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. Some of the LPE mechanism of action was also elucidated. Fifty adult rats were divided into five groups: 1) normal control, 2) lemon peel extract group supplemented with 0.5 g% LPE for 12 weeks, 3) high fat diet-fed for 12 weeks, 4) preventive group fed on high fat diet supplemented with LPE 0.5 g% for 12 weeks and 5) therapeutic group fed on high fat diet for 12 weeks then supplemented with LPE 0.5 g% further 6 weeks.

Methodology: Body weight gain, feed efficiency ratio, serum lipid profile, serum glucose, serum insulin, erythrocytes 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 LPE-treated groups. Additionally mRNA level of ACO in the liver was up-regulated in LPE-treated groups. compared with HFD.

Conclusion: These findings demonstrate that LPE prevent body weight gain and fat accumulation through improvement of lipid metabolism by up-regulating the activities of MCAD and ACO while down regulating the activity of glucose 6 phosphate dehydrogenase. In this context, the preventive effect of LPE was more pronounced than the therapeutic effect.

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

1. INTRODUCTION

The incidence of obesity and its related metabolic disorders has escalated dramatically worldwide, including the populations living in the developing countries, and is becoming a key public health issue with a massive burden to the healthcare system [1]. Obesity increases the morbidity rates of a number of metabolic disorders, such as type 2 diabetes mellitus, hypertension and cardiovascular diseases [1]. Obesity is a disease characterized by excess body weight, associated with a state of chronic subclinical inflammation, caused by an increased secretion of adipokines such as leptin and adiponectin that modulate certain responses in the body **[**2**].** In Egypt, obesity is raising among young adults, overtime these conditions, especially if associated with poor dietary habits, smoking or physical inactivity, will lead to cardiovascular diseases later in adulthood [3].

Because diets containing high-fat foods are becoming common, it is necessary to find suitable alternatives, such as phytochemicals, to ameliorate the effect of a high fat diet, thereby reducing the risk associated with obesity [4].

Over the years, many medications have been used to manage obesity, but most of them are now withdrawn due to their serious adverse effects [5].

In recent years, much attention has been paid to vegetables and fruits as novel protective and even therapeutic strategies for management of obesity and several diseases due to their phytochemical constituents [6]. Phytochemicals are known to possess antioxidant properties with the effective anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities with low adverse effects [7]. Phytochemicals such as flavonoids may help to prevent or reduce obesity through the regulation of different molecular pathways [8-9].

Citrus fruits contain various kinds of flavonoids such as flavanone glycosides, flavones glycoside and polymethoxy flavones [8]. Lemon plant (*Citrus aurantifolia*) belongs to the Rutaceae family, and is the third most important Citrus species after orange and mandarin. Crude extracts of different parts of lemon (leaves, stem, root and flower) rich in flavonoid glycosides and polyphenols that have a number of positive health effects in the prevention of lifestyle-related diseases and have antiobesity effects [9-10].

Previous studies have demonstrated the effects of Citrus limon flavonoids on lipid and glucose metabolism, specifically on lipid catabolism, glucose transport, the insulin-receptor function, and peroxisome proliferator-activated receptors (PPARs) activation, all of which play essential roles in weight control [11-12].

In this study, the effect of supplementation with 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 has been explored. In addition, LPE effect on the mRNA expression of the acyl coenzyme A oxidase (ACO) gene was evaluated.

2. MATERIALS AND METHODS

2.1 Lemon Peels Extract Preparation

Fresh lemons (*Citrus aurantifolia*) were obtained from the local market, washed with distilled water; then dried at 60°C, grounded peeled and finely extract was diluted in appropriate concentration 50% with 70% methanol a shaking water bath at 60°C. The extract was clarified by centrifugation at 3000 r.p.m for 20 minutes followed by four times filtrations. Finally, the supernatant was stored in the dark [13]. The constitutent LPE was analyzed by high performance liquid chromatography (HPLC).

2.2 RP-HPLC Analysis

The Polyphenolic components of LPE were identified by Agilent 1100 series Model HPLC apparatus (Germany) was coupled with an UV-V is multi-wavelength detector set at 280 nm. Chromatographic separation of dissolved extracts was performed on a Zorbax SB-C18 column (Agilent Technologies, USA). The mobile phase composed of acetonitrile: phosphate buffer (pH 4.5, 0.01M) in the ratio of (65:35; v/v) at a flow rate of 0.5 ml/min. Five µg of the stem extract were diluted in 1 ml of methanol (HPLC grade) using the protocol described by **Miyake** [14] & **Perdetzoglou et al.** [15] which occurred in Faculty of Agriculture-Faculty of Agriculture Research (FARP)-Cairo University**.** The injection volume was twenty µl and peaks were identified by comparing the retention times of the samples with those of the known standards.

2.3 Experimental Design and Dietary Treatment

Fifty adult male Swiss albino rats, weighing 100-120 g 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, Entomology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. After one week as an adaptation period, the animals were divided into five equal groups (10 rats each) as follows:

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

Lemon peel extract group (LPE): rats were fed a standard diet enriched with 0.5 g% (w/w) LPE for 12 weeks.

High fat diet group (HFD): rats were fed a high fat AIN-76 diet (55% carbohydrate, 15% fat, 20% protein, vitamins, minerals and fiber 5%) for 12 weeks serving as negative control.

Preventive group: rats were fed a high fat diet enriched with 0.5 g% (w/w) LPE for 12 weeks.

Therapeutic group: rats were fed a high fat diet for 12 weeks, then they were fed a high fat diet supplemented with LPE 0.5 g% (w/w) further 6 weeks.

2.4 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 fasted for 12 hours, anesthetized and sacrificed. All animal experiments were performed under protocol approved by the Local Institutional Animal Ethics Committee of Ain Shams University. 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 -80ºC until analysis.

2.5 Biochemical Assay

Feed efficiency ratio was calculated based on the following formula: **Feed efficiency ratio**= body weight gain/total food intake during experiment period [16]**.** Serum TC, HDL- C, TG and serum glucose levels were estimated using commercial kits supplied by Reactivos GPL, Espana [17-20] respectively. Atherogenic index was calculated based on the following formula: AI= (TC- HDL-C)/ HDL-C [21]. Serum low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) were calculated by Friedewald's formula [22]. Serum phospholipids were estimated using a commercial kit supplied by Spectrum-Diagnostic, Egypt [23]. Serum insulin was measured by ELISA kit [24]. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated based on the following formula: fasting insulin (μUI/mL) × fasting glucose (mM)/22.5 [25]. Serum leptin and adiponectin were determined using rat ELISA kits [26-27] respectively. Serum total antioxidant capacity was estimated using a kit supplied by Spectrum-Diagnositc, Egypt [28]. Erythrocytes glucose-6-phosphate dehydrogenase (G6PD) activity was determined using a kit supplied by Bio-Diagnostic, Egypt [29]. Serum acyl coenzyme A oxidase (ACO) and

serum medium chain acyl coenzyme A dehydrogenase (MCAD) activities were measured using ELISA kits [30-31] respectively.

2.6 RNA Isolation from Liver and Real Time PCR

Total RNA from the liver tissue was extracted with BIOZOL™ Reagent (BioFlux, South San Francisco, U.S.A) [32]. 1 µg of extracted RNA was used for reverse transcription by RevertAid™ First Strand cDNA synthesis Kit™ (Fermentas life science Co, Van Allen Way, Canada) [33] using oligo (dt) primer for 1 hr at 42˚C according to manufacturer instructions [33]. The relative expression of the ACO gene was analyzed by STRATAGENE MX3000P apparatus maxima SYBR Green/ROX qPCR master mix utilizing ACO gene specific (NCBI accession no. NM_017340) primer (5′-CTTTCTTGCTTGCCTTCCTTCTCC-3′, Forward p) and (5′- GCCGTTTCACCGCCTCGTA-3′, Reverse p). And including GAPDH gene as a reference gene to normalize the expression data (5′-TGCACCACCAACTGCTTAGC-3′, Forward p) and (5′-GGCATGGACTGTGGTCATGAG-3′, Reverse p) with cycling conditions for both genes as follows: denaturing step at 95˚C for 2 min, followed by 40 cycles of 95˚C for 15 s, and 60˚C for 1 min [34]. After completing the run melting curve analysis was done to ensure specific amplification (Fig.1). The relative expression and fold changes were calculated according to the formula $2^{-\Delta\Delta Ct}$ [35].

2.7 Histological Studies

Immediately after scarifying rats, livers were removed, washed with chilled physiological saline (0.9% w/v), dried between filter papers then stored in 10% neutral buffered formalin for histological examination. 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 analysis of variance (ANOVA) post hoc Duncan's test to determine the significant differences between means using SPSS program (version 19.0). 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 constituents was performed by RP-HPLC analysis. The chromatogram of lemon peels methanolic extract as shown in Table (1), Fig. (2) identified eleven polyphenolic compounds; pyrogallic (39.1 mg/100g), salicylic acid (11.26 mg/100g), luteoline (6.21 mg/100g), ρ -coumaric acid (3.92 mg/100g), eugenol (1.23 mg/100g), caffeic acid (1.19 mg/100g), resorcinol (1.05 mg/100g), quercetin (0.84 mg/100g), ρ -hydroxy benzoic acid (0.77 mg/100g), chrysin (0.50 mg/100g) and luteolin-3-methoxy-7-rutinoside (0.40 mg/100g).

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

Table (2) showed that rats fed on a standard diet and supplement with LPE had non significant changes in body weight, food intake or feed efficiency ratio compared to the normal control group. The high fat diet group showed highly significant increases ($P<0.01$) in body weight and food intake by 82.44 and 45.63%, respectively, and a significant elevation ($P<0.05$) in feed efficiency by 53.75 % compared to the normal control group. The LPE-treated groups showed significant decreases ($P<0.05$) in body weight, food intake and feed efficiency ratio by 35.30, 16.02 and 34.31%, respectively, for the preventive group, and by 33.63, 9.21 and 32.52% for the therapeutic group, respectively, as compared to the HFD-group. However, comparing the LPE-treated groups with the normal control group showed significant increases ($P<0.05$) in body weight and food intake by 18.04 and 22.30 %, respectively, in the preventive group, and by 21.09 and 32.22 %, respectively, in the therapeutic group, while showing a non significant change in feed efficiency ratio.

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 by the significant decrease in both serum TG and VLDL-C by 13.88 and 15.44%, respectively, compared to normal rats.

The HFD-group showed significant increases ($P<0.05$) in serum TG, TC, VLDL-C, LDL-C, AI and phospholipids by 100.17, 112.96, 100.14, 469.24, 455.77 and 176.96%, respectively, while showing a significant decrease ($P<0.05$) in HDL-C by 35.33% compared to the normal control group. In LPE-treated groups, there were significant decreases ($P<0.05$) in serum TG, TC, VLDL-C, LDL-C, AI and phospholipids levels, by 22.12, 41.52, 22.11, 80.38, 78.03 and 21.23%, respectively, in the preventive group, and by 7.84, 15.12, 15.11, 29.97, 61.07 and 16.96%, respectively, in the therapeutic group compared to the HFD-group. In

contrast, HDL-C level was significantly increased ($P<0.05$) by 72.42% in the preventive group and by 90.59% in the therapeutic group as compared to the HFD-group.

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

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

In the HFD-group, there were significant increases ($P<0.05$) in serum glucose level by 47.35%, serum insulin and HOMA-IR by 68.21 and 124.13%, respectively, compared to the normal control group. However, comparing the LPE-treated groups with the HFD-group showed significant decreases ($P<0.05$) in serum glucose, serum insulin and HOMA-IR by 31.53, 34.52 and 50.37%, respectively, in the preventive group, and by 23.86, 32.71 and 43.30%, respectively, in the therapeutic group.

3.5 Effect of Lemon Peel Extract on Serum Adiponectin and Serum Leptin Levels as well as Serum Total Antioxidant Capacity

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

The HFD-group showed a significant increase ($P<0.05$) in serum leptin by 81.87%, and significant decreases ($P<0.05$) in serum adiponectin and TAC by 23.23 and 29.09 %, respectively, compared to the normal control group. However, comparing the LPE-treated groups with the HFD-group showed a significant decrease ($P<0.05$) in serum leptin by 27.10% in the preventive group and by 24.12% in the therapeutic group. On the other hand, adiponectin and TAC levels showed significant increases by 18.01 and 34.61%, respectively, in the preventive group, and by 9.91 and 30.77%, in the therapeutic group, respectively.

3.6 Effect of Lemon Peel Extract on Some Lipid-Metabolizing Enzyme Activities

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

The HFD-group showed significant increases ($P<0.05$) in erythrocytes G6PD by 200.15%, serum ACO by 563.28% and serum MCAD by 90.41%, compared to the normal control group. The LPE-Treated groups showed a significant decrease ($P<0.05$) in G6PD by 59.83% in the preventive group, and by 24.38% in the therapeutic group, while showing significant increases ($P<0.05$) in serum ACO and MCAD by 79.78 and

167.07%, respectively, in the preventive group, and by 15.97 and 49.31%, respectively, in the therapeutic group as compared to the HFD-group.

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

ACO gene expression levels were measured in liver tissues by RT-PCR using STRATAGENE MX3000P instrument. Data presented in Figure (3 and 4) LPE showed that: a highly significant up-regulated in the expression of ACO mRNA level in the preventive group by 2-fold, p value=0.001 and in the therapeutic group by 1.5-fold, p value=0.001 as compared to the normal control group.

3.8 Histological examination of liver

Histopathological examination of normal control and LPE rats revealed normal histological structure of hepatic lobules, normal centrally round nucleus and homogeneous cytoplasm Figure (5 and 6). On the other side, liver of the HFD-group showed vacuolation of hepatic cells cytoplasm and some time appears foamy which indicate glycogen infiltration Figure (7). The effect of lemon peel extract (polypehonls) on hepatocyte cells of the preventive group revealed mild swelling of hepatocytes and granularity of cytoplasm Figure (8). Focal area of glycogen infiltration with narrowing of hepatic sinusoids was seen in the therapeutic group Figure (9).

4. Discussion

In the present study, HPLC analysis of the phenolic compounds in the LPE revealed the presence of eleven components; pyrogallic, salicylic acid, luteoline, ρ-coumaric acid, eugenol, caffeic acid, resorcinol, quercetin, ρ-hydroxy benzoic acid, chrysin and luteolin-3-methoxy-7-rutinoside. Pyrogallic was the major compound, while luteolin-3-methoxy-7-rutinos was the least (Table 1). Polyphenol (pyrogallic) and flavonoids (quercetin, luteolin and chrysin) have been linked to reduce obesity [36] and cardiovascular diseases [37]. Luteolin; a flavonoid with pharmacological benefits, including anti-oxidant, anti-inflammatory immuno-modulatory and anti-tumor effects [37]. Coumaric acid and caffeic acid are phenolic acids in many plant foods protecting against oxidative stress, inflammation, dyslipidemia and hypercoagulability [38]. Quercetin is the strongest anti-oxidant and has powerful anti-inflammatory benefits [37]. It has many beneficial health effects including improvement of cardiovascular health, preventing obesity-related diseases and protection against osteoporosis [37-38]. Quercetin induces apoptosis and influences protein and lipid kinase signaling pathways. It is also a candidate for preventing obesity-related diseases [37].

In this study, the reduction of body weight and food intake revealed that supplementation with LPE in the preventive group as well as the therapeutic group reduced body weight and food intake as compared to the HFD-group (Table 2). This may be due to the effect of pyrogallic acid and quercetin in LPE. These results are in agreement with **Shi et al.** [36] and **Huang et al.** [39] who reported that quercetin (black tea extract) and pyrogallic acid (walnut polyphenols) decreased body weight and fat accumulation.

Abnormality in the metabolism of lipids and lipoproteins are very common conditions that take place in obese populations. The increase in total cholesterol may potentially enhance the risk of fatty liver and atherosclerosis [40]. Oxidized LDL has been shown to be atherogenic and inhibition of the LDL oxidation by potent dietary antioxidants effectively attenuates atherosclerosis [41]. Oral supplementation of LPE effectively enhanced obesity induced variation in lipid profile in rats fed high fat diet. In this context, the preventive effect of LPE was more pronounced than the therapeutic effect (Table 3). This may be attributed to the anti-obesity effect of polyphenols in LPE (especially pyrogallic acid, coumaric acid and quercetin), which have been shown to inhibit cholesterol absorption and biosynthesis and to promote the expression of LDL-cholesterol receptors [36, 42]. These findings were supported by the hepatic histological studies (Fig. 8 and 9) that showed reductions in hepatic lipid accumulation and fat vacuoles in LPE-treated groups.

It has been reported that high fat diet increase the liver mitochondrial ROS production. ROS causes cell damage via the mechanism involving lipid peroxidation that leads to tissue injuries, especially in the liver [43]. In the current study the reduction in the levels of serum TAC (Table 4) together with the elevation in the lipid contents in the HFD-group of rats as compared to the normal control group indicated an increase in the lipid peroxidation rates. On the other hand, the increase in serum TAC resulted from the supplementation of LPE to the high fat diet either in preventive (34.61%) or therapeutic groups (30.77%) supported the antioxidant effect of its major component, pyrogallic (39.1mg/100g) rich in phenolic hydroxyl groups and consequently its ability to reduce oxidative stress. This finding confirmed that of **Cui et al.** [43] **& Hatia et al.** [44] **& Romelle et al.** [45] which showed that dietary polyphenols such as caffeic acid (Huangshan Maofeng green tea), ρ -coumaric acid, ρ -hydroxy benzoic acid, caffeic acid and quercetin (major dietary polyphenols) and quercetin (Statroltea) contain a number of phenolic hydroxyl groups and have demonstrated various beneficial effects, which is mainly due to their ROS scavenging activity.

The metabolic pathways of glucose and 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 [46]. Therefore, any transformation in hepatic insulin sensitivity is rapidly reflected in glucose homeostasis and TG levels [47]. The observed reduction in the level of serum glucose, serum insulin and HOMA-IR in LPE-treated groups (Table 4) may be through depletion of the adipose tissue triglycerides stores that ultimately results in reductions of lipid levels due to the presence of quercetin, caffeic acid, ρ-hydroxy benzoic acid and ρ–coumaric acid in LPE which has direct effects on glucose metabolism, increasing insulin secretion and improving in insulin resistance [48] and increased fatty acid β-oxidation pathway [49].

Obese people are said to be resistant to the effects of leptin, in the same way that people with type 2 diabetes are resistant to the effects of insulin. The high sustained concentrations of leptin from the enlarged adipose stores result in leptin desensitization. The pathway of leptin control in obese people might be flawed at some points, so the body doesn't adequately receive the satiety feeling subsequent to eating [47]. In this study, the observed increases in serum insulin and leptin levels in rats fed high fat diet compared to those fed standard diet may be related to the fact that serum leptin levels are directly proportional to adipose tissue weight [50]. However, serum insulin and leptin were significantly reduced ($P<0.05$) in LPE-treated groups, although the levels were higher than the normal control group (Table 4). These effects may be attributed 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 [51]. It is well known that circulating adiponectin levels are negatively correlated with obesity, particularly visceral obesity and insulin resistance [52]. 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 TG and TC [52].

One of the enzymes of pentose phosphate pathway is G6PD which convert Glucose-6-phosphate into 6-phosphglucono-δ-lactone, that supplied reducing energy to cells (such as erythrocytes) by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is 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 TG [48, 52-53]. The NADPH in turn maintains the level of glutathione in these cells that helps protect the red blood cells against oxidative damage [52]. In the present study, the reduction in erythrocytes G6PD activity (Table 5) found in LPE-treated groups along with the reduction in TG and TC levels supported the anti-obesity effect of LPE due to its content of ρ-hydroxy benzoic acid, ρ–coumaric acid, luteoline, caffeic acid and quercetin. These results are in agreement with **Chung et al.** [48] **& Adem et al.**

[52] **& Kumar et al.** [53] who stated that ρ-hydroxy benzoic acid, ρ–coumaric acid and caffeic acid (Cooked Giant Embryonic Rice), caffeic acid (some phenolic compound) and quercetin (Fenugreek Seed Extract) affects the pentose phosphate pathway by reducing G6PD activity, NADPH as a consequence; that is required for the biosynthesis of fatty acid and TC; leading to a rapid decline in fat stores.

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) [54]. Isoforms of 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 [55]. In the present study, the significant elevations in serum ACO and MCAD activities (Table 5) in the high fat diet-group compared to the normal control group may be attributed to the increase of fatty acids that exert as an auto-regulatory and coordinate effect over gene expression that could target peroxisomal or mitochondrial β-oxidation enzymes [56].

PPARs have many diverse functions, including the regulation of genes expression associated with glucose and lipid homeostasis [55]. 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 such as flavonoids [57]. The dramatic increase in the activities of ACO and MCAD was more pronounced in the preventive group (1092.44 and 408.53%, respectively) than the therapeutic group (699.18 and 184.30%, respectively). In addition, ACO mRNA expression levels (Fig. 3 and 4) showed the same pattern with 2-fold increase in the preventive group and 1.5-fold increase in the therapeutic group. This may be due to the presence of quercetin and caffeic acid in LPE which are known to activate and elevate PPARα and PPARδ expression which are known to increase expression of ACO leading to elevated energy expenditure, subsequently resulting in anti-obesity actions [58].

5. CONCLUSION

LPE may be a promising natural measure against obesity. The effect of LPE may protect rather than treat obesity consequently reducing risk of obesity associated diseases as atherosclerosis and diabetes mellitus. 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-regulating G6PD activity while up-regulating of MCAD and ACO activities; and ACO mRNA expression level.

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CAPTION:

Fig. 1- The melting curve analysis.

Fig. 2- HPLC Chromatogram analysis of lemon peel extract at 280 nm. The presented numbers on the chart indicats the lemon peel componants as follows: 1: pyrogallic; 2:caffeic acid; 3: eugenol ; 4: ρ-coumaric acid; 5: ρ -hydroxy benzoic acid; 6: resorcinol; 7: salicylic acid; 8: luteoline; 9:quercetin; 10: chrysin; 11: luteolin-3-methoxy-7-rutinoside.

Fig. 3- ACO expression levels in the liver tissues from the different studied groups.

Fig. 4- The fold changes of ACO gene expression was calculated according to the formula $2^{-\Delta\Delta Ct}$. The significance value of the fold change is included in Figure as pairwise comparison between the

different groups 1) * $p<0.05$, ** $p<0.01$versus the normal group, 2) # $p<0.05$, ## $p<0.01$ versus the HFD-group.

Fig. 5- Photomicrographs of H&E X200-stained heptatocyte sections of a normal histological structure of the hepatic lobule from the normal control rats.

Fig. 6- H&E X200-stained sections from the LPE-group showed a normal histological structure of the hepatic lobule.

Fig. 7- H&E X200-stained sections from the HFD-group showed diffuse glycogen infiltration.

Fig. 8- H&E X200-stained sections from the preventive group showed mild swelling and granularity of cytoplasm.

Fig. 9- H&E X200-stained sections from the therapeutic group showed focal areas of glycogen infiltration with narrowing of hepatic sinusoid.

Fig 1-

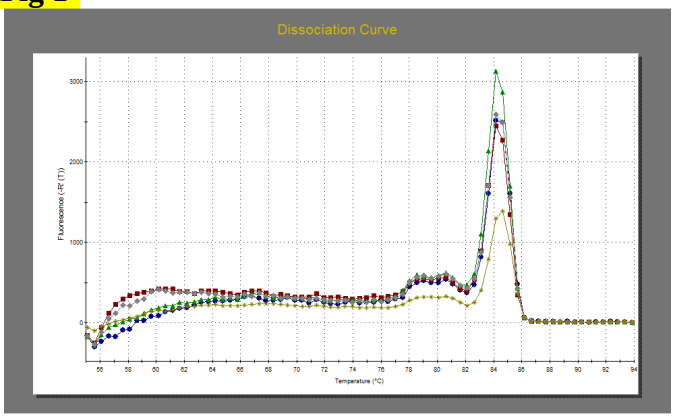

Fig 2-

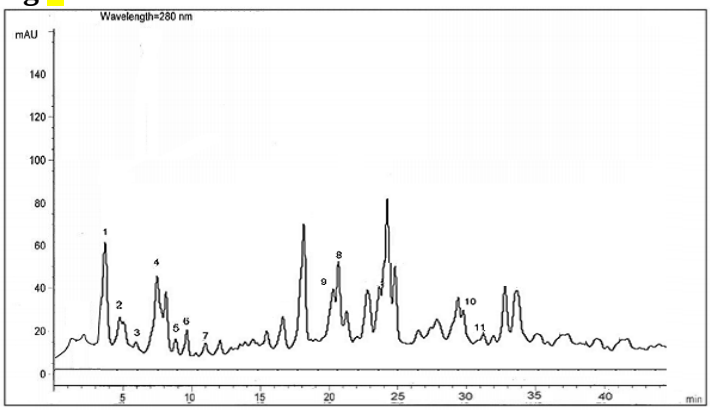

Fig 3-

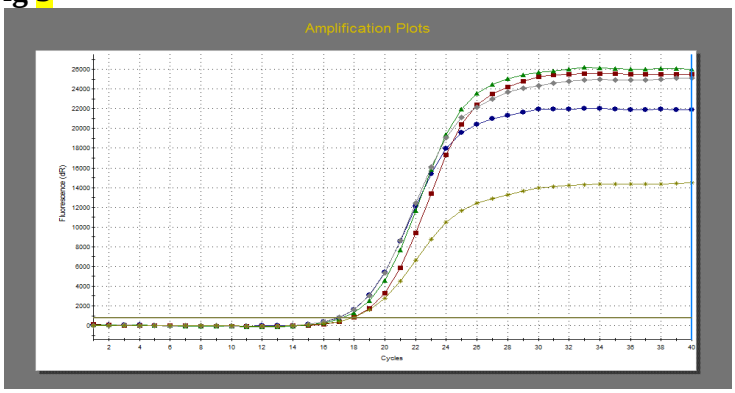

Fig 4-

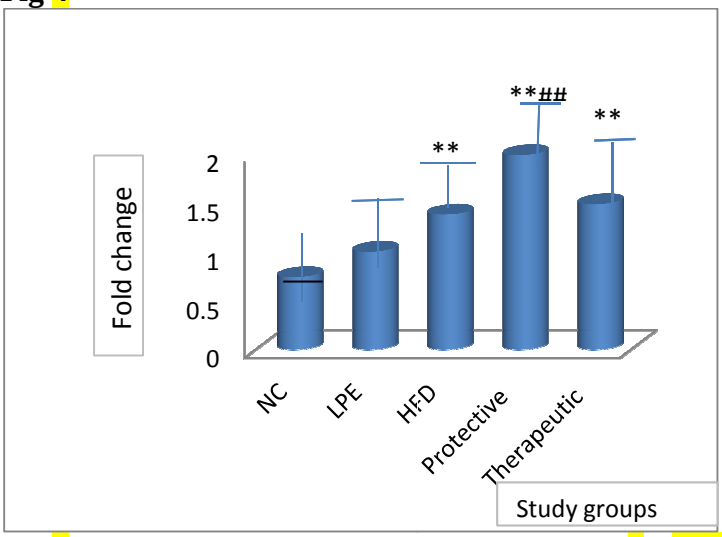

Fig 5- NC

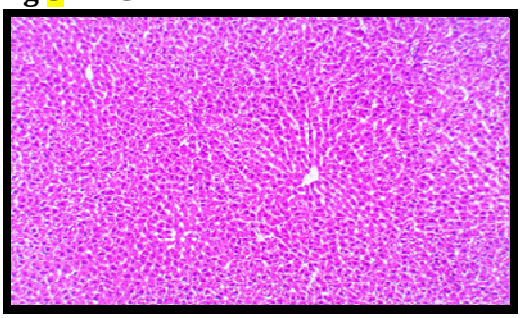

Fig 6- LPE

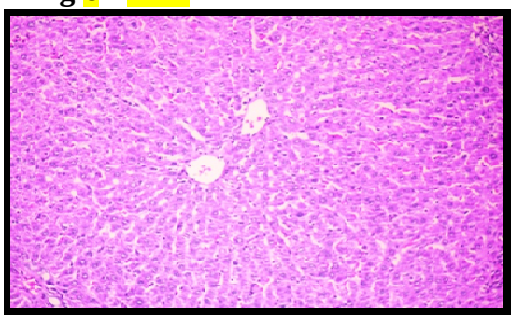

Fig 7-HFD

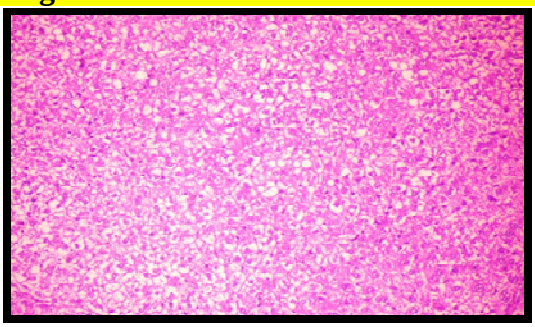

Fig 8- Preventive

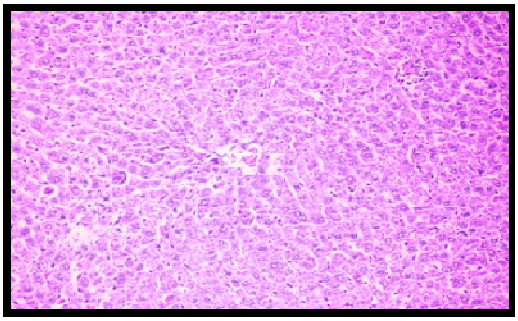

Fig 9- Therapeutic

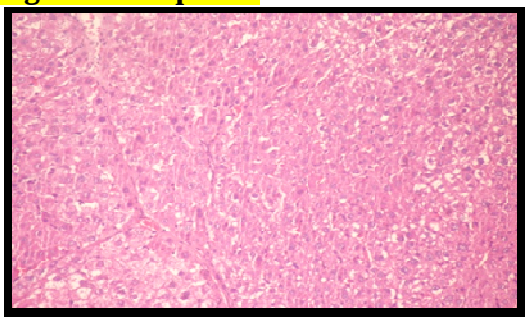

Table 1- HPLC analysis of lemon peel extract (polyphenols)

Peak No.	t_R (min)	Compound	Concentration (%)
1	3.4	Pyrogallic	39.1%
2	5.3	Caffeic acid	1.19%
3	6.0	Eugenol	1.23%
4	7.8	Salicylic acid	11.26%
5	8.1	ρ -hydroxy benzoic acid	0.77%
6	8.9	Resorcinol	1.05%
7	13.4	ρ -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 ratio in different studied groups.

Parameters	NC	LPE	HFD	Preventive	Therapeutic
Weight gain (g)					
mean ± SD	**61.63 ± 8.21**	**64.50± 9.17**	**112.44± 6.20 [a]**	**72.75± 6.01 [ab]**	**74.63± 7.46 [ab]**
%Changefrom NC		**4.66 ↑**	**82.44 ↑**	**18.04 ↑**	**21.09 ↑**
%Change from PC				**35.30 ↓**	**33.63 ↓**
Food intake (g/day)					
mean ± SD	**13.50 ± 0.93**	**15.31± 1.58**	**19.66 ± 1.26 [a]**	**16.51 ± 2.37 [ab]**	**17.85 ± 0.65 [a]**
%Changefrom NC		**13.41 ↑**	**45.63 ↑**	**22.30 ↑**	**32.22 ↑**
%Change from PC				**16.02 ↓**	**9.21 ↓**
Feedefficiency ratio					
mean ± SD	**4.00 ± 0.54**	**4.02± 0.96**	**6.15± 1.41 [a]**	**4.04± 0.81 [b]**	**4.15± 0.61 [b]**
%Changefrom NC		**0.50 ↑**	**53.75 ↑**	**1.00 ↑**	**3.75 ↑**
%Change from PC				**34.31 ↓**	**32.52 ↓**

(a) Statistical significantly from normal control group at *p*<0.05.

(b) Statistical significantly from positive control group at *p*<0.05.

NC: Normal Control. **PC: Positive control.** **LPE: Lemon peel extract.**

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

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

(a)Statistical significantly from normal control group at *p*<0.05.
(b) Statistical significantly from positive control group at *p*<0.05.
NC: Normal Control. PC: Positive control. LPE: Lemon peel extract.

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

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

(a)Statistical significantly from normal control group at *p*<0.05.
(b) Statistical significantly from positive control group at *p*<0.05.
NC: Normal Control. PC: Positive control. LPE: Lemon peel extract.

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

Parameters	NC	LPE	HFD	Preventive	Therapeutic
G6PD (U/g Hb)					
mean ± SD	**6.71 ± 1.10**	**7.14 ± 1.39**	**20.14 ± 7.17 [a]**	**8.09 ± 1.08 [ab]**	**15.23 ± 2.05 [ab]**
%ChangefromNC		**6.41 ↑**	**200.15 ↑**	**20.57 ↑**	**126.97 ↑**
%Changefrom PC				**59.83 ↓**	**24.38 ↓**
ACO (ng/ml)					
mean ± SD	**35.43 ± 8.48**	**40.47 ± 6.76**	**235.00 ± 63.52 [a]**	**422.48±128.14 [ab]**	**272.52 ± 63.20[ab]**
%ChangefromNC		**14.23 ↑**	**563.28 ↑**	**1092.44 ↑**	**699.18 ↑**
%Changefrom PC				**79.78↑**	**15.97 ↑**
MCAD (ng/ml)					
mean ± SD	**10.32 ± 2.32**	**12.64 ± 3.31**	**19.65± 9.95 [a]**	**52.48 ± 36.82 [ab]**	**29.34 ± 11.10 [ab]**
%ChangefromNC		**22.48 ↑**	**90.41 ↑**	**408.53 ↑**	**184.30 ↑**
%Changefrom PC				**167.07 ↑**	**49.31 ↑**
Hb (g/dl)					
mean ± SD	**14.25 ± 1.49**	**14.63± 1.30**	**12.25± 1.49 [a]**	**12.88± 2.10 [a]**	**12.38 ± 1.77 [a]**
%ChangefromNC		**2.67 ↑**	**14.04 ↓**	**9.61 ↓**	**13.12 ↓**
%Changefrom PC				**5.14 ↑**	**1.06 ↑**

(a)Statistical significantly from normal control group at *p*<0.05.

(b) Statistical significantly from positive control group at *p*<0.05.

NC: Normal Control. PC: Positive control. LPE: Lemon peel extract.