



Hypocholesterolemic Effect of Pumpkin Kernel and Defatted Meal in Mice Fed a High-Cholesterol Diet

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Abstract

This research evaluated the hypocholesterolemic impacts of pumpkin kernel and defatted meal in mice using three different types of pumpkin seeds: two Egyptian varieties (Kafr Saad and Kafr Al-Battikh) and a Chinese variety called Hongli. Six groups of mice were formed: a normal control group (NC), a hyperlipidemic control group (HC), a group of mice given defatted pumpkin meal (20 and 40%), and a group of mice given pumpkin kernels (20 and 40%). According to the results, pumpkin seeds at 20 and 40 % had a substantial impact on decreasing cholesterol more effectively than defatted flour, with the high cholesterol control group's total cholesterol dropping from 135 mg/dl to 106.43 mg/dl when the seeds at 40 % were consumed. These results stimulate the use of pumpkin seeds to prevent high cholesterol and its serious consequences for human health.

Introduction

The gourd squash known as pumpkin (*Cucurbita mixta*) is a member of the Cucurbitaceae family [1,2]. There are over 80 genera and about 800 species in the Cucurbitaceae family [3]. The pumpkin plant is a perennial that grows leafy green crops and can grow up to 12 meters tall [4]. All of a pumpkin seed's components including the hard shell, seeds, and even the flowers and leaves, are edible. Pumpkin seeds are useful in a variety of ways. One can boil, bake, steam, or roast ripe pumpkin fruit. Pumpkins can be consumed in a variety of ways, including raw or cooked as a vegetable, frozen or preserved [5]. Pumpkin flour has been reported to be used as a substitute for grain flour in baked products, soups, sauces, instant noodles, and dressings, and as a natural coloring in pasta, flour mixes and candies. Pumpkin seeds, commonly called 'pepitas', are flat and covered with yellow and white shells [6]. Although they also contain plant compounds, pumpkin seeds have significant antinutritional effects in certain essential amounts [7].

These substance include phytates, nitrates, cyanides, oxalates, and more. In various cultures, pumpkin seeds are eaten directly as a snack [4]. According to Murkovic et al. [8], roasting the seeds increases their nutritional value. When roasted seeds were compared to fresh seeds, a higher ratio of sterols and vitamin E was found. Pumpkin seeds contain high amounts of protein (25-52%). Bombardelli and Morazzoni et al. [9]. The oil content is as high as 40-60% [10]. Up to 60.8% of this consists of the fatty acids oleic (up to 46.9%), linolenic (up to 40.5%), palmitic and stearic (up to 17.40%). 0.60 to 0.75 grams of monounsaturated to polyunsaturated acids. The content of phytosterols, squalene and chlorophyll pigments is about 1% each. There are also phytosterols in free and bound forms. The content of minerals is 4-5%, including selenium, zinc, calcium, copper, iron, manganese, phosphorus, and potassium. 30% pectin content [9].

In Egypt, pumpkin seeds are an interesting alternative to Western popcorn eaten as part of the culture [6]. In many civilizations of the world, people eat pumpkin seeds raw as a snack. After salting and roasting, the seeds are very popular, especially in Arab countries [11]. In Austria, pumpkins are mainly grown for the production of salad oil or edible pumpkin seeds [12]. Pumpkin seeds (*Cucurbita maxima*) have long been used for medicinal, medicinal, and clinical purposes. They contain a number of useful functional elements. Pumpkin seed oil is used as a nerve tonic as well as a safe anthelmintic and diuretic [13, 14]. Pumpkin seed oil is a powerful antioxidant [15]. Health benefits include preventing enlargement and shrinkage of the prostate, delaying the development of hypertension, reducing pain associated with high cholesterol and arthritis, reducing bladder and urethral pressure, improving bladder compliance, reducing diabetes by promoting hypoglycemia effect and a reduced incidence of gastrointestinal diseases, enterocolitis, breast, lung and colon cancer [15,16]. In many countries, pumpkin seeds are used extensively for protein or oil production [17].

Pumpkin seeds are used in Austria, Slovenia, Hungary and Serbia to produce high quality salad oil [18]. Jams, marmalades and preserves can add flavor to dishes and are an effective way to use fruits that cannot be preserved or frozen [19]. In addition, pumpkin can be used as a natural coloring and processed into flour that has a longer shelf life and is used as a substitute for wheat flour in baked goods, soups, sauces, instant noodles, and dressings [20].

Material and Methods

Pumpkin Seed Samples

Samples of pumpkin seeds were used in this investigation, including two Egyptian cultivars-Kafir Saad and Kafir El-batikh-as well as one imported cultivar from China, which was purchased at a local market in Cairo City during the growing season. The seeds were freed from foreign contaminants, manually dehulled, and then crushed using an electronic grinder before being stored at -20 °C for the upcoming examination.

Analytical Methods

Pumpkin Oil Samples and Defatted Oil Cake

The total lipids in ground pumpkin seeds were extracted using a 2:1 v/v mixture of chloroform and methanol. The mixture was mechanically shaken for 16 hours, after which it was filtered. The filtrate was filtered since being washed three times with a chloroform and methanol solution. The resultant filtrate was allowed to dry outside at a temperature of around 25 °C. The recovered lipids were filtered over anhydrous sodium sulphate and kept at -20°C for further analysis in dark brown glass bottles. After being defatted and dried, the flour was crushed to pass through a 70 mesh screen and kept at 0°C for further examination [21].

Preparation of Pumpkin Defatted seed Meal

The pumpkin kernels were dried before lipid extraction. Lipid

extraction from the dry kernels was carried out by n-hexane extraction under the operating conditions specified in IUPAC methods no. 1.121.(1987) [22].

Gross Chemical Composition

Moisture, crude oil, total nitrogen, ash content and crude fiber of pumpkin seeds kernels were determined as outlined in A.O.A.C Carbohydrate was calculated by difference(1997) [23].

Animals Experimental Design

Adult female Swiss common bred albino mice purchased from Theodor Bilharz Institute, Giza, Egypt, with an average body weight of 25 to 30 g were used. Mice were randomly divided into six groups of rather similar total weight. Each group consisted of seven mice. The mice in each group were assigned to the corresponding experimental diet and were housed individually in cages in a controlled environment. Diets and water were supplied ad libitum throughout the study.

Diets

Rich Cholesterol Diet (RCD): Basal Diet + 2% cholesterol Mice groups fed during experimental time as follows:

Group I. Normal mice fed basal diet (negative control). for 30 days.

Group II. Normal mice fed basal diet+2%cholesterol for 30 days.

Groups III. Normal mice fed basal diet+2% cholesterol + % Kafir Saad pumpkin defatted meal for 30 days.

Group IV. Normal mice fed basal diet+2%cholesterol+ % Kafir Saad pumpkin defatted meal for 30 days.

Group V. Normal mice fed basal diet+2%cholesterol+ % Kafir Saad pumpkin whole kernel for 30 days.

Group VI. Normal mice fed basal diet+2%cholesterol+ % Kafir Saad pumpkin whole kernel for 30 days.

Blood Samples

Mice of each group were sacrificed after the end of treatment period, and the blood samples were collected and subjected to different laboratory assay, in which small part was separated for hematological determinations. The other blood portion was lifted to clotting, centrifuged at 3000 rpm for 15 minutes to separate serum. It was freshly used in the determination of some parameters and the other was quickly frozen till the biochemical assay was carried out.

Triglyceride determination

Triglycerides were enzymatically determined according to the method of Fossati and Principe et al., [24]. The developed color was measured at 546 nm against dist. H O as a blank sample. Calculation

Triglyceride concentration

$$(mg/dL) = (A_{sample} / A_{standard}) \times C_{St}$$

Where,

A_{sample} : Absorbance of sample.

$A_{standard}$: Absorbance of standard.

C_{St} : Cholesterol (200 mg/dL).

Total cholesterol determination

Total cholesterol was determined in serum samples according to the method of Richmond et al., [25]. The developed color was measured at 500 nm against dist. H O as a blank sample. Calculations

$$\text{Concentration of concentration (mg/dL)} = (A_{sample} / A_{standard}) \times C_{St}$$

Where,

A_{sample} : Absorbance of sample.

$A_{standard}$: Absorbance of standard.

C_{St} : Cholesterol (200 mg/dL).

Estimation of HDL cholesterol in serum

HDL cholesterol concentrations were measured colorimetrically with commercially available kits (HDL-cholesterol test, ELITECH diagnostics French), According to the quantitative estimation of HDL cholesterol was done using HDL cholesterol precipitating reagent in combination with enzymatic colorimetric assay kit for total cholesterol. Chylomicrons, very low density lipoprotein (VLDL) cholesterol, and low density lipoprotein (LDL) cholesterol fractions are precipitated from serum or plasma by means of phosphotungstic acid and magnesium ions, according to Lopes-Virella et al., [26] After centrifugation, high density lipoprotein HDL) cholesterol is then determined in the supernatant fluid. using a cholesterol reagent and the derived dilution factor in the calculation (Table 1).

Table 1: Basal Diet (BD): Normal diet (Moon et al). Provided from animal house.

Ingredients/Diet	Ratio%
Casein	20.5
D,L-methionine	0.3
Corn starch	15
Sucrose	49.5
Cellulose powder	5
Corn oil	5
Mineral mixture	2
Vitamin mixture	3.5
Choline bitartrate	0.1

$$\text{Concentration of concentration (mg/dL)} = (A_{sample} / A_{standard}) \times C_{St}$$

Where,

A_{sample} : Absorbance of sample.

$A_{standard}$: Absorbance of standard.

C_{St} : Conc standard (200 mg/dL).

Estimation of LDL and VLDL cholesterol in serum:

The concentration of LDL cholesterol was calculated as the equation

of Fried Ewald et al as follows:

$$[LDL\ chol] = [Total\ chol] - [HDL\ chol] - [TG / 5]$$

$$[VLDL\ chol] = Triglyceride \times 0.20$$

Results

Weight of seeds , kernel and peels

The data in Table 2 indicated that there were significant differences between the studied cultivars in their contents of seeds. However, KafrSaad and Hongli cultivars consider a good source of seeds among the studied cultivars, while Kafr El-batikh cultivar is the least in their content of seeds (Table 2).

Table 2 : Weight of seeds, kernel and seed peels of pumpkin.

Varieties	Weight 1000. seeds (g)	Weight 1000 kernel (g)	peels weight (g)	peels %
Kafr El - batikh	390.90a	259.60 a	131.30 a	33.59%
Kafr Saad	518.90b	348.30b	170.60b	32.88%
Hongli	597.50c	437.50b	160.00 b	26.78%
LSD	22.93	21.64	15	15

Gross chemical composition

(Table 3) shows the approximate chemical make-up of pumpkin seeds. The results of Onimawo et al., [27-29] who reported that the moisture content was 3.7, 5.11, and 5.20%, respectively, were in agreement with the moisture content range of 3.38 to 5.53%. In contrast to local varieties, the imported Chinese Hongli cultivar had a somewhat lower moisture content. Also, the lower moisture content is crucial for maintaining quality while in storage. The range of the pumpkin seeds under investigation's protein

content was 39.05%. (on dry weight basis). The calculated protein content values agreed with data from Alfawaz et al., [30-33]. According to Table, the examined pumpkin seeds' crude oil content ranged from 35.20 to 41.20%. The examined pumpkin varieties' seeds had a high oil content. Similar findings were also reported by Gohari Ardabili et al., [29, 34,35]. Data in Table 2 revealed that, Hongli pumpkin cultivar seeds have the highest value of crude fiber (9.69%) followed by Kafr saad cultivar (9.16%) finally, Kafr El-batikh cultivar (7.72%).

Table 3: Gross chemical composition of pumpkin seed kernel.

Characteristics (g / 100g on dry weight) *	Pumpkin cultivars		
	Kafr El batikh	Kafr Saad	Hongli
Moisture	4.67b	5.53c	3.38a
Protein	36.15b	39.05c	34.19a
Crude oil	35.20a	36.25b	41.20c
Crude Fiber	7.72a	9.16b	9.69b
Ash	5.30b	5.21b	4.22b
**Nitrogen free extract	10.96c	4.80a	7.33b

Values with different subscripts on the same column are significant ($p > 0.05$)

*calculated by differences*On dry weight basis%

** Calculated by differences

Similar results for crude fiber was reported by Abd El- Ghany et al., [28]. On other hand, the data revealed that, the Nitrogen free extract was ranged from 4.80 to 10.96% in the studied pumpkin seeds. Moreover, similar results were reported by Hegazy and El Kinawy et al., [32,34,36]. The ash content was ranged from 4.22 to 5.30 % as indicated in Table 1. Similar results for ash content of various cultivars of pumpkin seeds were reported by Gohari Ardabili et al., [29,32,34].

Nutritional Utilization of Pumpkin Seeds

Hypercholesterolemia is a major cause of cardiovascular disease (CVD), such as atherosclerosis and coronary heart disease. CVD is the most common cause of mortality and morbidity worldwide. Hyperlipidemia refers to either increased serum total cholesterol level or serum triglyceride level or both. Hyperlipidemia or hypercholesterolemia leads to the development of atherosclerosis

and later leads to the progression of Coronary Heart Disease (CHD), which will cause cardiovascular morbidity and mortality. Although several factors, such as cigarette smoking, high-fat diet, high blood pressure, physical inactivity, age, and heredity have significant roles in causing CVD, high blood cholesterol is mainly responsible for the onset of CVD [37]. Lowering serum cholesterol levels by drug or dietary interventions could reduce the risk of CVD. Therefore, it is worthwhile to develop new safe and effective cholesterol-lowering agents from natural products. Pumpkin seed contain phytosterol, which help for reduce hypercholesterolemia. The presence of a high amount of cholesterol in the diet has been demonstrated to elevate total cholesterol and may increase the risk of cardiovascular complications. Agents that can lower serum cholesterol and scavenge or inhibit free radical formation have gained wide therapeutic value. Great efforts have been made to reduce the risk of cardiovascular disease (CVD) through the regulation of cholesterol, and the ther-

apeutic benefits of plants have been the focus of many extensive dietary studies [38].

Serum Lipid Profile

In the present study, the lipid-lowering effect of pumpkin defatted meal and whole seed in mice fed a high- cholesterol diet was investigated. Kafr Saad variety was chosen for the experiment because it has the highest percentage of mono and polyunsaturated fatty acids , the active ingredients responsible for its biological effects. Mice were fed a high cholesterol diet for four weeks. Notably, the mice fed with the dietary cholesterol showed a significant increase in serum triglycerides (TG), total cholesterol (TC) and low density lipoprotein (LDL) levels compared to those of the normal group (normal diet). On the other hand, the mean of HDL levels were decreased as shown in Table 4. Serum cholesterol was elevated significantly in the high cholesterol diet group compared with the control group. In the addition, mice treated with

pumpkin defatted meal 20 and 40% mice group had no significant difference compared to high cholesterol diet group. While there was significant lowering in 20 and 40% whole seed groups and 21.16%); respectively compared to that of the high cholesterol-diets group. The results of the Table 1 added that, the mean levels of triglycerides were significantly improved by decrease (6.30%) in mice treated with 40% pumpkin defatted meal, and 5.54 and 17.44% in mice treated with 20 and 40% whole seed groups respectively . LDL was significantly decreased by (19.91 and 35.64%) in the mice treated with 20 and 40% whole seed groups respectively compared to high cholesterol diet group. Simultaneously, the mean levels of HDL were significantly increased by (13.03%) of mice treated 40% whole seed group, when the levels were compared those with the mice fed with high cholesterol diet. VLDL was significantly decreased by (17.45%) in the mice treated with 40% whole pumpkin seed groups. The obtained results were in agreement with that reported by Al showayman et al.[39-41].

Table 4: Effect of pumpkin defatted powder and kernels on Serum lipid profile in mice fed high cholesterol diet.

Parameters	Control	high cholesterol diet	defatted Meal 20 %	defatted Meal 40 %	Seed kernels 20 %	Seed kernels 40 %	LSD
Total cholesterol (mg / dl)	88.89a	135.00b	132.00b	130.40b	120.14c	106.43d	7.94
HDL (mg / dl)	39.36a	29.32b	29.88b	30.92b	31.34b	33.14bc	2.43
LDL (mg / dl)	31.34a	76.68b	73.24b	72.31b	61.41c	49.35d	4.8
Triglyceride (mg / dl)	90.97a	145.00b	144.41b	135.86c	136.96c	119.71d	7.14
VLDL (mg / dl)	18.19a	29.00b	28.88b	27.17b	27.39b	23.94c	2.05

Values were expressed as mean (n = 7)

Means significantly different from Hypercholestrolemi group at P < 0.05.

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