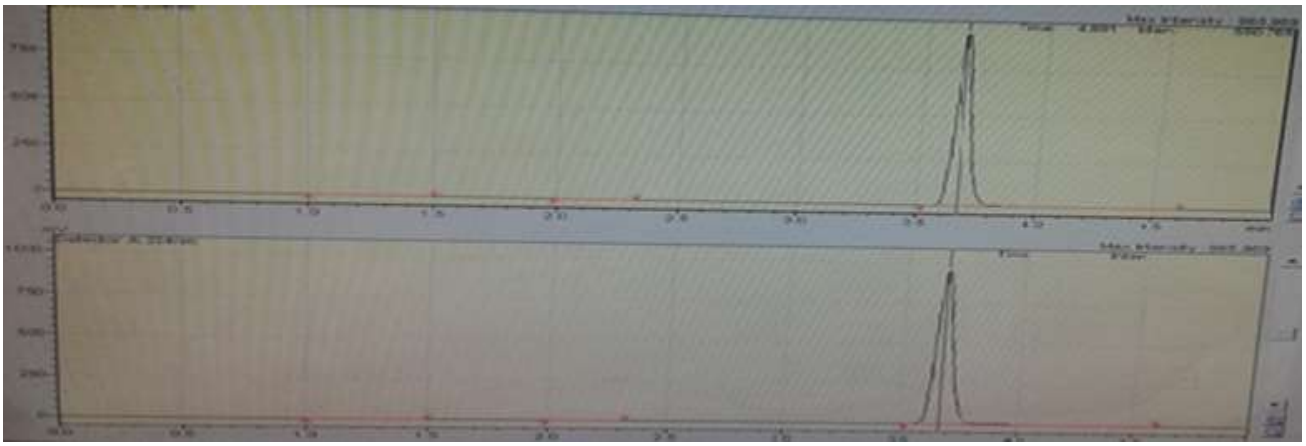

Fig.4: Retention time of Ascorbic Acid in standard and samples of DOE at 254 nm

The levels of ascorbic acid that determination in serum of rabbits of G4 in the first and the end of six weeks of experiment is showing by the following Figure(5).

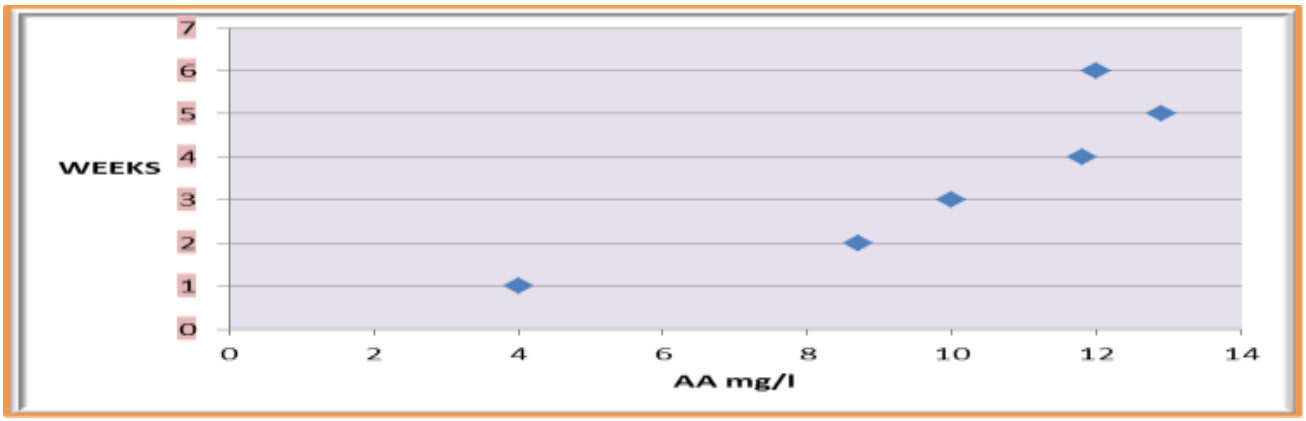

Fig.5: levels of Ascorbic Acid during times of experiment in G4

Discussion

Okra is one of the members of family Malvaceae. Okra is known by many names, such as lady's fingers, bamyah (in Iraq), and its pods are eaten raw as well as cooked as a vegetable. Okra is of African origin where it has been cultivated for more than 4000 years [13]. Nowadays is also grown in different tropical and warm temperature regions of the world, like Iraq, Iran, Egypt, and India.

The present study indicated the occurrence of large amount of ascorbic acid extract from the dry okra extract and this is very benefits to removing the free radicals and work as antioxidant agent. One of the important ascorbic acid is well-known for its physiology functions, such as, anti-microbial, anti-microbial, anti-inflammatory, anti-cancer activities etc. It also lowers cholesterol level in serum and increases sperm viability [14]. Many studies have suggested that the flavonoids found in okra like phenolic acids are well-known for its anti-inflammatory, anti-allergic, anti-thrombitic, hepatoprotective, anti-spasmodic and anticancer properties [15].

The previous studies were suggests that oxidative stress plays an important role in the development of chronic diseases such As cardiovascular disease developed by non-control hypercholesteremia [16, 21]. In the present study, oral feeding with DOE for six weeks significantly reduced the levels of (TCH, LDL/HDL and VLDL/HDL), suggesting that DOE has antioxidant effect on rabbits. The other finding from this study was increasing in ascorbic acid that has antioxidant activity in serum of rabbits by increasing the time of feeding. The oxidative damage could be indicated by level of MDA, a product of lipid peroxidation, and the activity of antioxidant enzymes such as ascorbic acid and TAOC is enhanced by DOE. Numerous previous literatures have proved that phenolic acids and flavonoids showed cellular keeping activity due to their antioxidant effects [22, 24]. Okra extract has health benefit in genitourinary disorders, cholesterol controlling, chronic dysentery, hypertension, spermatorrhoea, and ulcer, relieves hemorrhoids and possesses anti-inflammatory properties [25, 28]. In the present study we found positive correlation between the levels of ascorbic acid with increase time and dose of DOE during the progress period of experiment (data not showed).

Conclusion

The aqueous extract of dry okra may be stimulating the conversion of cholesterol into bile acids by the liver during activation of an enzyme α-hydroxylase. We suggesting the DOE may be have inhibition action on HMG-COA synthase that responsible on endogenous synthesis of cholesterol in liver.

Declaration of Interest

The authors state no conflict of interest and agree to publishing of this paper.

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