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Investigation of the Effect of Lycopene, Hesperidin, Essential Oil and their Relative Nano-Formulation Form on Experimentally Obese –Induced Rats

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Abstract

The study was designed to investigate the effect of lycopene, hesperidin, and essential oil and their nano-formulation form in high-fat diet (HFD) -induced obese Wistar rats. To achieve this, a total of ninety adult Wistar male rats were used. The animals were made obese by feeding rats' high-fat diet (HFD, cholesterol, and bile acid). Cholesterol dose, 0.3 mL olive oil containing 30 mg cholesterol/ 1 kg body weight was orally administrated (five times per week) for 8 consecutive weeks. Obesity was confirmed by the percentages of body weight gain. Animals were divided into negative control groups (normal rats received normal diet) and positive control groups (normal rats received HFD) and treatment groups (obese rats received different samples) of five animals each. Dose of lycopene and its nanoparticles 50 mg/kg b. wt., hesperidin, essential oil, and their nano-formulation form (100 mg/kg b.wt). All samples and standard drug (orlistat 12 mg/kg b. wt.) were orally administered once daily for four weeks. At the end of experiement, all animal groups were sacrificed and blood samples were collected. Different parameters including total cholesterol, triglycride, HDL-cholesterol, total lipid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinene and urea were determined in the serum. The liver tissue was excised for the determination of hepatic antioxidant biomarkers (catalase and GSH) and oxidative stress (lipid peroxide). Histopathological examination of liver tissue of obese rats showed diffuse fatty liver, circumscribed hepatic vacuoles with prepheralnucleous. However, obese rats- treated with all different samples declaring amelioration in all the measured parameters. The results obtained illustrated that the nano-formulation form are more effective than a native form of lycopene, hesperidin, and essential oil. Besides, the lycopene and its nanoparticles were more effective than hesperidin, essential oil, and their nano-formulation forms. So, it could be concluded that , oral supplementation with lycopene, hesperidin, essential oil, and their relative nano-formulation forms can treat hepatic dysfunction in obese rats.

Keywords: obesity, lycopene, hesperidin, essential oil, nano-formulation form, lipid profile, liver function, kidney function, antioxidant biomarkers, oxidative stress, and Histopathology.

1. Introduction

Obesity is a metabolic disease that refers to high body fat stores. In this disease an imbalance between energy intake and consumption in which energy intake is higher than consumption. Therefore, an increase in body fat and body weight. Obesity is a gateway to serial diseases, affecting adults, children, and adolescents. For instance, cancer, cardiovascular, metabolic, and respiratory disorders. Obesity treatment and control has attracted investigators expressed by multiple studies, which have shown that detection and counseling rates among physicians remain low [1-2]. It has been reported that the global obese or population has reached 30% of the global population, mainly due to lifestyle changes, fast foods, urbanization, and genetic factors, such as eating habits and lack of exercise. The composition and the amount of food is a critical factors inducing obesity. thus, high fat foods leads to an increase in the number and size of body fat cells (hyperplasia and hypertrophy), respectively [3-4]. In addition to increasing the inability to store triglycerides under excessive feeding, which leads to a metabolic dysfunction attendanting adipokines secretion, and therefore inflammatory initiation and apoptotic induction. However, the inflammatory cells cascades affect various tissues, by cell-cell interactions and finaly induce organ dysfunction [5]. In this context, adipose tissue is regulating energy balance and nutrient homeostasis. Two typs of adibose tissue were identified, white adipose tissue (WAT)

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which store the excess energy in the form of triglycerides, while brown adipose tissue (BAT) with multi-room fat cells contains large amounts of mitochondria. In response to high-fat diets, the content of mitochondria in WATs increases dramatically, a process called “browning”. Thus, it can prevent lipid accumulation through brown-like adipocyte formation induction [6-7]. Accordingly, the composition of the dietary intake can be a limiting factor to obesity rescue. Natural products have been implicated in obesity treatment, such as carotenoids and their derivatives flavonoids, alkaloids, etc [8]. Lycopene is a carotenoid found in tomatoes and tomato products whish display an antioxidant effects. It is useful for the prevention of several inflammation-coupled diseases, including cancer, cardiovascular diseases, and obesity [9-10]. Due to their chemical structure, lycopene is mainly stored in adipose tissue in humans [11]. It has been reported that, higher intake of lycopene was associated with lower fat accumulation. The effect of lycopene has an antioxidative and anti-inflammatory role in adipose tissue, based on several observations. Several studies has revealed that lycopene reduces mRNA expression levels of pro-inflammatory cytokines in the adipose tissue in animal models feeded by high-fat (HF) diet-fed [12]. Additionally, lycopene reduced TNF-α secretion in lipopolysaccharide-stimulated macrophages (LPS-stimulated RAW 264.7), which was associated with attenuated NF-κB and mitogen-activated protein kinase (MAPK) signaling. Thus, lycopene seems to preclude the crosstalk between adipocytes and macrophages and thereby prevent obesity-associated adipose inflammation. However, the role of lycopene in obesity manipulation and modulation the inflammatory status of the tissue remains unclear [13]. Flavonoids also are a class of phenolic compounds that investigated in the form of free state and glycoside [14] and have some other biological activity in that manner including antioxidant [15], anticancer and anti-inflammatory effects [16]. Hesperidin is a flavonoid glycoside that wiediel distributed in citrus fruit peel. Citrus essential oil is a natural plant extract, mainly exists in the citrus fruit peel, which belongs to the genus Citrus of the family Rutaceae [17]. Citrus essential oil is a by-product of citrus fruit processing. It has been broadly used in food and beverage products as a natural food flavour [18]. Citrus essential oil exhibited many bioactivities including antioxidant, anticancer, antimicrobial and anti-allergy activities, anti-inflammatory, as well as cardiovascular effect, neuroprotective effect, and hepato-protective effect as it has been reported by several articles [17, 19]. D-limonene is the main component of citrus essential oil has been used effectively in the treatment of obesity [20]. In this study, we used agro-waste derived compounds as a new obesity treatment strategy. In our previous study [21], nanoparticales were prepared using lycopene of tomato pomace, hesperidin and essential oil of sweet orange peels.We had revealed that the nanoparticales increased the solubility and bioavailability of lycopene, hesperidin and essential oil. The purpose of this study is to evaluate the weight loss effect of native lycopene, hesperidin and essential oil and their nanoformulation form on obese rats, to investigate novel dietary supplement to reduce body weight.This can achieved through measuring total cholesterol, triglycride, HDL-cholesterol, total lipid, ALT, AST, ALP, creatinene, urea, catalase, GSH, lipid peroxide and histopathological examination.

2. Materials and methods

2.1. Plant materials:

All chemicals used in this study were purchased from Sigma (USA) and Fluka (Switzerland) analytical grade. The tomato's pomaces were obtained from Heinz industrial, 6 October-City, Egypt. The peels and essential oil (Essential oil extraction at cold-pressing technique) of orange were obtained from El Marwa Food Industries, 6th of October Giza-Egypt. Then the tomato pomace and peels of orange air drying of and grinding to obtain a homogenous sample. Extraction and preparation of nano-formulation form: Extraction and preparation of nano-formulation form has been previously described [21].

2.2. Experimental animals

Experiments were carried out in male albino rats (n = 90) weighted (150±20 g) was carried out. The animals supplied from the Animal House of the National Research Centre (NRC) were allowed to acclimatize for seven days in the laboratory. They were housed under 3 °C temperature cycles (26−29 °C) with fixed light cycles. Rats were allowed free access to water ad libitum and food. Handling of the animal was performed according to the Committee of Ethics, NRC, Egypt (with ethical approval no: 33458). Obesity was conducted according to Adaramoye et al. [22], by feeding rats' high-fat diet (HFD, cholesterol, and bile acid). A dose of cholesterol (30 mg/0.3 mL olive oil /1 kg) was orally administrated (five times per week) for 8 consecutive weeks. The HFD diet (Table 1) was mixed with lard fat and bile acid to increase the cholesterol absorption (five kg of the diet were mixed with one kg of animal lard with the addition of 2.5 g of bile acid). The diet from Cairo Oil and Soap Company (Egypt) was used (4.39 kcal/g). Obesity was demonstrated by measuring the percentages of body weight gain.

Table 1. Composition of the high-fat diet (HFD) used in the study (4.39 kcal/g).

Ingredients	Amount
Lard Fat	20 %

Bile Acid	25 %
Casein Purified High Nitrogen	21–23 %
DL-Methionine	0. 3 %
Sucrose	60 %
Corn Starch	20 %
Coconut Oil (Hydrogenated)	20 %
Vitamin D3	10 MIU
Vitamin E	25 MIU
Calcium	0.8–1.2 %
Vitamin A	10 MIU

MIU: Milli-international units

2.3. Doses and routes of administration

Orlistat (12 mg/kg b.wt.) was used as a standard drug against obesity). The drug was dissolved in distilled water for oral administration for four consecutive weeks to obese-induced rats [23]. Also, lycopene and its nanoparticles: 50 mg/kg b.wt. [24], hesperidin, essential oil, and their nano-formulation forms: 100 mg/kg b.wt. were administered to obese rats for eight successive weeks [25-26].

2.4. Experimental design

Male Wistar rats weighing between 150-170 g (mean ± SD), which is the rat's weight at the day received from the supplier post period of adaptation to the environment, were used. All animals were randomly allocated into eighteen major groups (1 to 18) of 5 rats each. Group (1): control received distilled water by gavage and a normal diet (ND). Group (2): normal rats received a normal diet and were treated with 50 mg/ kg b.wt. of native lycopene consecutive for four weeks. Group (3): normal rats received a normal diet and were treated with 50 mg/ kg b.wt. of lycopene-NPS consecutive for four weeks. Group (4): normal rats received a normal diet and were treated with 100 mg/ kg b.wt. of native hesperidin consecutive for four weeks. Group (5): normal rats received a normal diet and were treated with 100 mg/ kg b.wt. of hesperidin-NPS consecutive for four weeks. Group (6): normal rats received a normal diet and were treated with 100 mg/ kg b.wt. of native essential oil consecutive for four weeks. Group (7): normal rats received a normal diet and were treated with 100 mg/ kg b.wt. of essential oil-NPS consecutive for four weeks. Group (8): normal rats treated with 100 mg/ kg b.wt. of PEG-NPs (polyethylene glycol-nanoparticales) consecutive for four weeks. Group (9): normal rats received a normal diet and were treated with standard drug; orlistat (12 mg/kg b.wt.) consecutive for four weeks. Group (10): rats feeding HFD for 8 weeks. Group (11): obese rats medicated for 4 weeks with 50 mg /kg b.wt. of native lycopene. Group (12): obese rats medicated for 4 weeks with 50 mg /kg b.wt. of lycopene-NPs. Group (13): obese rats medicated for 4 weeks with 100 mg /kg b.wt. of native hesperidin. Group (14): obese rats medicated for 4 weeks with 100 mg /kg b.wt. of hesperidin-NPs. Group (15): obese rats medicated for four weeks with 100 mg /kg b.wt. of native essential oil. Group (16): obese rats medicated for 4 weeks with 100 mg /kg b.wt. of essential oil-NPs. Group (17): obese rats medicated for 4 weeks with 100 mg /kg b.wt. of PEG-NPs. Group (18): obese rats medicated for 4 weeks with 12 mg /kg b.wt. of the standard drug; orlistat. The Health and behavior conditions of all rats were monitored daily and no adverse events were observed throughout the study. All experiments and biochemical analyses were conducted using 90 rats with triplicate measurements.

2.5. Blood sample

At the end of treatment (12 weeks), blood collection from the sublingual vein was obtained. The animals were overnight fasting under light anesthesia with an oral administration of pentobarbital 30 mg/kg. Separation of serum was carried out by centrifugation (4000 rpm, 10 min) and was kept at −80 °C for further analysis [26]. Post treatments (8 and 4 weeks respectively), the rats were sacrificed by decapitation and the liver tissue was removed for biochemical analysis. In a saline solution, a part of liver tissue was homogenized to yield 10 %. The other part was fixed in formalin (10 %), for histopathological examination.

3. Biological analysis

3.1. Lipid profile

Total blood lipids (TL) were determined spectrophotometrically according to the method of Zollner and Kirsch (1962) [27]. The triglycerides (TG) in serum was estimated spectrophotometrically according to the method of Fossati and Prencipe (1982) [28]. The method of Allian et al. (1974) [29] was used to determine total cholesterol (TC). High Density Lipoprotein –cholesterol (HDL-c) was determined according to the study of Burstein (1970) [30].

3.2. Renal function

Creatinine level was measured according to the method described by Patton and Crouch (1977) [31]. Urea content is determined according to the method described by Patton and Crouch (1977) [32].

3.3. Liver function

The activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured as described by Reitman and Frankel (1957) [33]. The activity of alkaline phosphatase (ALP) was determined according to the method described by Belfield and Goldberg (1971) [34].

3.4. Antioxidant biomarkers and oxidative stress

Reduced glutathione (GSH)was estimated according to the method of Beutler *et al*. (1963) [35]. Catalase (CAT) activity was determined according to the method of Fossati *et al.* (1980) [36]. Lipid peroxide

(MDA) was measured according to the method described by Kei (1978) [37].

3.5. Histological examination

Preservation of the slides of liver tissue silices in 10 % buffer formalin was carried out. Hematoxylin and eosin (H&E) stains have been used for standard paraffin sections (4 pm) [38].

4. Statistical analysis

Data were statistically analyzed using Co-stat statistical package data according to Anonymous, (1989) [39].

5. Results and discussion

Effect of native lycopene, hesperidin, and essential oil and their nano-formulation form on lipid profile.

Hyperlipidemia referes to an excess of fatty substances in blood including lipids, largely cholesterol, and triglycerides. These fatty substances are traveling in the blood after attachment to proteins as the LDL-c (low-density lipoprotein-cholesterol) and HDL-c (high-density lipoprotein-cholesterol) lipoproteins which allow to these fatty substances to remain dissolved [40]. The data in Table (2) showed high significant level (P≤0.05) of total lipid TL (907.41 mg/dl), triglycerides TG (258.97mg/dl), total cholesterol TC (373.08 mg/dl) and low significant level (P≤0.05) of high density lipoprotein HDL- c (27.93 mg/dl) in obese rats compared to control (580, 117.09, 155.13 and 58.14 mg/dl respectively). Lipid profile abnormalities is a strong and direct predisposing issue to obesity diseases, specifically hypertriglyceridemia and low significant levels (P≤0.05) of HDL-c [7, 8]. The elevated values of lipid profile from normal rats to obese rats (P≤0.05) may explained on the basis of low activity of cholesterol biosynthesis enzymes or low level of lipolysis [41]. Treatment of obese rats (P≤0.05) with lycopene; hesperidin, essential oil, and these relative nanoformulation forms ameliorate all of those detected parameters compared to obese non-treated rats. The results also illustrated that Orlistat as the standard drug recorded the highest percentage of improvement (TC 169.60%, HDL-c 49.07%, TG 132.85% and TL 70.21 %) followed by lycopene-NPs on TC (140.50%), HDL- c (43.46%), TG (110.95%) and TL (61.70%) compared to native lycopene (138.02%, 24.18%, 91.24% and 41.49% respectively). While, hesperidin-NPs showed the highest percentage of improvement on TC (121.49%), HDL- c (25.41%), TG (76.64%), and TL (59.57%) levels compared with native hesperidin (104.96%, 18.95%, 56.20%, and 34.04% respectively). In addition, essential oil-NP-s has the highest percentage of improvement on TC (144.63%), HDL- c (30.72%), TG (100.73%), and TL (36.17%) levels compared with native essential oil (128.10%, 26.80%, 83.94% and 40.43% respectively). In contrast, PEG-NPS (polyethylene glycol-nanoparticales) showed the lowest percentage of improvement (TC 17.36%, HDL-c 2.94%, TG 13.87%, and TL 1.06%). Lycopene has a potential activity to reduce cholesterol levels via cholesterol synthesis suppression. As well as, increase LDL degradation level, and inhibition of the hydroxy-methyl-glutaryl-coenzyme A (HMGCoA)-reductase enzyme [42]. Hesperidin also inhibits cholesterol synthesis and absorption and regulating C-FABP and H-FABP mRNA expression which they are regulating fatty acid uptake and intracellular transport [43]. On the other hand, orange peels essential oil contains terpenes such as α-pinene (0.761 %), β-pinene (2.397 %) and d-limonene (94.742 %). Terpenes are effective compounds to reduce plasma cholesterol levels. D-limonene (the major component of orange peels essential oil), which is a monocyclic monoterpene, has a hypocholesterolemic effect and control of serum cholesterol levels [44]. Also, a similar effect was found for triglycerid because low-density lipoproteins comprise a core of cholesterol esterified to linoleic acid and triglycerides, via downregulation of PPARα and LXRβ [45].

Effect of native lycopene, hesperidin, and essential oil and their nano-formulation form on renal function.

The biochemical kidneys parameters tested in this study are kidney toxicity markers and used for the assessment of tissue damage [46]. Hypercholesterolaemia accelerates kidney disease progression. Urea and creatinine levels are used as an indicator of nephrotoxicity as low clearance of these substances indicates the diminished or impaired ability of the kidney to clear the body's waste products [47]. Blood levels (P≤0.05) of creatinine and urea were different between control (0.36 and 41mg/dl respectively) and obese rats (0.83 and 58.78 mg/dl respectively). The results showed that no significant difference between control and normal rats groups treated with all different treatments (Table 2). Also, no significant difference in creatinine level (P≤0.05) between control rats (0.36 mg/ dl) and obese rats treated with lycopene (0.43 mg/ dl), lycopene-NPs (0.36 mg/ dl), essential oil (0.34 mg/ dl), essential oil-NPs (0.31 mg/ dl) and drug (0.35 mg/ dl). On the other hand, it was observed a significant difference in creatinine level (P≤0.05) between control rats and obese rats treated with hesperidin (0.55mg/dl), hesperidin-NPs (0.56mg/dl), and polyethylene glycol-nanoparticales (PEG-NPS) (0.87 mg/dl). Essential oil-NPs showed

the highest percentage of improvement in urea level (63.86%) followed by essential oil (52.86%), lycopene (53.49%), drug (50.63%), and lycopene-NPs (44.19%), while PEG-NPs showed the lowest percentage of improvement (1.52%) followed by hesperidin (26.74%) and hesperidin-NPs (38.10%). Treatment of HFD rats with lycopene significantly reduced urea and creatinine levels to 36.62 and 0.43 mg/ dl, respectively. These results are points to a nephroprotective effect reole of lycopene against hyperlipidemia. Renal histopathological examinations indicated that lycopene protects the renal parenchyma from oxidative damage and modulates the endogenous cholesterol synthesis and metabolism as well as improves the intensity of hyperlipidemia-based nephrotoxicity [48]. While, hesperidin significantly increases the level of α-Klotho (α-KL) in serum, liver, and kidney tissues and significantly reduces the levels of blood urea nitrogen (BUN) and creatinine in fibroblast growth factor-23 (FGF-23) in kidney tissues and serum [49]. On the other hand, D-limonene of essential oil orange peels may increase GFR following the amelioration of renal histopathological alterations and finally lowering serum levels of urea and creatinine [50].

Effect of native lycopene, hesperidin, essential oil and their nano-formulation forms on liver function.

Liver function evaluation was assessed through using liver enzymes AST, ALT and ALP activities. Both AST and ALT are hepatocellular markers. ALT is more specific biomarker of liver pathology and is found mainly in the liver. However, AST and ALP are less specific biomarkers of liver function (Lee *et al*., 2003) [51]. Hepatic AST, ALT, and ALP (U/L) activities were increased significantly in obese rats ($P \leq 0.05$) (194.54, 108.901, and 166.14 U/L, respectively) compared to control rats (128.50, 85.01 and 128.41 U/L, respectively) as presented in Tables 3. In obese rats, an increase in these enzymes activities indicates a cellular leakage and liver dysfunction. A significant increase in the activities of AST, ALT, and ALP in this study may be also interpreted as a result of the liver cell destruction or changes in the membrane permeability indicating the severity of hepatocellular damage [52]. These enzymes were restored towards the control level post the treatments with all different treatments except PEG-NPs. AST biomarker showed the highest improvement in obese rats upon treatments with drug (42.89%) followed by lycopene (36.50%), essential oil-NPs (35.90%), lycopene-NPs (31.49%), hesperidin-NPs (30.24%), essential oil (28.96%) and hesperidin (25.22%). Essential oil-NPs showed the highest improvement for ALT (40.17%) followed by lycopene-NPs (32.78%), hesperidin-NPs (29.37%), lycopene (23.75%), standard drug (24.05%), essential oil (23.38%), and hesperidin (22.12%). The percentage of improvement for ALP was the highest for lycopene-NPs (25.88%) and the lowest for essential oil (15.93%) compared to the reference drug (28.36%). This observation coincides with earlier findings of Aydın *et al.* [53] suggests that lycopene emhamces parenchymal cell regeneration in liver, thus protecting membrane fragility thereby decreasing enzyme leakage and restoring the normal liver cell integrity. While, hesperidin significantly increases the level of α-Klotho in serum, liver, and kidney tissues of diabetic rats, and consequently significantly reduce the levels of AST and ALT, so it helps in maintaining normal function by accelerating the regenerative capacity of liver cells [54]. The anti-oxidative protective effects of essential oil might be associated with its abilities to scavenge ROS and/or induce the gene expression of antioxidant enzymes [50].

Effect of native lycopene, hesperidin, essential oil and their relative nano-formulation forms on antioxidant biomarkers and oxidative stress.

Due to oxidative stress, the balance between the ROS generation and antioxidant are disrubted. Lipid peroxides and their products induces damage to membrane-bound enzymes and other macromolecules. In addition, it causes degradation of unsaturated fatty acids and cholesterol in lipid bilayers, membrane fluidity and permeability, finally leads to membrane structure disruption and dysfunction [55, 56]. MDA is an important indicators of lipid peroxidation associated with oxidative stress [56]. The levels of GSH was measured due to oxidative stress has a promising role in the development of nonalcoholic fatty liver disease (NAFLD), and in the protection of tissues from the deleterious effects of oxidative damage. It helps to protect biological membranes, which are susceptible to peroxidation [57].

Free radical scavenging enzymes like CAT protect the biological systems from oxidative stress. Catalase, traditionally considered a peroxisomal protein, was found to be present in cardiac mitochondria and significantly increased in content and activity in response to high fat feeding. In fact, oxidation of fatty acid enhances mitochondrial H_2O_2 production, and increase the subcellularlocalized of catalase to consume excessive mitochondrial H_2O_2 produced by increased fat metabolism [58]. Keeping rats on HFD lead to a significant increase ($P \leq 0.05$) in MDA (132.35 nmol/g) and CAT (902.65U/g) while a remarkable reduced level of GSH (79.59 mg/g) compared to control (65.44 nmol/g, 581.12 U/g and 116.35 mg/g respectively) (Table 3). The results showed that no significant difference between control rats and obese rats ($P \leq 0.05$) treated with all different treatments except PEG-NPs (137.99 nmol/g, 888.20 U/g, and 71.51 mg/g respectively).

Table 2. Effect of native lycopene, hesperidin, and essential oil and their nano-formulation form on lipid profile and renal function.

	Lipid profile								Renal function			
	Cholesterol	**%**	**HDL- Cholesterol**	**%**	**Triglyceride**	**%**	**Total lipid**	**%**	**Creatinine**	**%**	**Urea**	**%**
Groups	**Level (mg/dl)**											
Nor. Con	155.13^{e}±5.88	------	58.14^{ab}± 0.57	------	117.09^{hijk}± 3.92	------	580.25^{de}± 42.77	------	0.36^{c}± 0.15	------	41.42^{bc}± 6.05	------
Nor. Lyc	153.85^{e} ± 6.70	------	57.00^{abc}± 0.60	------	105.98^{kl}± 6.45	------	549.38^{def}± 56.58	------	0.32^{c}± 0.18	------	39.70^{bcd}± 8.32	------
Nor. Lyc-NPs	156.41^{e}±8.01	------	54.53^{bc}± 2.37	------	122.22^{ghi}± 2.96	------	567.90^{def}± 10.69	------	0.38^{c}± 0.09	------	40.63^{bcd}± 5.34	------
Nor. Hes	157.69^{e}±3.85	------	57.76^{ab}± 2.00	------	124.79^{gh}± 9.00	------	611.11^{cd}± 49.00	------	0.39^{c}± 0.03	------	39.56^{bcd}± 2.80	------
Nor. Hes-NPs	151.28^{e}± 4.44	------	58.71^{a}± 3.02	------	114.53^{hijkl}± 3.92	------	534.57^{efj}± 40.80	------	0.35^{c}± 0.09	------	38.37^{cde}± 6.29	------
Nor. Esse	155.13^{e}± 8.07	------	54.91^{abc}± 2.63	------	110.26^{jkl}± 2.56	------	614.81^{cd}± 19.60	------	0.37^{c}± 0.08	------	42.78^{bc}± 5.75	------
Nor. Esse-NPs	155.13^{e}± 12.36	------	54.72^{abc}± 3.47	------	111.97^{ijkl}± 3.90	------	548.15^{def}± 36.48	------	0.33^{c}± 0.04	------	43.07^{bc}± 2.29	------
Nor. PEG-NPs	152.56^{e}± 8.46	------	54.91^{abc}± 3.14	------	120.51^{ghij}± 5.13	------	570.99d^{ef}± 29.76	------	0.36^{c}± 0.08	------	41.00^{bc}± 5.20	------
Nor. Drug	105.13^{f}± 4.43	------	56.81^{abc}± 3.34	------	119.66^{ghij}± 5.34	------	472.22^{g}± 16.04	------	0.32^{c}± 0.04	------	30.89^{e}± 2.58	------
HFD	373.08^{a}± 7.69	------	27.93^{f}± 2.28	------	258.97^{b}± 8.88	------	907.41^{a}± 55.56	------	0.83^{a}± 0.07	------	58.78^{a}± 3.31	------
HFD. Lyc	158.97^{e}± 4.41	138.02	41.99^{de}± 1.74	24.18	152.14^{ef}± 6.44	91.24	666.67^{bc}± 18.52	41.49	0.43b^{c}± 0.04	109.30	36.62^{cde}± 1.52	53.49
HFD. Lyc-NPs	155.13^{e}± 2.22	140.50	53.2^{c}± 2.81	43.46	129.06^{g}± 7.83	110.95	549.38^{def}± 46.60	61.70	0.36^{c}± 0.08	130.23	40.48^{bcd}± 7.28	44.19
HFD. Hes	210.26^{c}± 9.68	104.96	38.95^{e}± 2.30	18.95	193.16^{c}± 9.71	56.20	709.88^{b}± 21.38	34.04	0.55^{b}± 0.05	76.74	47.70^{b}± 5.94	26.74
HFD. Hes-NPs	184.62^{d}± 3.85	121.49	42.75^{de}± 2.27	25.49	169.23^{d}± 7.69	76.64	561.73^{def}± 28.29	59.57	0.56^{b}± 0.07	74.42	43.00^{bc}± 6.26	38.10
HFD. Esse	174.36^{d}± 5.88	128.10	43.51^{d}± 3.44	26.80	160.68^{de}± 7.83	83.94	672.84^{bc}± 10.69	40.43	0.34^{c}± 0.12	134.88	36.89^{cde}± 2.85	52.86
HFD. Esse-NPs	148.72^{e}± 2.21	144.63	45.79^{d}± 0.87	30.72	141.03^{f}± 10.26	100.73	697.53^{b}± 53.46	36.17	0.31^{c}± 0.04	144.19	32.33^{de}± 7.14	63.86
HFD. PEG-NPs	346.15^{b}± 10.18	17.36	26.22^{f}± 2.61	2.94	275.21^{a}±12.12	13.87	913.58^{a}± 101.99	1.06	0.87^{a}± 0.06	11.63	59.41^{a}± 4.30	1.52
HFD. Drug	111.54^{f}± 3.81	168.60	56.81^{abc}± 1.19	49.67	103.42^{l}± 3.00	132.85	500.00^{fg}± 32.08	70.21	0.35^{c}± 0.11	132.56	37.81^{cde}± 3.11	50.63
LSD	11.23	------	4.03	------	11.68	------	71.25	------	0.14	------	8.51	------

All values are represented as mean ±S.D.

Means with different letters are significantly different($p<0.05$).

(%).): Improvment, (Nor.): normal, (Con.): control, (Lyc.): lycopene, (Hes): hesperidin, (Esse.): essential oil, (NP-s): nanoparticles, (HFD): high-fat diet and (PEG): polyethylene glycol.

Lycopene-NPs showed the highest improvement with MDA (111.42%) followed by essential oil (102.26%), essential oil-NPs (100.37%), and lycopene (95.51%) while hesperidin and hesperidin-NPs showed the lowest improvement of MDA (92.88 and 85.39%, respectivaly) compared to standard drug (100.00%). In the present study the level of GSH was significantly decreased in obese rats (79.59 mg/g), compared to control rats (116.35 mg/g), while, no significant difference between control rats, normal -treated rats, and obese rats -treated with all different treatments except PEG-NPs (71.51 mg/g). The result showed that the highest improvement of GSH was 37.85% for essential oil and the lowest improvement was 24.31% for hesperidin compared with Orlistat standard drug (39.06%). The presented data indicated that the obese rats exhibited significantly higher CAT expression level (902.65 U/g) compared to control group (581.12 U/g). No significant difference between control, normal –treated rats, and obese rats -treated with all different treatments except PEG-NPs (888.20 U/g). The decrease in liver tissue MDA and catalase, while increase of GSH level after being treated with lycopene may be related to the ability of lycopene to affect the antioxidant defense system by reduce of reactive oxygen species (Figure1) [59-61]. Also, hesperidin displayed potential antioxidant activity and prevent the increase of reactive oxygen species (ROS) production in rats by exhaustive exercise, and pervent catalase activity inhibition in the thymus and spleen, which effectively inhibit the formation of superoxide and oxygen. Hesperidin reduces ROS, MDA, caspase-9, caspase-3, and Bax/Bcl-2 levels and inhibits apoptosis, thereby protecting RGC-5 cells from high glucose-induced oxidative stress [62]. The anti-oxidative protective effects of D-limonene (the main component of orange peels essential oil) might be associated with its abilities to scavenge ROS and/or induce the gene expression of antioxidant enzymes [63].

Histopathological examination of hepatic rats.

Histopathological examination (Figure 2) of liver sections of normal control rats (Control) showed normal hepatic parenchyma; note the healthy polyhedral hepatocytes with central basophilic nucleus. Obese control group (HFD) showed diffuse fatty liver; note the diffuse circumscribed hepatic vacuoles with a peripheral nucleus. Normal rats treated with lycopene (Nor. Lyc), hesperidin (Nor. Hes), essential oil (Nor. Esse), lycopene nanoparticales (Nor. Lyc-NPs) and polyethylene glycol nanoparticales (Nor. PEG-NPs) showed no histopathological alterations, while hesperidin-NPs (Nor. Hes-NPs) and essential oil-NPs (Nor. Esse-NPs) showed congestion of the central vein compared to drug (Nor. Drug). On the other hand, obese rats treated with lycopene (HFD-Lyc) showed moderately improved hepatocytes with regressed fat vacuoles from many hepatocytes. Howeve, treatment with lycopene nanoparticales (HFD-Lyc-NPs) showed markedly improved hepatocytes with complete absence of fat vacuoles, the appearance of normal hepatocytes and central vein showed slight congestion. Also, treatment with hesperidin (HFD-Hes) showed slightly improved hepatocytes with regressed fat vacuoles from some hepatocytes. Hesperidin nanoparticales (HFD- Hes-NPs) showed markedly improved hepatocytes with complete absence of fat vacuoles and appearance of normal hepatocytes. The essential oil (HFD-Esse) showed slightly improved hepatocytes with regressed fat vacuoles from some hepatocytes. Essential oil nanoparticales (HFD- Esse-NPs) showed markedly improved hepatocytes with complete absence of fat vacuoles and appearance of normal hepatocytes like hesperidin nanoparticales compared to standard drug (HFD- Drug). In conclusion, the histopathological examination of hepatic rats showed that the nano-formulation forms were improved hepatocytes more than the native form of lycopene, hesperidin, and essential oil, which may be attributed to, nano-sized materials owe superior alteration in the physical and chemical properties, small object, quantum size and surface effects [64]. The usage of lycopene as an antioxidant produced exhibited hepatic protection that markedly ameliorated the severity of hepatic lesions and subsequently the hepatic functions which may be explained on the basis of its antioxidant, anti-inflammatory, and free radical scavenging properties. Therefore, hesperidin is a citrus flavonoid, which protect against many toxicological situations including CCl4 toxicity caused by oxidative stress [65]. In this context, it is suggesting that hesperidin may be clinically used in human health as a radical scavenger agent [66]. While the previous study found that dietary supplementation with D-limonene (2.00%) for four weeks reversed the HFD induced changes and restored pathological alterations of the liver [67]. In this study, we confirmed that the nano-formulation form of lycopene, hesperidin, and essential oil was more effective than their relative native forms.

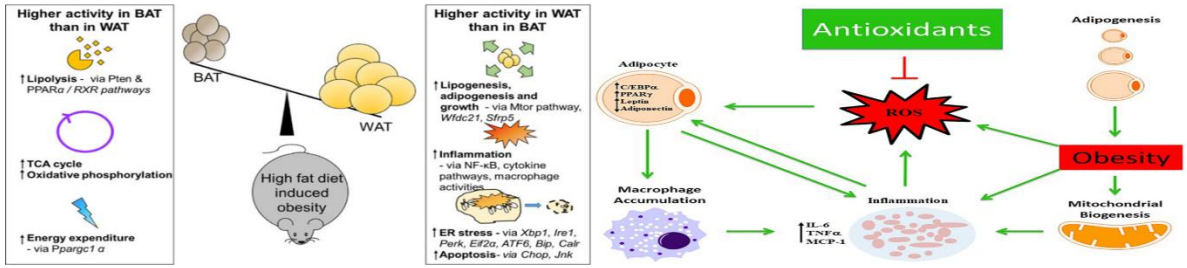


Figure 1: mechanism of obesity induced high fat diet and anti-obesity using antioxidants, Obesity mediates excessive ROS production and inflammation which is further exacerbated by altered mitochondrial biogenesis and activation of macrophages from dysfunctional adipocytes. The antioxidant defense mechanism scavenges ROS and ameliorates obesity phenotype (adapted by Tun *et al*. 2020 and Wijayatunga *et al*. 2018)

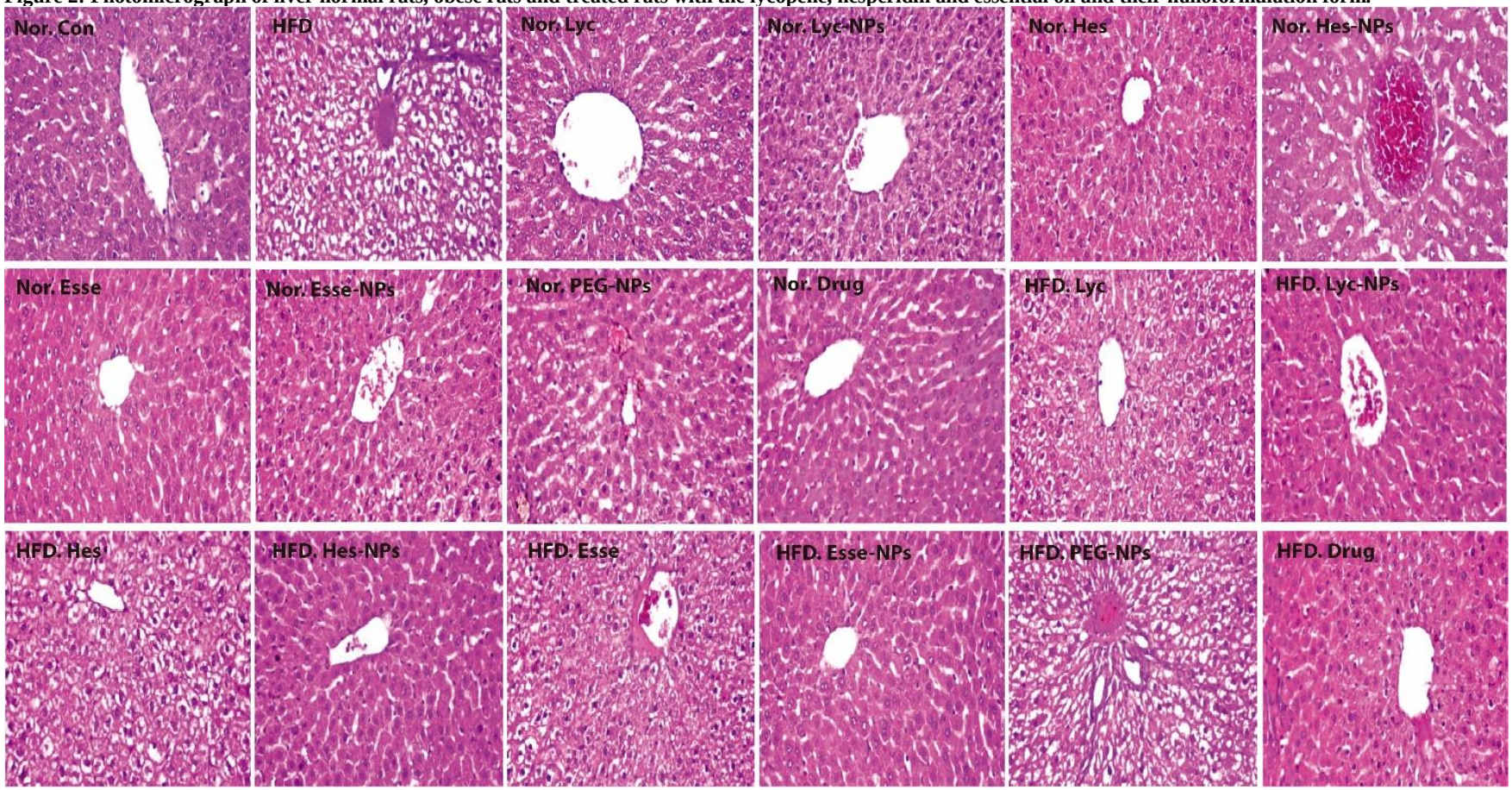
Table 3. Effect of native lycopene, hesperidin, and essential oil and their nano-formulation form on liver function, antioxidant biomarkers, and oxidative stress.

	Liver function						Oxidative stress		Antioxidant biomarkers			
	AST	%	ALT	%	ALP	%	MDA	%	CAT	%	GSH	%
Groups	**Level (IU/L)**		**Level (IU/ L)**		**Level (IU/L)**		**Level (nmol/g)**		**Level (U/g)**		**Level (mg/g)**	
Nor. Con	128.50^{f}± 11.71	-----	85.01^{bc}± 1.43	-----	128.41^{ef}± 7.50	-----	65.44cd± 3.37	-----	581.12b± 75.26	-----	116.35abcde± 3.64	-----
Nor. Lyc	129.04^{f}± 12.00	-----	84.94^{bc}± 5.74	-----	123.98^{f}± 3.32	-----	73.04bc± 5.90	-----	557.52b± 58.03	-----	113.93cde± 3.24	-----
Nor. Lyc-NPs	127.88^{f}± 1.63	-----	86.08^{bc}± 11.74	-----	124.24^{f}± 6.28	-----	67.65cd± 3.37	-----	587.02b± 35.77	-----	117.56abcd± 2.64	-----
Nor. Hes	128.11^{f}± 3.92	-----	92.43^{b}± 8.96	-----	135.01^{de}± 1.23	-----	68.14c± 3.77	-----	589.97b± 33.50	-----	112.07de± 3.05	-----
Nor. Hes-NPs	129.74^{f}±4.45	-----	86.52^{bc}± 4.81	-----	126.82^{ef}± 4.09	-----	65.44cd± 4.57	-----	584.07b± 30.66	-----	111.30de± 5.50	-----
Nor. Esse	132.29^{ef}± 4.59	-----	87.14^{b}± 2.27	-----	133.64^{def}± 2.29	-----	64.95cd± 5.52	-----	578.17b± 90.82	-----	113.73de± 6.19	-----
Nor. Esse-NPs	136.33^{def}± 10.12	-----	89.28^{b}± 2.29	-----	126.32^{ef}± 3.48	-----	72.55bc± 2.36	-----	533.92b± 74.22	-----	115.34bcde± 5.43	-----
Nor. PEG-NPs	133.70^{def}± 6.74	-----	92.43^{b}± 9.59	-----	132.11^{def}± 9.74	-----	69.85bc± 8.82	-----	557.52b± 58.03	-----	111.50de± 6.83	-----
Nor. Drug	133.61^{def}± 7.00	-----	91.99^{b}± 8.82	-----	124.89^{f}± 3.16	-----	67.40cd± 3.47	-----	590.01b± 48.74	-----	123.22abc± 7.28	-----
HFD	194.54^{a}± 1.63	-----	108.91^{a}± 11.39	-----	166.14^{a}± 2.72	-----	132.35a± 6.74	-----	902.65a± 8.85	-----	79.59f± 9.28	-----
HFD. Lyc	147.64^{bcde}± 11.87	36.50	88.72^{b}± 6.72	23.75	141.36^{bcd}± 6.54	19.29	69.85bc± 7.68	95.51	560.47b± 36.84	58.88	120.19abcd± 3.94	34.90
HFD. Lyc-NPs	154.08^{bc}± 21.67	31.49	81.04^{bc}± 4.98	32.78	132.90^{def}± 10.42	25.88	59.56d± 3.89	111.24	581.12b± 67.01	55.32	118.17abcd± 3.21	33.16
HFD. Hes	162.14^{b}± 2.91	25.22	90.10^{b}± 4.60	22.12	146.19^{b}± 7.38	15.53	71.57bc± 5.16	92.88	610.62b± 26.55	50.25	107.87e± 4.58	24.31
HFD. Hes-NPs	155.71^{b}± 6.53	30.24	83.94^{bc}± 6.47	29.37	144.89^{bc}± 6.42	16.55	76.47b± 5.84	85.39	595.87b± 31.08	52.79	113.93cde± 5.39	33.20
HFD. Esse	157.33^{b}± 11.24	28.96	89.03^{b}± 4.25	23.38	145.68^{b}± 2.03	15.93	65.20cd± 1.53	102.62	554.57b± 39.90	59.90	123.62ab± 6.15	37.85
HFD. Esse-NPs	148.42^{bcd}± 3.20	35.90	74.75^{c}± 6.80	40.17	135.57^{cde}± 6.40	223.81	66.67cd± 2.58	100.37	551.62b± 56.89	60.41	118.37abcd± 6.07	33.91
HFD. PEG-NPs	202.68a± 12.43	6.33	112.99^{a}± 10.30	4.81	166.70^{a}± 3.90	0.44	137.99a± 3.32	8.61	888.20a± 35.84	2.49	71.51f± 5.78	6.94
HFD. Drug	139.43^{cdef}± 8.85	42.89	88.47^{b}± 9.34	24.05	129.72^{ef}± 7.86	28.36	66.91cd± 5.84	100.00	595.87b± 13.52	52.79	125.04a± 7.89	39.06
LSD	15.42	-----	12.20	-----	9.73	-----	8.91	-----	83.40	-----	9.32	-----

All values are represented as mean ±S.D.

Means with different letters are significantly different (p<0.05). (%).): Improvment, (Nor.): normal, (Con.): control, (Lyc.): lycopene, (Hes): hesperid in, (Esse.): essential oil, (NP-s): nanoparticles, (HFD): high-fat diet and (PEG): polyethylene glycol.

Figure 2: Photomicrograph of liver normal rats, obese rats and treated rats with the lycopene, hesperidin and essential oil and their nanoformulation form.



(Nor.Con): normal control group, (HFD): obese control group, (Nor. Lyc): normal rats treated with lycopene, (Nor. Lyc-NPs): normal rats treated with lycopene nanoparticales, (Nor. Hes) normal rats treated with hesperidin, (Nor. Hes-NPs) normal rats treated with hesperidin nanoparticales, (Nor. Esse) normal rats treated with essential oil, (Nor. Esse-NPs): normal rats treated with essential oil nanoparticales, (Nor. PEG-NPs): normal rats treated with polyethylene glycol nanoparticales, (Nor. Drug): normal rats treated with drug, (HFD-Lyc): obese rats treated with lycopene, (HFD-Lyc-NPs): obese rats treatmed with lycopene nanoparticales, (HFD-Hes): obese rats treatmed with hesperidin, (HFD- Hes-NPs): obese rats treatmed with hesperidin nanoparticales, (HFD-Esse) obese rats treated with essential oil, (HFD- Esse-NPs): obese rats treated with essential oil nanoparticales, (HFD. PEG-NPs): normal rats treated with polyethylene glycol nanoparticales and (HFD- Drug): obese rats treated with drug.

6. Conclusions

Besides its valuable nutritional benefits of bioactive compounds (lycopene, hesperidin, and essential oils), the nano- formulation form was more effective than a native form of lycopene, hesperidin, and essential oils. Also, the result showed that lycopene and its nanoparticles were more effective than hesperidin, essential oil, and their nano-formulation form. In conclusion, oral supplementation with lycopene, hesperidin, essential oil, and their nano-formulation form can treat dysfunction in obese rats.

AUTHOR CONTRIBUTIONS: Habiba A. Ahmed, Salah H. Salem and Hanan F; Performed experiments and analyzed data. Zeinab A. Salama and Hanan F. Aly, Faten Aboul-Elella, Ahmed M. Aboul-Enein; Supervised the research and Designed and analyzed data. Habiba A. Ahmed and Salah H. Salem; Writing—original draft, Zeinab A. Salama, Faten Aboul-Elella, Ahmed M. Aboul-Enein, Hanan F. Aly; review & editing. Ahmed M. Aboul-Enein; Overall management, funding acquisition, review & editing, review progress and provide recommendations.

7. Conflicts of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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