THE POSSIBLE EFFECTS OF OLEUROPEIN ON ADIPOSE TISSUE BROWNING IN TREADMILL EXERCISED OBESE RATS

By

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ABSTRACT

Background: Obesity is considered to be a pandemic that has increased during the last decades. The energy burning capacity of brown adipose tissue (BAT) makes it an attractive target in anti obesity therapies. Several strategies are being examined to activate and recruit BAT with no side effects. **Objective:** To use olive tree waste (leaves) as a source of medicinally important phenolic compound oleuropein and to evaluate the possible effects of oleuropein on browning of white adipose tissue in exercised obese Rats. **Material and methods:** Sixty adult male Wister albino rats were divided into six equal groups. Group I: Normal control, group II: Oleuropein treated group, group III: High fat diet (HFD), group IV: HFD plus oleuropein, group V: HFD plus exercise training, group VI: HFD plus exercise training and oleuropein. At the end of the experiment, the body weight and the serum levels of lipid profile, GSH, MDA, IL-6 and irisin hormone were determined. The abdominal adipose tissues were excised for the measurement of gene expression of adipose tissue uncoupling protein 1 (UCP1), and CD137. **Results:** Oleuropein treatment significantly decreased body weight. Serum cholesterol, triglycerides and LDL significantly decreased, while HDL significantly increased. Furthermore, oleuropein suppressed the increase in serum IL-6 significantly. **Conclusion:** Oleuropein supplementation may provide an effective therapeutic option for combating obesity as it possessed antioxidant and anti-inflammatory activities, as well as increased expression UCP1 and CD137 which promoted additional browning in BAT

Key words: Oleuropein, HFD, Exercise, Browning of adipose tissue.

INTRODUCTION

Inactivity and sedentary lifestyle is one of the primary causes for the development of obesity, cardiac dysfunction, cardio-vascular aging and type 2 diabetes mellitus (T2DM) **(Sanchez-Delgado et al., 2015).** The adipose tissue, which is greatly expanded in obese persons, acts as secretory endocrine organ of cytokines, hormones and proteins that affect the functions of cells and tissues all over the body **(Gavrieli and Mantzoros, 2016).** It is composed of white (WAT) and brown (BAT) adipose tissues. Both are different in morphology, distribution, gene expression and functions **(Mathieu et al., 2010).** Brown adipocytes are highly vascular, contain high number of mitochondria and innervated by the sympathetic nervous system **(Tews and Wabitsch, 2011).** Studies suggested that identification of BAT in healthy adults have opened up new opportunities for the

development of therapeutics for metabolic diseases like obesity and type 2 DM (**Sanchez-Delgado** *et al.,* **2015**).

Regular exercise training is an effective approach for prevention and treatment of obesity (**Gamas** *et al.,* **2015**). Many of the molecular events responsible for the curative and protective role of exercise remain elusive (**Roca-Rivada** *et al.,* **2013**).

Skeletal muscles represent about 40% of the body weight and constitute a large reservoir for the production of signaling molecules. It is considered to be the largest organ determining whole-body insulin sensitivity and metabolic homeostasis (**Lu** *et al.,* **2016**). It is capable of communicating with other tissues through myokines, which are hormones released into the circulation during exercise. These myokines includes irisin, myostatin, IL-4, IL-6, IL-7, IL-15, leukemia inhibitory factor, myonectin, fibroblast growth factor 21 (FGF-21), brain-derived neurotrophic factor, insulin-like growth factor, follistatin-related protein 1, erythropoietin, musclin and β-amino isobutyric acid (**Stranska and Svačina, 2015 and Panati** *et al.,* **2016**).

Dietary consumption of olive oil is considered a key component to explain the association of Mediterranean diet with a lowered incidence of metabolic disorders and cardiovascular diseases (**Widmer** *et al.,* **2015**). The beneficial action of extra virgin olive oil is mainly attributed to its phenolic compounds as oleuropein which is derived from olive oil and olive leaf (**Tavafi and Ahmadvand, 2011**). Researchers have reported that oleuropein is one of the important antioxidant (**Khalatbary and Ahmadvand, 2012**).

The pharmacological benefits of oleuropein in preventing and protecting from diseases are known. It has in addition to anti-oxidant, antihistaminic, cardioproductive and hypolipidemic effects, anti-inflammatory effects, the effect of inhibiting thrombocyte aggregation, anti-atherogenic effect, anticancerogenic effect, antimicrobic properties against gram-positive and gram-negative bacteria, neuroprotective effect and antiviral effects (**Andreadou** *et al.,* **2006 and Bazoti** *et al.,* **2006**). Despite the benefits attributed to the consumption of olive oil, its use in enhancing thermogenesis has sparked interest in the scientific research.

The aim of the present study was to isolate oleuropein in pure form from cheap olive tree waste (leaves) and evaluate the possible mechanisms by which oleuropein and/or exercise can combat obesity-associated metabolic alterations.

MATERIAL AND METHODS

Plant material: The leaves, collected from trees of Olive (*Olea europaea* L.) Chemlali cultivar, were grown in the Siwa oasis, at North West of Egypt in the middle of April 2015. The authentication of the plant was kindly confirmed by Professor Dr. M. El-Gendy professor of Taxonomy. The leaves were air-dried, reduced to fine powder and kept in tightly closed amber colored glass containers. Voucher specimens were kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Animals: Sixty adult male albino rats of local strain (7-8-weeks old, weighing 130-180 g) were purchased from Helwan Farm (Cairo, Egypt). They were housed in

standard cages (3 rats/25X30X30 cm cage), under specific pathogen-free conditions in facilities maintained at controlled room temperature (21-24°C) and under normal dark–light cycles. All animals had free access to rat chow diet and water *ad libitum* and were acclimated for two weeks prior to initiation of the experiment in the laboratory of in the National Research Centre. All procedures were approved by the Animal Care Committee of the National Research Centre. The "Principles of laboratory animal care" were followed, and also the specific national laws were applicable.

Diets: Commercial rat chow diet (balanced diet), containing 67% carbohydrates, 10% fat, and 23% protein as the energy sources (overall calories: 3.6 kcal/g), was purchased from El Gomhorya company (Cairo, Egypt). High fat diet (HFD), consisting of 88% of standard pellet animal diet, 10% lard and 2% cholesterol was prepared and used to induce hyperlipidemia. The major composition of the diets used in this study was previously characterized by **Xu *et al.* (2010) and Hussain *et al.* (2016).** The HFD was composed of the following energy sources: 52% carbohydrates, 30% fat and 18% protein (overall calories: 4.8 kcal/g) (**Hussain *et al.*, 2016).**

Extraction and isolation of oleuropein: 500g of dried olive leaves in powder form were extracted until exhaustion with ethanol 80 %. The extracts were then filtered through a Whatman No.1 filter (Whatman,UK). The filtrate was then evaporated in rotary evaporator at room temperature under vacuum. The dried ethanolic extract was further fractionated with 4 x 300 ml ethyl acetate. Ethyl

acetate fractions were pooled together and evaporated by rotatory evaporator under reduced pressure at 40 °C then dried on anhydrous sodium sulphate 12g of a dry extract were obtained. Ethyl acetate dry residue was chromatographed with HPLC to determine its content of oleuropein quantitatively and further purified by silica gel flash column chromatography, using methylene chloride: methanol: water / (14:3:1) as an isocraticeluent to yield 5.1 g of oleuropein as amorphous powder in pure form.

Qualitative and quantitative determination of oleuropein in olive leaf extracts by HPLC: Qualitative and quantitative determinations of oleuropein in plant under investigation were carried out using reversed phase HPLC. Stationary phase was silca-based C18 bonded phase column (C18, 250 mm×4.6 ID). Mobile phase consisting of a mixture of water and acetonitrile (70/30 volume ratio) containing 1% acetic acid at a flow rate of 1.0 mL/min. UV detector at 240 nm was used for oleuropein detection and determination. The injection volume used was 20.0 µl for both standard and sample solutions. Identification of isolated oleuropein and quantitative determination of oleuropein in olive ethyl acetate fraction was based on retention times in comparison with standard of oleuropein. The concentration of oleuropein in ethyl acetate fraction was calculated using peak area. The amount of oleuropein was calculated as % weight / weight.

Experimental Design: The rats were divided into 6 equal groups. **Group I:** Rats were assigned to normal control group and given normal balanced chow (10% fat) and supplemented orally with

saline (10 mg/kg/day) for 12 weeks. **Group II:** Rats were given normal balanced chow (10% fat) and supplemented orally with oleuropein (50 mg/kg/day) by gastric gavage tube (**Hadrich** *et al.,* **2016**) for 12 weeks. **Group III** (HFD group): Rats were given HFD (30% fat) and orally supplemented with saline for 12 weeks and left without exercise (**Xu** *et al.,* **2010 and Hussain** *et al.,* **2016**). **Group IV** (HFD-oleuropein group): Rats were kept on HFD for 6 weeks, and then were given oleuropein (50 mg/kg/day) by gastric gavage (**Hadrich** *et al.,* **2016**), with HFD for 6 weeks. **Group V** (HFD-exercise group): Rats were kept on HFD for 6 weeks. At the beginning of the 6th week of HFD intake, the rats were acclimated to exercise (5 meter/minute, for five minutes) for 1week according to **Cechettia** *et al.* **(2012)** to minimize stress. By the beginning of the 7th week, the exercise training program began 30 minutes (5 times/week) for the next 6 weeks according to **Kim** *et al.* **(2015)** *and* **Mahjoub** *et al.* **(2016)** which were continued with HFD intake. **Group VI** (HFD-oleuropein-exercise group): Rats received HFD for 6 weeks with acclimation to the exercise (5 meter/minute, for five minutes) by the beginning of 6th week for one week. On the beginning of the 7th week, rats received oleuropein orally at a daily dose of 50 mg/kg for 6 weeks, and exercised by the same program of group V to the end of 12th week.

The animals of control and sedentary groups (II, III and IV) were transported to the experimental room and handled exactly as the exercised animals. They were maintained in the turned off treadmill for 5 minutes without forcing them to run. This was designed to minimize any possible effects of external factors as handling and shape of treadmill (**Bernardi** *et al.,* **2013).**

Treadmill Exercise training program: 47302 - Rodent Treadmill NG, Ugo Basile, Varese, Italy, Model was used. The exercise training program began on the 7th week of experiment at morning between 10.00 and 12.00 Am (**Bae** *et al.,* **2016**) by sessions of gradually increasing speed training 30 minutes, 5 times/week (5 meter/minute for 5 minutes increased to 12 meter/minute for 5 minutes, and finally 18 meter/minute for 20 minutes, once daily, 5 times per week) for 6 weeks (**Kim** *et al.,* **2015 and** *Mahjoub et al.,* **2016).**

The body weight of each rat was measured and recorded weekly for all groups. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins.

At the end of the experiment, after overnight fasting, rats were anesthetized in the morning, and blood samples were collected from retro-orbital venous plexus by capillary tubes under light ether anesthesia. The blood was then centrifuged at 3000 rpm for 15 minute for serum collection. Serum was separated in aliquots in Eppendorf tubes and stored frozen at -80°C until analysis. The separated serum was analyzed for estimation of the levels of lipid profile, oxidative stress markers, inflammatory markers and irisin hormone. The abdominal adipose tissues were excised for the measurement of gene expression of adipose tissue uncoupling protein 1 (UCP1), and CD137.

Biochemical analysis: total serum cholesterol and HDL were measured by quantitative enzymatic colorimetric determination using biomed diagonstic assay kits (**Khairunnuur *et al.,* 2010**). Serum triglycerides were measured by quantitative enzymatic colorimetric determination using Cayman colorimetric assay kit (**Khairunnuur *et al.,* 2010**). Serum LDL cholesterol was calculated from the values of total cholesterol (TC), HDL and triglycerides using Friedewald equation: LDL (mg/dl) = TC - HDL – $(TG/5.0)^2$ (**Ahmadi *et al.,* 2008**). Serum reduced GSH was determined using Cayman's GSH assay kit (**Rahman *et al.,* 2007**). Serum MDA has been identified by reaction of thiobarbituric acid with MDA in acidic medium at temperature of 95 °C for 30 minutes to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at wave length 534nm (**Zeb and Ullah, 2016**). Serum IL-6 was measured by commercial ELISA kits (Ray Bio® Rat, Ray Biotech, Norcross, GA, USA) (**Gaber *et al.,* 2013**). Serum Irisin was measured by commercial ELISA kits (**Bostrom *et al.,* 2012**).

Detection of uncoupling protein-1 (UCP-1) and CD137 Gene expression in abdominal adipose tissue: RNA was extracted, reversely transcribed into cDNA and amplified by PCR and then detected using agarose gel electrophoresis. Western blot analysis for adipose p38 MAPK total protein was extracted from the tissue using a protein extraction kit. Total protein levels were determined by using the Bradford method (**Han *et al.,* 2014**).

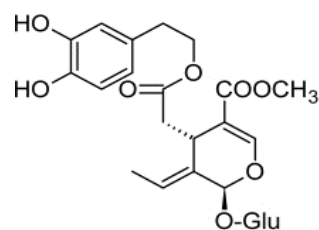**Quantitative real-time reverse transcription PCR analysis:** Total RNA was extracted from the tissue using TRIzol Reagent (Invitrogen, San Diego, California, USA).The concentration of total RNA was measured by absorbance at 260/280nm. The reverse transcription reaction for the first-strand cDNA synthesis was carried out with reverse transcriptase (Bio-Rad, Hercules, California, USA) using 2?g of total RNA. Real-time PCR was initiated on a Step One Plus Real time**.** PCR System (ABI Applied Biosystems, San Francisco, USA) using Power SYBR Green PCR Master Mix (**Pfaffl, 2001**).

Statistical Analysis: All values are presented as means ± standard error of the means (SE). Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey HSD test for multiple comparisons. Graphpad Prism software, version 5 (Inc., San Diego, USA) was used to carry out these statistical tests. The difference was considered significant when $p < 0.05$.

RESULTS

Qualitative and quantitative determination of oleuropein in olive leaf extracts by HPLC:

HPLC results showed that the concentration of oleuropein in ethyl acetate fraction is 40.1% w/w. HPLC also confirmed identification & authentication of isolated compound as pure oleuropein by comparing to retention time of standard oleuropein.

Oleuropein

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Effect of exercise and/or oleuropein on body weight and serum lipid profile:

High fat diet for 12 weeks induced a robust increase in body weight by 48% on comparison to normal control rats. Body weight increase was ameliorated upon use of exercise, oleuropein administration and their combination by 30%, 26% and 32% respectively. Furthermore, the induced dyslipidemia in HFD group was presented by a significant elevation of TG and TC by 1.47 and 1.48 folds, respectively, and decrease in HDL-C by 56.18 % as compared to normal control. Notably, exercise, administration of oleuropein or a combination of both successfully ameliorated the aforementioned deleterious effects as compared with HFD group **(Table 1).**

Table (1): Effect of exercise and/or oleuropein on body weight and serum lipid profile.

Groups / Parameters	Normal control	Oleuropein (50 mg/kg)	HFD	HFD + exercise	HFD + Oleuropein	HFD + exercise + Oleuropein
Body weight (g)	216.9 ± 0.1	214.35 ± 0.8	321.1 ± 3.4^a	224.7 ± 2.33^{ab}	237.54 ± 1.25^{abc}	$218.28^{a,b,c,d} \pm 2.50^{abcd}$
Total cholesterol (mg/dl)	146.29 ± 1.56	153.70 ± 3.43	217.70 ± 0.79^a	183.82 ± 1.58^{ab}	193.36 ± 0.97^{abc}	175.12 ± 2.35^{abcd}
Triglyceride (mg/dl)	79.67 ± 1.12	84.07 ± 1.77	117.70 ± 0.79^a	96.28 ± 0.81^{ab}	108.36 ± 1.72^{abc}	99.28 ± 0.96^{abd}
HDL (mg/dl)	63.21 ± 1.25	62.81 ± 0.91	27.70 ± 0.79^a	35.48 ± 1.65^{ab}	44.24 ± 0.29^{abc}	49.32 ± 0.50^{abcd}

[a] Significantly different from normal control (Saline) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD + exersice, d significantly different from HFD + oleuropein at *P<0.05*.

Effect of exercise and/or oleuropein on oxidative stress and inflammation:

MDA levels elevated after HFD supplementation by about 19.39 fold and GSH activity showed reduction about 70.82 % These effects were associated with disturbance in the pro-inflammatory markers by causing a prominent elevation in serum levels of pro-inflammatory marker IL-6, by 4.60 fold as compared to normal control. On the other hand, exercise and/or oleuropein administration showed significant decline in MDA and IL-6 serum levels and elevation in GSH activity as compared with HFD group **(Figure 1&2).**
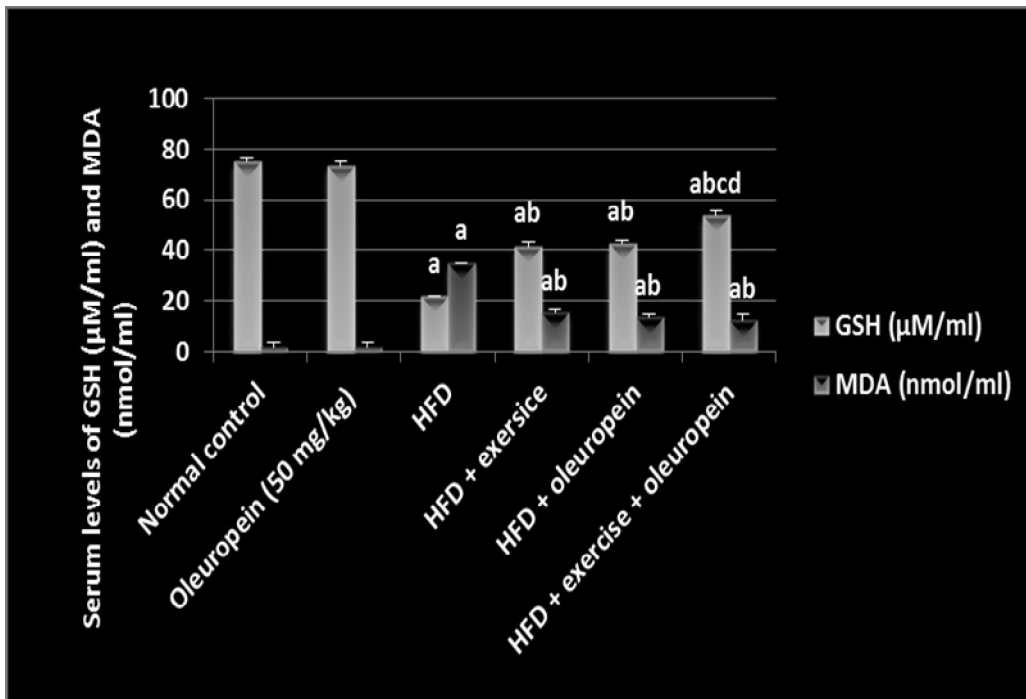
Figure (1): Effect of exercise and/or oleuropein on serum levels of GSH and MDA.
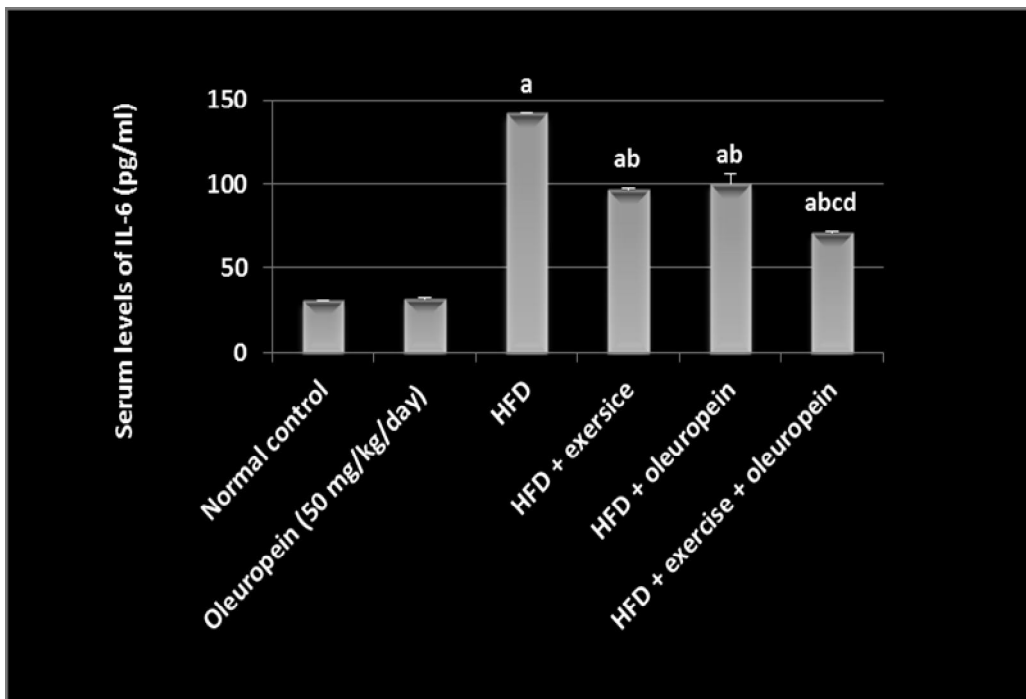

Figure (2): Effect of exercise and/or oleuropein on serum IL-6.

[a] Significantly different from normal control (Saline) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD + exersice, [d] significantly different from HFD + oleuropein at *P<0.05*.

Effect of exercise and/or oleuropein on serum irisin level:

HFD showed marked reduction in serum irisin level by 70.6%, from the control level. Conversely, exercise and its combination with oleuropein administration concomitantly with HFD increased serum irisin level by 1.23 and 1.38 fold respectively, while oleuropein administrated with HFD did not change serum irisin level as compared with HFD group **(Figure 3).**
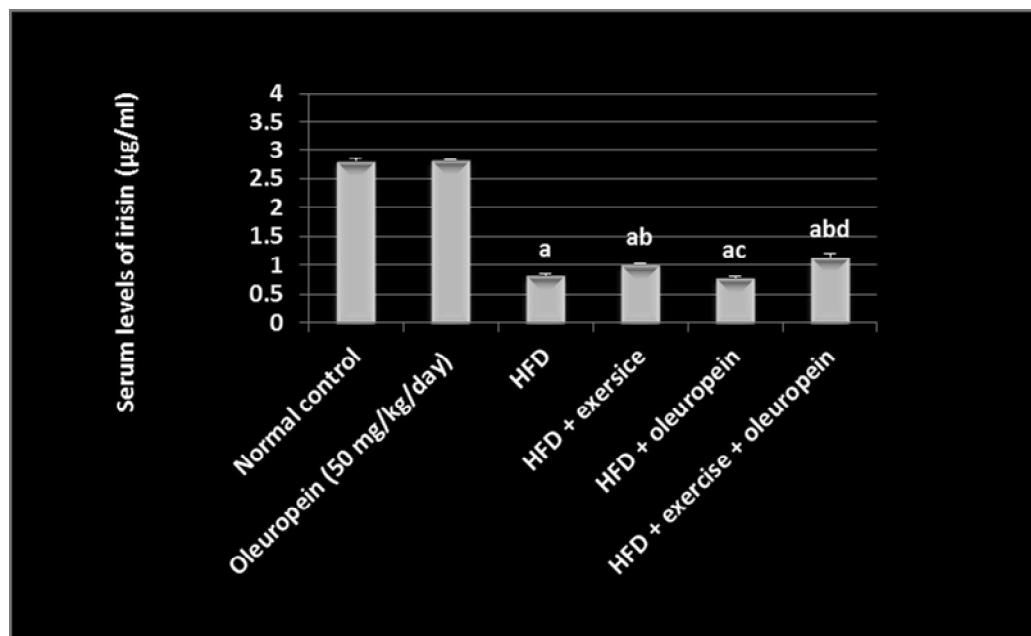

Figure (3): Effect of exercise and/or oleuropein on serum levels of Irisin.

[a] Significantly different from normal control (Saline) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD + exersice, [d] significantly different from HFD + oleuropein at *P<0.05*.

Effect of exercise and/or oleuropein on adipose tissue UCP1 and CD137 gene expression:

HFD produced a significant reduction of the browning marker UCP1 gene expression by 70.4% and beige fat-specific marker CD137 gene expression by 97.2 % in adipose tissue compared to their respective controls. On the other hand, exercise, oleuropein administration and a combination of both concomitantly with HFD increased UCP1 expression by 2.13, 1.71 and 2.04 folds, respectively, as well as increased CD137 expression by 25.71, 21.75 and 26.59 folds, respectively, as compared with HFD group **(Figure 4).**
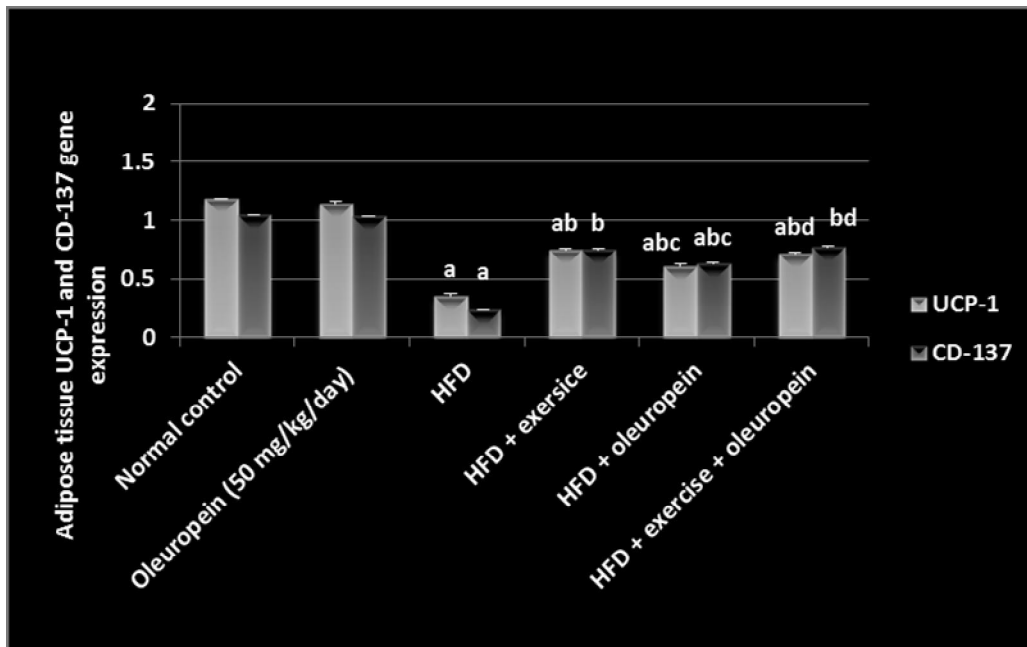
Figure (4): Effect of exercise and/or oleuropein on adipose tissue UCP1 and CD137 gene expression.

[a] Significantly different from normal control (Saline) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD + exersice, [d] significantly different from HFD + oleuropein at *P<0.05*

DISCUSSION

Current drug treatments for obesity produce small and usually unsustainable decrease in body weight with the risk of major adverse effects. Lifestyle modification including the use of functional foods could produce a reliable decrease in obesity and its comorbidities **(Brown *et al.*, 2015).** These have aroused interest in studying nutrients rich in phenolic compounds, especially olive leaves which contain oleuropein as a potential irisin inducer, and its combination with chronic treadmill exercise as an agent that could stimulate the adipose tissue browning for correction of obesity.

In the present study, twelve weeks supplementation of rats with high fat diet (30% fat) induced significant elevation of body weight. This elevation was in agreement with previous studies of **Ahn & Go (2017) and Marics *et al.* (2017)** who showed that HFD increase the body weight significantly. **Yang *et al.* (2015)** explained this elevation to the digestion of fat to monoglycerides and fatty acids by lipase and absorbed fat is accumulated in adipose tissue through excessive adipocyte differentiation. Another explanation was reported by **Dodd *et al.* (2015)** who stated that the elevation of body weight may be attributed to hyperphagia and consequently high energy intake induced by adipocyte-derived leptin hormone secretion.

Increased physical activity results in the control of body weight, after considering

energy supply according to energy consumption and expenditure associated with hormones **(Harris *et al.,* 2015 and Chen *et al.,* 2016).** On the basis of these findings, our results revealed that chronic regular treadmill training exercise for six weeks significantly reduced the body weight. These results were in consistence with the study of **Chen *et al.* (2017)** who observed that treadmill exercise to mice significantly reduce the body weight and insulin resistance, and explained this reduction to that exercise can alter food preference in obese mice, which may be mediated by dopaminergic plasticity of the ventral tegmental area-nucleus accumbens and enhanced insulin sensitivity. Another observation was reported by **Rocha-Rodrigues *et al.* (2016 a)** that eight-weeks of exercise training decrease body weight, visceral adiposity and adipocyte size, plasma non-esterified fatty acids (NEFA) and glycerol levels in obese rats. Oleuropein supplementation induced significant decrease of body weight in the present study. This reduction was supported by the study of **Hadrich *et al.* (2016)** who attributed this decrease to reduction of adipose tissue mass and triglyceride with increased serum adiponectin concentration, which is an important hormone with fatty-acid oxidation properties. In addition, our work reported a decrease of body weight in exercise and oleuropein combination group.

The current study showed a marked increase in the serum lipid profile level (total cholesterol, triglycerides and LDL) in HFD group, while serum HDL level significantly decreased. These results were consistent with the other studies indicating altered lipid metabolism associated with HFD intake **(Galaly *et al.,* 2014 and Tanko *et al.,* 2016).** The cholesterol level in plasma and liver of hyperlipidemic rats increased due to the increased uptake of exogenous cholesterol and subsequent its deposition, in addition to the decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids **(Barakat and Mahmoud, 2011)**. Chronic exercise for six weeks significantly reduced the serum levels of lipid profile in exercised obese rats compared with normal sedentary rats, which is supported by the study of **Przyborowski *et al.* (2017).** This improvement of lipid profile after exercise may be attributed to increased serum levels of irisin as reported by **Tekin *et al.* (2017)** that irisin causes decrease in LDL, triglycerides and cholesterol levels, while increases HDL levels. Also, **Xiong *et al.* (2015)** reported that irisin could improve dyslipidemia and hepatic steatosis in mice. Oleuropein treatment induced improvement of altered lipid profile. In agreement with this results, **Ahmadvand *et al.* (2016)** reported that, high fat diet induced alteration of serum paraoxonase 1 (PON1) which improved by oleuropein supple-mentation. PON1 has the ability to protect lipoprotein particles from free radical oxidation, and it can hydrolyze oxidized cholesteryl esters, phosphatidyl choline core aldehydes, and degrade hydrogen peroxide. So, it has antiatherogenic properties. Another mechanism was reported by **Poudyal *et al.* (2010)** that oleuropein enhances the decreased blood lipid concentrations due to its agonist action on bile acid-activated TGR5, a metabotropic G-protein–coupled receptor. Bile acids increase energy expenditure by

a TGR5-mediated increase in intracellular activation of thyroid hormone, hence preventing the development of obesity and insulin resistance in mice fed a high-fat diet **(Sato et al., 2007).** Moreover, our results showed a decrease of serum lipid level in exercise and oleuropein combination group that may be explained by increased serum levels of irisin.

The current work assessed the major compounds involved in the down-regulation of substances formed during oxidative stress MDA, up-regulation of GSH scavenging activity (antioxidant activity), and reduction of pro-inflammatory cytokines, like IL-6, in order to investigate the effect of oleuropein and exercise on obese rats. Significant elevation of serum levels of MDA and IL-6 as well as decreased serum GSH activity after twelve weeks of HFD supplementation was observed in the present work. These results were consistent with the study of **Viggiano et al. (2016)** who reported that over nutrition can induce inflammatory responses in peripheral tissues and in the hypothalamus, resulting in a defective control of food intake and energy expenditure. Hypothalamic inflammation affects whole body energy homeostasis mostly by controlling neural inputs to specific organs **(Thaler et al., 2010 and 2013).** On the other hand, **Alcal? et al. (2015)** observed that HFD to mice induced marked insulin resistance, hypertriglyceridemia and smaller adipocytes surrounded by a fibrotic extracellular matrix and an increased macrophage infiltration, with the consequent release of pro-inflammatory cytokines. Chronic exercise to HFD supplemented rats in the current study

induced correction of oxidative stress markers and inflammatory marker (IL-6). This was in agreement with **Macpherson et al. (2015)** who stated that adipose tissue is impaired in obesity and is associated with inflammation, macrophage infiltration, and polarization toward a pro-inflammatory phenotype, Acute 0exercise can reduce markers of adipose inflammation, including IL-6. Oleuropein supplementation and their combination with exercise reduced oxidative stress and adipose inflammation. These results were explained by **Ebaid et al. (2010)** who stated that oleuropein is a free radical scavengers, have antioxidant activity as found in other antioxidants, such as vitamin E and butylated hydroxytoluene (BHT).

In the present work, the irisin serum levels significantly reduced in HFD treated rats. This reduction was corrected significantly by exercise and its combination with oleuropein. The study of **Belviranli et al. (2016)** showed that irisin levels decreased with obesity. **Yang et al. (2015)** explained this reduction by down-regulation of FNDC5/irisin expression in adipose tissues observed in HFD-induced obese mice. **Winn et al. (2017)** reported that single bout of moderate and high intensity afternoon exercise induces modest increases in circulating irisin concentrations during exercise in obese females. **Huh et al. (2014)** observed that an acute bout of vibration exercise increases circulating irisin, whereas chronic training does not change the values of irisin in humans.

The adipocyte expression of the browning markers UCP1 and the beige fat-specific marker CD137 in the present

study significantly reduced in obese rats, and elevated by exercise and oleuropein interventions, either individually or together. The present findings were supported by those of **Tanaka-Yachi** *et al.* **(2017)** who reported that the gene expression levels of UCP1 and CD137 increased with agents that promote thermogenic adipocyte differentiation in mammalian white adipose tissues as the α-tocopherol. Exercise causes an increase in energy expenditure through augmentation in brown fat and the browning of white fat (**Bostr?m** *et al., 2012*). Oleuropein supplementation to obese rats significantly elevated the serum irisin level and increased co-expression of the beige fat-specific marker CD137 and the browning marker UCP1 in all types of white fat, while its supplementation to obese exercised rats induced marked elevation in there levels to reach nearly the normal levels. These findings were supported by that of **Rocha-Rodrigues** *et al.* **(2016 b)** who revealed that the adipocyte hypertrophy induced by HFD was attenuated by exercise treadmill.

CONCLUSION

Browning of WAT and thermogenesis promoted in rats with diet-induced obesity by oleuropein supplementation a major component of olive leaves through increased expression of the brown fat marker UCP1 in all types of adipose tissues, together with activation of CD137-positive beige fat formation of WATs which promoted additional browning in BAT. This suggested that targeting a white adipose tissue in obesity by oleuropein supplementation provide an effective therapeutic option for combating obesity and its related metabolic disorders.

On the other hand, although both olive leaves & fruit oil are rich in oleuropein but leaves was selected in this work as economical cheap waste product of this tree. This work finds application using this antioxidant phenolic Oleuropein molecule to design cheap drug for the treatment of obesity, and acts as a curative to complications of fat accumulation on tissues.

REFERENCES

1. **Ahmadi, S.A.; Boroumand, M.A.; Gohari-Moghaddam, K.; Tajik, P. and Dibaj, S.M. (2008):** The impact of low serum triglyceride on LDL-cholesterol estimation. Arch Iran Med., 11(3):318-21.

2. **Ahmadvand, H**.; **Bagheri, S.; Tamjidi-Poor, A.; Cheraghi, M.; Azadpour, M.; Ezatpour, B.; Moghadam, S.; Shahsavari, G. and Jalalvand, M. (2016):** Biochemical effects of oleuropein in gentamicin-induced nephro-toxicity in rats. ARYA Atheroscler., 12(2):87-93.

3. **Ahn, H. and Go, G.W. (2017):** Pinus Densiflora Bark Extract (Pinexol) Decreased Adiposity in Mice by Down-Regulation of Hepatic de novo Lipogenesis and Adipogenesis in White Adipose Tissue. J Microbiol Biotechnol. Jan 12.

4. **Alcal?, M.; S?nchez-Vera, I.; Sevillano, J.; Herrero, L.; Serra, D.; Ramos, M.P. and Viana, M. (2015):** Vitamin E reduces adipose tissue fibrosis, inflammation, and oxidative stress and improves metabolic profile in obesity. Obesity (Silver Spring), 23(8):1598-606.

5. **Andreadou, I.; Iliodromitis, E.K.; Mikros, E.; Constantinou, M.; Agalias, A.; Magiatis, P.; Skaltsounis, A.L.; Kamber, E.; Tsantili-Kakoulidou, A. and Kremastinos, D.T. (2006):** The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. J Nutr., 136(8):2213-9.

6. **Bae, J.Y.; Shin, K.O.; Woo, J.; Woo, S.H.; Jang, K.S.; Lee, Y.H. and Kang, S. (2016):**

Exercise and Dietary Change Ameliorate High Fat Diet Induced Obesity and Insulin Resistance via mTOR Signaling Pathway. J Exerc Nutrition Biochem., 20 (2), 28-33.

7. **Barakat, L.A.A. and Mahmoud, R.H. (2011):** The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. N Am J Med Sci., 3(9): 411- 417.

8. **Bazoti, F.N.; Bergquist, J.; Markides, K.E. and Tsarbopoulos, A. (2006):** Noncovalent interaction between amyloid-beta-peptide (1-40) and oleuropein studied by electrospray ionization mass spectrometry. J Am Soc Mass Spectrom., 17(4):568-75.

9. **Belviranli, M.; Okudan, N. and Celik, F. (2016):** Association of Circulating Irisin with Insulin Resistance and Oxidative Stress in Obese Women. Horm Metab Res., 48(10):653-657.

10. **Bernardi, C.; Tramontina, A.C.; Nardin, P.; Biasibetti, R.; Costa, A.P.; Vizueti, A.F.; Batassini, C.; Tortorelli, L.S.; Wartchow, K.M.; Dutra, M.F.; Bobermin, L.; Sesterheim, P.; Quincozes-Santos, A.; de Souza, J. and Carlos Alberto Gonçalves, C.A. (2013):** Treadmill Exercise Induces Hippocampal Astroglial Alterations in Rats. Neural Plasticit, Volume 2013, Article ID 709732, 10 pages

11. **Bostr?m, P.; Wu, J.; Jedrychowski, M.P.; Korde, A.; Ye, L.; Lo, J.C.; Rasbach, K.A.; Bostr?m, E.A.; Choi, J.H.; Long, J.Z.; Kajimura, S.; Zingaretti, M.C.; Vind, B.F.; Tu, H.; Cinti, S.; H?jlund, K.; Gygi, S.P. and Spiegelman, B.M. (2012):** A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature, 481:463–468.

12. **Brown, L.; Poudyal, H. and Panchal, S.K. (2015):** functional foods as potential therapeutic options for metabolic syndrome. Obes. Rev., 16 (11): 914-941.

13. **Cechetti, F.; Worma, P.V.; Elsner, V.R., Bertoldi, K.; Sanches, E.; Ben, J.; Siqueira, I.R. and Netto, A.C. (2012):** Forced treadmill exercise prevents oxidative stress and memory deficits following chronic cerebral hypoper-

fusion in the rat. Neurobiology of Learning and Memory, 97: 90–96.

14. **Chen, N.; Li, Q.; Liu, J. and Jia, S. (2016):** Irisin, an exercise-induced myokine as a metabolic regulator: an updated narrative review. Diabetes Metab Res Rev., 32:51–59.

15. **Chen, W.; Wang, H.J.; Shang, N.N.; Liu, J.; Li, J.; Tang, D.H and Li, Q. (2017):** Moderate intensity treadmill exercise alters food preference via dopaminergic plasticity of ventral tegmental area-nucleus accumbens in obese mice. Neurosci Lett., 3940(17):30075-30077.

16. **Dodd, G.T.; Decherf, S.; Loh, K.; Simonds, S.E.; Wiede, F.; Balland, E.; Merry, T.L.; Münzberg, H.; Zhang, Z.Y. and Kahn, B.B. (2015):** Leptin and insulin act on POMC neurons to promote the browning of white fat. Cell, 160: 88–104.

17. **Ebaid, G.M.; Seiva, F.R.; Rocha, K.K.; Souza, G.A. and Novelli, E.L. (2010):** Effects of olive oil and its minor phenolic constituents on obesity-induced cardiac metabolic changes. Nutr J., 19; 9: 46.

18. **Gaber W, Ghada S. Azkalany, Tamer A. Gheita, Mohey A. and Sabry, R. (2013):** Clinical significance of serum interleukin-6 and _174G/C promoter polymorphism in Rheumatoid arthritis patients. The Egyptian Rheumatologist, 35: 107–113

19. **Galaly, S.R.; Hozayen, W.G.; Amin, K.A. and Ramadan, S.M. (2014):** Effects of Orlistat and herbal mixture extract on brain, testes functions and oxidative stress biomarkers in a rat model of high fat diet. Beni-Suef University. Journal of Basic and Applied Sciences, 3(2): 93–105.

20. **Gamas, L.; Matafome, P. and Seiça1, R. (2015):** Irisin and Myonectin Regulation in the Insulin Resistant Muscle: Implications to Adipose Tissue: Muscle Crosstalk. Journal of Diabetes Research, 2015 (Article ID 359159): 8 pages.

21. **Gavrieli, A. and Mantzoros, C.S. (2016):** Novel Molecules regulating energy homeostasis: physiology and regulation by macronutrient intake and weight loss., 31(3):361-372.

22. **Hadrich, F.; Mahmoudi, A.; Bouallagui, Z.; Feki, I.; Isoda, H.; Feve, B. and Sayadi, S. (2016):** Evaluation of hypocholesterolemic effect of oleuropein in cholesterol-fed rats. Chem Biol Interact., 25; 252:54-60.

23. **Han, L.; Zi, X.; Garmire, L.X.; Wu, Y.; Weissman, S.M.; Pan, X. and Fan, R. (2014):** Co-detection and sequencing of genes and transcripts from the same single cells facilitated by a microfluidics platform. Scientific Reports, 4(6485): 9 pages.

24. **Harris, L.; Hankey, C.; Murray, H. and Melville, C. (2015):** The effects of physical activity interventions on preventing weight gain and the effects on body composition in young adults with intellectual disabilities: systematic review and meta-analysis of randomized controlled trials. Clin Obes., 5: 198–210.

25. **Huh, J.Y.; Mougios, V.; Skraparlis, A.; Kabasakalis, A. and Mantzoros, C.S. (2014):** Irisin in response to acute and chronic whole-body vibration exercise in humans. Metabolism, 63(7):918–921.

26. **Hussain, M.A; Abogresha, N.M.; Hassan, R.; Tamany, D.A. and Lotfy, M. (2016):** Effect of feeding a high-fat diet independently of caloric intake on reproductive function in diet-induced obese female rats. Arch Med Sci., 12(4): 906–914.

27. **Khairunnuur, F.A.; Zulkhairi, A.; Hairuszah, I.; Azrina, A. and Nursakinah, I. (2010):** Hypolipidemic and weight reducing properties from Tamarindus indica L. pulp extract in diet-induced obese rats. Int J Pharmacol., 6: 216-223.

28. **Khalatbary, A.R. and Ahmadvand, H. (2012):** Neuroprotective effect of oleuropein following spinal cord injury in rats. Neurol Res.; 34(1):44-51.

29. **Kim, Y.M.; Ji, E.S.; Kim, S.H.; Kim, T.W.; Ko, I.G.; Jin, J.J.; Kim, C.J.; Kim, T.W. and Kim, D.H. (2015):** Treadmill exercise improves short-term memory by enhancing hippocampal cell proliferation in quinolinic acid-induced Huntington's disease rats. J Exerc Rehabil., 11:5–11

30. **Lu, Y.; Li, H.; Shen, S-W.; Shen, Z-H.; Xu, M.; Yang, C-J.; Li, F.; Feng, Y-B.; Yun, J-T.; Wang, L. and Qi, H-J. (2016):** Swimming exercise increases serum irisin level and reduces body fat mass in high-fat-diet fed Wistar rats. Lipids Health Dis., 15: 93.

31. **Macpherson, R.E.; Huber, J.S.; Frendo-Cumbo, S.; Simpson, J.A and Wright, D.C. (2015):** Adipose Tissue Insulin Action and IL-6 Signaling after Exercise in Obese Mice. Med Sci Sports Exerc., 47(10):2034-42.

32. **Mahjoub, S.; Ghadi, A.; Pourbagher, R.; Hajian-Tilaki, K. and Masrour-Roudsari, J. (2016):** Effects of Regular Treadmill Exercise on a DNA Oxidative-Damage Marker and Total Antioxidant Capacity in Rat Hippocampal Tissue. J Clin Neurol., 12(4): 414-418.

33. **Marics, B.; Peitl, B.; P?zm?ndi, K.; B?csi, A.; Németh, J.; Oszl?cs, O.; Jancs? G. and Dux, M. (2017):** Diet-Induced Obesity Enhances TRPV1-Mediated Neurovascular Reactions in the Dura Mater. Headache, 2017 Jan 30.

34. **Mathieu, P.; Lemieux, I. and Després, J.-P. (2010):** Obesity, inflammation, and cardiovascular risk. Clinical Pharmacology and Therapeutics, 87(4): 407–416.

35. **Matteis, R.D.; Lucertini, F. and Guescini, M. (2013):** Exercise as a new physiological stimulus for brown adipose tissue activity. Nutrition, Metabolism & Cardiovascular Diseases, 23 (6): 582–590.

36. **Panati, K.; Suneetha, Y. and Narala, V.R. (2016): risin/FNDC5--An** updated review. Eur Rev Med Pharmacol Sci., 20(4): 689-97.

37. **Pfaffl, M.W. (2001):** A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res., 29:e45 .

38. **Poudyal, H.; Campbell, F. and Brown, L. (2010):** Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. J Nutr., 140(5):946-53.

39. **Przyborowski, K.; Kassassir, H.; Wojewoda, M.; Kmiecik, K.; Sitek, B.; Siewiera, K.; Zakrzewska, A.; Rudolf, A.M.; Kostogrys, R.; Watala, C.; Zoladz, J.A. and Chlopicki,**

S. (2017): Effects of a Single Bout of Strenuous Exercise on Platelet Activation in Female ApoE/LDLR-/- Mice. Platelets, 28(7):657-667.

40. Rahman, I., Kode, A. and Biswas, S.K. (2006): Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nature Protocols, 1(6):3159-65.

41. Roca-Rivada, A.; Castelao, C.; Senin, L.L.; Landrove, M.O.; Baltar, J.; Belén Crujeiras, A.; Seoane, L.M.; Casanueva, F.F. and Pardo, M. (2013): FNDC5/irisin is not only a myokine but also an adipokine. PLoS One, 8 (4):e60563.

42. Rocha-Rodrigues, S.; Rodr?guez, A.; Becerril, S.; Ram?rez, B.; Gonçalves, I.O. Beleza, J.; Frühbeck, G.; Ascens?o, A. and Magalh?es, J. (2016a): Physical exercise remodels visceral adipose tissue and mitochondrial lipid metabolism in rats fed a high-fat diet. Clin Exp Pharmacol Physiol. , 44(3):386-394.

43. Rocha-Rodrigues, S.; Rodr?guez, A. Gouveia, A.M.; Gonçalves, I.O. Becerril, S.; Ram?rez, B.; Beleza, J.; Frühbeck, G., Ascens?o, A. and Magalh?es, J. (2016 b): Effects of physical exercise on myokines expression and brown adipose-like phenotype modulation in rats fed a high-fat diet. Life Sci., 165: 100-108.

44. Sanchez-Delgado, G.; Martinez-Tellez, B.; Olza, J.; Aguilera, C.M.; Gil, A. and Ruiz, J.R. (2015): Role of Exercise in the Activation of Brown Adipose Tissue. Ann Nutr Metab., 67:21–32.

45. Sato, H.; Genet, C.; Strehle, A.; Thomas, C.; Lobstein, A.; Wagner, A.; Mioskowski, C.; Auwerx, J. and Saladin, R. (2007): Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. Biochem Biophys Res Commun., 362:793–8.

46. Seldin, M.M. and Wong, G.W. (2012): Regulation of tissue crosstalk by skeletal muscle-derived myonectin and other myokines. Adipocyte, 1(4):200-202.

47. Stranska, Z. and Svacina, S. (2015): Myokines - muscle tissue hormones. Vnitr Lek., 61(4):365-8.

48. Tanaka-Yachi, R.; Takahashi-Muto, C.; Adachi, K.; Tanimura, Y.; Aoki, Y.; Koike, T. and Kiyose, C. (2017): Promoting Effect of α-Tocopherol on Beige Adipocyte Differentiation in 3T3-L1 Cells and Rat White Adipose Tissue. J Oleo Sci., 66 (2): 171-179.

49. Tanko, Y.; Jimoh, A.; Ahmed, A.; Mohammed, A. and Ayo, J.O. (2016): Resveratrol Protects Rabbits Against Cholesterol Diet-Induced Hyperlipidaemia. Niger J Physiol Sci., 31(1):71-5.

50. Tavafi, M. and Ahmadvand, H. (2011): Effect of rosmarinic acid on inhibition of gentamicin induced nephrotoxicity in rats. Tissue Cell., 43(6):392-7.

51. Tekin, S.; Erden, Y.; Ozyalin, F.; Cigremis, Y.; Colak C. and Sandal, S. (2017): The effects of intracerebroventricular infusion of irisin on feeding behaviour in rats. Neurosci Lett., 645:25-32.

51. Tews, D. and Wabitsch, M. (2011): Renaissance of brown adipose tissue. Horm Res Paediatr., 75: 231-9.

52. Thaler, J.P.; Choi, S.J.; Schwartz, M.W. and Wisse, B.E. (2010): Hypothalamic inflammation and energy homeostasis: resolving the paradox. Front Neuroendocrinol., 31(1):79-84.

53. Thaler, J.P.; Guyenet, S.J.; Dorfman, M.D.; Wisse, B.E. and Schwartz, M.W. (2013): Hypothalamic inflammation: marker or mechanism of obesity pathogenesis? Diabetes, 62(8):2629-34.

54. Viggiano, E.; Mollica, M.P.; Lionetti, L.; Cavaliere, G.; Trinchese, G.; De Filippo, C.; Chieffi, S.; Gaita, M.; Barletta, A.; De Luca, B.; Crispino, M. and Monda, M. (2016): Effects of High-Fat Diet Enriched in Lard or in Fish Oil on the Hypothalamic Amp-Activated Protein Kinase and Inflammatory Mediators. Front Cell Neurosci., 10:150 .

55. Widmer, R.J.; Flammer, A.J.; Lerman, L.O. and Lerman, A. (2015): The mediterranean diet, its components, and cardiovascular

disease. The American Journal of Medicine, 128(3):229–238.

56. **Xiong, X.Q.; Chen, D.; Sun, H.J.; Ding, L.; Wang, J.J.; Chen, Q.; Li, Y.H.; Zhou, Y.B.; Han, Y.; Zhang, F.; Gao, X.Y.; Kang, Y.M. and Zhu, G.Q. (2015):** FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. Biochim. Biophys. Acta., 1852:1867–1875.

57. **Xu, Z.; Fan, J.; Ding, X.; Qiao, L. and Wang, G. (2010):** Characterization of high-fat, diet-induced, non-alcoholic steatohepatitis with fibrosis in rats. Dig Dis Sci., 55: 931–40.

58. **Yang, Z.; Chen, X.; Chen, Y. and Zhao, Q. (2015):** Decreased irisin secretion contributes

to muscle insulin resistance in high-fat diet mice. Int J Clin Exp Pathol., 8(6):6490-7.

59. **Zeb A, Ullah F. (2016):** A Simple Spectrophotometric Method for the Determination of Thiobarbituric Acid Reactive Substances in Fried Fast Foods. Journal of Analytical Methods in Chemistry Volume 2016, Article ID 9412767, 5 pages**.**

60. **Zhou, X.; Li, R.; Liu, X.; Wang, L.; Hui, P.; Chan, L.; Saha, P.K. and Hu, Z. (2016):** ROCK1 reduces mitochondrial content and irisin production in muscle suppressing adipocyte browning and impairing insulin sensitivity. Sci Rep., 6:29669.

التأثيرات المحتملة للأوليوروبين علي إسمرار الأنسجة الدهنية في الجرذان السمينة المعرضة لتمرينات التريدميل

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خلفية البحث: تعد السمنة مرضاً متفشياً في العقود الأخيرة . ومقدرة الأنسجة الدهنية البنية علي حرق الدهون تجعلها هدف مغري في علاج السمنة. ومن هذا المنطلق يتم دراسة العديد من الإستراتيجيات لتفعيل الإستفادة من هذه الدهون البنية لعلاج السمنة بدون ظهور اي أعراض جانبية.

الهدف من البحث: دراسة التاثيرات المحتملة للأوليوروبين علي إسمرار الأنسجة الدهنية في الجرذان السمينة المعرضة لتمرينات التريدميل.

مواد وطرق البحث: تم تنفيذ الدراسة الحالية على 60 من ذكور الجرذان الذكور البالغة البيضاء تم تقسيمهم إلى ستة مجموعات متساويةعلى النحو التالي :

المجموعة الأولى: المجموعة الضابطة

المجموعة الثانية: مجموعة تمت معالجتها بالأوليوروبين

المجموعة الثالثة: مجموعة الطعام عالي الدهون

المجموعة الرابعة: مجموعة الطعام عالي الدهون والأوليوروبين

المجموعة الخامسة: مجموعة الطعام عالي الدهون والتمرينات الرياضية

المجموعة السادسة: غذاء عالي الدهون والأوليوروبين بجانب التمرينات الرياضية

وفى نهاية التجربة تم تحديد تغيرات وزن الجسم، وقياس مستويات دهنيات الدم (نسب الكوليستيرول الكلي والكوليستيرول في البروتين الدهني منخفض الكثافة والكوليستيرول في البروتين الدهني عالي الكثافة والدهون الثلاثية)، ومعامل الأكسدة (الجلوتاثيون والمانولدهيد) هذا بالإضافة إلي دلالات الإلتهابات (الإنترليكون-6) وهرمون الأيريزين في المصل. هذا بالإضافة إلى إستئصال الأنسجة الدهنية من منطقة البطن لكل جرذ لقياس التعبير الجيني لبروتين فك الإرتباط وجين سي دي 137 في الأنسجة الدهنية.

النتائج: تتلخص نتائج البحث الحالي في أن إستخدام الأوليوروبين نتج عنه إنخفاضاً ذو دلالة إحصائية لوزن الجسم ، ومستويات الكوليستيرول الكلي والدهون الثلاثية والكوليستيرول في البروتين الدهني منخفض الكثافة، بينما إرتفع الكوليستيرول في البروتين الدهني عالي الكثافة، بالإضافة إلى انخفاض معـدلات الأكسـدة فـي الـدم والتـى وضـحت بانخفـاض مسـتويات المانولدهايـد وإرتفـاع مسـتويات الجلوتـاثيون بالإضافة الي انخفاض دلالات الاورام (لانترليكون 6). اضيف إلى ذلك تحفيز وتنشيط التعبير الجيني لبروتين فك الإرتباط وجين سي دي 137 في الأنسجة الدهنية.

الإستنتاج: اثبتت نتائج هذه الدراسة ان تناول اللأوليوروبين ربما يعطي خياراً علاجياً فعالاً للتخلص من السمنة حيث أثبتت هذه الدراسة قدرته الفعالة كمضاد للأكسدة والإلتهابات بالإضافة إلى تنشيط كلاً من معامل تحويل الأنسجة الدهنية البيضاء إلى أنسجة بنية والمعروف بالتعبير الجيني لبروتين فك الإرتباط والجين المسئول عن إكتشاف الخلايا الجينية والمعروف بالسي دي 137.