

EFFECT OF LEMON PEELS AS A SOURCE OF PECTIN ON HYPERCHOLESTEROLEMIC RATS

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ABSTRACT: *Hyperlipidemia is defined as increase in the lipid content in blood. The objective of this study was investigate effect of lemon peels on high cholesterol fed diet rats. Thirty five male albino rats were used. The rats were divided into 5 groups. Group (1): Rats were fed on basal diet as a control group (control negative), group (2): Fed on basal diet with 2% cholesterol as a control positive. Three groups fed on high cholesterol diet with lemon peels at the levels 5, 10 and 20. At the end of the experiment, the weight gain, food intake and feed efficiency were calculated. The results showed that high cholesterol fed diet rats exhibited significant increase in body weight, total serum cholesterol, triglycerides, low density lipoprotein, and significant decrease in high density lipoproteins. Treatment with lemon peels significantly decreased body weight, total serum cholesterol, triglycerides, low density lipoprotein, and increased in high density lipoproteins. The level 20% was the best level followed by 10 and 5%. So Hypolipidemic activity of lemon peels may be attributed due to the presence of high fiber.*

Key words: *Flavonoids, phenolics, pectin, lemon peels and hyperlipidemia*

INTRODUCTION

Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases. World health organization reports that high blood cholesterol contributes to approximately 56% of cases of cardiovascular diseases causes about 4.4 million deaths each year (Dhuley *et al.*, 1999). Hyperlipidemia is a metabolic disorder, specially characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and triglycerides (TG) with a concomitant decrease in the concentrations of high density lipoprotein cholesterol (HDL-C) in the blood circulation (Kaliara and Dedoussis, 2009). Currently, the use of alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal and fruit peels medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use (NCEP, 2002).

Lemon represent a major proportion of the fruit supply throughout the year in most Western countries because of various factors: availability in the market, diversity of cultivars and variety of conditionings (fresh fruit and juice.). It has been estimated that lemon could provide 13–20% of the per capita consumption of fruit polyphenols in the United States (Vinson *et al.*, 2001) as well as 10–30% of the daily intake of fiber and potassium, depending on individual eating habits. Most of the investigations on the health effects of lemon have focused on their lipid-lowering effects (Aprikian *et al.*, 2001).

Lemon peels contain about 5 to 10 times more vitamins than lemon juice. Lemon peels are also an excellent source of fiber, potassium, magnesium, calcium, folate, and beta carotene. Lemon peels improve bone health too. Since lemon peels contain high amounts of calcium and vitamin C, lemon peels have been shown to aid preventing osteoporosis, inflammatory polyarthritis, and rheumatoid arthritis. Moreover, Some bioflavonoid compounds are associated with the prevention of chronic diseases such as cancer and hyperlipidemia. Citrus fruits contain various bioflavonoids. Naringin and

hesperidin, glycosylated citrus flavonoids, are two major bioflavonoids identified in tangerine-peel extract. The citrus industry produces large quantities of peels and seed residue, which may account for up to 50% of the total fruit weight. Citrus industry by-products, if utilized optimally could be major sources of phenolic compounds as the peels, in particular, have been found to contain higher amounts of total phenolics compared to the edible portions. Citrus peels are waste materials, obtained after extraction of juice from citrus fruit. Methanolic extract of citrus peel is known to have different antioxidative compounds. The fiber you will get from lemon peels 3.5 oz of lemon peels contains 10.6 grams of fiber (Emin *et al.*, 1994; Bok *et al.*, 1999 and Bocco *et al.*, 2007).

The fiber in lemon, which is thought to play a major role in its lipid-lowering capacities, is not found in especially high concentration (7–13 g/100 g), and soluble fibers such as pectin represent <50% of the fiber in lemon. Nevertheless, it has been reported that this fraction probably contributes to the effects of lemon peel on lipid metabolism (Cara *et al.*, 1991). In fact, lemon peel also contain a variety of secondary plant metabolites such as polyphenols, to which have been ascribed a multiplicity of metabolic effects, including anti-oxidative properties but also, in some cases, more direct effects on lipid metabolism (Tomás-Barberán and Clifford, 2000). The basic structure of pectin is a polymer of galacturonic acid units, with variable degrees of methyl ester groups. In most plants, the basic galacturonic acid polymer has side chains composed of galactose, arabinose, xylose and rhamnose. The molecular weights of pectin molecules usually range from 60.000 to 90.000. Pectin has a considerable water holding capacity and thus it can form strong gell. They can also bind cations and some organic materials such as bile acids (Visser and Voragen, 1996). Pectin is found in most fruits such as citrus, apples, grapes and berries (Keppler *et al.*, 2006). Pectin, a polyanionic heterogeneous mixture of complex carbohydrates found in the primary

cell of plants, when supplemented in the diet of laboratory animals as well as human volunteers, causes lowering of serum and/or liver cholesterol levels (Kay and Truswell 1999). However, the chemical basis to help explain the observed hypocholesterolemic effects of dietary pectin remains elusive.

Epidemiological studies show a strong relationship between elevated levels of serum cholesterol and subsequent development of atherosclerosis. Cholesterol is carried in plasma bound to various lipoprotein fractions primarily by low-density lipoprotein (LDL) (Miettinen, and Tarpila, 2007). Atherosclerotic lesions are characterized by intimal proliferation of smooth muscle cells accompanied by accumulation of large amounts of connective tissue components such as collagen, elastin and glycosaminoglycans. In atherosclerosis, evidence for complex formation between LDL and glycosaminoglycans which like pectin are also polyanionic complex carbohydrates found in connective tissues, and demonstration of a correlation between severity of atherosclerosis and the amount of LDL present in the intimal have been presented by several investigators such as Lindahl and Hook, (2002).

It was of interest to us to investigate the interaction of polyanionic pectin with lipoproteins in order to gain an insight into the elusive biochemical basis by which dietary pectin causes lowering of serum/liver cholesterol levels. Elusive biochemical basis by which dietary pectin causes lowering of serum/liver cholesterol. So, the present research work was undertaken to investigate the antihyperlipidemic activity of different levels of lemon peels by studying *in vivo* effects on cholesterol induced hyperlipidemia in rats (Keppler *et al.*, 2006).

MATERIALS AND METHODS

Lemon used in this research was obtained from local market, washed carefully, peeled with knife and then dried in oven at temperature 50°C for four days and kept in polyethylene bags in refrigerator for later use. Cholesterol casein, vitamins, minerals and cellulose were obtained from El-Gomhariya Pharm. and Chem. Ind.

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Comp., Cairo, Egypt. While starch and corn oil were obtained from local market.

Animals

Male albino rats (110 ±5g) of Sprague Dawley strain were obtained from the Laboratory of Animal Colony, Ministry of Health and Population, Helwan, Cairo, Egypt. The rats were kept under controlled conditions in plastic cages.

Diets

The basal diet consists of casein (12%), com oil (10%), methionin (0.3%) choline chloride (0.2%), vitamin mixture (1 %) according to Campbell (1963), cellulose (5%), salt mixture(4%) according to Hegsted *et al.* (1941) and corn starch (up to 100%),

Induction of Hyperlipidemia:

High cholesterol diet was prepared by mixing 2% cholesterol with standard animal diet. The diet was placed in the cage carefully and was administered for seven days (Pandya *et al.*, 2006).

Experimental design

The experiment was conducted in the Agricultural Research Center, Animal Production Research Institute, Giza - Egypt. Rats were housed in wire cages in a room maintained at 25±2°C and kept under normal healthy conditions. All rats were fed on basal diet for one week before starting the experiment for acclimatization. After one-week period, the rats were fed on the hypercholestermic diet except the first group fed on basal diet (negative control or group 1) and the other rats divided into 4 groups as follow:

- Group 2 :(Control positive) Rats fed on basal diet + 2% cholesterol.
- Group 3: Hyperlipidemic rats fed on basal diet + 5% lemon peels.
- Group 4: Hyperlipidemic rats fed on basal diet +10% lemon peels.
- Group 5: Hyperlipidemic rats fed on basal diet + 20% lemon peels.

Collection of blood: On the 28 day, blood was collected by retero orbital sinus puncture, under mild ether anesthesia after

8 hr fasting and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out.

Biological evaluation:

At the end of the experiment, biological evaluation of the different diets carried out by determination of daily feed intake (consumption), relative organs weights (% of body weight), body weight gain% (BWG %) and feed efficiency ratio (FER) according to Champan *et al.* (1959) using the following formulas.

$$\text{BWG} = \frac{\text{Final weight} - \text{initial weight} \times 100}{\text{Initial weight}}$$

$$\text{Food efficiency ratio (FER)} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}}$$

Enzymes and metabolites assay:

Total lipids, total cholesterol, total triglycerides and LDL cholesterol were determined according to Jacobs and Van Denmark (1960). GOT (glutamate – oxaloacetate transaminase) and GPT (glutamate –pyruvate transaminase) were determined according to Tietz, (1976) and Yound (1975. sodium and copper were determined according to A.O.A.C., (1995).

Statistical analysis:

The obtained data were statistically analyzed as reported by Snedecor and Cochran, (1967), Interactions were analyzed by the general linear model procedure and Duncan's Multiple Range Test was used to separate means. Differences with *P* values ≤ 0.05 were considered significant

RESULTS AND DISCUSSION

Effect of the tested doses of lemon peel on the body weight gain, feed intake and feed efficiency ratio:

The effect of feeding 5, 10 and 20 gm lemon peel on feed intake, feed efficiency ratio and body weight gain of hypercholestermic rats are shown in table (1). Concerning the feed intake, it were 12.9±0.38 g / day for negative control.

However, the feed intake for groups 3, 4 and 5 increased compared to positive control. There is no significant between groups 3, 4,5 and the two control groups . Results of body weight gain were 42.27 ± 2.51 g. for negative control group and 61.58 ± 5.71 for positive control. Body weight gain decreased gradually for groups 3, 4, and 5 respectively. The values were 55.12 ± 3.46 , 43.17 ± 4.82 and 28.28 ± 4.69 g respectively. This decrease is statistically significant ($P \leq 0.05$). This finding matched with the results obtained by Prockop and Kivirikko (1995).

The feed efficiency ratio was 0.12 ± 0.011 for negative control, while the values increase in groups positive control, G3 and G4 more than the negative control. There was statistically significant between positive control , group 4 and group 5. There is no significant between negative control and group 4. This find was coincided with the results by Aprikian et al. (2001) who found that lemon peel decreased the body weight, feed efficiency ratio and increased the feed intake than the obese control group.

Data in table (2) represents the effect of feeding 5, 10 and 20% lemon peel on liver, kidney and spleen organs weight. The control liver weight was based on body weight 2.03 ± 0.04 g, there is no significant difference between G2 and groups 3 and 4. Also, there is no significant difference between G1 and group 5. The values of liver weight were 3.06 ± 0.35 , 2.88 ± 0.28 and 2.63 ± 0.14 g for

groups 2, 3 and 4 respectively. However, the kidney weight for control group (G1) was 0.59 ± 0.02 g, there is no significant difference between control (G2) and group 3, between control (G1) and group 4. The pancreas weight of the control group (G1) was 0.34 ± 0.02 g, there is no significant difference between group 3 and 4, whereas there was significant differences between both of controls and groups 4 and 5. These results are in agreement with the results obtained by Diplock (1999) who reported that lemon peels protected the organs as liver and spleen from toxins and damage.

The effect of feeding 5, 10, and 20 % lemon peel on kidney functions is illustrated in table (3). Concerning creatinine, control group(G1) showed a level of 0.69 ± 1.31 mg / dl, all group showed a higher values than negative control group. However, there is no statistical difference between G3 and G4. The negative control presented a level of 3.84 ± 0.152 mg / dl for albumin. All groups 4, and 5 values showed a low statistical difference compared to negative control group. The control group (G1) showed a level 2.35 ± 0.15 mg/dl for uric acid . For group (5), there is no statistical difference between this group and controls groups for uric acid and groups 3 and 4. The obtained results are in line with those found by Hassan *et al.* (2003) who stated that supplementation with lemon peel at low doses for a long time can improve the kidney functions .

Table (1). Effect of the tested doses of lemon peel on the body weight gain, feed intake and feed efficiency ratio.

Parameters	Animal groups				
	Negative control (G1)	Positive control (G2)	Hypercholesterolemic rats with levels of lemon peels		
			5% (G3)	10% (G4)	20% (G5)
Food intake g/ day	$12.9^a \pm 0.38$	$9.83^a \pm 0.03$	$9.88^a \pm 0.38$	$10.54^a \pm 0.35$	$11.14^a \pm 0.35$
BWG g/28 days	$42.27^c \pm 2.51$	$61.58^a \pm 5.71$	$55.12^b \pm 3.46$	$43.17^c \pm 4.82$	$28.28^d \pm 4.69$
FER g/day	$0.12^b \pm 0.011$	$0.22^a \pm 0.02$	$0.20^a \pm 0.007$	$0.15^b \pm 0.016$	$0.09^c \pm 0.031$

Data are mean \pm SD, $n = 6$ rats . values with different superscripts differ significantly, $P \leq 0.05$. Same letter means non-significant, $P \geq 0.05$.

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Table (2): Effect of the tested doses of lemon peel on liver, kidney and spleen organ weight (gram).

Parameters	Animal groups				
	Negative control (G1)	Positive control (G2)	Hypercholesterolemic rats with levels of lemon peels		
			5% (G3)	10% (G4)	20% (G5)
Liver weight /Body weight g/100g	2.03 ^b ±0.04	3.06 ^a ±0.35	2.88 ^a ±0.28	2.63 ^a ±0.14	2.22 ^b ±0.14
Kidney weight /Body weight g/100g	0.59 ^b ±0.02	0.64 ^a ±0.29	0.60 ^{a±} 0.19	0.56 ^b ±0.01	0.50 ^c ±0.21
Spleen weight /Body weight g/100g	0.34 ^d ±0.02	0.53 ^a ±0.21	0.46 ^b ±0.03	0.44 ^b ±0.02	0.40 ^c ±0.07

Data are mean ±SD, $n = 6$ rats . values with different superscripts differ significantly, $P \leq 0.05$. Same letter means non-significant, $P \geq 0.05$.

Table (3). Effect of the tested doses of lemon peel on kidney functions (mg / dl).

Parameters	Animal groups				
	Negative control (G1)	Positive control (G2)	Hypercholesterolemic rats with levels of lemon peels		
			5% (G3)	10% (G4)	20% (G5)
Creatinine mg/100ml	0.69 ^c ±0.31	1.70 ^a ±0.14	1.50 ^a ±0.07	1.33 ^a ±0.21	1.17 ^b ±0.02
Albumin mg/100ml	3.84 ^a ±0.152	3.82 ^a ±0.01	3.80 ^a ±0.05	3.79 ^b ±1.32	3.78 ^b ±2.31
Uric Acid mg/100ml	2.35 ^b ±0.15	2.56 ^b ±1.2	2.82 ^a ±1.00	2.75 ^a ±1.5	2.55 ^b ±0.11

Data are mean ±SD, $n = 6$ rats . values with different superscripts differ significantly, $P \leq 0.05$. Same letter means non-significant, $P \geq 0.05$.

Blood lipid profile was also affected by feeding 5, 10, and 20 % mg lemon peel as shown in table (4). The negative control G1 (-) presented a level of 86.26 ± 1.19 mg/dl for total cholesterol. Groups G2 (+), 3,4 and 5 showed significantly higher values than negative control. The values were 286.2 ± 0.12 , 266.1 ± 0.13 , 237.7 ± 3.21 , and 206.8 ± 3.21 mg/dl, respectively. The control group (G1) showed a level of 6.48 ± 0.13 mg/dl for triglycerides, there is no significant between positive control G2 and group 3, while there is significant between these groups and the others. The control G1 presented a level 53.94 ± 0.12 mg/dl for high density lipoprotein cholesterol (HDL-C), all groups values showed a lower statistical difference in relation to control. The control group G1 showed a level of 20.2 ± 1.17 mg/dl for low

density lipoprotein cholesterol (LDL-C). There is no significant between positive control G2 and group 3, between groups (4) and (5). However, all groups values showed a lower statistical difference in relation to negative control G1. The obtained results are in agreement with the results mentioned by Miettinen, and Tarpila (2007) who found that lemon peel improve the content of lipid profile in serum and maintain these component with normal levels. Generally, the results of this study confirm the earlier hypolipidemic effects reported for lemon peels (Kay and Truswell 1999). A high hypocholesterolemic effect of lemon peels was observed previously in rats fed a high-cholesterol diet in the presence of 20% of lemon peels (Kannell *et al.*, 1999).

Table (4). Effect of the tested doses of lemon peel on blood lipid profile (mg / dL).

Serum lipids	Animal groups				
	Negative control (G1)	Positive control (G2)	Hypercholesterolemic rats with levels of lemon peels		
			5% (G3)	10% (G4)	20% (G5)
Total cholesterol	86.26 ^e ±1.19	286.2 ^a ±0.12	266.1 ^b ±0.13	237.7 ^c ±3.21	206.8 ^d ±3.21
Triglycerides	6.48 ^d ±0.13	9.68 ^a ±0.63	9.4 ^a ±2.01	8.96 ^b ±1.56	7.36 ^c ±0.02
HDL-cholesterol	53.94 ^a ±0.12	35.92 ^c ±0.03	36.89 ^c ±0.04	40.94 ^b ±0.05	43.90 ^b ±0.97
LDL-cholesterol	20.2 ^c ±1.17	33.1 ^a ±0.91	30.5 ^a ±0.74	25.1 ^b ±0.91	23.8 ^b ±0.24

Data are mean ±SD, *n* = 6 rats . values with different superscripts differ significantly, *P* ≤ 0.05. Same letter means non-significant, *P* ≥ 0.05.

Also, Pectin content existed in lemon peels could be considered as a polyanionic heterogeneous mixture of complex carbohydrates found in the primary cell of plants, when supplemented in the diet of laboratory animals as well as human volunteers, causes lowering of serum and/or liver cholesterol levels. However, the chemical basis to help explain the observed hypocholesterolemic effects of dietary pectin remains elusive. Epidemiological studies show a strong relationship between elevated levels of serum cholesterol and subsequent development of atherosclerosis. Based on our knowledge of the facts that lipoproteins are carriers of cholesterol in blood streams and they are involved in atherogenesis. pectin, when supplemented in diet, causes lowering of serum and/or liver cholesterol levels in man as well as a number of laboratory animals, and that polyanionic glycosaminoglycans interact with lipoprotein (Lindahl and Hook, 2002).

CONCLUSION

It is concluded that antihyperlipidemic effect of lemon peel especially at the level 20% may be attributed to presence of flavonoids, phenolics and pectin.

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تأثير قشور الليمون كمصدر للبكتين على الفئران المصابة بإرتفاع الكوليستيرول

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المخلص العربى:

يعرف إرتفاع الكوليستيرول على أنه زيادة فى محتوى الدهون فى الدم . و تهدف هذه الدراسة إلى إستخدام قشور الليمون لمعرفة تأثيراتها على الفئران المصابة بإرتفاع نسبة الكوليستيرول و لتحقيق ذلك تم إستخدام 35 فأرا و قد تم تقسيمها إلى 5 مجموعات , المجموعة الأولى مجموعة الفئران التى تتغذى على الوجبة الضابطة (كمجموعة ضابطة سالبة) و مجموعة الفئران التى تغذت على الوجبة الضابطة مضاف لها 2% من الكوليستيرول (مجموعة ضابطة موجبة) و ثلاث مجاميع أخرى تغذت على الوجبة الضابطة مضاف لها 5 , 10 , 20 % قشر الليمون . وقد أظهرت النتائج أن إرتفاع الكوليستيرول أدى إلى زيادة الوزن و زيادة الدهون الثلاثية و الليبوبروتينات منخفضة الكثافة وإنخفاض فى الليبوبروتينات عالية الكثافة و لكن بإضافة قشور الليمون أدى إلى تقليل الوزن و إنخفاض الدهون الثلاثية و الليبوبروتينات منخفضة الكثافة وزيادة فى الليبوبروتينات عالية الكثافة و كان تركيز 20% أفضل النسب يليه 10 % ثم 5 % و لذلك نجد أن قشور الليمون تساعد على خفض الكوليستيرول فى الدم و قد يعزى ذلك إلى وجود الفلافونيدات و الفينولات و البكتين.