Hypolipidemic Effect of Persimmon in Rats Fed High Fat Diet and its Use in Juice and Nectar Preparation

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ABSTRACT

The objective of this study is to investigate the hypolipidemic effects of persimmon fruit on rats fed on high fat diet. Three groups of male Wister rats were fed on: normal control (NC), high-fat (HF), and high fat supplemented with whole dried persimmon (Pers/HF, 7%, wt/wt) diets. The results revealed that, the rat's body weight gain and relative liver weight were significantly higher in the group fed on HF than the other two groups. The animals fed on diets supplemented with persimmon had improved lipid profile as compared to high fat group. Furthermore, the supplementation with persimmon induced a significant increase in levels of reduced glutathione and a reduction in malondialdehyde level when compared to high fat group. In addition, the histopathological examination of the heart and aorta in rats group fed on high fat showed severe histological changes as compared to supplemented persimmon group, these results reflecting the protective effect of persimmon against atherosclerosis.

In this study, juice and nectar were prepared from fresh persimmon fruit as well as from persimmon powder. The dehydration process improved the sensory attributes of rehydrated persimmon juice and nectar as compared to fresh samples.

Keywords: persimmon, hypolipidemic, lipid peroxidation, rats, juice, nectar.

INTRODUCTION

Cardiovascular diseases (CVD) are currently the leading cause of death in the Western world (Roberts *et al.*, 2007). This pathology has a complex origin and results from a combination of many factors. Among the risk factors identified, we can mention elevated plasma total cholesterols and low-density lipoprotein (LDL)-cholesterol, and decrement of high density lipoprotein (HDL)-cholesterol (Samaha *et al.*, 2007). Thus, a reduction of plasma cholesterol is a critical component in both CVD prevention and treatment. Oxidative damage is also a major contributor to CVD development (Münzel *et al.*, 2008).

Diet is the first therapeutic approach to hypercholesterolemia. Combination of antioxidants and hypocholesterolemic agents in diets are crucial to restrict the development of atherosclerotic lesions. In the past several years, much attention has been drawn on the study of persimmon (*Diospyros kaki*) due to its cholesterol-lowering, antioxidant and anti-atherogenic activity as well as for the identification of its active ingredients (Yugarani *et al.*, 1992, Gorinstein *et al.*, 1998a, Gorinstein *et al.*, 1998b, Matsumoto *et al.*, 2006).

Persimmon pulp is rich in some nutrients such as vitamin C (70 mg/100 g), vitamin A (65 mg/100g), calcium (9 mg/100 g) and iron (0.2 mg/100 g) (Tous & Ferguson, 1996). It is also rich in phenolic compounds other than tannins having antioxidant effects which reduce the risk of chronic disease by protecting against free radical mediated damage (Gorinstein *et al.*, 1994, Gorinstein *et al.*, 1999, Gorinstein *et al.*, 2000).

The fruit is mainly eaten fresh, and can also be frozen, canned or dried. Temperatures commonly applied during drying lead to tannin degradation. Also, sugars present in the fruit exude to the surface where they crystallize. The result is a sweet, tasteful and nonastringent dried product (Marder & Schoemaker, 1995, Mallavadhani *et al.*, 1998).

The present study was designed to assess the hypolipidemic effects of whole dried persimmon fruit on the lipid profile, oxidative stress parameter and histopathological alteration in rats fed high fat diet. The effect of dehydration process on sensory characteristics of rehydrated persimmon juice and nectar compared to fresh state was also investigated.

MATERIALS AND METHODS

Materials

Persimmon fruits (*Diospyros kaki* L.) were obtained from the local market, Egypt, commercial kits used for determining total cholesterol, triglycerides (TG), high density lipoprotein (HDL), low Density Lipoprotein (LDL), malonaldehyde (MDA) and reduced glutathione (GSH) were purchased from Biodiagnostic Co. Dokki, Egypt.

Methods

Preparation of samples

To prepare persimmon for use in this experiment, whole fruits of the seedless were washed, sliced into 1cm thick rings and then were dehydrated at 60°C in a cabinet dryer for 12 hr and powdered (Akyidiz *et al.*, 2004). Persimmon powder was mixed with the basal diet before the rats were fed, based on the biological experiment it was investigated in human diet such as juice and nectar preparation.

Animals and diets

The experiment was conducted on 18 male Wistar rats with an average weight of 120 g. The rats were obtained from the Organization of Biological Products and Vaccines (Helwan Farm, Cairo, Egypt). They were housed in screen-bottomed aluminum cages in rooms maintained at 25±1°C with alternating cycles of light and dark of 12hr duration. The animals were fed on the control diet for three consecutive days. Rats were then randomly divided into three groups, each consists of 6, the first group was (the control) fed on the normal control diet (NC), the second was fed on high fat diet (HF) and the third was fed on high fat diet supplemented with 7% (wt/wt) powdered whole persimmon (HF/Pers) for 4 weeks. The composition of the experimental diet is shown in Table (1) according to (Gorinstein *et al.*, 1998b, Gorinstein *et al.*, 1999, Gorinstein *et al.*, 2000).

The animals were given food and distilled water ad-libitum during the experimental period. The changes in body weight were recorded weekly. Blood samples were also obtained from the retro-orbital plexus of the eyes from all animals of each group at the end of the experiment, the organs were excised immediately after bleeding for weight. Plasma was obtained from blood samples by centrifugation at 1500 rpm for 15 min at ambient temperature.

Lipid profiles parameter:

Enzymatic determination of total cholesterol (TC) was carried out according to Allain, (1974). Fully enzymatic determination of total triglycerides (TG) in plasma was measured colorimetrically at 546 nm, according to Fossati & Principe, (1982), low density lipoproteins (LDL-C) were determined by enzymatic methods of Wieland & Seidel, (1983). While, the high density lipoproteins (HDL-C) was determined according to the method of Lopez-Virella *et al.*, (1977).

Table 1: Compositions of the experimental diets (%)

Component %	NC[1]	HF[2]	HF/Pers[3]
Corn starch	63	48	48
Casein	15	15	15
Corn oil	10	10	10
Cellulose	7	7	-
Salt mixture	4	4	4
Vitamin mixture	1	1	1
Whole dry persimmon	-	-	7
Cholic acid	-	0.2	0.2
Lard	-	14.8	14.8

[1] Normal control diet

[2] High fat diet

[3] High fat with whole dried persimmon diet

Oxidative stress parameter

The extent of lipid peroxidation in plasma was determined by measurement of malondialdehyde (MDA) formation at 534 nm using the thiobarbituric acid reactive substances (TBARS) method as described by Ohkawa *et al.*, (1979). The reduced glutathione (GSH) in the plasma was estimated by its reaction with dithio–bis–2–nitrobenzoic acid (DTNB) that gave a yellow coloured complex with maximum absorption at 412 nm, according to the method described by Beutler *et al.*, (1963).

Histopathology

Autopsy samples were taken from hearts of the different groups of rats and fixed in 10% formol saline for twenty four hours. Washing was done with tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stains for histopathological examinations through the light microscope (Banchroft *et al.*, 1996).

Preparation of persimmon juice and nectar

The fresh persimmon fruits were juiced after washing and peeling, and then the puree of persimmon fresh juice was mixed with water, citric acid and sugar in order to achieve nectar of 25% puree, 14 °Brix. While, the persimmon powder was rehydrated to prepare nectar of 14 °Brix (Gössinger *et al.*, 2009).

Sensory evaluation

A panel of 10 judges from the staff of Food Science Department Ain Shams University were asked to evaluate the prepared juice and nectar samples for their taste, astringency and coulor, giving numerical scores to each of there attributes from 10, using a report sheet according to (Watts *et al.*, 1989)

Statistical analysis

All data were expressed as mean values ± SD for 6 rats in each group. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test with $P<0.05$ being considered statistically significant. Statistical analysis was conducted with SAS program (SAS, 1996).

RESULTS AND DISCUSSION

Effect of experimental diet on body weight gain and relative weight of internal organs in rats

Initial body weights of the three animals groups were not significantly different, however, after 30 days of feeding, body weights were significantly higher in rats group fed on HF diet as compared to other groups (Table 2). It was clear that the weight gain was influenced by the type of dietary fat fed to animals. It seems that the lipids were accumulated in rats bodies fed on HF without persimmon supplementation, while rats fed on the same high fat diet supplemented with persimmon showed significant less weight gain and statistically equal to those

Table 2: Effects of experimental diets on body weight gain and relative weight of organs in rats fed on high fat diets.

Parameters	NC[1]	HF[2]	HF/Pers[3]
Initial weight (g)	120.00 ± 1.79^{a}	120.17 ± 1.75^{a}	120.50 ± 1.87^{a}
Average weight gain (g/rat)	137.33 ± 1.60^{b}	160.33 ± 1.63^{a}	142.58 ± 1.69^{b}
Relative weight of organs (g/100 g BW)			
Liver	4.264 ± 0.27^{b}	5.779 ± 0.15^{a}	4.168 ± 0.12^{b}
Heart	1.016 ± 0.14^{a}	1.045 ± 0.05^{a}	0.977 ± 0.02^{a}

[1] Normal control diet

[2] High fat diet

[3] High fat with whole dried persimmon diet

Means having different letters (superscript) in the same row are significantly different ($P < 0.05$).

fed on control diet. These findings are in agreement with the results obtained by Hill *et al.*, (1993).

The heart relative weights were not significantly affected by the type of diet in all the groups. However, the relative weights of liver were significantly higher in the HF group than in the NC and persimmon supplemented group. This result may be attributed to cholesterol deposition in the liver of HF group as compared with other groups (Table 2).

Lipid profile parameters:

Concentrations of plasma lipid profile are shown in Table (3). The concentration of total cholesterol (TC) and low density lipoproteins (LDL-C) in plasma of both animal groups fed on HF and HF/Pers diets was greater than rats fed on NC diet. Rats fed on high fat diet, TC and LDL-C were significantly greater than those fed on HF/Pers diets. Therefore, the persimmon-supplemented diet significantly reduced plasma TC concentration by 25.84% and LDL-C by 27.03%. These findings are in agreement with those obtained by Gorinstein *et al.*, (1998a).

The greater concentration of TG in rats fed on HF diet than in the other groups indicated that the persimmon-supplemented diet significantly prevented the rise of TG in rats fed on high fat diet (18.77% vs. HF group). On the other, hand Plasma HDL-C was significantly decreased in rats receiving high fat diet. Addition of persimmon did not completely restore HDL-C level to that value of the normal control group, however, a small rise was observed (NS), (Bozena, 1998).

The plasma total cholesterol/HDL-cholesterol ratio significantly increased in animals fed on HF diet while it decreased statistically in animals fed on persimmon supplemented diet and control group. For this reason, atherogenic index (AI) was significantly higher in the HF group than in the NC and persimmon groups.

The total cholesterol/HDL-cholesterol and atherogenic index are also predictors of coronary risk (Goldstein & Brown, 1987). A significant increase in total cholesterol/HDL-cholesterol and atherogenic index were observed in high fat fed rats that has an effect on cardiovascular diseases. Persimmon may play an important protective role against atherosclerosis and cardiovascular disease in mammals, which explained the reduction of these ratios in HF/Pers group.

Oxidative stress parameters

Lipid peroxidation (MDA) is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer, toxicity of xenobiotics and aging (Bandyopadhyay *et al.*, 1999). In the present study, plasma Lipid peroxidation concentrations were significantly greater in both rat groups fed on the HF/Pers and HF diets as compared with those fed on the NC diet (Table 4). This concentration was also significantly greater in HF than in HF/Pers fed rats (57.5%), thus persimmon again reduced the cholesterol-induced increase in plasma Lipid peroxidation.

Table 3: Effect of experimental diets on plasma lipids profiles in rats fed on high fat diets

Parameters	NC[1]	HF[2]	HF/Pers[3]
Triglycerides (mmol/L)	0.667±0.01[b]	0.847±0.09[a]	0.688±0.03[b]
Total cholesterol (mmol/L)	2.662±0.15[c]	4.788±0.17[a]	3.551±0.11[b]
HDL-cholesterol (mmol/L)	1.603±0.02[a]	1.528±0.04[b]	1.544±0.04[b]
LDL-cholesterol (mmol/L)	1.309±0.03[c]	3.074±0.44[a]	2.243±0.06[b]
Total cholesterol / HDL-cholesterol ratio	1.661±0.09[c]	3.129±0.17[a]	2.301±0.10[b]
Atherogenic index[4]	0.678±0.01[c]	2.139±0.02[a]	1.229±0.01[b]

[1] Normal control diet

[2] High fat diet

[3] High fat with whole dried persimmon diet

[4] Atherogenic index = (Total cholesterol - HDL-cholesterol)/HDL-cholesterol

Means having different letters (superscript) in the same raw are significantly different ($P < 0.05$).

Table 4: Effect of experimental diets on plasma MDA and GSH in rats fed on high fat diets

Diets	MDA[4] (nmol/ml)	GSH[5] (mg/dl)
NC[1]	0.133 ± 0.02[c]	22.923 ± 1.49[a]
HF[2]	0.512 ± 0.03[a]	9.782 ± 1.19[c]
HF/Pers[3]	0.327 ± 0.03[b]	16.338 ± 1.26[b]

[1] Normal control diet
[2] High fat diet
[3] High fat with whole dried persimmon diet
[4] Malondialdehyde
[5] Reduced glutathione
Means having different letters (superscript) in the same column are significantly different ($P < 0.05$).

Changes in reduced glutathione (GSH) as an antioxidant biomarker content of different groups has been shown in Table (4).The levels of non-enzymatic antioxidant (GSH) were significantly lowered in HF group by about 57.3 % as compared to those of normal control group. In contrast, treatment with persimmon significantly improved the level of glutathione compared with HF treated group by 67.02 %. Furthermore, it reduced the levels of MDA and enhancement of reduced glutathione indicated strong antioxidant properties of persimmon. These observations agreed with (Gorinstein *et al.*, 1994, Gorinstein *et al.*, 1999, Gorinstein *et al.*, 2000).

Histopathological examination

Data in Figures (1) to (6) reveal the histopathological examination of semi-thin sections of heart and aorta of rats stained with hematoxylin and eosin.

The group fed on normal control (NC) diet had no histopathological altreration observed in the myocardium (m), Fig (1), as well as in the tunica intima (i) and media (m) of the aorta Fig (2).

Treatment with high fat group (HF) caused sever congestion with focal haemorrhages in the myocardium (h), Fig (3), associated with sloughing of the endocardial (arrow) endothelial cells and sub-endocardial oedema (s) as well as hyalinization of the myocardium Fig (4). There was sloughing in the endothelial cells lining the intima of the aorta with hyalinization (h) of the underlying media Fig (5).

Supplementation of whole dried persimmon (HF/Pers) for rats which were fed on high fat diet had mild hypertrophy in the wall of the myocardium blood vessels (v), Fig (6). These findings are in harmony with those obtained by Bansal *et al.*, (2002) who reported that atherosclerotic lesions are significantly more developed in animal experimental group fed on high fat diet than other groups. Therefore, the results of the present investigation could justify the inclusion of supplementation of whole dried persimmon in atherosclerosis protective diets.

Sensory characteristics of fresh and rehydrated persimmon juice and nectar

The sensory evaluation of fresh and rehydrated persimmon juice and nectar was carried out and the mean score values of samples evaluated for their taste, astringency and colour are represented in Table (5). No significant differences were noticed between treatments in the taste characteristic, while, significant differences were found in the astringency and

Table 5: Mean values of the organoleptic characteristics of persimmon juice and nectar

Treatment	Characteristics		
	Taste	Astringency	Colour
Fresh juice	8.2[a]	6.8[a]	9.4[a]
Fresh nectar	8.0[a]	6.8[a]	9.0[a]
Rehydrated juice	7.8[a]	8.6[b]	6.0[b]
Rehydrated nectar	8.2[a]	8.8[b]	5.8[b]

Means having different letters (superscript) in the same column are significantly different ($P<0.05$).

Fig. 1: Heart of a normal control rat showing the normal histological structure of myocardium (H & E, 160X)

Fig. 2: Aorta of a normal control rat showing the normal histological structure of the intima and media (H & E, 64X)

Fig. 3: Heart of rat in hypercholesterolemia showing the sever congestion and focal haemorrhage in myocardium (H & E, 160X)

Fig. 4: Heart of rat in hypercholesterolemia showing the sloughing of the endocardial endothelium with subendocardial oedema (H & E, 160X)

Fig. 5: Aorta of rat in hypercholesterolemia showing the sloughing of the endothelial lining the intima with hyalinization of the media (H & E, 160X)

Fig. 6: Heart of rat protected group from hypercholesterolemia showing the mild hypertrophy in the myocardial blood vessel (H & E, 64X)

colour. These results reflected the improvement effect of dehydration processing at 60°C on the astringency and palatability characteristics as compared to fresh juice and nectar (Akyidiz *et al.*, 2004). These results confirm the suitability of persimmon in the production of healthy and simply applicable form in the human diet such as juice and nectar.

CONCLUSION

Based on the above results, it could be concluded that the persimmon-supplemented diet significantly hindered increases in TC, TG and LDL-C levels. Therefore, the hypolipidemic and antioxidative effect of persimmon should be investigated in the human diet for application in preventing atherosclerosis.

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تأثير الكاكا كعامل خافض لليبيدات في فئران التجارب المغذاة على وجبة مرتفعة الدهن واستعمالها فى تحضير العصير والنكتار

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تهدف هذه الدراسة إلى الكشف عن تأثير التدعيم بثمار الكاكا كعامل خافض للبيدات في الفئران المغذاه على وجبة مرتفعة الدهن. غذيت ثلاث مجموعات من ذكور الفئران على ثلاث وجبات مختلفة: وجبة عادية، وجبة مرتفعة الدهن ووجبة مرتفعة الدهن مدعمة بثمار الكاكا المجففة ٧٪ وقد أظهرت النتائج ارتفاعا معنوياً فى معدل الزيادة فى وزن جسم الفئران ووزن الكبد للمجموعة المغذاة على وجبة مرتفعة الدهن مقارنة بالمجموعة الضابطة والمجموعة المرتفعة في الدهن ومدعمة بالكاكا المجففة. وقد حدث تحسن في حالة الليبيدات في الحيوانات المغذاة على وجبة مدعمة بالكاكا مقارنة بالمجموعة المرتفعة في الدهن. علاوة على ذلك، أدى التدعيم بالكاكا إلى زيادة معنوية فى الجلوتاثيون المختزل وانخفاض فى المالونالدهيد مقارنة بالمجموعة المغذاة على وجبة مرتفعة الدهن. كما أظهرت الدراسات الهستوباثولوجية للقلب والأورطى في المجموعة المغذاة على غذاء مرتفع الدهن تغييرات خطيرة مقارنة بالمجموعة المدعمة بالكاكا وهذه النتائج تعكس التأثير الوقائي للكاكا ضد تكوين الجلطات.

وقد أشارت هذه الدراسة إلى أن عملية التجفيف لثمار الكاكا أدت إلى تحسين الخواص الحسية لعصير وشراب الكاكا المسترجع مقارنة بالعينات الطازجة.