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Effect of feeding sweet orange peels on bloodglucose and lipid profile in Diabetic and hypercholesterolemic rats

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ABSTRACT

range peels (Citrus sinensisL) contain fiber and antioxidant which are beneficial to our health. Presentstudy aimed to investigate the effects of different doses of orange peels on blood glucose, lipid profile and some physiological parameters as liver and kidney functions in diabetic and hypercholesterolemic rats. Rats were divided into 3 main groups, first main group negative control, second main group diabetic rats and third main group hypercholesterolemia. Second and third main groups were divided into four sub- groups (six rats / group) and fed with different diet levels of orange peels (5%, 7.5%, and 10%) for 28 days. Bodyweight gain, feed intake, feed efficiency ratio and relative weight of some organs were calculated at the end of experiment. Fasting blood sample were taken for determination of serum glucose, total cholesterol, triglycerides, creatinine, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). There was a significant reduction in both serum total cholesteroland Triglycerides in all treated groups with orange peels. The higher peels doses improved liver and kidney functions. However, the highest reduction was achieved by feeding diabetic rats with 10% orange peels. Study concluded orange peels ameliorated blood glucose, lipid profile and liver, kidney function.

Key words: - orange peel, blood glucose, lipid profile, liver and kidney functions

INTRODUCTION

Oranges are widely grown in warm climates worldwide, and the flavors of oranges vary from sweet to sour. The fruit is commonly peeled and eaten fresh, or squeezed for its juice (Bender and Bender. 2005).

However orange peels contain compounds that are beneficial to our health. The peel of one medium orange contains over 60 flavonoids and 170 different phytonutrients (Myers, 2011).

Flavonoids that consist ofterpenoidssuch mainly limonene.linalool and other volatile oils are the major ingredients of orange peels. Pectin is the of type carbohydrate in orange peels. Orange peels provide 139 mg of vitamin C per100g. It vitamin provides Α and Bcomplex and minerals such as calcium, selenium, manganese and zinc Morton, (1987).

Diabetes mellitus, is one of the most common metabolic disorders has caused significant morbidity and mortality (Patel et al., 2012). WHO (2017)defined diabetes is a

chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Raised blood sugar is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

Since 1932 diabetes mellitus has been among the top 10 leading causes of death in America. It is a major cause of blindness. renal failure. congenital malformation, lower extremity amputation (ADA 2014). Hyperlipidemia and hypercholesterolemia important risk factors for the development of atherosclerosis and coronary artery disease (Gielen et al 2009).

The main pathogenic bloodparameters are increased concentrations of cholesterol bound to low-density lipoprotein (LDL-C), total cholesterol (T. Chol) and triglycerides (TG)(Jones, 2008). Majority of therapeutic protocols rely on drugs that belong to statin

family. **Statins** inhibit the activity of 3-hydroxy-3methylglutaryl-CoA (HMG-CoA) reductase, which catalyzes the rate-limiting step mevalonate biosynthesis, a key intermediate in cholesterol metabolism.

This is associated with a decrease in T. Chol and a switch from LDL-C to high-density lipoprotein (HDL-C) fraction. Despite the significant clinical benefits provided by statins many patients, in particular those with metabolic syndrome, do not achieve the recommended low-density and High-density lipoprotein (LDL, HDL) cholesterol target goals with statins (Jones 2008). Moreover, the use of statins is forbidden in more than 40% of patients eligible for this therapeutic approach, mostly for the occurrence of side effects including muscle pain (myalgia), muscle weakness (myopathy) or liver diseases in more severe (Alsheikh-Ali cases Karas2009), Joy and Hegele (2009) whoreported limits the use of statins and suggests the need of other alternative therapy.

Wolf, (2010) reported that orange peels are a source of health-promoting carbohydrates. Peels also contain healthy polymethoxylatedflavones (PMF), which are plant pigment compounds, present in all citrus fruits.

Several authors found that the PMF compounds in citrus peels have the potential to lower cholesterol when included diet as well as LDL in cholesterol without the side effects of mainstream cholesterol drugs. Orange peel and pulp contain hesperidin, a flavonoid that helps to lower cholesterol and triglycerides. Orange peel being rich in pectin which is a natural fiber helps to reduce cholesterol levels (Youssef, et al.2013)

AIM OF THE STUDY

This study aimed to evaluate the effect of sweet orange peels on blood glucose level, lipids profile and some physiological parameters as liver and kidney functions in diabetic and Hypercholesterolemic rats.

MATERIALS & METHOD:

Materials:

peels (Citrus **Orange** sinensisL):- orange peels were obtained from local market. Orange peels were cleaned from impurities and washed with tap water. For drying, air dryer oven at (45 °C)was used for 48 hours, and thenpeels were ground in aMulti Mill apparatus and passed through a 0.5-mm mesh sieve to obtain a fine peel powder.

Rats: - fifty four healthy adult albino male rats "Sprague Dawley strain" whose weight between 200-210 g obtained from research institute of ophthalmology medical department, Giza. analysis Egypt. The animals kept in single wire cages with wire under bottoms hygienic conditions and controlled laboratory conditions temperature (25°C), lighting and ventilation. Food and tap water were provided ad-libitum and checked daily...

Diet:The standard diet prepared as described by **Reeves et al.**

(1993), (AIN1993). The vitamins mixture and salt mixture were prepared according to (AIN,1977).

Experimental design:

Adult male albino rats fed on standard diet for one week for adaptation then, they were divided into three groups (n=18). The first group (A) fed on standard diet only and served as control group. Secondgroup (B): (diabetic groups). Diabetes was induced in normal healthy adult male rats by injection of alloxan 150mg/ kg body weight according the method to described by

DesaiandBhide, (1985).

Sixhours after the injection of alloxan, fasting blood samples were obtained by retro-orbital method to estimate fasting serum glucose. Rats having fasting serum glucose more than 200mg/dl considered were diabetics (NDDG,1994). Then was divided to subgroups as the Subgroups Control following: B: Fed on basal diet as the positive diabetic control. Subgroups B1, B2 and B3 were fed on basal diet +5%, 7.5% and

10% orange peel respectively replaced equal amount of starch. Third group (C)(hypercholesterolemic groups):Hypercholesterolemia was induced in normal healthy adult male albino rats by feeding on hyperlipidemia diet (1.5% cholesterol and +10% lard) for 2 weeks. then fasting sample was obtained estimateserum total cholesterol and TG level. When insure rats have hypercholesterolemia then divided into subgroups as the following: Subgroups Control C: Hypercholesterolemia positive control fed on basal diet; Subgroups C1, C2 and C3 were fed on basal diet + 5%, 7.5% and 10% orange peel replaced respectively amount of starch for 28 days.

At the end of experiment period, the animals were sacrificed after being fasted (overnight)under anesthetized and blood samples collected in centrifuge tubes hepatic portal vein. The organs (liver, kidney, and spleen) of each animal was quickly removed by careful dissection, washed in saline solution (0.9%), dried using filter paper then rapidly weighed separately to calculate the absolute and relative organs weight. Serum separated by centrifugation of blood at 4000 rpm (round per minute) for 15 minutes at room temperature and kept in plastic vial at -20° c till analysis.

Methods:

Chemical analysis of peels:-

Crude protein, fiber, fat, and ash content were determined following the method described by (AOAC, 1995).

Biochemical parameters:

Enzymatic colorimetric method used to determineserum glucose according to Kaplan (1984). Determination of serum Cholesterol was made according (Allain et al., 1974). Enzymatic determination triglycerides in serum was conducted according to(Fossati and Prancipe, 1982). Creatinine was determined according to the method described by (Bohmer 1971). Urea was determined according to the method described by(Patton and **1977**).(AST) Crouch and

(ALT)activities were measured according to the method described by(Reitman and Frankel 1957).

Statistical analysis:

The data were expressed as means ± standard deviation (mean \pm SD). All variables were tested for normal distribution using one way analysis of variance (ANOVA) (P<0.05). If the groups showed significant differences, Turkey's multiple comparison tests was performed with Snedecor and Cochran (1972). Statistical analysis was carried out using the program of Statistical Package for the Social Sciences (SPSS), PC statistical software (Version 16; Untitled-SPSS Data Editor).

RESULTS & DISCUSSION

Data in table (1) indicated that fiber was 9.33 g/100g dried orange peels represents approximately one-third of the recommended daily intake. Dietary fiber that is fundamental and intact in fiber-rich foods (eg, fruits, vegetables, legumes, whole grains) is widely recognized to

have beneficial effects on health when consumed at recommended levels (25 g/d for adult women, 38 g/d for adult men)(McRorie 2015). Johansson et al., (2014) concluded that high dietary fiber intake help to prevent the risk of cardiovascular disease.

Data in table 2 showed that, the mean values of feed intake of diabetic group fed on basal diet and treated with orange peels (B1,B2,B3)ranged between 10.65 ± 0.28 , 9.65 ± 0.28 and 8.67 ± 0.03 g/day. The values of feed intake of diabetic rats that treated with diets containing different doses of orange peels weredecreased significantly than that fed on Basel diet (negative control). Data also showed that, the mean values of feed intake of hypercholesterolemia group fed on basal diet and treated with orange peels ranged between 12.21 to 13.93 g/day. It could be noted that the differences in values of feed intake among all treated groups were not considerable

compared with the positive control group, as the obtained data showed a slight variation in feed intake between treated groups. These results are in accordance with those reported by Wiley and Sons (2008)who said that the variation in feed intake between treatments may be due to the active components of the added materials.

Feeding rats on basal diet containing orange peel B1and B3 resulted in non-significant changing in FER as compared to positive control group (B). Although the mean values of feed intake were almost the same, feed efficiency ratio anon-significant showed compared with increase the negative control group. This may be due to the increase of body weight during the experimental period as a result of highly utilization of the added materials and its positive effect. The obtained data in table (2) also revealed that feeding hypercholestrolemia rats on basal diet containing orange peels C1,C2 and C3 treatments showed a highly significant positive correlation vis FER and BWG while a non-significant negative correlation was found vis FI.The obtained results were inagreement with those reported by youssef, et al. (2013) who indicated that when the treatments increased in its amounts (to a limit value), FER and BWG increased.

Table 3 illustrated that in diabetic rats a gradual increase in relative organs weight when the doses of orange peels increased. The statistical showed analysis a highly significant positive correlation between treatments and organs ratio. This may be due to good antioxidant action of orange peels against the free radicals. These results are in accordance with those of oluremi et al. (2008); Wiley and Sons (2008) who found that there increase in kidney/body weight ratio. Theobtained data fromhypercholestrolemic ratsfedon basal diet containing orange peels by groups C1, C2 and C3 asignificant negative correlation found was concerning spleen while other

organs showed non-significant correlation. This means that the treatments affected spleenwith no effect on other organs. These results were in agreement with those reported by Hossin et al. (2009) Moreover, It suggested that. consumption of or its powder extract may modify the risk hypercholesterolemia and it have as health more potential a supplement rich natural in antioxidants.

Table 4 observed that blood glucose showed gradual decrease in diabetic rats after 4 weeks (as an experimental period) with the increase of supplement dose. These results are in agreement with those reported by Youssef et al. (2013) who reported that, peels marked protection, it brought the level of blood down sugar. Chifai et al. (2003) who suggested that, glucose lowering effects are most often associated with viscous fiber that lies in the soluble dietary fiber content of peels. Also these results were in accordance with Spandana, et al (2016) who found that orange peels and orange peel extract can provide benefits to diabetic patients and may reduce overeating. This is due to the natural fiber in orange peels as a natural source of pectin which helps in reducing blood sugar.

Serum total cholesterol triglycerides and Serum diabetic rats fed different doses of orange peel in table (5) showed a gradual decrease as the level of supplement increased. Data of hypercholestrolemic rats feeding on basal diet containing orange peels also showed that, total cholesterol andtriglycerides levels (mg/dl) were increased significantly (P<0.05) for rats fed on hypercholesterolemia diet (group C), compared to (group A) the negative control $(206.55\pm12.38 \text{ and } 120.7 \pm3.11)$ (78.39 ± 0.78) VS. and 97.68±1.76).Total cholesterol and triglycerides groups C1 and C2 decreased significantly (P<0.05) compared with group (C). The analysis showed a statistical decreasein significant total cholesterol and triglycerides of all treated groups with different doses of nutritional peels when compared with control positive group. On the other hand, it showed a negative correlation between total cholesterol. doses triglycerides and nutritional peel as seen in group C3 but still lower than positive control, this may be due to the increase of food intake. The study denotes that treatments under the study reduced serum cholesterol and triglycerides concentration and the highest reduction was achieved by hypercholesterolemic feeding rats on C2. This present results were in the same line with that reported by Youssef et al. (2013) who saidthat fortified with biscuits citrus peels powders reduced the levels of serum cholesterol, triglycerides and LDL cholesterol, both of which are known to contribute to disorders such as diabetes, obesity and increase risks of heart disease. The polymethoxylated flavones (PMF) in orange peels have cholesterol-lowering properties. Meanwhile, fortified biscuits with citrus peels powders raised HDL cholesterol level, which is

beneficial because it can counteract the high level of the cholesterol (LDL cholesterol) than some prescription drugs without the risk of side effects. Orange's peels may be more effective at lowering cholesterol than other citrus fruits because they contain PMFs and another flavonoid, hesperidin, which also help to cholesterol. lower Fortified biscuits with 10% Abo-Sora peels powders orange are recommended for caloric for reduced diet obese. overweight and diabetic persons. Also these results acceptance with Wilev and Sons (2008) who revealed that citrus sinensis peels decreased the concentration of different serum lipids such as total cholesterol (TC), triglycerides (TG), lowdensity lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C).Also. results were agreement with kelawala and Ananthanaryan (2004), Seham et al. (2011), who reported that orange peels had more favorable effects on blood lipids and plasma lipoproteins as well as on

the number and lipid content of LDL-c subtractions.

Table (6) shows gradual decrease of (AST) levels with the increase in the supplement dose. ALT levels decreased gradually when the supplement dose increased.In both diabetic rats control (B) and hypercholesterolemia rats control (C) showed highlysignificant increase both AST and ALT enzyme levels compared with the healthy rats control (A).Data in the same table showed that serum AST andALT levels were decreased significantly (p<0.05) in treated groups compared with the control (B) and (C). These results may be attributed to the fact that orange peels are rich in polyphenols which exhibit antioxidant and antiinflammatory act capacities in vitro. The obtained results were in agreement with those of Abdel-Rahim, et al.(2013) who found that fruit peels did not cause any adverse effect on AST and ALT. but these results did match with study not by**Ochuko**et al. (2012)who

mentioned that higher AST and lower ALT activities were observed in orange peel oil fed groups.

indicated Table (7) thatcreatinine and urea levels in both diabetic control group (B) and hypercholesterolemic group(C) increased control compared with the healthy control group (A). A gradual decrease of serum creatinine and Urea were observed, as the feeding dose of orange peel increased. These results were in the same line with Parkar and **Addepalli(2014)** who found that using of orange peel extract improved renal functions and significantly prevented increase in creatinine, urea and blood urea nitrogen levels.Also the effect of orange peels in the present study is similar to those described by Spandana, et al (2016) who mentioned that serum urea and creatinine levels were reduced due to the active components in orange peels.

CONCLUSION:

Study concluded that orange peels as a rich source of

fiber that is intrinsic and intact in whole foods and antioxidant as active components. Study observed a significant reduction in blood glucose level, hypercholesterolemic and liver, kidney function after intake high dose (10g) from orange peels in diabetic and hypercholesterolemicrats.

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Table (1): Chemical composition of dried orange peels (g /100gm)

Constituents	Protein	Fat	Fiber	Ash	T. Carb.	Total
	(g)	(g)	(g)	(g)	(g)	(g)
Material						
Orange peels	1.41	2.1	9.33	6.78	80.38	100

 $\label{eq:table constraints} Table \ (2) \hbox{: Effect of feeding different doses of orange peels onfeed} \\ in take \ (FI), feed \ efficiency \ ratio \ (FER) \ and \ Body \ weight \ gain \ (BWG) \\ in \ diabetic \ and \ hypercholesterolemic rats \ (Mean \pm SD) \\$

Groups	A	В	B1	B2	В3	С	C1	C2	C3
	(-ve)	(+ve)	5%	7.5%	10%	(+ve)	5%	7.5%	10%
Parameter									
FI	14.32	9.67	10.65	9.65	8.67	12.2	12.68	13.14	13.93
g / day	±	±	±	±	±	±	±	±	±
·	0.38	0.03	0.28	0.28	0.03	0.35	0.35	0.03	0.28
	a	ab	ab	ab	ab	ab	b	ab	ab
FER	0.105	0.108	0.11	0.119	0.12	0.14	0.118	0.113	0.276
	±	±	±	±	±	±	±	±	±
	0.01 ^a	0.09 a	0.02^{b}	0.02 b	0.09 a	0.02^{g}	0.01^{g}	0.01^{g}	0.02 ^e
BWG	42.27	29.12	32.83	32.23	29.12	47.87	41.92	41.49	107.75
(g / period)	±	±	±	±	±	±	±	±	±
	2.15 ^a	2.32 a	7.31 b	7.31 b	2.32 a	7.57 ^f	5.72 ^f	8.71 ^f	5.26 ^b

Elsayed M Hammad; Magda R Kostandy and DoaaHEl-Sabakhawi

Data are expressed as mean \pm SD. Values within a row having different superscripts are significantly different ($p \le 0.05$);

Table (3): Effect of feeding different doses of orange peels on relative weight of the organs in diabetic and hypercholesterolemic rats

	A	В	B1	B2	В3	С	C1	C2	C3
	(-ve	(+ve)	5%	7.5%	10%	(+ve)	5%	7.5%	10%
Groups)								
Parameter									
Liver	3.15	2.30	2.43	2.48	2.53	4.09	3.89	3.63	3.89
relative	±	±	±	±	±	±	±	±	±
weight	0.18^{g}	0.01^{a}	0.02^{a}	0.03^{a}	0.02^{b}	0.06^{a}	0.21 ^b	0.19^{d}	0.12 ^b
Kidney	0.57	1.28	1.33	1.40	1.48	0.64	0.59	0.58	0.59
relative	±	土	±	土	±	±	±	土	±
weight	0.01^{c}	0.04^{a}	0.02^{b}	0.02^{b}	0.01^{b}	0.01^{a}	0.22^{c}	0.03^{c}	0.03 ^c
Spleen	0.17	0.41	0.43	0.46	0.48	0.27	0.20	0.18	0.19
relative	±	±	±	±	±	±	±	±	±
weight	0.01 ^f	0.02 ^a	0.01 ^a	0.01 ^a	0.02 ^b	0.02 ^a	0.01 ^{cd}	0.01 ^e	0.01 ^{dg}

Table (4): Effect of feeding different doses of orange peels on glucose level in diabetic rats (mg/dl)

Groups	GA	GB	GB1	GB2	GB3
Item					
Blood glucose	89.80	388.20	288.30	257.70	218.50
level	±	±	±	±	±
	0.87	4.50	3.50	1.70	3.50
	f	a	b	b	b

Table (5): Effect of feeding different doses of orange peels on lipids profile in diabetic andhypercholesterolemic rats

Groups	A	В	B 1	B2	В3	С	C1	C2	C3
	(-ve)	(+ve)	5%	7.5%	10%	(+ve)	5%	7.5%	10%
Lipid profile									
Total	78.39	120.9	111.54	103.35	93.99	206.55	121.29	115.12	140.78
cholesterol	±	±	±	±	±	±	±	±	±
(mg/dl)	$0.78^{\rm e}$	0.78^{a}	0.39^{b}	1.17 ^a	0.78^{d}	12.38 ^a	6.92 ^h	4.71 ⁱ	1.72 ^d
Triglycerides	97.68	142.56	124.96	122.32	107.3	120.70	53.40	44.30	80.00±
(mg/dl)	±	±	±	±	±	±	±	±	3.82 ^b
	1.76 ^c	2.64 ^a	0.88 ^b	0.88 ^b	1.76 ^c	3.11 ^a	2.04 ^f	1.44 ^{gh}	

Table (6): Effect of feeding different doses of orange peels on liver functions in diabeticand hypercholesterolemic rats

Groups	A	В	B1	B2	B3	С	C1	C2	C3
Liver enzymes	(-ve)	(+ve)	5%	7.5%	10%	(+ve)	5%	7.5%	10%
AST(U/L)	18.06	32.10 ±	30.10 ±	27.10	24.10 ±	37.25	28.66	24.87	22.31
	±	0.10^{a}	0.10^{a}	±	0.35^{b}	±	±	±	±
	1.07 ^f			0.11^{b}		5.82 ^a	1.76 ^b	0.42 ^d	1.80 ^e
ALT(U/L)	9.51	29.40 ±	26.40 ±	24.80	22.10	16.10	11.29	11.79	10.44
	±	2.50 ^a	2.01 ^b	±	±	±	±	±	±
	0.94 ^h			2.20 ^b	2.10 ^c	1.10 ^a	0.26 ^{ef}	2.28 ^f	0.79 ^g

Table (7): Effect of feeding different doses of orange peels on kidney functions in diabetic and hypercholesterolemicrats

Groups	A	В	B1	B2	В3	C	C1	C2	C3
	(-ve)	(+ve)	5%	7.5%	10%	(+ ve)	5%	7.5%	10%
kidney function									
Creatinine	0.69	0.74	0.70 ±	0.66 ±	0.63	1.88	1.74	1.53	1.42
(mg/dl)	±	±	0.20^{b}	0.20^{b}	<u>±</u>	±	±	±	±
	0.31^{g}	0.10^{a}			0.20^{b}	0.12^{a}	0.09^{b}	0.11 ^c	0.05 ^{df}
Urea	14.70 ±	26.60 ±	20.60 ±	20.90 ±	22.60	47.35	44.15	42.10	38.00 ±
(mg/dl)	0.90 ^h	2.20 ^a	1.40 ^{cd}	0.60°	±	±	±	±	0.02^{b}
					1.90 ^b	0.10 ^a	0.20 ^a	0.30 ^b	

تأثير استخدام قشور البرتقال على مستوى سكر ودهون الدم في الجرذان المصابة بالسكر وارتفاع الكوليسترول في الدم

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الملخص العربي

يعتبر البرتقال من الفواكه المنتشرة والمعروفة في مصر والمتناولة بكميات كبيرة و نظرا لارتفاع محتواه من المواد المضادة للاكسدة والالياف خاصة في الجزء الغير مأكول مثل القشور فان هذة الدراسة تهدف الى قياس تأثير تناول تركيزات مختلفة من قشور البرتقال على مستوى سكر ودهون الدم وكذلك تقييم بعض التاثيرات الفسيولوجية مثل وظائف الكلى والكبد لدى الجرذان المصابة بالسكر وارتفاع الكوليسترول بالدم. وقد قسمت الجرذان الى ثلاث مجموعات رئيسية المجموعة الاولى هي المجموعة الضابطة السالبة والمجموعة الثانية هي المجموعة المصابة بالسكر والمجموعة الثالثة هي المجموعة المصابة بارتفاع الكوليسترول بالدم . وقد قسمت ي كل من المجموعة الثانية والثالثة الي اربع مجموعات فرعية كل مجموعة 6 فئران (مجموعة ضابطة موجبة تم تغذيتها على الوجبة الاساسية وثلاث مجموعات تم تغذيتها على الوجبة الاساسية مضاف لها تركيزات مختلفة من قشور البرتقال 5% و 7,5 % و 10 % من الوجبة الإساسية) وذلك لمدة 28 يوم . وفي نهاية التجربة تم حساب معدل اكتساب الوزن ومعدل كفاءةالطعام . كما تم جمع عينات الدم لقياس مستوى سكر الدم والكوليسترول الكلي والدهون الثلاثية ووظائف الكلي والكبد . و قد أشارت النتائج إلى إنخفاض معنوي في مستوى الجلوكوز بالدم بالنسبة للفئران المصابة بالبول السكري والتي تم إعطائها قشور البرتقال في التركيز العالي (10%) وذلك بمقارنتها بالمجموعات الأخرى والمجموعة الضابطة الموجبة كما اوضحت نتائج الدراسة وجود انخفاض معنوي في مستوى الكوليسترول الكلي والدهون الثلاثية في كل المجموعات المعالجة . وقد لوحظ زيادة التحسن في وظائف الكبد والكلي مع زيادة تركيز قشور البرتقال. وكانت افضل النتائج مع استخدام تركيز 10% من قشور البرتقال.

الكلمات الدالة: قشور البرتقال, مستوى سكر الدم, وظائف الكبد و الكلى, صورة الدهون