

Biological Effect of Lemon Peels Powder on Hyperlipidemic Rats

Heba S. Abdelhaliem and Hanady G. Sheha

Clinical Nutrition Dept - National Nutrition Institute, (NNI) - Cairo, Egypt



ABSTRACT

Hyperlipidemia is defined as increase in the lipid content in blood. This study aimed to evaluate the effect of supplementation of hyperlipidemic diet with different levels of lemon peels on lipid profile and other biochemical parameters of hyperlipidemic rats. Results showed that rats fed diet with high cholesterol exhibited significant increasing in body weight, liver weight, total serum cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and significant decreasing in high density lipoproteins-cholesterol (HDL-C). Treatment with lemon peels significantly decreased body weight, total serum cholesterol, triglycerides, low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C) and increased the level of high density lipoproteins (HDL-C). The level 10% recorded the best results followed by 5 and 2.5%. Plasma calcium was the high affected by increasing the level of lemon peels. Finally, it can be concluded that lemon peels improved lipid profile and the other biochemical parameters without increasing the using level above 10%.

Keywords: Lemon peels, Anti hyperlipidemia, Cholesterol, HDL, LDL, Triglycerides.

INTRODUCTION

Hyperlipidemia is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. It is also called hypercholesterolemia / hyperlipoproteinemia. Although elevated low density lipoprotein cholesterol (LDL-C) is thought to be the best indicator of atherosclerosis risk, dyslipidemia (abnormal amount of lipids in the blood) can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL-C) (Amit *et al.*, 2011). Medicinal plants have an important role for the health of individuals and communities. These plants have a great medicinal value that lies various chemical substances which produce physiological action on the human body. Medicinal plants contain many chemical compounds such as alkaloids, flavonoids, glycosides, saponins, resins, oleoresins, sesquiterpene, phenolic compounds, fats and oils (Devanooru *et al.*, 2015). Citrus fruit is an important medicinal plant of the family Rutaceae, It is used mainly for its alkaloids, which are having anticancer activities. Citrus fruits peels contain considerable amounts of minerals (calcium, selenium, manganese, and zinc ... etc and vitamins (C, A, and B-complex) several fold than its pulp. The citrus peels are rich in many phytochemicals; these are β and γ -sitosterol, glycosides and volatile oils. (Ain *et al.*, 2012) reported that lemon peels are a source of health-promoting carbohydrates. Peels also contain healthy polymethoxylated flavones (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 in our diet as well as LDL cholesterol without the side effects of mainstream cholesterol drugs. Lemon peel and pulp contain hesperidin, a flavonoid that helps lower cholesterol and triglycerides. Lemon peel is also a source of pectin, a natural fiber that helps reduce cholesterol levels and in a joint study with the US department of Agriculture identified a class of compounds isolated from lemon peels that shows promise in animal studies as a potent natural alternative for lowering LDL cholesterol, without the possible side effects, such as liver disease and muscle weakness, of conventional cholesterol lowering drugs (Sohn *et al.*, 2016 and Pultrin *et al.*, 2017).

Pectin in lemon peels, 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 explains 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. Lipoproteins is 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 glycosaminoglycan interact with lipoprotein (Lindahl and Hook, 2002).

(Archibald, 2005) reported that lemon peels had a powerful antioxidant as alpha, delta and gamma tocotrienols and other constituents which had been shown to lower total cholesterol and triglyceride levels. They outlined the potential dietary benefits of lemon peels pectin and fiber. (Liu, 2017) stated that lemon peel's hydroxycinnamic acids inhibited human LDL oxidation in vitro. The present investigation was designed to evaluate anti hypercholesterolemic activity of lemon peels powders on albino induced hypercholesterolemic rats as well as their effect on their other biological parameters.

MATERIALS AND METHODS

Materials

Baladi lemons were procured from local market in Cairo. Cholesterol, casein, vitamins mixture, minerals mixture and cellulose were obtained from El-Gomhariya Pharm. and Chem. Ind. Comp., Cairo, Egypt. While starch and corn oil were obtained from local market in Cairo.

Animals

Thirty male adult albino rats (140 \pm 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 according to (Ilwy, 2003) consists of casein (12%), corn oil (10%), methionine (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%.

Methods

The lemons were washed, peeled and the obtained peels were dried in oven at temperature 50°C for four days to complete dryness. The dried peels were milled by hammer mill to produce lemon peels powders. The citrus peel powder was kept in glass containers at 4°C in the refrigerator till the analysis.

Induction of Hyperlipidemia

High cholesterol diet was prepared by mixing 2.5% cholesterol and 2% animal saturated fat with standard basal diet. The diet was placed in the cage carefully and was administered for two weeks (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 following:

- Group 2: Rats received hyperlipidemic diet as positive control.
- Group 3: Rats received hyperlipidemic diet plus 2.5% lemon peels.
- Group 4: Rats received hyperlipidemic diet plus 5% lemon peels.
- Group 5: Rats received hyperlipidemic diet plus 10% lemon peels.

Lemon peels added instead of corn starch

Blood Sampling

At the end of the experiment, rats were fasted overnight and anesthetized. Blood samples were collected from all animals from the retro-orbital plexus of each group into clean, dry and labeled tube. The tubes contained heparin (10.01 U/ml) as anticoagulant. Blood was centrifuged (3500 rpm for 15 min) to separate plasma which was tightly kept in sealed aliquot tubes at -20°C until biochemical assay was carried out.

Organs

The organs including liver, kidney and spleen were removed, washed and weighted to calculate the organ's relative weight.

Relative organ weight = Organ weight (g)/Final Body weight

Biological evaluation:

At the end of the experiment, feeding and growth parameters of the different diets were evaluated by determination of daily feed intake (consumption), relative organs weights (% of body weight), body weight gain (BWG) and feed efficiency ratio (FER) according to Chapman *et al.* (1959) using the following formulas.

BWG = Final weight- initial weight

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

Minerals content:

The minerals of blood serum were determined by using AAS Atomic Absorption Spectrophotometer (PYE unican 929) according to the method of (Jorhem and Engman, 2000).

Biochemical analysis of serum

Blood glucose was determined according to Trinder (1969), Triglycerides according to (Fassati and Prencipe, 1982), Plasma total cholesterol, HDL-c, LDL-c and VLDL-c according to (Friedewald, and Fredrickson., 1972) and (Jacobs and Van Denmark, 1960) respectively. Albumin according to (Drupt, 1974). Creatinine according to (Tietz, 1976). Uric acid according to (Barham, D. and P. Trinder, 1972) and (Fossati, Prencipe and G. Berti, 1980). AST and ALT were determined according Yound, (1975).

Statistical analysis:

Statistical analyses were performed by using computer program. Statistical Package for social Science (SPSS), and compared with each other using the suitable tests (SPSS, 2008).

RESULTS AND DISCUSSION

1. Effect of different levels of lemon peel on feeding and growth parameters:

The effect of feeding 2.5, 5 and 10 g. lemon peel on feed intake, feed efficiency ratio and body weight gain of hyperlipidemic rats are shown in Table (1). Concerning the feed intake, it was 12.23±0.38 g / day for negative control and 9.48±0.03 g / day for positive control group. However, the feed intake for groups 3, 4 and 5 significantly decreased as compared to negative control group. There are no significant changes among groups 3, 4 and positive control group (G2). For body weight gain, the mean levels decreased gradually for groups 3, 4, and 5. There are no significant changes between group fed on 2.5% lemon peel and the positive control and also there is no significant changes between the negative group and the group fed on 5% lemon peels. For FER, the group fed on 10% lemon peels was decreased when compared with the other groups. This decrease is statistically significant (P<0,05) This finding matched with those of Prockop and Kivirikko (1995) who reported that lemon peel is also a source of pectin, a natural fiber that helps reduce of body weight, Also (Aprikanet *al.*, 2001) found that lemon peel can decrease the body weight and decrease the feed intake when the rats fed on high fat diet

Table 1. Effect of feeding different levels of lemon peel on feeding and growth parameters:

Parameters Groups	Feed intake (g/ day)	BWG (g/28 days)	FER (g/day)
G1 (negative)	12.23 ^a ±0.38	47.27 ^b	0.12 ^b ±0.011
G2 (positive)	9.48 ^c ±0.03	51.58 ^a ±5.71	0.19 ^a ±0.02
G3 (2.5 %)	9.87 ^c ±0.38	50.12 ^a ±3.46	0.18 ^a ±0.007
G4 (5 %)	10.24 ^c ±0.05	43.17 ^b ±4.82	0.15 ^b ±0.016
G5 (10 %)	11.24 ^b ±0.35	28.28 ^c ±4.69	0.089 ^c ±0.031

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

2. Effect of different levels of lemon peel on some relative organs weight

Table (2) represents the effect of feeding 2.5, 5 and 10% lemon peel with high fat diet on some relative organs

weight. The liver weight of negative control was $2+03 \pm 0.04$ g, there is no significant difference among positive control (+), groups 3 and 4. Also, there is no significant differences between negative control group (-) and group (5) which fed on high fat diet with 10% lemon peels. However, the kidney weight for negative control group was $0.50 \pm 0, 02$ g, there is no significant difference between positive control group (+) and groups 3 and 4, also, there is no significant changes between negative control (-) and group (5). The relative spleen weight of the negative control group was 0.34 ± 0.02 g> there is no significant difference among all tested groups which fed on different levels of lemon peels, whereas there were significant differences between both of controls and groups 3, 4 and 5. These results are in accordance with those of (Aprikian *et al.*, 2001) who reported that lemon peels protected the organs as liver and spleen from toxins and damage.

Table 2. Effect of feeding different levels of lemon peel on some relative organs weight

Parameters Groups	liver weight (gm %)	Kidney weight (gm %)	Spleen weight (gm %)
G1 (negative)	2.03 ^b ±0.04	0.50 ^b ±0.02	0.34 ^a ±0.02
G2 (positive)	3.06 ^a ±0.35	0.64 ^a ±0.29	0.53 ^a ±0.21
G3 (2.5 %)	3.00 ^a ±0.28	0.60 ^a ±0.19	0.46 ^a ±0.03
G4 (5 %)	2.63 ^a ±0.14	0.58 ^a ±0.01	0.44 ^b ±0.02
G5 (10 %)	2.22 ^b ±0.14	0.50 ^b ±0.21	0.41 ^b ±0.07

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

3. Effect of feeding different levels of lemon peel on blood glucose

The results of Table (3) showed the effect of feeding different levels of lemon peels at levels of 2.5, 5 and 10 % on plasma glucose of hyperlipidemic rats. The rats administrated the basal diet with lemon peels at doses of (2.5, 5 and 10 %) showed significantly decreased in serum glucose when compared with the positive control group. Feeding rats on 10% lemon peels led to decrease the level of blood glucose to reach the glucose level of negative control group. The hypoglycaemic effect observed in this study corroborates the reports of Sohn *et al.*, (2016). The presences of phytochemicals with recognizable hypoglycaemic effects, as well as the presence of soluble fiber and carbohydrates in lemon peel may contribute to this effect.

Table 3. Effect of different levels of lemon peel on blood glucose

Parameters Groups	Blood glucose
G1 (negative)	94.08 ^a ± 3.35
G2 (positive)	148.50 ^a ± 3.41
G3 (2.5 %)	122.16 ^a ± 3.44
G4 (5 %)	112.58 ^c ± 9.68
G5 (10 %)	93.83 ^d ± 3.03

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

4. Effect of feeding different levels of lemon peel on kidney functions :

The effect of feeding 2.5, 5 and 10 % lemon peels on kidney functions is illustrated in Table (4). Concerning creatinine, control group showed a level of $0+69 \pm 0,31$ mg /dL all groups showed higher values than negative control group creatinine of all groups' values showed a high statistical difference as compared to negative control group. The negative control group showed a level 1.35 ± 0.15 mg/dL for uric acid. There is no significant difference between the plasma uric acid of group 2,3,4,5 and the negative control. The obtained results are matched with these of Hassan *et al.*, (2003), who stated that supplementation with lemon peel at low doses for a long time can improve the kidney functions.

Table 4. Effect of feeding different levels of lemon peel on kidney functions (mg/dl) of hyperlipidemic rats

Parameters Groups	Creatinine (mg/ml)	Uric Acid (mg/ml)
G1 (negative)	0.69 ^c ± 0.31	1.35 ^b ± 0.15
G2 (positive)	1.70 ^a ± 0.14	2.99 ^a ± 1.2
G3 (2.5 %)	1.50 ^a ± 0.07	2.98 ^a ± 1.00
G4 (5 %)	1.23 ^b ± 0.21	2.45 ^a ± 1.5
G5 (10 %)	1.17 ^b ± 0.02	2.30 ^a ± 0.11

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

5. Effect of feeding different levels of lemon peel on lipid profile of hyperlipidemic rats.

Blood lipid profile was affected by feeding 2.5, 5 and 10 % of lemon peels shown in Table (5). The negative control (G1) presented a level of 86.26 ± 1.19 mg/dL for total cholesterol. Groups 3,4,5 and positive group showed significantly higher values than that of negative control. The negative control group showed a level $90 48 \pm 0.13$ mg/dL for triglycerides, there is no significant between group 3 and 4 while there were significant different between negative control group and the others. Also, there were significant among the groups fed at different levels of lemon peels. The negative control group presented a level 53.94 ± 0.12 mg/dL for high density lipoprotein cholesterol (HDL-C), all lemon peels groups values showed a lower statistical difference in relation to negative control and statically higher than positive control group.

The control group showed a level 12.2 ± 1.17 mg/dL for low density lipoprotein cholesterol (LDL-C). There were significant reductions for LDL-C of different levels of lemon peels as compared with positive control group. For VLDL-C, the level of reduction were 11.44, 16.35 and 29.43% for groups fed on 2.5, 5 and 10% lemon peels respectively when compared with positive control group. The obtained results are in the same line of the study of Sohn *et al.* (2016), who found that lemon peel improve the content of lipid profile in serum and maintain these component with normal levels. 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 7.5% of lemon peels (Kannellitf at, 1999).

Table 5. Effect of feeding different levels of lemon peel on blood lipid profile (mg / dL)

Parameters Groups	Total cholesterol	Triglycerides	HDL -cholesterol	LDL -cholesterol	VLDL -cholesterol
G1 (negative)	86.26 ^a ± 1.19	90.48 ^d ± 0.13	53.94 ^a ± 0.12	12.2 ^c ± 1.17	18.02 ^b ± 1.09
G2 (positive)	186.2 ^a ± 0.12	149.68 ^a ± 0.63	32.92 ^c ± 0.03	123.14 ^a ± 0.91	29.9 ^a ± 2.96
G3 (2.5 %)	166.1 ^a ± 0.13	132.4 ^b ± 2.01	33.89 ^c ± 0.04	105.5 ^b ± 0.74	26.48 ^a ± 3.65
G4 (5 %)	137.7 ^c ± 3.21	125.00 ^b ± 1.56	40.94 ^b ± 0.05	71.6 ^c ± 0.91	25.01 ^a ± 4.61
G5 (10 %)	106.8 ^d ± 3.21	109.36 ^c ± 0.02	43.90 ^b ± 0.97	41.8 ^d ± 0.24	21.1 ^b ± 2.54

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

6. Effect of feeding different levels of lemon peel on liver functions (mg / dL) of hyperlipidemic rats.

Results of aspartateaminotransferase (AST) and alanineamine transferase (ALT) are presented in Table (6). Hyperlipidemic rats (positive control) showed highly significant increase in both AST and ALT enzyme levels as compared with the healthy rats (negative control). Data showed that serum AST levels were decreased significantly (P<0.05) in all treated groups that fed on 2.5, 5 and 10% lemon peels compared with the control (+). The lowest levels of AST enzymes were found in group of rats that fed on hyperlipidemic diet containing 10% lemon peels.

Results obtained from this table showed a significant increase (P<0.05) in the mean values of ALT enzyme in the group fed on Hyperlipidemic diet (control +) as compared with the most treated groups. On the other hand, significant reduction in the levels of ALT enzyme in hyperlipidemic rats were found due to feeding with lemon peels when compared with control (+). From the above mentioned data, it could be concluded that, feeding rats with basal diet with lemon peels at the level 10% was the best group, because these treatments led to highest reduction in AST and ALT enzymes, as compared to other groups.

The negative control presented a level of 3.84 ± 0.152 g/dl for albumin. There are no significant differences between different groups in plasma albumin (Hassan *et al.* 2003)

Table 6. Effect of different levels of lemon peels on liver functions (AST and ALT) of hyperlipidemic rats

Parameters Groups	AST (IU/L)	ALT (IU/L)	Albumin (g/dL)
G1 (negative)	28.6 ^d ± 1.07	29.51 ^c ± 0.94	3.84 ^a ± 0.152
G2 (positive)	47.25 ^a ± 5.82	46.10 ^a ± 1.10	3.82 ^a ± 0.01
G3 (2.5 %)	38.66 ^b ± 1.76	35.29 ^b ± 0.26	3.80 ^a ± 0.05
G4 (5 %)	34.87 ^c ± 0.42	35.79 ^b ± 2.28	3.80 ^a ± 1.32
G5 (10 %)	32.31 ^c ± 1.80	32.44 ^b ± 0.79	3.78 ^a ± 2.31

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

7. Effect of feeding different levels of lemon peel on some plasma minerals of hyperlipidemic rats.

The effect of feeding different levels of lemon peels on plasma mineral profile of hyperlipidemic rats is presented in Table (7). Plasma calcium of positive control group was 1.9±G.02 mmol/L. Data obtained show a slight increase of serum calcium as the supplement level increased of lemon peels especially at the level 10%.

Plasma phosphorous of positive control group was 0.69±0.01 mmol/L. regarding lemon peel feed levels 2+5%, 5% and 10%, phosphorous levels were 0.71±0.2 , 0.74±0.1 and 0.76±0.1mmol/L5 respectively. From the above results, it could be observed that a slight increase of serum

phosphorous as the supplement levels of lemon peels increased.

In case of plasma magnesium of rats fed on positive control diet was 0.71±0.02 mmol/L. There were significant differences among the groups fed on lemon peels (at the levels 5 and 10%) and both of controls. There are no significant differences in case of 2.5% of lemon peels and both of controls. The table illustrates a gradual increase of serum magnesium as the supplement level of lemon peels increased. Plasma zinc of positive control group was 12.9±0.2 mmol/L. Rats fed the diet supplemented with lemon peel at levels 2.5%, 5% and 10% showed a slight increase of plasma zinc. There are no significant differences between plasma zinc at the levels of lemon peels. This may led to contain lemon peels with zinc (Kannell *et al*M 1999).

Table 7. Effect of different levels of lemon peels on same serum minerals of hyperlipidemic rats

Parameters Groups	Serum Ca (mmol/L)	Serum Ca (mmol/L)	Serum Ca (mmol/L)	Serum Ca (mmol/L)
G1 (negative)	1.7 ^c ± 0.02	0.66 ^c ± 0.01	0.68 ^b ± 0.03	12.6 ^b ± 0.3
G2 (positive)	1.9 ^b ± 0.02	0.69 ^b ± 0.01	0.71 ^b ± 0.02	12.9 ^b ± 0.2
G3 (2.5 %)	1.2 ^d ± 0.03	0.71 ^b ± 0.02	0.70 ^b ± 0.03	13.6 ^a ± 0.1
G4 (5 %)	1.9 ^b ± 0.02	0.74 ^a ± 0.01	0.73 ^a ± 0.02	13.7 ^a ± 1.3
G5 (10 %)	2.1 ^a ± 0.02	0.76 ^a ± 0.01	0.76 ^a ± 0.03	13.8 ^a ± 1.2

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

CONCLUSION

It can be concluded that lemon peel level 10% had high hypercholesterolemia lowering effect on body weight, blood glucose, lipid profile, liver and kidney function.

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التأثير الحيوي لقشور الليمون على الفئران المصابة بارتفاع نسبة الدهون والكوليستيرول في الدم

هبه سعيد عبد الحليم و هنادي جابر شيحة

قسم التغذية الاكلينيكية - المعهد القومي للتغذية - القاهرة

يتم تعريف زيادة الدهون في الدم علي انها اضطراب في محتوى الدهون في الدم. هدفت هذه الدراسة إلى تحديد تأثير إضافة مستويات مختلفة من قشور الليمون على مستوى دهون الدم والمعاملات الحيوية الأخرى للفئران المصابة بارتفاع دهون الدم. أظهرت النتائج أن الفئران التي تغتذت على النظام الغذائي مع ارتفاع الكوليستيرول أظهرت زيادة معنوية في وزن الجسم ووزن الكبد، والكوليستيرول الكلي في الدم، والدهون الثلاثية، والبروتين الدهني منخفض الكثافة، والبروتين الدهني منخفض الكثافة جدا، وانخفاض ملحوظ في البروتينات الدهنية عالية الكثافة. العلاج مع قشور الليمون انخفضت بشكل ملحوظ من وزن الجسم، والكوليستيرول الكلي في الدم، والدهون الثلاثية، والبروتين الدهني منخفض الكثافة، والبروتين الدهني منخفض الكثافة جدا وزيادة مستوى البروتينات الدهنية عالية الكثافة. سجل المستوى 10% أفضل النتائج تليها 5 و 2.5%. كان الكالسيوم في الدم هو الأكثر تأثراً بزيادة مستوى قشور الليمون. الخلاصة: خلصت هذه الدراسة إلى أن قشور الليمون قد حسنت من مستوى الدهون و المعاملت الحيوية الأخرى دون زيادة مستوى استخدامها إلى أكثر من 10%.