

Original Research Article

Protective and Curative Antiobesity Potential of Lemon Peel Extract in Rats Fed on High Fat Diet: Mechanism of Action.

ABSTRACT

Aim: This study was to investigate the effect of dietary lemon peel extract (LPE) on high fat diet induced obesity in rats, and elucidate some of its mechanism of actions. Fifty adult rats were divided into five groups: Gr.1 normal control (NC), Gr.2 negative control supplemented with 0.5gm% LPE (LP), Gr.3 positive control group fed on HFD (PC), Gr.4 protective group fed on HFD and supplemented with LPE 0.5gm% for 12 weeks (Prot.) and Gr.5 curative group fed on HFD for 12 weeks then treated with LPE 0.5gm% for 45 days (Curat).

Methodology: Body weight gain, feed efficiency ratio, serum lipid profile, blood glucose, serum insulin, blood glucose-6-phosphate dehydrogenase (G6PD) activity, serum adiponectin, leptin, acyl coenzyme A oxidase (ACO) and medium chain acyl coenzyme A dehydrogenase (MCAD) activities were measured.

Results: Body weight gain, lipid profile, glucose, insulin, G6PD activity and leptin were significantly suppressed by the effect of LPE in treated groups. LPE also up-regulated ACO and MCAD activities in the treated groups. Additionally mRNA level of ACO in the liver was up-regulated in treated groups with LPE compared with PC.

Conclusion: These findings demonstrated that LPE prevent body weight gain and fat accumulation due to improvement in lipid metabolism through up-regulations of the activities of MCAD, ACO and its mRNA

level in the liver and down regulation of the activity of G6PD and normalization of total antioxidant capacity.

In this context the protective effect of LPE was more pronounced than the curative effect.

Keywords: Lemon peel extract; ACO; G6PD; MCAD; Leptin; Adiponactin.

1. INTRODUCTION

The incidence of obesity and its related metabolic disorders has escalated dramatically worldwide including populations living in developing countries, and is becoming a key public health issue with a massive burden to the healthcare system[1]. Obesity increase the morbidity rates of a number of metabolic disorders, such as type2-diabetes, hypertension and cardiovascular diseases[1]. In Egypt, obesity is rising among young adults, overtime these conditions especially if associated with poor dietary habits, smoking or physical inactivity; will lead to cardiovascular disease later in adulthood[2].

Because diets containing high-fat foods are becoming common, it is necessary to find suitable alternatives, such as phytochemicals, to ameliorate the effects of a high fat diet, thereby reducing the risk associated with obesity[3].

Over the years, many medications have been used to manage obesity, but most of them are now withdrawn due to their serious adverse effects[4].

In recent years, much attention has been paid to vegetables and fruits based food as novel protective and even therapeutic strategies for management of obesity and several diseases due to their phytochemical constituents[5]. Phytochemicals are known to possess antioxidant properties with effective anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities with low adverse effects[6]. Phytochemicals such as flavonoids may help to prevent or reduce obesity through the regulation of different molecular pathways[7].

Citrus fruits contain various kinds of flavonoids such as flavonone glycoside, flavonoes glycoside and polymethoxy flavones. The flavonoids in lemon fruits have been reported to have a number of positive health effects in the prevention of lifestyle-related diseases, and to have antiinflammatory, anticancer, and antiviral activities based on their antioxidant activity[8]. Furthermore, previous studies demonstrated that flavonoids in lemon fruits has been known to have antiobesity and antihypertensive effects in animal models[9] and human studies[10]. These previous reports may lead to the speculation that LPE modulates the development of obesity through the regulation of gene expression of some of lipid metabolic enzyme.

In this study, the effect of dietary LPE was investigated on high-fat diet-induced obesity in rats. To achieve this aim the regulatory effect of LPE administration on lipid profile, glucose, insulin, leptin, adiponectin and some lipid metabolism regulatory enzymes. In addition, LPE effect on the expression of the acyl coenzyme A oxidase gene was evaluated to elucidate some of its mechanism of action.

2. MATERIALS AND METHODS

2.1 Lemon Peels Extract Preparation

Lemons were obtained from local market, washed with distilled water; then dried at 60°C, grounded and finely extracted with 70% methanol in a hot water in a shaking bath at 60°C. The extract was clarified by centrifugation at 3000r.p.m for 20 minutes and a number of filtrations. Finally, the supernatant was stored in the dark[11]. The purity of LPE was analyzed by high performance liquid chromatography(HPLC).

2.2 Experimental Design and Dietary Treatment

Fifty adult male Swiss albino rats, weighing 100-120g obtained from Helwan farm, Egypt were used throughout this study. Rats were housed individually in stainless steel cages fitted with wire mesh bottoms and fronts in care of Laboratory animal house in the Faculty of Science-Ain Shams University Cairo, Egypt. Rats were housed under controlled conditions, temperature(23±1°C)and relative humidity ~60%, on a daily photoperiods of 12h light/dark cycle, and had free access to food and tap water. After one week as adaptation period, rats were divided into five groups(10 rats each)as follows:

Normal control group(NC): rats were fed on a standard diet AIN-76 diet(65%carbohydrate, 5%fat, 20%protein, vitamins, minerals and fiber5%)for 12 weeks.

Negative control group(LP): rats were fed on standard diet with 0.5%w/w LPE for 12 weeks.

Positive control group(PC): rats were fed on high fat AIN-76 diet(55%carbohydrate, 15%fat, 20%protein, vitamins, minerals and fiber5%)for 12 weeks.

Protective group(Prot): rats were fed on high fat diet together with 0.5%w/w LPE for 12 weeks.

Curative group(Curat): rats were fed on high fat diet for 12 weeks, then they were given LPE(0.5% w/w)for 45 days.

2.3 Body Weight, Food Intake and Blood Collection

The body weight of each rat was recorded twice weekly. Food intake was measured on per cage, basis 3 times per week and averages of food consumed were calculated weekly. At the end of the experimental period, rats were fasten for 12 hours, anesthetized and sacrificed. Blood samples were collected from the portal hepatic vein in dry clean centrifuge tubes with or without EDTA, for whole blood and serum samples respectively. Serum samples were stored at -80°C until analysis.

2.4 Preparation of Tissue, RNA Isolation and Real-Time PCR

Liver samples were taken in BIOZOL™ for the isolation of total mRNA and stored at -80°C for the single-step of RNA isolation method[28].

2.5 Biochemical Assay

The Polyphenolic components of LPE were separated by reversed phase HPLC using Agilent 1100 series column 18 (C18)[12-13]. Serum TC, HDL- cholesterol, triacylglycerol and blood glucose levels were estimated using commercial kits supplied by Reactivos GPL, Espana[14-16]respectively. Atherogenic index was calculated based on the following formula: $AI = (TC - HDL-C) / HDL-C$ [17]. Serum low density lipoprotein-cholesterol(LDL-C)and very low density lipoprotein-cholesterol(VLDL-C)were calculated by Friedewald's formula[18]. Phospholipid was estimated using a commercial kit supplied by Spectrum-Diagnostic, Egypt[19]. Insulin was measured by ELISA kit[20]. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated based on the following formula: $\text{fasting insulin } (\mu\text{UI/mL}) \times \text{fasting glucose (mM)} / 22.5$ [21]. Leptin and adiponectin were determined using ELISA kits[22-23]respectively. Total antioxidant capacity was estimated using a kit supplied by Spectrum-Diagnositc, Egypt[24].

2.6 Enzymes Assay

Glucose-6-phosphate dehydrogenase(G6PD)activity was determined using a kit supplied by Bio-Diagnostic, Egypt[25]. Acyl coenzyme A oxidase(ACO)and medium chain acyl coenzyme A dehydrogenase(MCAD)activities were measured using ELISA kits[26-27]respectively.

2.7 Histopathological Studies

The fixed tissues were then cut into suitable sections and processed for preparation of 5µm-thick paraffin sections. These sections were sequentially stained with Hematoxylin and Eosin. The slides were examined and photographed under a light microscope at a magnification power of x200.

2.8 Statistical Analysis

The results were expressed as mean±SD of ten rats per group and statistical significance was evaluated by one way ANOVA using SPSS(version 19.0)program. Values were considered statistically significant at *p* value less than 0.05.

3. Results

3.1 Lemon Peel Polyphenols Extract

Qualitative and quantitative determination of citrus LPE were performed by RP-HPLC analysis. The chromatogram of lemon peel methanolic extracts identified eleven polyphenols compounds; pyrogallol(39.1 mg/100g), salicylic acid(11.26 mg/100g), luteoline(6.21 mg/100g), *p*-coumaric acid (3.92 mg/100g), euganol(1.23 mg/100g), caffeic acid(1.19 mg/100g), resorcinol(1.05 mg/100g), quercetin(0.84 mg/100g), *p*-hydroxy benzoic acid(0.77 mg/100g), chrysin(0.50 mg/100g) and luteolin-3-methoxy-7-rutinos(0.40 mg/100g)(Table 1).

3.2 Effect of Lemon Peel Extract on Body Weight, Food Intake and Feed Efficiency Ratio

Table(2)showed that rats fed on a normal diet and supplement with LPE had non significant changes in body weight, food intake or feed efficiency ratio compared to normal control.

Positive control group showed highly significant increases($P<0.01$)in body weight and food intake by82.44 and 45.63%respectively, and significant elevation($P<0.05$)in feed efficiency by53.75 % compared to normal control group. Treated groups showed significant decreases($P<0.05$)in body weight, food intake and feed efficiency by35.30, 16.02 and 34.31%respectively for protective and by33.63, 9.21 and 32.52%for curative group respectively as compared to positive control group.

However, comparing the treated groups with normal control showed significant increases ($P<0.05$)in body weight and food intake by18.04 and 22.30%respectively in protective group and by 21.09 and 32.22 % respectively in curative group and non significant change in feed efficiency.

3.3 Effect of Lemon Peel Extract on Serum Lipid Profile

Table(3)revealed that supplementation with LPE to standard diet resulted in a moderate hypolipidemic effect manifested from the significant decrease in both TAG and VLDL-C by13.88 and 15.44% respectively compared to normal rats.

Positive control group showed significant increases($P<0.05$)in serum TC, TAG, VLDL-C, LDL-C, AI and phospholipids by112.96, 100.17, 100.14, 469.24, 455.77 and 176.96%respectively, and a significant decrease($P<0.05$)in HDL-C by35.33%compared to normal control group.

In treated groups, LPE induced significant decreases($P<0.05$)in the levels of TC, TAG, VLDL-C, LDL-C, AI and phospholipids levels, by41.52, 22.12, 22.11, 80.38, 78.03 and 21.23%respectively for protective group and by7.84, 15.12, 15.11, 29.97, 61.07 and 16.96%respectively for curative group compared to positive control group. In contrast, HDL-C level was significantly increased ($P<0.05$)by72.42% in the protective group and by90.59% in the curative group as compared to positive control group.

3.4 Effect of Lemon Peel Extract on Blood Glucose, Serum Insulin and the Homeostasis Model Assessment Insulin Resistance

Table(4)showed that group of rats fed on standard diet and supplement with LPE had non significant changes in glucose, insulin and the homeostasis model assessment insulin resistance(HOMA-IR) compared to normal control group.

In positive control group, there was a significant increases($P<0.05$)in blood glucose level by47.35%, serum insulin and HOMA-IR by68.21 and 124.13%respectively compared to normal control group.

However, comparing the treated groups with positive control showed significant decreases ($P<0.05$)in blood glucose, serum insulin and HOMA-IR by31.53, 34.52 and 50.37%respectively for protective group and by23.86, 32.71and 43.30%respectively for curative group.

3.5 Effect of Lemon Peel Extract on Hormones and Total Antioxidant Capacity

Table(4)demonstrated that rats fed on normal diet and supplement with LPE showed non significant changes in leptin, adiponectin and total antioxidant capacity(TAC)compared to normal control.

Positive control group showed a significant increase($P<0.05$)in leptin by81.87%, and significant decreases($P<0.05$)in adiponectin and TAC by23.23 and 29.09%respectively compared to normal control group.

However, comparing the treated groups with positive control showed a significant decrease ($P<0.05$) in leptin by 27.10% for protective and by 24.12% for curative group. On the other hand, adiponectin and TAC showed significant increases by 18.01 and 34.61% respectively for protective group, and by 9.91 and 30.77% for curative group respectively.

3.6 Effect of Lemon Peel Extract on Enzyme Activities

Table (5) revealed that rats fed on normal diet and supplement with LPE showed non significant changes in G6PD, ACO and MCAD activities compared to normal control.

Positive control group showed significant increases ($P<0.05$) in G6PD by 200.15%, in ACO by 563.28%; and in MCAD by 90.41% compared to normal control group.

Treated groups showed a significant decrease ($P<0.05$) in G6PD by 59.83% for protective group and by 24.38% for curative group; and significant increases ($P<0.05$) in serum ACO and MCAD by 79.78 and 167.07% respectively for protective and by 15.97 and 49.31% respectively for curative group as compared to positive control group.

3.7 Effect of Lemon Peel Extract on Acyl Coenzyme A Oxidase (ACO) Gene Expression

Data presented in Figure (1) showed that: LPE up-regulated the level of ACO mRNA in protective group with (2.69-fold induction) and in curative group with (2-fold induction) as compared to normal control group.

4. Discussion

In Egypt, obesity and cardiovascular risk factors are very prevalent among young adults [2]. Recent reports have outlined that administration of an efficient anti-obesity drug may decrease energy/food intake and increase energy expenditure, decrease preadipocyte differentiation and proliferation, decrease lipogenesis, and increase lipolysis and fat oxidation [3].

In the present study, the effect of dietary LPE on high-fat diet-induced obesity in rats was investigated. To achieve this aim, the regulatory effect of LPE administration on lipid profile, glucose, insulin, leptin, adiponectin and some lipid metabolism regulatory enzymes. In addition, LPE effect on the expression of the acyl coenzyme A oxidase gene was evaluated to elucidate some of its mechanism of action.

Polyphenolic compounds are a subject of considerable scientific and therapeutic interest mainly due to their antioxidant properties and scavenging of reactive oxygen species and free radicals as well as their implication in the prevention of many pathological diseases[29].

HPLC analysis of the phenolic compounds in the LPE revealed the presence of eleven components. Pyrogallol was the major compound, while luteolin-3-methoxy-7-rutinos was the least (Table 1). Flavonoids (Pyrogallol, quercetin, luteolin and chrysin) have been linked to reduce cancer, cardiovascular diseases, osteoporosis and many other health benefits[30]. Luteolin; a flavonoid with pharmacological benefits, including anti-oxidant, anti-inflammatory immuno-modulatory and anti-tumor effects[30]. Coumaric acid and caffeic acid are phenolic acids in many plant foods; protect against oxidative stress, inflammation, dyslipidemia and hypercoagulability[31]. Quercetin is the strongest anti-oxidant and has powerful anti-inflammatory benefits[30]. It has many beneficial health effects including improvement of cardiovascular health, reducing risk for cancer, protection against osteoporosis. This phytochemical modulates the expression of specific enzymes (glutathione transferase, aldo-keto reductase, glutamylcysteine-synthetase (GCS), glutathione-peroxidase and glutathione-reductase). Quercetin induces apoptosis and influences protein and lipid kinase signaling pathways. It is also a candidate for preventing obesity-related diseases[30].

Data presented in this study revealed that LPE in protective group reduced body weight and food intake (Table 2), this reduction may be caused by the active compounds of polyphenols such as pyrogallol, caffeic acid, quercetin and *p*-coumaric acid. Caffeic acid and quercetin are responsible for the suppressive effect on the body weight increase and fat accumulation by increasing the activities of different molecular pathways, which controls the expression of many genes involved in fatty acid oxidation[32]. These results are in agreement with **Shi et al.**[33] who stated that administering of pyrogallol acid (walnut polyphenols) decreased body weight and fat accumulation.

Abnormality in the metabolism of lipids and lipoproteins are very common conditions that take place with obese populations. The increase in cholesterol may potentially enhance the risk of fatty liver and atherosclerosis[34]. Oxidized LDL has been shown to be atherogenic and inhibition of the LDL oxidation by potent dietary antioxidants effectively attenuates atherosclerosis[35]. Oral supplementation of LPE effectively enhanced the variation in lipid profile in rats fed on high fat diet, in this context the protective effect of LPE was more pronounced than the curative effect (Table 3). This may be attributed to the anti-obesity effect of polyphenols in LPE, which have been shown to inhibit

cholesterol absorption and biosynthesis and to promote the expression of LDL-cholesterol receptors[33,36]. These findings were supported by the hepatic histopathological studies(Fig.5and 6) that showed reductions in hepatic lipid accumulation, fat vacuoles in groups consuming LPE.

High fat diet has been known to increase the liver mitochondrial reactive oxygen species (ROS) production. ROS causes cell damage via the mechanism involving lipid peroxidation that leads to tissue injury, especially in the liver[37]. Dietary polyphenols contain a number of phenolic hydroxyl groups and have demonstrated various beneficial effects, which is mainly due to their ROS scavenging activity[37]. The relation found between obesity and oxidative stress in this study showed that obesity induced by ingestion of high fat diet is the primary cause of lipid peroxidation. Therefore, the possible reason for improvement in obesity with LPE may be due to reduction in oxidative stress in HFD fed rats. In the present study, HFD-induced obesity showed decreased activities of total antioxidant capacity(Table4); therefore, it may be concluded that HFD causes the induction of oxidative stress in the hepatic and cardiac tissue that may lead to the consequences like fatty liver disease and atherosclerosis[37,38]. One of the constituent of LPE is pyrogalllic acid. It has better radical scavengers than many monomeric flavones and flavonols, due to the presence of pyrogalllic moiety as privileged radical-scavenging sites[39]. Caffeic acid has been employed as a natural antioxidant for inhibiting oxidation of lipids present in different food. Additionally, there are important redox cycles between caffeic acid and the endogenous reducing agents ascorbic acid and tocopherol which play an important role in the protective effects[40]. Administration of LPE showed a significant increase($P<0.05$)in total antioxidant capacity and decrease in the atherogenic index, in this context the protective effect of LPE was more pronounced than the curative effect. This could be explained that pyrogalllic acid, quercetin, luteolin, caffeic acid and *p*-coumaric acid have an antioxidant benefits and radical scavenging properties through their structure of molecules by hydroxycinnamates or hydroxyphenyl to inhibit oxidation of lipids[29].

The many metabolic pathways of glucose and the lipid metabolism in the liver are controlled by insulin, which regulates the hepatic glucose output and lipid synthesis[1]. With the increase in adipose tissue fat deposits, as in obesity, the ability of insulin to stimulate glucose transport and metabolism in adipocytes and skeleton muscle is impaired resulting in insulin resistance[41].

Therefore, any transformation in hepatic insulin sensitivity is rapidly reflected in glucose homeostasis and TG levels[42]. The observed reduction in the level of blood glucose, serum insulin and

HOMA-IR in LPE treated groups(Table4) may be due to the presence of quercetin in LPE which have direct effects on glucose metabolism, increasing insulin secretion and improvement in insulin resistance by promoting pancreatic β -cells regeneration[43].

Adipose-derived hormones, such as adiponectin and leptin play an important role in the regulation of lipid metabolism and the development of insulin resistance in human and rodents[42]. In this study, the observed increases in serum insulin and leptin levels in rats fed on high fat diet compared to those fed on standard diet may be related to the fact that serum leptin levels are directly proportional to adipose tissue weight[44]. However, serum insulin and leptin were significantly reduced($P<0.05$)in groups consuming LPE, although the levels were higher than the normal control group(Table4). These effects might be attributable to caffeic acid in LPE probably through reduction in the expression level of leptin in white adipose tissue, improvement of insulin resistance and increased fatty acid β -oxidation pathway[45].

Circulating adiponectin levels are negatively correlated with obesity, particularly visceral obesity and insulin resistance[46]. Results of this study revealed the enhancement in adiponectin concentration found in LPE fed groups relative to high fat group suggested that the expression and secretion of this adipokine was influenced by the reduced amount of body fat, leading to the decrease in the concentrations of plasma triglyceride and cholesterol[46].

One of the enzymes of pentose phosphate pathway is G6PD, which produce NADPH essential for the biosynthesis of fatty acid and cholesterol. Reduced activity of G6PD could limit the availability of fatty acids required for the synthesis of triglycerides[46-48]. In the present study, the reduction in G6PD activity(Table5)found in treated groups along with the reduction in triacylglycerol and total cholesterol levels; may be due to luteoline, caffeic acid and quercetin which affects the pentose phosphate pathway by reducing enzyme G6PD, NADPH as consequence; that is required for the biosynthesis of fatty acid and total cholesterol; leading to a rapid decline in fat stores[46-48].

In the last decade, it has become apparent that the expression of many lipid-metabolizing enzymes, including ACO and MCAD, is transcriptionally regulated by peroxisome proliferator-activated receptors (PPARs)[49]. Isoforms of the PPAR family of nuclear receptors are involved in the systemic regulation of lipid metabolism and serve as a sensor for fatty acids, prostanoid metabolites, eicosanoids and related molecules[50]. In this study the significant elevations in ACO and MCAD

activities(Table5)in the positive control group compared to normal may be attributed to fatty acid exerted as an autoregulatory and coordinate effect over gene expression, that could target peroxisomal or mitochondrial β -oxidation enzymes[51].

Peroxisome proliferator-activated receptor(PPAR)have many diverse functions, including the regulation of genes expression associated with glucose and lipid homeostasis[50]. The expressions of ACO and MCAD in liver, which play a key role in fatty acid oxidation, are regulated by several compounds derived from food as flavonoids[52]. The dramatic increase in the activities of ACO and MCAD was more pronounced in the protective group(1092.44 and 408.53%respectively) than curative group(699.18 and 184.30%respectively). In addition, ACO mRNA expression levels(Table5 and Figure1)showed the same pattern with 2.69-folds in protective group and 2-fold in curative group. These results may be due to the effect of quercetin and caffeic acid in LPE that activate and elevate PPAR α and PPAR δ expression which is known to increase expression of ACO leading to elevated energy expenditure, subsequently resulting in anti-obesity actions as stated by **Ali et al.**[53].

5. CONCLUSION

In conclusion, LPE may be a viable and promising prophylactic measure against obesity. The effect of LPE may protect rather than treat obesity consequently reducing risk of associated diseases as diabetes mellitus and atherosclerosis. The mechanism of action by which LPE induces its anti-obesity effect may be related to decreasing lipid accumulation, enhancing insulin sensitivity, total antioxidant capacity, down-regulation of G6PD and up-regulation of MCAD,ACO and its mRNA level.

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CAPTION:

Figure(1): The expression level of peroxisomal liver gene involved in β -oxidation, mRNA level of ACO in the liver in different studied groups. The expression level of gene was obtained by RT-PCR and calculated by the $2^{-\Delta\Delta C_t}$ method.

Figure(2): Photomicrographs of H&EX200-stained hepatocyte sections of a normal histological structure of the hepatic lobule for normal control rats.

Figure(3): H&EX200-stained sections of negative control group showed a normal histological structure of the hepatic lobule.

Figure(4): H&EX200-stained sections of positive control group showed diffuse glycogen infiltration.

Figure(5): H&EX200-stained sections of protective group showed mild swelling and granularity of

cytoplasm.

Fig (6): H&EX200-stained sections of curative hepatic lobule showed focal areas of glycogen infiltration with narrowing of hepatic sinusoid

Fig 1-

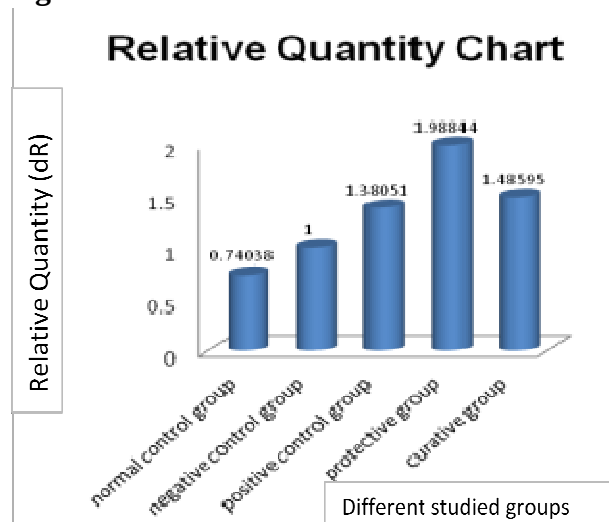


Fig 2-NC

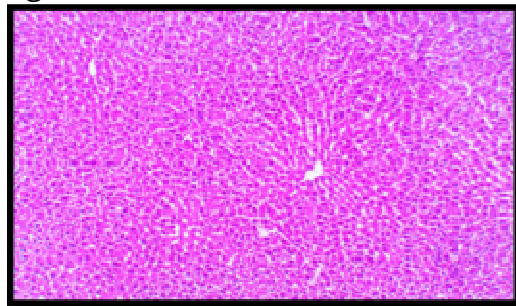


Fig 3-LP

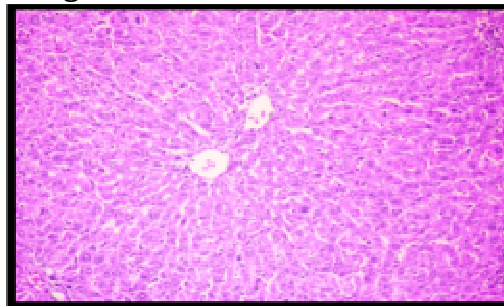


Fig 4-PC

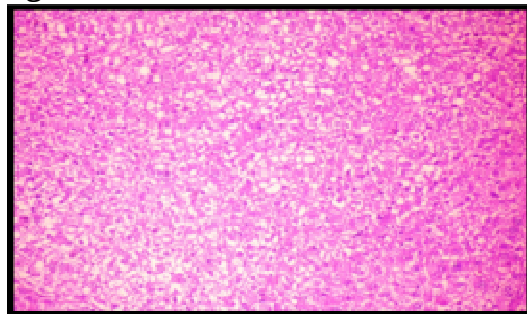


Fig 5-Prot.

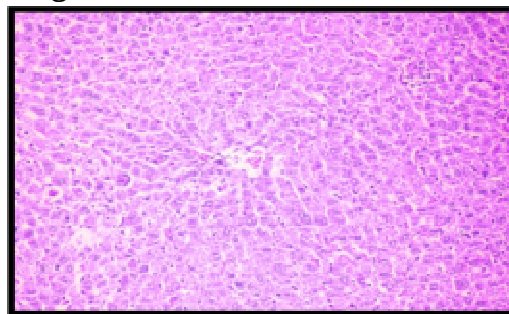


Fig 6-Curat.

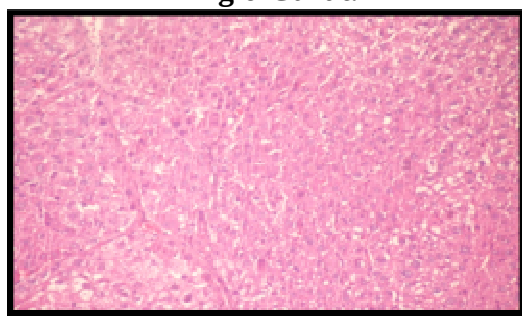


Table 1- HPLC analysis of lemon peel extract (polyphenols)

Peak No.	t _R (min)	Compound	Concentration (%)
1	3.4	Pyrogalllic	39.1%
2	5.3	Caffeic acid	1.19%
3	6.0	Euganol	1.23%
4	7.8	Salicylic acid	11.26%
5	8.1	p -hydroxy benzoic acid	0.77%
6	8.9	Resorcinol	1.05%
7	13.4	p -coumaric acid	3.92%
8	18.0	Luteoline	6.21%
9	18.3	Quercetin	0.84%
10	30.2	Chrysin	0.50%
11	31.1	Luteolin-3-meth-oxy-7- rutinoside	0.40%

Table 2- Weight gain, food intake, feed efficiency in different studied groups.

Parameters	NC	LP	PC	Prot	Curat
Weight gain (g)					
mean $\pm$ SD	61.63 $\pm$ 8.21	64.50 $\pm$ 9.17	112.44 $\pm$ 6.20 ^a	72.75 $\pm$ 6.01 ^{ab}	74.63 $\pm$ 7.46 ^{ab}
%Change from NC		4.66 $\uparrow$	82.44 $\uparrow$	18.04 $\uparrow$	21.09 $\uparrow$
%Change from PC				35.30 $\downarrow$	33.63 $\downarrow$
Food intake (g/day)					
mean $\pm$ SD	13.50 $\pm$ 0.93	15.31 $\pm$ 1.58	19.66 $\pm$ 1.26 ^a	16.51 $\pm$ 2.37 ^{ab}	17.85 $\pm$ 0.65 ^a
%Change from NC		13.41 $\uparrow$	45.63 $\uparrow$	22.30 $\uparrow$	32.22 $\uparrow$
%Change from PC				16.02 $\downarrow$	9.21 $\downarrow$
Feed efficiency ratio					
mean $\pm$ SD	4.00 $\pm$ 0.54	4.02 $\pm$ 0.96	6.15 $\pm$ 1.41 ^a	4.04 $\pm$ 0.81 ^b	4.15 $\pm$ 0.61 ^b
%Change from NC		0.50 $\uparrow$	53.75 $\uparrow$	1.00 $\uparrow$	3.75 $\uparrow$
%Change from PC				34.31 $\downarrow$	32.52 $\downarrow$

(a) Statistical significant from normal control group at $p < 0.05$.

(b) Statistical significant from positive control group at $p < 0.05$.

NC: Normal Control.

PC: Positive control.

LP: Lemon peel.

Prot: Protective.

Curat: Curative.

Table 3- Level of serum lipid profile in different studied groups.

Parameters	NC	LP	PC	Prot.	Curat.
TAG (mg/dl)					
mean $\pm$ SD	144.13 $\pm$ 51.95	124.13 $\pm$ 33.50	288.50 $\pm$ 10.17 ^a	224.69 $\pm$ 11.60 ^{ab}	244.88 $\pm$ 48.36 ^{ab}
%Changefrom NC		13.88 $\downarrow$	100.17 $\uparrow$	55.89 $\uparrow$	69.90 $\uparrow$
%Change from PC				22.12 $\downarrow$	15.12 $\downarrow$
T-CHL (mg/dl)					
mean $\pm$ SD	99.45 $\pm$ 8.17	101.40 $\pm$ 10.30	211.79 $\pm$ 8.59 ^a	123.86 $\pm$ 3.95 ^{ab}	195.18 $\pm$ 5.12 ^{ab}
%Changefrom NC		1.96 $\uparrow$	112.96 $\uparrow$	24.54 $\uparrow$	96.26 $\uparrow$
%Change from PC				41.52 $\downarrow$	7.84 $\downarrow$
VLDL-C (mg/dl)					
mean $\pm$ SD	28.83 $\pm$ 10.39	24.38 $\pm$ 6.70	57.70 $\pm$ 2.03 ^a	44.94 $\pm$ 2.32 ^{ab}	48.98 $\pm$ 9.67 ^{ab}
%Changefrom NC		15.44 $\downarrow$	100.14 $\uparrow$	55.88 $\uparrow$	69.89 $\uparrow$
%Change from PC				22.11 $\downarrow$	15.11 $\downarrow$
LDL-C (mg/dl)					
mean $\pm$ SD	21.49 $\pm$ 2.80	20.55 $\pm$ 9.96	122.33 $\pm$ 10.53 ^a	24.00 $\pm$ 3.44 ^b	85.67 $\pm$ 13.97 ^{ab}
%Changefrom NC		4.37 $\downarrow$	469.24 $\uparrow$	11.68 $\uparrow$	298.65 $\uparrow$
%Change from PC				80.38 $\downarrow$	29.97 $\downarrow$
HDL-C (mg/dl)					
mean $\pm$ SD	49.11 $\pm$ 4.40	52.17 $\pm$ 5.72	31.76 $\pm$ 4.45 ^a	54.76 $\pm$ 3.83 ^b	60.53 $\pm$ 5.85 ^{ab}
%Changefrom NC		6.23 $\uparrow$	35.33 $\downarrow$	11.50 $\uparrow$	23.25 $\uparrow$
%Change from PC				72.42 $\uparrow$	90.59 $\uparrow$
Atherogenic Index					
mean $\pm$ SD	1.04 $\pm$ 0.25	0.96 $\pm$ 0.28	5.78 $\pm$ 0.92 ^a	1.27 $\pm$ 0.12 ^{ab}	2.25 $\pm$ 0.32 ^{ab}
%Changefrom NC		7.69 $\downarrow$	455.77 $\uparrow$	22.12 $\uparrow$	116.35 $\uparrow$
%Change from PC				78.03 $\downarrow$	61.07 $\downarrow$
Phospholipids (mg/dl)					
mean $\pm$ SD	103.13 $\pm$ 62.22	101.25 $\pm$ 63.13	285.63 $\pm$ 58.26 ^a	225.00 $\pm$ 35.58 ^{ab}	237.19 $\pm$ 72.38 ^{ab}
%Changefrom NC		1.82 $\downarrow$	176.96 $\uparrow$	118.17 $\uparrow$	129.99 $\uparrow$
%Change from PC				21.23 $\downarrow$	16.96 $\downarrow$

(a)Statistical significant from normal control group at $p<0.05$.

(b) Statistical significant from positive control group at $p<0.05$.

NC: Normal Control.
Curative.

PC: Positive control.

LP: Lemon peel.

Prot: Protective.

Curat:

Table 4- Levels of total antioxidant capacity, blood glucose, serum insulin, homeostasis model assessment insulin resistance (HOMA-IR), serum leptin and adiponectin in different studied groups.

Parameters	NC	LP	PC	Prot.	Curat.
TAC (mM/L)					
mean $\pm$ SD	1.10 $\pm$ 0.37	1.16 $\pm$ 0.17	0.78 $\pm$ 0.19 ^a	1.05 $\pm$ 0.14 ^b	1.02 $\pm$ 0.09 ^b
%ChangefromNC		5.45 $\uparrow$	29.09 $\downarrow$	4.55 $\downarrow$	7.27 $\downarrow$
%Changefrom PC				34.61 $\uparrow$	30.77 $\uparrow$
Glucose (mg/dl)					
mean $\pm$ SD	89.31 $\pm$ 5.46	91.55 $\pm$ 5.09	131.60 $\pm$ 5.46 ^a	90.11 $\pm$ 3.55 ^b	100.20 $\pm$ 5.86 ^{ab}
%ChangefromNC		2.51 $\uparrow$	47.35 $\uparrow$	0.90 $\uparrow$	12.19 $\uparrow$
%Changefrom PC				31.53 $\downarrow$	23.86 $\downarrow$
Insulin (uIU/ml)					
mean $\pm$ SD	24.63 $\pm$ 5.18	26.88 $\pm$ 8.48	41.43 $\pm$ 8.72 ^a	27.13 $\pm$ 8.97 ^b	27.88 $\pm$ 5.17 ^b
%ChangefromNC		9.14 $\uparrow$	68.21 $\uparrow$	10.15 $\uparrow$	13.20 $\uparrow$
%Changefrom PC				34.52 $\downarrow$	32.71 $\downarrow$
HOMA-IR					
mean $\pm$ SD	5.43 $\pm$ 1.34	6.08 $\pm$ 2.09	12.17 $\pm$ 2.48 ^a	6.04 $\pm$ 2.00 ^b	6.90 $\pm$ 0.92 ^b
%ChangefromNC		11.97 $\uparrow$	124.13 $\uparrow$	11.23 $\uparrow$	27.07 $\uparrow$
%Changefrom PC				50.37 $\downarrow$	43.30 $\downarrow$
Leptin (ng/ml)					
mean $\pm$ SD	27.63 $\pm$ 9.29	27.88 $\pm$ 11.37	50.25 $\pm$ 6.36 ^a	36.63 $\pm$ 7.60 ^{ab}	38.13 $\pm$ 8.43 ^{ab}
%ChangefromNC		0.90 $\uparrow$	81.87 $\uparrow$	32.57 $\uparrow$	38.00 $\uparrow$
%Changefrom PC				27.10 $\downarrow$	24.12 $\downarrow$
Adiponectin (μg/ml)					
mean $\pm$ SD	173.58 $\pm$ 3.70	176.97 $\pm$ 2.40	133.25 $\pm$ 4.77 ^a	157.25 $\pm$ 4.81 ^a	146.54 $\pm$ 6.28 ^a
%ChangefromNC		1.95 $\uparrow$	23.23 $\downarrow$	9.41 $\downarrow$	9.97 $\downarrow$
%Changefrom PC				18.01 $\uparrow$	9.91 $\uparrow$

(a)Statistical significant from normal control group at $p<0.05$.

(b) Statistical significant from positive control group at $p<0.05$.

NC: Normal Control. PC: Positive control. LP: Lemon peel. Curative.

Prot: Protective.

Curat:

Table 5- Glucose-6-phosphate dehydrogenase(G6PD), peroxisomal acyl-coenzyme A oxidase(ACO), mitochondria medium-chain acyl-coenzyme A dehydrogenase(MCAD) enzymatic activities and hemoglobin level in different studied groups.

Parameters	NC	LP	PC	Prot.	Curat.
G6PD (U/g Hb)					
mean $\pm$ SD	6.71 $\pm$ 1.10	7.14 $\pm$ 1.39	20.14 $\pm$ 7.17 ^a	8.09 $\pm$ 1.08 ^{ab}	15.23 $\pm$ 2.05 ^{ab}
%ChangefromNC		6.41 $\uparrow$	200.15 $\uparrow$	20.57 $\uparrow$	126.97 $\uparrow$
%Changefrom PC				59.83 $\downarrow$	24.38 $\downarrow$
ACO (ng/ml)					
mean $\pm$ SD	35.43 $\pm$ 8.48	40.47 $\pm$ 6.76	235.00 $\pm$ 63.52 ^a	422.48 $\pm$ 128.14 ^{ab}	272.52 $\pm$ 63.20 ^{ab}
%ChangefromNC		14.23 $\uparrow$	563.28 $\uparrow$	1092.44 $\uparrow$	699.18 $\uparrow$
%Changefrom PC				79.78 $\uparrow$	15.97 $\uparrow$
MCAD (ng/ml)					
mean $\pm$ SD	10.32 $\pm$ 2.32	12.64 $\pm$ 3.31	19.65 $\pm$ 9.95 ^a	52.48 $\pm$ 36.82 ^{ab}	29.34 $\pm$ 11.10 ^{ab}
%ChangefromNC		22.48 $\uparrow$	90.41 $\uparrow$	408.53 $\uparrow$	184.30 $\uparrow$
%Changefrom PC				167.07 $\uparrow$	49.31 $\uparrow$
Hb (g/dl)					
mean $\pm$ SD	14.25 $\pm$ 1.49	14.63 $\pm$ 1.30	12.25 $\pm$ 1.49	12.88 $\pm$ 2.10	12.38 $\pm$ 1.77
%ChangefromNC		2.67 $\uparrow$	14.04 $\downarrow$	9.61 $\downarrow$	13.12 $\downarrow$
%Changefrom PC				5.14 $\uparrow$	1.06 $\uparrow$

(a)Statistical significant from normal control group at $p<0.05$.

(b) Statistical significant from positive control group at $p<0.05$.

NC: Normal Control. PC: Positive control. LP: Lemon peel.

Prot: Protective.

Curat: Curative.