

Potential Effect of Sour Orange Peel (Citrus Aurantium) Supplementation on Lipid Profile and Liver Function of Albino Rats Fed on High Fat Diet

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Abstract

The present study was carried out to investigate the effects of supplemented high fat diet (HFD) with two levels (1.5 and 3%) from dried unripe or ripe sour orange peel on serum leptin, glucose, lipid profile and liver functions of albino rats. A total of 36 male albino rats weighing (40±5g) were used. The rats were divided into two main groups. The first main group (n=6) fed on basal diet (BD) and used as a negative control group (-ve). The second main group 30 rats fed on high fat diet (HFD) and divided into five subgroups. The first subgroup fed on (HFD) all over the experimental period and used as a positive control group (+ve). The other (4 subgroups) were fed on (HFD) supplemented with two levels (1.5 or 3%) from dried unripe sour orange peel (DURSOP) or dried ripe sour orange peel (DRSOP) for 8 weeks. At the end of experiment, rats were sacrificed and blood samples were collected, then serum was separated, glucose,

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leptin level, lipid profile as well as AST, ALT and ALP enzymes were determined. The obtained results revealed that feeding 25 days of age albino rats on (HFD) supplemented with (3% from DURSOP or DRSOP) induced a significant decrease in serum leptin, glucose, total cholesterol (TC), triglycerides (TG), LDL-c, VLDL-c, AST, ALT and ALP, on the other hand significant increase in serum HDL-cholesterol occurred in albino rats fed on HFD supplemented with (3%) from (DURSOP) or (DRSOP) followed by group fed on (HFD) supplemented with (1.5%). In conclusion, the results showed that administration of citrus unripe or ripe sour orange peel at levels (3 or 1.5 %) to rats fed on (HFD) could assist in management of obesity and its complications.

Introduction

Over weight and obesity are the fifth leading risk for global death. At least 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease and between 7 and 41 % of certain cancer burdens are attributable to overweight and obesity (**WHO, 2012**). In developing countries with emerging economics (Classified by the world Bank as lower and middle -income countries), the rate of increase of childhood, overweight and obesity has been more than 30 % higher than that developed countries (**WHO, 2015**).

Obesity is a significant and increasing public health problem worldwide. Even through there are several treatments, such as surgery and drugs, there seems to be no efficient treatment without potential side effects., thus considering a life style modification as the best option. In addition to a lifestyle modification, natural alternatives may provide increased health expectancy. Several plants possess anti-obesity potential and have been poorly studied, while others are not even promoted (*Claudia et al., 2015*).

Polymethoxy flavones the major components of orange peel have been found to have health benefits including anti-inflammatory, anti-carcinogenic, anti-viral anti-oxidant, anti-thrombogenic and anti-atherogenic properties (*Li et al., 2009*). Therefore, the present study was carried out to investigate the effects of supplemented HFD with (DURSOP) or (DRSOP) on serum leptin, glucose, lipid profile and liver enzymes of albino rats.

Material and methods

Materials

Sour orange (*Citrus aurantium*) were obtained from El About market. The raw orange ripe and unripe were washed carefully and peel cut into small pieces to be exposed to solar energy at national research center and ground to fine powder.

Chemicals

Casein, vitamins, minerals, cellulose, choline chloride were obtained from Elgomhoria Company, Cairo, Egypt.

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Kits for biochemical analysis were obtained from Gamma trade Co- for Pharmaceutical and chemicals. Dokki, Egypt.

Rats

Thirty-six male albino rats of Sprague Dawleystain 25 days age (40 ± 5 g b.wt) were obtained from the laboratory of animal's colony, ministry of health and population, Helwan, Cairo, Egypt.

Experimental animals Design

Rats were housed in individual cages under hygienic laboratory conditions and were fed on basal diet ad libitum for one week for adaptation in the animal house of faculty of Home Economics, Helwan University. The basal diet in the preliminary experiment consists of 14 % casein (protein >85%) , corn oil 4%, salt mixture 3.5%, vitamins mixture 1%, choline chloride 0.25%, cellulose 5% and (72.25%) corn starch. **Reeves et al., (1993).**

The salt mixture and vitamin mixture were prepared according to **Hegsted et al., (1941)** and **Campbell (1963)**. After a period of adaptation on basal diet, rats were divided into two main groups .The first main group (6 rats) fed on basal diet (negative group). The second main group: thirty rats were fed on high fat diet (HFD) all over the experimental period containing (14% protein from casein, 20% fat "19% saturated fat and 1% unsaturated fat", 5% cellulose, 3.5 % salt mixture, 1% vitamin mixture, 10% sucrose , 0.25% choleinchloride and the remainder is cornstarch). Supplementation of diet with dried unripe or ripe dried peel of sour orange was at the expense of starch. Rats of the second main group were divided into five subgroups. one of them 6 rats was fed on (HFD) used as a positive control group and the other four subgroups were fed on (HFD) containing 1.5%

dried unripe peel (DURSOP), 3% (DURSOP), 1.5% dried ripe peel DRSOP and 3% DRSOP, respectively. During the experimental period body weight and food consumption were measured twice a week and total feed intake of the experimental period (8 weeks) and body weight gain % were calculated.

Biochemical analysis of serum

At the end of the experiment period, the rats were starved for 12h and then sacrificed under ether anaesthetized. Blood samples were collected from hepatic portal vein by the means of fine capillary glass tubes **Schermer, (1967)**. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m. to separate serum. Serum was carefully separated into dry clean Wasserman tubes, using a Pasteur pipette and kept frozen at (-20° C) till estimation of some biochemical parameters. Serum samples used for determination of total cholesterol (**Allain et al., 1974**), triglycerides (**Foster and Dumns., 1973**), high density lipoprotein cholesterol HDL-C (**Lopes-Virliet et al., 1977**). While serum low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated according to the equation of **Friedwald et al., (1972)**. Serum Aspartate Amino Transferase (AST) and Alanine Amino Transferase (ALT) (**Reitman and Frankel, 1957**) and Alkaline phosphatase (ALP) (**Belfield and Goldberg, 1971**). Statistical analysis: was carried out using SPSS statistical software version 11 (**SAS., 2004**).

Results and Discussion

Effect of sour orange peel(citrus aurantium) on lipid profile of Albino Rats fed on high fat diet

Table (1&2) Shows the effect of high fat diet (HFD) supplemented with two levels (1.5 or 3%) from dried unripe sour orange peel (DURSOP) or dried ripe sour orange peel (DRSOP) on serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and high density lipoprotein cholesterol (HDL-C) in albino rats 25 days of age fed on (HFD). The mean values of serum total cholesterol and triglycerides significantly ($P < 0.05$) increased for positive control group, while the mean values of (HDL-c) significantly decreased ($P < 0.05$), the mean values of (LDL-c) and (VLDL-C) recorded significantly increased ($P < 0.05$) in the control positive group (fed on high fat diet), as compared to the negative control (fed on BD). In this respect *Wu et al., (2013)* reported that (HFD) induced increased serum (TG) and (TC) levels compared to those fed on the (LFD).

Our results revealed that all animals groups which fed on (HFD) supplemented with (1.5 or 3%) from (DURSOP or DRSOP), recorded a significant decrease ($P < 0.05$) in (TC), as compared to the (+ve) control group. Results showed a significant difference between the (-ve) control group and animal group fed on (HFD) supplemented

with (3%) from (DURSOP) or (DRSOP) followed by groups fed on (HFD) supplemented with (1.5%) from (DRSOP).

Concerning the (TG) results revealed that groups fed on (HFD) supplemented with (1.5 or 3%) from (DURSOP or DRSOP) recorded significantly decrease ($P < 0.05$) in (TG) levels. The best results was recorded by group fed on (HFD) supplemented with (3%) from (DRSOP) followed by (3%) from (DURSOP).

The phenolic and flavonoids compounds extracted from (DURSOP and DRSOP) it could be identify 18 fraction, characterizes with high amounts of Naringin, Hesperidin, Apig 6 rhamnose 8-glucose, Rutin and Quercetrin to be the predominant compounds. Concerning phenolic compounds resulted in 19 fraction, while (DRSOP) showed 21 fraction. The predominant phenolic in (DRSOP) was pyrogallol, which amounted 9456.49 vs 3541.46 in (DURSOP). The predominant phenolic in (DURSOP), isoFerulic, Benzoic, Ferulic, catechin, P-OH-benzoic, caffeine and 3,4, 5 methoxy-cinnamic. Therefore, our results revealed that Egyptian sour orange peel have considerable number of healthy compounds namely polyphenols and flavonoids. Biological results also showed that the supplementation of (HFD) with (DURSOP or DRSOP) at levels (3 or 1.5%) induced a significant reduction in body weight gain %, organs weight /body weight and peritoneal fat pad %.

In this respect, *lee et al., (2015)* reported that citrus peel has a potential benefit in preventing obesity through the inhibition of

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lipogenesis and adipogenesis, Lee suggested that the B-oxidation – related genes were increased in rats fed combination composed of citrus unshiu peel extracts compared to that of animal fed on (HFD).

In this concern *kelleya et al., (2013)* reported that citrus flavonoids have positive effects against obesity such as lower in LDL-C and (TG). Citrus peel treatment reduced body weight gain and decreased epididymal fat, mesenteric fat, plasma and hepatic (TG) levels (*Karagozlu et al., 2016*).

Concerning serum lipoprotein cholesterol result presented in table (2) showed the effect of (HFD) supplemented with (1.5 or 3%) from (DURSOP or DRSOP) on serum lipoprotein cholesterol fraction of 25 days albino rats. Our results revealed that all animal groups fed on (HFD) supplemented with 1.5 or 3 % from (DURSOP or DRSOP) showed a significant decrease in LDL-C and VLDL-C, as compared to control (+ve) fed on (HFD). The best results recorded by supplementation of (3%) from (DRSOP or DURSOP). On the other hand result showed that all groups fed on (HFD) supplemented with (1.5 or 3%) from (DURSOP or DRSOP) recorded a significant increase ($P < 0.05$) in HDL –C level, while (+ve) control fed on (HFD) recorded a significant decrease ($P < 0.05$) in HDL-C.

It is known that there is a correlation between lipoprotein cholesterol changes and obesity. In obesity HDL-clevel and elevation

of HDL-C is one of the targets for ant-obesity treatment (*Vozarova et al., 2002*).

Effect of sour orange peel (*Citrus aurantium*) on serum glucose, leptin and liver functions in albino rats fed on high fat diet

Table (3) illustrate the effect of two levels (1.5 or 3%) from (DURSOP or DRSOP) on serum glucose, leptin, aspartate amino transferase (AST), alanine amino transferase (ALT) and alkalainphosphatase (ALP) levels in serum rats fed on (HFD). The results revealed that the mean values of serum glucose, leptin, AST, ALT and ALP levels for (+ve) control group fed on (HFD) showed highly significant increase ($P < 0.05$), as compared to the negative control group (139.293 ± 5.320 mg/dl, 17.925 ± 1.474 ng/ml, 148.423 ± 5.678 u/l, 78.476 ± 2.392 u/l and 168.487 ± 4.752 u/l, vs. 77.830 ± 4.508 mg/dl, 5.986 ± 0.297 ng/ml, 53.884 ± 2.749 u/l, 20.598 ± 2.035 u/l and 86.967 ± 1.966 u/l, respectively).

Animal groups fed on (HFD) supplemented with (1.5 or 3%) from (DURSOP or DRSOP) recorded a significant decrease ($P < 0.05$) in serum levels of glucose, leptin, AST, ALT and ALP, as compared to the (+ve) control group. The best results recoded by groups fed on (HFD) supplemented with 3% from (DRSOP).

In this respect (*Alam et al., 2014*) reported that naringin prevented the increase in hepatic marker enzymes activities (AST, ALT and ALP) and reduced the accumulation of lipid deposition and fibrosis in the liver of high carbohydrate, high fat diet fed obese rats. Naringin supplementation also improved the mitochondrial respiration in these rats, suggesting an improvement in mitochondrial compartment dysfunction and lipid energy expenditure by liver.

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In conclusion the results taken together indicated that the dried Egyptian sour orange peel (*Citrus aurantium*) as a good source of phenols and flavonoids incorporated into (HFD) could contribute to potential management of obesity, being beneficial to alleviate the complications present in obesity.

Table (1): Effect of high fat diet supplemented with dried unripe or ripe sour orange peel on serum total cholesterol and triglycerides of 25 days albino rats:

parameter		Cholesterol mg/dl	Triglycerides mg/dl
Groups			
	Control (-ve) group fed on (BD)	79.574 ^e ±3.224	39.058 ^f ±3.359
	Control (+ve) group fed on (HFD)	150.585 ^a ±6.577	79.933 ^a ±2.600
High fat diet containing	1.5% dried un ripe peel (DURSOP)	138.971 ^b ±6.891	70.915 ^b ±4.024
	3% dried un ripe peel (DURSOP)	125.005 ^c ±2.351	61.583 ^d ±2.467
	1.5% dried rip peel (DRSOP)	130.017 ^c ±3.673	66.255 ^c ±4.322
	3% dried ripe peel (DRSOP)	116.540 ^d ±2.685	57.162 ^e ±2.889

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05.

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Table (2): Effect of high fat diet supplemented with dried unripe or ripe sour orange peel or serum lipoproteins cholesterol fractions of 25 days albino rats:

parameter		HDL-c mg/dl	LDL-c mg/dl	VLDL-c mg/dl
Groups				
Control (-ve) group		44.755 ^a ±3.091	27.008 ^f ±1.213	7.811 ^f ±0.671
Control (+ve) group fedon (HFD)		18.788 ^f ±0.823	115.810 ^a ±6.225	15.986 ^a ±0.519
High fat diet containing	1.5% dried un ripe peel (DURSOP)	24.594 ^e ±1.338	100.193 ^b ±5.891	14.183 ^b ±0.804
	3%dried un ripe peel (DURSOP)	32.167 ^c ±0.983	80.521 ^d ±2.525	12.316 ^d ±0.493
	1.5% dried rip peel (DRSOP)	26.982 ^d ±0.687	89.784 ^c ±2.356	13.251 ^c ±0.864
	3% dried ripe peel (DRSOP)	34.939 ^b ±0.563	70.168 ^e ±1.565	11.432 ^e ±0.577

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05.

Table (3): Effect of sour orange peel on serum glucose, leptin and liver functions in albino rats fed on high fat diet:

parameter		Glucose mg/dl	Leptin ng/ml	Liver functions		
				AST U/L	ALT U/L	ALP U/L
Groups						
Control (-ve) group		77.830 ^e ±4.508	5.986 ^d ±0.297	53.884 ^e ±2.749	20.598 ^f ±2.035	86.967 ^d ±1.966
Control (+ve) group fedon (HFD)		139.293 ^a ±5.320	17.925 ^a ±1.474	148.423 ^a ±5.678	78.476 ^a ±2.392	168.487 ^a ±4.752
High fat diet containing	1.5% dried un ripe peel (DURSOP)	122.196 ^b ±3.766	14.594 ^b ±1.774	128.037 ^b ±3.159	68.341 ^b ±3.503	147.749 ^b ±5.610
	3%dried un ripe peel (DURSOP)	102.381 ^c ±3.766	10.278 ^c ±1.364	112.369 ^c ±2.979	59.034 ^d ±2.308	137.158 ^c ±4.461
	1.5% dried rip peel (DRSOP)	124.193 ^b ±2.556	15.024 ^b ±0.859	126.777 ^b ±1.009	65.005 ^c ±2.879	147.897 ^b ±3.005
	3% dried ripe peel (DRSOP)	95.732 ^d ±4.537	9.690 ^c ±1.048	105.364 ^d ±4.114	53.811 ^e ±2.615	133.436 ^c ±2.150

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05

AST: Aspartate Amino Transferase

ALT: Alaine Amino Transferase

ALP: Alkaline phosphatas

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**التأثير المحتمل للتدعيم بقشور موالح البرتقال الحامضي على صورة الدهون
وظائف الكبد في فئران الالبينو التي تتغذى على غذاء عالي الدهون**

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الملخص العربي

أجريت هذه الدراسة لمعرفة تأثير تدعيم الغذاء عالي الدهون بمستويين (١,٥ و ٣٪) من قشر البرتقال الحامضي الغير ناضج أو الناضج على مستوى الليبتين والجلوكوز وصورة الدهون ووظائف الكبد في فئران الالبينو. استخدمت في هذه الدراسة عدد ٣٦ فأر من نوع الالبينو أوزانهم (٤٠ ± ٥ جرام). تم تقسيم الفئران الي مجموعتين رئيسيتين. المجموعة الرئيسية الأولى (٦ فئران) تم تغذيتها على غذاء أساسي وتم استخدامها كمجموعة ضابطة سالبة. المجموعة الرئيسية الثانية (٣٠ فأر) تم تغذيتها على غذاء عالي الدهون، وتم تقسيمهم الي خمس مجموعات فرعية. المجموعة الفرعية الأولى تم تغذيتها على غذاء عالي الدهون طوال فترة التجربة وتم استخدامها كمجموعة ضابطة إيجابية. المجموعات الفرعية الأربع الباقية تم تغذيتها لمدة ٨ أسابيع على غذاء عالي الدهون والمدعم بمستويين (١,٥ و ٣٪) قشور برتقال حامضي مجفف (ناضج وغير ناضج). في نهاية التجربة، تم تخدير الفئران وتم تجميع عينات الدم، ثم فصل مصل الدم، وتم تقدير الجلوكوز والليبتين وصورة دهون الدم بالإضافة الي انزيمات الكبد المتضمن (AST, ALT and ALP). أشارت النتائج المتحصل

عليها إلى أن تغذية فنران الالبينو التي تبلغ من العمر ٢٥ يوما على غذاء عالي الدهن والمدعم بمستوي (٣% قشور برتقال حامضي مجفف غير ناضج أو ناضج) أحدثت تناقصا معنويا في مستوى الليبتين والجلوكوز والكوليسترول والجلسريدات الثلاثية و الكوليسترول الليبوبروتينات منخفضة الكثافة والكوليسترول الليبوبروتينات منخفضة الكثافة جدا ومستويات انزيمات الكبد (AST, ALT and ALP) ، ومن ناحية اخري حدث تزايدا معنويا في مستوى كوليسترول الليبوبروتينات عالية الكثافة في فنران الالبوا التي تغذت علي غذاء عالي الدهن والمدعم بمستوى ٣% قشور برتقال حامضي مجفف (ناضج وغير ناضج)، يليها المجموعة التي تم تغذيتها علي غذاء عالي الدهن والمدعمة بمستوى ١,٥%. استخلصت النتائج أن تناول الفنران غذاء عالي الدهن المدعم بقشور البرتقال الحامضي المجفف (الغير ناضج والناضج) بالمستويين (٣% أو ١,٥%) يمكن ان يساعد في التحكم في السمنة ومضاعفاتها.