

Effect of orange peel powder (*Citrus sinensis* L.) on lipids profile in normal and experimentally - induced oxidative stress albino mice

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Abstract

The effect of orange peel powder on lipids profile in normal and oxidative stress albino mice was studied. Fifty-four albino mice were randomly divided into six groups (nine animals in each group). The first group was considered as control, the second, third and fourth groups were supplied with pellets containing 0.1, 0.2 and 0.4% orange peel powder for 21 days. The fifth group was subjected to H₂O₂ (0.1%) in drinking water for ten days. The last group was subjected to H₂O₂ (0.1%) and supplied with pellets containing the optimum concentration of orange peel powder for 14 days. The results showed that there was a significant ($P < 0.05$) decrease in lipid profile parameters, except HDL-C concentration which increased significantly ($P < 0.05$) in the three groups treated with orange peel powder and orange peel powder + hydrogen peroxide compared with stress animals. It is concluded that orange peel powder decreases serum TC, TG, LDL-C, VLDL-C levels and atherosclerosis index in the experimental animals. Therefore, the orange peel powder is recommended to prevent the development of hypercholesterolemic and coronary heart diseases.

Key words: orange peel, oxidative stress, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein.

Introduction

The plants are used as medicaments in the form of traditional preparations or as derivatives from pure active. In every region, based on the climate and geographic conditions, special medicinal plants grow and many of them have unique medicinal properties in the treatment of diseases [1].

One of these plants is orange that probably originated in Southeast Asia and have been cultivated in China since 2000 BC [2].

Several studies reported that wastes and by-products of fruits may be an abundant source of antioxidant polyphenols [3]. Peels of Citrus fruits are known

to have antioxidative, anti-inflammatory, anticancer, and antibacterial activities[١].

Orange peel contains high concentrations of phenols. Its extract contains significant amount of beta-carotene and is a good source of vitamin C [٢]. The main constituent of orange peel essential oil is d-limonene (present to the extent of at least ٩٠ %), which is the only hydrocarbon present [٣]. However, limonene is known as a chemopreventive agent with potential value as a dietary anti-cancer tool in humans [٤]. In addition, the principal odoriferous constituent of the orange peel essential oil is *n*-decyclic aldehyde, which was believed to be the only aldehyde present then until capraldehyde and citral have been identified[٥].

Research studies show that oranges have a wide variety of phytonutrients. They include citrus flavanones, anthocyanins, hydroxycinnamic acids, and a variety of polyphenols [٦, ٧]. These constituents act as antioxidants which have been reported to possess multi-beneficial effects in disease prevention [٨]. Furthermore, epidemiologic data suggested that the intake of complex carbohydrates and dietary fibers, which are available in citrus peels, is associated with an inverse manner to risk of coronary artery disease [٩].

Despite the great importance of orange peel, its effects on serum lipids have rarely been investigated. The importance of alterations in the plasma lipid profile and lipid metabolism in atherogenesis is well established [١٠]. Therefore, the present study was conducted to evaluate the effect of orange peel powder on lipid profile in normal and experimentally- induced oxidative stress mice.

Material and Method

Instruments

The instruments that used in this experiment were laboratory mill (Enaaz International, Taiwan), centrifuge (Griffin & Geory, England), spectrophotometer (CECIL, England), incubator (Memmert, Germany) and Sensitive balance (Sartorius, Germany).

Preparation of orange peel powder

Fresh orange fruit (*Citrus sinensis* L.) were obtained from local market. They were washed and peeled using fruit knife. The peels were sun-dried for ٤٨h. The dried peels were ground into a fine powder in laboratory mill. The ground powder was passed through a sieve and packed into bags until used [١١, ١٢, ١٣].

Experimental animals

Fifty-four albino mice with an average age of about ٧ - ٨ weeks and weight between ٢٨ - ٣٠ g were used. They were bred in special cages in National Center for Drug Control and Research, fed pellets (contain ٢٠ % crude protein and ١١% crude fibre, rich in protein and energy) and given tap water *ad libitum* during the experimental period. The conditions of their living

were as following: average temperature about 30°C. and the light cycle was divided into 12 hours light: 12 hours dark [3, 4].

Design of the experiment

The mice were randomly divided into six groups (nine mice in each group). The first group was considered as control and supplied with pellets and water. The second, third and fourth groups were supplied with food (pellets) containing 0, 1, 2 and 4% orange peel powder for 21 days. The fifth group was subjected to experimentally-induced oxidative stress by the *ad libitum* supply of drinking water containing 0.1% H₂O₂ for ten days. The last (sixth) group was subjected to H₂O₂ (0.1%) and supplied with pellets containing the optimum concentration of orange peel powder for a period of 14 days [3, 4].

Blood sample collection

After the period of the experiment was elapsed, blood was collected by killing the animals. The blood was collected in glass tubes and expressed slowly into the vial to reduce the risk of hemolysis after removing of the needles from syringes [3, 4].

Serum was separated by putting the tubes in the centrifuge at 3000 rpm for 10 min at 4°C. Serum samples were stored at -4°C until biochemical tests were performed [4].

Biochemical parameters

Total cholesterol, HDL cholesterol and triacylglycerides were determined using assay kits (Biomaghreb Company, Tunis) for *in vitro* diagnosis use [4, 5, 6]. The total cholesterol and triglycerides assays depend on enzymatic hydrolysis and oxidation. On the other hand, the HDL cholesterol assay depends on the precipitation reaction and supernatant formation.

Friedewald equation was used to obtain the concentration of LDL cholesterol as following:

$$[LDL-Chol] = [Total-Chol] - [HDL-Chol] - [VLDL-Chol]$$

While VLDL-Chol concentration was calculated as below:

$$[VLDL-Chol] = TG/5 [6].$$

The atherosclerosis index was calculated by dividing the values of LDL-Chol on HDL-Chol concentrations [6].

Statistical analysis

Statistically, the results were analyzed using analysis of variance (ANOVA) applicable to a completely randomized design. Using SPSS program (version 17), the significance among means was tested depending on Duncan Multiple Range Test [7].

Results and discussion

Table (1) illustrates the effect of orange peel powder on cholesterol and triglycerides concentration in normal and oxidative stress albino mice. The

results showed that there was a significant ($P < 0,05$) decrease in cholesterol and triglyceride concentrations in all groups treated with orange peel powder and orange peel powder + hydrogen peroxide compared with stress animals. In addition, there was no significant difference in cholesterol and triglyceride concentrations in the group treated with orange peel powder (0,1%) and orange peel powder + hydrogen peroxide compared with control animals, while there was a significant ($P < 0,05$) decrease in cholesterol and triglyceride concentrations in the groups treated with orange peel powder (0,2% and 0,4%) compared with control group. The cholesterol concentration means were 101,77, 98,66, 95,11, 103,00, 104,33 and 146,11 mg/dl for orange peel powder (0,1), 0,2 and 0,4%), orange peel powder + stress, control and stress groups, respectively. While the means of triglycerides concentration for these groups were 62,11, 60,00, 57,22, 64,89, 64,22 and 97,33 mg/dl, respectively. (Table 1).

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Table (١): Effect of orange peel powder on cholesterol and triglycerides concentration in normal and oxidative stress albino mice.

Experimental Groups	Cholesterol concentration (mg/dl)	Triglycerides concentration (mg/dl)
Control	b ١٠٤,٣٣ ± ١,٣٥	b ٦٤,٢٢ ± ١,١٧
Orange peel powder (٠,١%)	bc ١٠١,٧٧ ± ١,٢٨	bc ٦٢,١١ ± ١,١٢
Orange peel powder (٠,٢%)	c ٩٨,٦٦ ± ١,٠٨	cd ٦٠,٠٠ ± ١,١٢
Orange peel powder (٠,٤%)	d ٩٥,١١ ± ٠,٨٢	d ٥٧,٢٢ ± ١,٠٩
Hydrogen peroxide (٠,١٥%)	a ١٤٦,١١ ± ١,٦٦	a ٩٧,٣٣ ± ١,٣٠
Hydrogen peroxide + orange peel powder	b ١٠٣,٠٠ ± ٠,٩٣	b ٦٤,٨٩ ± ١,٠٩

*Similar letters indicate no significant differences and different letters indicate significant differences at $p < ٠,٠٥$.

On the other hand, the results in Table (٢) revealed that there was a significant ($P < ٠,٠٥$) increase in HDL-C concentration in all groups treated with orange peel powder and orange peel powder + hydrogen peroxide compared with stress animals. The HDL-C means were ٦٩,٨٨, ٧١,٤٤, ٧٣,٥٥, ٦٧,٠٠ and ٥٥,٤٤ mg/dl, respectively for orange peel powder (٠,١, ٠,٢ and ٠,٤%), orange peel powder + hydrogen peroxide and stress animals. The results also showed that there was no significant difference in HDL-C means in the group treated with orange peel powder (٠,١ and ٠,٢%) and the group treated with orange peel powder + hydrogen peroxide compared with control group. While there was a significant ($P < ٠,٠٥$) increase in HDL-C concentration in the group treated with orange peel powder (٠,٤%) compared with control group. The means of HDL-C were ٧٣,٥٥ and ٦٨,١١ mg/dl, respectively for orange peel powder (٠,٤%) and control animals. In addition, these results demonstrated that there was a significant ($P < ٠,٠٥$) decrease in LDL-C and VLDL-C concentrations as well as atherosclerosis index in all groups treated with orange peel powder and orange peel powder + hydrogen peroxide compared with stress animals, while there was no significant difference in these parameters in the group treated with orange peel powder (٠,١%) and orange peel powder + hydrogen peroxide compared with control animals. In addition, there was a significant ($P < ٠,٠٥$) decrease in LDL-C and VLDL-C concentrations as well as atherosclerosis index in the groups treated with orange peel powder (٠,٢ and ٠,٤%) compared with control group. The means of LDL-C in orange peel powder (٠,١, ٠,٢ and ٠,٤%), orange peel powder + hydrogen peroxide, control and stress groups were ١٩,٤٦, ١٥,٢٤, ١٠,١١, ٢٣,٠٢, ٢٢,٣٨ and ٧١,٢٠ mg/dl, respectively. For these groups mentioned, the means of VLDL-C were ١٢,٤٢, ١٢,٠٠, ١١,٤٤, ١٢,٩٧, ١٢,٨٤ and ١٩,٤٦ mg/dl, respectively. Furthermore, the means of atherosclerosis index in these groups were ٠,٢٨, ٠,٢١, ٠,١٤, ٠,٣٤, ٠,٣٣ and ١,٢٣, respectively.

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Table (٧): Effect of orange peel powder on lipoprotein concentrations and atherosclerosis index in normal and oxidative stress albino mice.

Experimental Groups	HDL-C concentration (mg/dl)	LDL-C concentration (mg/dl)	VLDL-C concentration (mg/dl)	Atherosclerosis index
Control	bc ٦٨,١١ ± ١,٣٨	b ٢٢,٣٨ ± ١,٤٠	b ١٢,٨٤ ± ٠,٢٣	b ٠,٣٣ ± ٠,٠٢
Orange peel powder (٠,١%)	abc ٦٩,٨٨ ± ١,٤٤	b ١٩,٤٦ ± ١,٣٠	bc ١٢,٤٢ ± ٠,٢٢	bc ٠,٢٨ ± ٠,٠٢
Orange peel powder (٠,٢%)	ab ٧١,٤٤ ± ١,٤٧	c ١٥,٢٤ ± ١,٥٢	cd ١٢,٠٠ ± ٠,٢٢	cd ٠,٢١ ± ٠,٠٣
Orange peel powder (٠,٤%)	a ٧٣,٥٥ ± ١,١٤	d ١٠,١١ ± ١,٣٠	d ١١,٤٤ ± ٠,٢٢	d ٠,١٤ ± ٠,٠٢
Hydrogen peroxide (٠,١٥%)	d ٥٥,٤٤ ± ١,٣٠	a ٧١,٢٠ ± ١,٩٣	a ١٩,٤٦ ± ٠,٢٦	a ١,٢٣ ± ٠,٠٥
Hydrogen peroxide + orange peel powder	c ٦٧,٠٠ ± ١,٣٤	b ٢٣,٠٢ ± ٠,٩٧	b ١٢,٩٧ ± ٠,٢١	b ٠,٣٤ ± ٠,٠٢

* Similar letters indicate no significant differences and different letters indicate significant differences at $p < 0,05$.

It is postulated from these results that high polyphenolic and flavonoid compounds concentrated in this preparation could partly explain the underlying mechanism of its cholesterol and triacylglycerol concentrations lowering properties in animals [١, ٣].

Some studies have shown hesperidin (a flavanone) may have cholesterol lowering effects [٧]. Various studies have shown that diets containing elevated contents of pectin decrease the concentration of blood serum cholesterol, particularly low-density lipoprotein (LDL-C), and other cholesterol-related lipids. Pectin obtained from orange, is referred to as citrus pectin. Pectin is frequently regarded as a very potential carbohydrate to decrease serum cholesterol [٨]. In animal studies, it has been found that supplementation with flavones, hesperitin, and naringin found in orange peel powder, reduced low-density lipoprotein (LDL) cholesterol, apolipoprotein B (apo B) and triglycerides [٩].

The hypocholesteromic effects of orange peel powder were obtained by inhibiting β -Hydroxy- β -methyl-glutaryl coenzyme A reductase and increasing the expression of LDL receptors in the liver [١٣].

In addition, the transfer of lipids to HDL is an essential metabolic step for reverse cholesterol transport in which cholesterol from peripheral tissues is shuttled back to the liver and excreted in the bile [٩].

Furthermore, the atherosclerosis index markedly decreased due to a significant decrease of LDL levels the treated groups [١, ٨].

The increase in HDL levels observed in our study might be due to the stimulation of pre-HDL and reverse cholesterol transport as shown by previous

findings. In this respect, it is suggested that the lipid lowering effect of orange powder in this study can be attributed mainly to a combination of the enriched phenolic contents and enhanced biosynthesis of hepatic-HDL resulting in increased plasma HDL concentration [1]. Furthermore, the increases in HDL cholesterol observed during the treatment with orange peel powder suggest that the beneficial alterations occurred mostly in HDL_x, a subclass of HDL containing greater proportions of cholesterol than another major HDL subclass, HDL_r. This response could provide an additional cardioprotective effect because previous studies reported a reduction in HDL_r but no changes in HDL_x in individuals with coronary heart disease [6].

Another important application of plasma lipid markers that is relevant to CVD risk is the association between the LDL to HDL ratio known as atherosclerosis index (AI). Our results showed that orange peel powder had markedly decreased the atherosclerosis index due to a significant decrease of LDL levels in treated groups [1].

The reduction in the LDL-HDL cholesterol ratio observed during treatment with the highest dose of orange juice was entirely due to changes in HDL-cholesterol concentrations and to long-term effect of the peel powder components, possibly flavonoids, on hepatic lipoprotein metabolism [7, 8].

Furthermore, it was hypothesized that orange peel powder would improve the transfer of lipids (free cholesterol, cholesteryl esters, phospholipids and triglycerides) to HDL [13].

In this study, however, the treatment of oxidative stress animals concomitantly with orange peel powder showed a protective effect against free radical attack as indicated by a reduced plasma lipid peroxidation biomarker [1].

It was found that the radical- scavenging activities of this powder increased with increasing concentration. Phenols and polyphenolic compounds, such as flavonoids have been shown to possess significant antioxidant activities [14]. These agents inhibit peroxidation reactions and significantly reduce the oxidative stress [3, 4].

Furthermore, it is known that only flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [14].

In addition, it has been found that hesperidins, the most important flavanone of orange peel, have antioxidant in rats. Furthermore, its constituents may counteract enzymatic lipid peroxidation processes [4].

It is concluded from this study that orange peel powder has an overall healthy effect on lipid metabolism. Where it decreases serum TC, TG, LDL-C, VLDL-C levels and atherosclerosis index in the experimental animals. Therefore, the orange peel powder is recommended to prevent the development of hypercholesterolemic and coronary heart diseases.

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تأثير مسحوق قشور البرتقال (*Citrus sinensis* L.) في نسق الدهون للفئران البيض الطبيعية وفئران الإجهاد التأكسدي المستحدث تجريبيا

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الخلاصة

تم دراسة تأثير مسحوق قشور البرتقال في نسق الدهون للفئران البيض الطبيعية وفئران الإجهاد التأكسدي. قسمت أربع وخمسون فأرة بصورة عشوائية إلى ست مجموعات بواقع تسعة حيوانات في المجموعة الواحدة. اعتبرت المجموعة الأولى كمجموعة سيطرة، أما المجموع الثانية والثالثة والرابعة فقد جهزت بالعليقة الحاوية على ٠,١ و ٠,٢ و ٠,٤ % مسحوق قشور البرتقال لمدة ٢١ يوما، فيما خضعت المجموعة الخامسة لبيروكسيد الهيدروجين (٠,١٥%) في ماء الشرب لمدة عشرة أيام. اما المجموعة الأخيرة فقد خضعت لبيروكسيد الهيدروجين (٠,١٥%) وزودت بالعليقة الحاوية على امثل تركيز من مسحوق قشور البرتقال ولمدة ١٤ يوما. أظهرت النتائج بأن هناك انخفاضا معنويا ($P < ٠,٠٥$) في معايير نسق الدهون ما عدا تركيز البروتين الدهني عالي الكثافة (HDL) الذي اظهر ارتفاعا معنويا ($P < ٠,٠٥$) وذلك في المجموع المعرضة للتراكيز الثلاثة من مسحوق قشور البرتقال ومجموعة مسحوق قشور البرتقال + بيروكسيد الهيدروجين المذكورة مقارنة بحيوانات الإجهاد. يستنتج من ذلك بأن مسحوق قشور البرتقال يقلل من مستويات الكوليستيرول والكليسيريدات والدهون الواطئة الكثافة ودليل تصلب الشرايين في الحيوانات التجريبية، لذا فإنه يوصى مسحوق قشور البرتقال لمنع تطور امراض فرط الكوليستيرول وامراض القلب التاجية.

الكلمات المفتاحية: قشور البرتقال، الإجهاد التأكسدي، الكوليستيرول، الشحوم الثلاثية، البروتين الدهني عالي الكثافة، البروتين الدهني واطى الكثافة.