International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Olive Leaves (Olea europaea, L.) Improve Insulin-like Growth Factor (IGF) System Implications Induced by High Fat-High Sucrose Diet

Amira Abd El-Rhman[1], Eman Hassan[2]

Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education, Ain Shams University

Abstract: ***Insulin-like growth factors system consists of insulin-like growth factors (IGF-I and –II) as ligands, IGF receptors and family of six high-affinity IGF binding proteins (IGFBPs). The aim of this study is to determine the effect of olive leaves administration either in the form of aqueous (AOLE) or ethanolic (EOLE) extracts on implications of IGF system induced by feeding on high fat-high sucrose diet. Animals were randomly separated into four groups. The first group fed on balanced diet as a control group, the second group fed on high fat-high sucrose diet, the third group fed on HF-HS diet + AOLE in oral dose of (500mg/kgbw) and the fourth group fed on HF-HS diet + EOLE in oral dose of (500mg/kgbw). Serum glucose, insulin, lipogenesis factors as well as inflammatory biomarkers and TAC, serum GH, IGF-I, IGFBP-1, IGFBP-3 were estimated. Results indicated that feeding on HF-HS diet significantly increases serum glucose, insulin and results in bad lipid profile with increased inflammatory markers and reduced TAC, in addition to disturbance in serum GH and IGF system parameters level. While, consuming either AOLE or EOLE significantly increased insulin sensitivity, and improved IGF system implications and associated inflammation, furthermore, EOLE had better effect than AOLE.***

Keywords: IGF, insulin growth factor, IGFBP, insulin resistance, olive leaves, AOLE, EOLE

1. Introduction

Insulin-like growth factor (IGF) pathway consists of three hormones (IGF-I, IGF-II, and insulin), several IGF binding proteins (IGFBP 1-6), three receptors [IGF-I receptor (IGF-IR), IGF-II receptor (IGF-IIR) and insulin receptor (IR)], and their downstream signaling cascades. IGF-I is a peptide hormone predominantly secreted by the liver in response to pituitary-derived growth hormone (GH). In the blood, approximately 90% of IGF-I is complexed with IGFBP-3 and acid labile subunit in a 1:1:1 ratio which increases the half-life of IGF-I (Rohrmann et al., 2010). Circulating IGF-I and IGFBP-3 are markers of biological activity of the cellular level IGF pathway (Yeap et al., 2010). IGF-I stimulates a number of cellular pathways by activating IGF-IR which affects the overall functioning of the cell. The intracellular part of the IGF pathway involves the adapter proteins insulin-receptor substrate (IRS) -1 and -2 through which the phosphatidylinositol 3' kinase (PI3K) /Akt pathway and the mitogen-activated protein kinase (MAPK) pathways are activated. The mitogenic and anti-apoptotic properties of IGF-I are mediated through IRS- 1 and the MAPK and P13-kinase pathways, while IRS-2 mediates IGF-I's metastatic mobility effects. The signaling proteins in these pathways transduce the IGF-I signal via phosphorylation reactions to a variety of intracellular proteins, including transcription factors (Ren and Anversa, 2015). IGF-I and IGFBP-3 levels are nutritionally regulated and depend on the composition of diet. Serum levels of IGF-I and IGFBP-3 elevate with high fat consumption. Both IGF-I and IGFBP-3 stimulate the activity of glycerol-3-phosphatedehydrogenase during differentiation of visceral and subcutaneous preadipocytesisolated from adipose tissue of obese children. In adipocytes from visceral adipose tissue, IGFBP-3 was found to reduce insulin-stimulated glucose uptake this lead to diet-induced insulin resistance. By contrast,IGFBP-1 and IGFBP-2 inhibit in vitro adipose differentiation by IGF-I, probably by preventing IGF-I binding to the IGF1R. Systemic over expression of IGFBP-1 or IGFBP-2 was found to protect mice from diet-induced or age-induced obesity and insulin resistance (Garten et al., 2012).

Dietary factors, such as high-fat or high-sucrose diets have been implicated in the development of hepatic and peripheral tissues insulin resistance in rats. Insulin's ability to stimulate glucose metabolism is severely diminished in the adipose tissue of rats fed a high-fat high sucrose diet as compared to that of rats fed a balanced diet(Sumiyoshi et al., 2006). Numerous studies showed that a HF and/or HS diet induces insulin resistance in rodents. It was reported that excess circulating free fatty acids (FFA) and glucose may contribute to insulin resistance which is characterized by hyperinsulinemia. On the other hand, it was reported that adipocytokines such as leptin, adiponectin, tumor necrosis factor (TNF)-α and IL-6 are secreted from adipose tissue (Chun et al., 2010).

Olive leaves have been widely used in traditional remedies in European and Mediterranean countries. They have been used in the human diet as extracts, herbal teas, and powder. It contains several potentially bioactive compounds that may have antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties (Wainstein et al., 2012). The main active component in olive leaf is oleuropein, several studies have shown that oleuropein possesses a wide range of pharmacologic and health promoting properties (Zoair, 2014). In addition, Poudyal et al., (2010) reported that olive leaf extract (OLE) containing polyphenols reversed the chronic inflammation and oxidative stress by diet-induced obesity and diabetes in cardiovascular, hepatic, and metabolic symptoms in rats. The concentration of olive plant polyphenols is far greater in the leaves than in the fruit or fruit oil, and supplementation with olive leaf extract was

reported to significantly improve insulin sensitivity and pancreatic β-cell secretory capacity (de Bock et al., 2015).

The main aim of this study was to investigate the potential effect of olive leaf as an improvement factor for implications of IGF system caused by high fat-high sucrose diet and comparing the effect of olive leaf water extract with olive leaf ethanolic extract.

2. Materials and Methods

2.1. Plant materials

Olive leaves were purchased from, local market, Cairo, Egypt. Aqueous olive leaf extract (AOLE) was prepared according to Hung et al., (2006) by boiling olive leaves (300g) in water (3000 ml) for 60 minutes then filtered through Whatman filter paper No.2 and the filtrate was dried, the yield of dried extract was about 30g which dissolved in 300ml water (final concentration was 0.1g/ml). Rats were given (AOLE) orally by gavage as (500mg/kgbw) daily for continuous 28 days. Ethanolic olive leaves extract (EOLE) was prepared according to Babu et al., (2003) with some modification. One gram of the powdered dry leaves was mixed with 10ml of 80% ethanol. The mixture was stirred using magnetic stirrer in air tight container for about 1hr then filtered. The filtrate was evaporated in rotary evaporator to remove alcohol at (40-50^{o}C and 250 r.p.m.). The alcohol free sample was weighed and suspended in distilled water to give (0.1 g/ml). Rats were given (EOLE) orally by gavage as (500 mg/kg.bw) daily for continuous 28 days.

2.2. Animals and experimental design

Thirty two adult male albino rats "Sprague Dawely" 90-110 g were kept in stainless steel cages in the well-ventilated animal house. The rats had been kept in the room for 1 week prior to the beginning of the experiment for acclimatization. They had access to 12h cycle of light/dark and provided with standard diet prepared by AIN (1993) and tap water ad libitum. Animals then separated into control group (8 rats) that fed standard balanced diet according to AIN-1993 (Reeves et al., 1993) and the remaining (24rats) fed on high fat-high sucrose diet containing 21% fat and 55% carbohydrates(37%sucrose and 18 %starch), for induction of obesity, according to Yang et al., (2012) for 28days, then separated into groups as follow:

Group1: control group fed on standard balanced diet + plain water given daily using oral gavage.
Group2: fed on HF-HS diet + plain water given daily using oral gavage.
Group3: fed on HF-HS diet + AOLE in oral dose of (500mg/kg.bw) given daily by oral gavage.
Group4: fed on HF-HS diet + EOLE in oral dose of (500mg/kg.bw) given daily by oral gavage.

2.3. Sample collection

At the end of the experimental period, animals were sacrificed under ether anesthesia after 12 hr fasting. Blood was collected from portal vein in centrifuge tubes, and then left to clot and serum was separated by centrifugation at 3000 r.p.m. for 10 min at 4°C.Samples were kept in microtubes at -70°C until analysis.

2.4. Determination of Total phenolic content of olive leaf extracts by Folin-Ciocalteu assay

TPC content was measured using Folin-Ciocalteu assay according to the procedure described by Stintzing et al., (2005). The results were expressed as mg equivalent of gallic acid (GAE) per 100ml extract, according to calibration curve, build on the range of 0.02 - 0.10 mg gallic acid used as a standard.

2.5. Determination of Antioxidant activity of olive leaf extracts by DPPH assay:

The assay was performed according to the method described by Kivrak et al., (2009). The radical scavenging activities of the tested samples, was expressed as percentage inhibition of DPPH.

2.6. Determination of carbohydrate metabolism markers

Serum glucose was determined using the enzymatic colorimetric technique (GOD/ POD/ PAP) by Diagnosticum Zrt Kit, according to the method of Trider (1969). Serum insulin was determined using ELISA kit (IBL-America)according to the method of Frosig et al., (2007). HOMA-IR and QUIKI were calculated according to the following equations:
HOMA-IR= fasting insulin (µIU/ml) x fasting glucose (mg/dl)/405 (Stumvoll et al., 2001)
QUIKI=1/log fasting insulin (µIU/ml) + log fasting glucose (mg/dl) (Katz et al., 2000)

2.7. Determination of lipogenesis markers and atherogenic index

Serum total cholesterol (TC) was determined using the enzymatic colorimetric technique (PAP) by Diagnosticum Zrt Kit, according to the method of Allain (1974). Serum triacylglycerols (TAGs) was determined using the enzymatic colorimetric technique (PAP) by Diagnosticum Zrt Kit, according to the method of Annoni et al., (1982). Serum HDL-C was determined using the precipitating reagent by Diagnosticum Zrt Kit, according to the method of LopesVirella (1977). LDL-C, VLDL-C and atherogenic index (AI) were calculated applying the Friedwald's equation (Friedewald. 1972).
VLDL-C= TG/5
LDL-C=TC-(HDL-C+VLDL-C)
AI=LDL-C/HDL-C

2.8. Determination of inflammatory markers and total antioxidant capacity

Serum (IL-6) was determined using ELISA kit (BioVision-USA) according to the method of Pradhan et al., (2001).Serum TNF-α was determined using ELISA kit (CUSABIO- USA)according to the method of D'Haens, (2003). Serum CRP was determined using ELISA kit (BD-America) according to the method of Banerjee et al., (2003).

Serum TAC was determined using colorimetric kit (BioVision- USA) according to the method of Koracevic et al., (2001).

2.9. Determination of growth hormone and some IGF system parameters

Serum (GH) was determined using ELISA kit (CUSABIO - USA)according to the method of Span et al., (2000).Serum (IGF-I) was determined using ELISA kit (KAMIYA-USA)according to the method of Janssen et al., (1998).Serum (IGFBP-1) was determined using ELISA kit (CUSABIO -USA)according to the method of Wang et al., (2007).Serum (IGFBP-3) was determined using ELISA kit (CUSABIO -USA)according to the method of Eggert et al., (2014). Molar ratio of IGF-I/IGFBP-3 was calculated according to the equation of Morimoto et al., (2005) Molar ratio= IGF-I X 0.13/IGFBP-3 X 0.036

2.10. Statistical Analysis

Results were expressed as mean ± Standard deviation (S.D.) of the mean. Differences among means were tested for statistical significance by one-way analysis of variance using SPSS package version 19. Statistical significance was considered when P <0.05.

3. Results

3.1. Total phenolic content and antioxidant activity of olive leaf extracts:

From table (1) the colorimetric Folin-Ciocalteu assay is the most used and rapid quantitative technique for the determination of total polar phenolics in olive leaves. Solvents could significantly affect total phenolics due to differences in solvent polarities, which might influence the solubility of various constituents present in olive leaves. Hence, the extraction capacity of ethyl alcohol showed a better effect on extraction of olive leaf phenolics (3.37meq of gallic acid) than water (1.73 meq of gallic acid).Ethanol extracts exhibited higher antiradical activity. The radical scavenging activity of ethanol olive leaves extract was (65.75% of inhibition of DPPH) higher than the antioxidant activity of water extract (30.90% of inhibition of DPPH).The previous results confirmed that EOLE has higher phenolic content and antioxidant activity than AOLE.

3.2 Effect of olive leaf consumption (either aqueous or ethanolic extract) on some impaired carbohydrates metabolism markers

From table (2) it is clear that consuming HF-HS diet increased significantly (P<0.05) serum glucose and insulin levels by about 134.76% and 49.82% respectively compared with rats fed on balanced diet. Hyperglycemia and hyperinsulinemia were interpreted as an increase in HOMA-IR value from (1.49±0.03) to (5.25±0.22) and decrease in QUICKI value from (0.36±0.001) to (0.30±0.002) which indicated the negative effect of consuming HF-HS diet on insulin sensitivity. Moreover, administration of AOLE with HF-HS diet decreased significantly (P<0.05) serum glucose, insulin levels and HOMA-IR value by about 25.96%, 9.71% and 33.14% respectively, while QUIKI value increased significantly by about 6.67% in comparison with rats fed on HF-HS diet solely. Consuming HF-HS diet with EOLE decreased significantly (P<0.05) serum glucose, insulin levels and HOMA-IR value by about 42.45%, 21.48% and 54.86% respectively, while QUIKI value increased significantly by about 10% compared with rats fed on HF-HS diet solely. The previous results confirmed a better effect of EOLE than AOLE for hypoglycemia and insulin sensitivity.

3.3 Effect of olive leaf consumption (either aqueous or ethanolic extract) on lipogenesis markers and atherogenic index

Considering the serum levels of TC, TAGs, LDL-C, VLDL-C and AI presented in table (3) all of them showed a significant increase (P<0.05) in rats fed on HF-HS diet by about 57.90%, 80.28%, 281.55%, 80.32% and 609.30% respectively compared with rats fed on balanced diet. With respect to serum HDL-C level there was a significant decrease (P<0.05) with consuming HF-HS diet by about 47.15%compared with rats fed on balanced diet. Administration of AOLE with HF-HS diet significantly decreased TC, TAGs, LDL-C, VLDL-C and AI by about 13.98%, 26.24%, 22.48%, 26.62% and 41.64% respectively, while HDL-C serum level increased significantly (P<0.05) by about 32.65% compared with rats fed on HF-HS only. Also consuming EOLE with HF-HS diet showed a significant (P<0.05) decrease in serum levels of TC, TAGs, LDL-C, VLDL-C and AI by about 27.21%, 35.97%, 53.21%, 38.71% and 78.69% respectively, while HDL-C serum level increased significantly by about 70.79% compared with rats fed on HF-HS only. Previous results confirmed a better effect of EOLE than AOLE on lipogenesis markers and atherogenic index.

3.4 Effect of olive leaf consumption (either aqueous or ethanolic extract) on some inflammatory markers and total antioxidant capacity

From the results tabulated in table (4) it is clear that consuming HF-HS diet increased significantly (P<0.05) serum inflammatory markers IL-6, TNF-α and C-RP by about 53.77%, 72.89% and 60.70% respectively, while serum TAC decreased significantly by about 46.73%compared with rats fed on balanced diet. Administration of AOLE with HF-HS diet reduced significantly (P<0.05)serum inflammatory markers IL-6, TNF-α and C-RP by about17.38%, 23.76% and 35.34% respectively, while serum TAC increased by about 48.24% compared with rats fed on HF-HS only. With respect to consuming EOLE with HF-HS diet there were significant decreases in serum inflammatory markers IL-6, TNF-α and C-RP by about21.17%, 33.19% and 35.52% respectively, while serum TAC increased by about 63.68% compared with rats fed on HF-HS only. The previous data confirmed better effect of EOLE on reducingTNF-α level and increasing TAC than AOLE while serum IL-6 and C-RP levels showed non-significant changes between two treatments.

3.5 Effect of olive leaf consumption (either aqueous or ethanolic extract) on growth hormone and some IGF system parameters

From table (5) it is clear that serum level of GH decreased significantly (P<0.05) in rats fed on HF-HS diet by about 53.89%, while IGF-I serum level increased by about 86.03% compared with rats fed on balanced diet. With respect to binding proteins IGFBP-1 decreased significantly, while IGFBP-3 increased significantly (P<0.05) with consuming HF-HS diet by about 46.66% and 37.37% respectively compared with rats fed on balanced diet. According to this results the molar ratio between IGF-I and IGFBP-3 increased significantly by 35.53% compared with rats fed on balanced diet. Treating with AOLE showed significant increases in GH and IGFBP-1serum levels by about 58.73% and 52.13% respectively. While IGF-I, IGFBP-3 and MR decreased significantly by about 35.39%, 19.65% and 19.44% respectively compared with rats fed on HF-HS only.Also consuming EOLE with HF-HS diet showed significant (P<0.05) increases in serum levels of GH and IGFBP-1 by about 73.32% and 69.54% respectively. While IGF-I, IGFBP-3 and MR decreased significantly by about 38.61%, 23.93% and 19.15% respectively compared with rats fed on HF-HS only. From previous data EOLE showed better effect on GH, IGFBP-1 and IGFBP-3 than AOLE, and while in IGF-I and MR there were no significant differences between two treatments.

Table 1: Total phenolic content and antioxidant activity of aqueous and ethanolic extract

Extract	TPC (meq of gallic acid)	Antioxidant activity (% of inhibition of DPPH)
Aqueous extract	1.73	30.90
Ethanolic extract	3.37	65.75

Table 2: Effect of olive leaves consumption (either aqueous or ethanolic extract) on some impaired carbohydrates metabolism markers

parameters / Groups	Glucose (mg/dl)	Insulin (µIU/ml)	HOMA-IR	QUICKI
Control group	a 109.83±2.07	a 5.50±0.12	a 1.49±0.03	d 0.36±0.001
HF-HS	d 257.84±6.41	d 8.24±0.18	d 5.25±0.22	a 0.30±0.002
HF-HS+AOLE	c 190.93±8.88	c 7.44±0.11	c 3.51±0.19	b 0.32±0.002
HF-HS+EOLE	b 148.40±11.69	b 6.47±0.16	b 2.37±0.18	c 0.33±0.004

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column (P<0.05).

Table 3: Effect of olive leaves consumption (either aqueous or ethanolic extract) on lipogenesis markers and atherogenic index

parameters / Groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	AI
Control group	a 52.33±2.15	a 66.07±2.58	d 27.53±1.56	a 11.60±3.30	a 13.21±0.52	a 0.43±0.14
HF-HS	d 82.63±2.77	d 119.11±4.49	a 14.55±0.71	d 44.26±3.33	d 23.82±0.90	d 3.05±0.31
HF-HS+AOLE	c 71.08±2.41	c 87.38±3.22	b 19.30±0.56	c 34.31±2.48	c 17.48±0.64	c 1.78±0.17
HF-HS+EOLE	b 60.15±1.75	b 76.27±3.56	c 24.85±0.80	b 20.71±2.33	b 14.60±1.93	b 0.83±0.09

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column (P<0.05).

Table 4: Effect of olive leaves consumption (either aqueous or ethanolic extract) on some inflammatory markers and total antioxidant capacity

parameters / Groups	IL-6 (Pg/ml)	TNF-α (Pg/ml)	CRP (ng/ml)	TAC (nmol/µl)
Control group	a 279.37±10.29	a 51.35±3.20	a 391.58±5.18	d 41.09±1.84
HF-HS	c 429.58±28.58	d 88.78±1.82	b 629.25±3.07	a 21.89±2.01
HF-HS+AOLE	b 354.90±16.67	c 67.69±4.66	a 406.90±45.62	b 32.45±2.34
HF-HS+EOLE	b 338.63±13.34	b 59.31±1.00	a 408.77±2.15	c 35.83±0.71

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column (P<0.05).

Table 5: Effect of olive leaves consumption (either aqueous or ethanolic extract) on growth hormone and some IGF system parameters

parameters / Groups	GH (ng/ml)	IGF-I (ng/ml)	IGFBP-1 (Pg/ml)	IGFBP-3 (ng/ml)	Molar ratio
Control group	d 202.90±6.65	a 4.01±0.06	d 392.11±5.28	a 2.89±0.12	a 5.01±0.28
HF-HS	a 93.56±3.19	c 7.46±0.47	a 209.15±4.99	d 3.97±0.09	c 6.79±0.34
HF-HS+AOLE	b 148.51±5.92	b 4.82±0.19	b 318.18±3.83	c 3.19±0.08	b 5.47±0.24
HF-HS+EOLE	c 162.16±4.19	b 4.58±0.13	c 354.59±6.10	b 3.02±0.09	b 5.49±0.25

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column ($P<0.05$).

3. Discussion

Olive leaf is an important source of naturally occurring phytochemicals and antioxidant compounds (de Bock et al., 2013). Filip et al., (2015) concluded that olive leaves are rich in polyphenolic compounds of highly radical scavenging activity in both water and ethanolic extracts. Overall, ethanol was found to be the best solvent for extraction of antioxidant compound from olive leaves. The present results are similar to the results of Martin et al., (2013) who examined the antioxidant activity of olive leaves ethanolic and water extracts. They concluded that the greatest radical scavenging and antioxidant activity were associated with ethanolic extracts.

Insulin resistance (IR) is one of the major causes of type 2 diabetes and plays a critical role in the pathogenesis of cardiovascular diseases (CVDs) (Deveci et al., 2009). The results of the present study showed that consuming HF-HS diet increased significantly serum glucose and insulin levels compared with rats fed on balanced diet. Hyperglycemia and hyperinsulinemia were interpreted as an increase in HOMA-IR value and decrease in QUICKI value, which indicated the negative effect of consuming HF-HS diet on insulin sensitivity. Olive leaves have been long used as a traditional antidiabetic and antihypertensive herbal intervention and have been used to treat these conditions as well as infectious diseases. Administration of AOLE or EOLE with HF-HS diet decreased significantly serum glucose, insulin levels and HOMA-IR value while QUIKI value increased. EOLE supplementation was associated with a reduction in the glucose and insulin excursion after oral glucose challenge, suggesting an improvement in both pancreatic β-cell function and insulin sensitivity. The olive leave extract has been shown to accelerate the cellular uptake of glucose, leading to reduced plasmaglucose (Wainstein et al., 2012). Since OLE contains polyphenols that are glycoside in their structure, it could actually access a glucose transporter such as a sodium-dependent glucose transporter (SGLT1) found in the epithelial cells of the small intestine, therefore permitting its entry into the cells. In accordance with the present work, olive leaf extracts especiallyethanolic extract have been found to decrease blood sugar in the animal studies through several mechanisms (Jemai et al., 2009).It was reported to slow digestion of starches into simple sugars, and so allows absorption of those sugars from the intestine to go in gradual fashion, and increase the uptake of glucose into tissues from the blood (Wainstein et al., 2012). EOLE protects tissues from the oxidative damage caused by the interaction between glucose and proteins in the process called glycation and increases the levels of other natural antioxidant systems in the body, which increase the degree of protection(Al-Azzawie and Alhamdani. 2006). The current study is in accordance with Rossetti. (1990) who confirmed that, rats fed on high fat high sucrose diet showed high values of serum glucose level. This finding was associated with the development of insulin resistance in peripheral tissues owing to impairment of both insulin secretion and insulin sensitivity. The biochemical basis for insulin resistance induced by hyperglycemia may be attributed to modifications in structure of insulin receptors and the glucose transport system, resulting in impaired signal transmission (Ordonez, 2007).

Plasma lipids revealed high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL, low HDL levels and a marked elevation in the ratio of LDL/HDL that increases the risk of coronary heart disease in rats fed on HF-HS diet which are well known as risk factors for cardiovascular diseases and affect patients with diabetes (Ravi, 2005). A study by Kinosian, (1995) showed that, the changes in TC/HDL and LDL/HDL ratios were better predictors of coronary heart disease than the changes in LDL alone. Results of the present work showed that, EOLE significantly amelioratedsera lipid profiles by reducing the values of TC, TG, LDL, VLDL and LDL/HDL ratio and elevating HDL levels. This indicates that, EOLE has a potential role in preventing formation of atherosclerosis and coronary heart disease in diabetic patients. EOLE contains a biofunctional component oleuropein, which may play as a regulatory lipid agent and have anti-atherosclerotic effect (Wang et al., 2008). These results are similar to the results of (Poudyal et al., 2010) who found that when rats were fed a high-fat, high-carbohydrate diet, they developed all the signs of metabolic syndrome (excessive abdominal fat, hypertension, abnormal lipid profile, and impaired glucose tolerance).But when these animals were fed that unhealthy diet along with olive leaf extracts, all of the metabolic abnormalities improved or, in some cases, normalized. Coni et al., (2000) found that the addition of OLE to the standard diet lowers plasma levels of total cholesterol and decreases the oxidation of low density lipoprotein (LDL) in rabbits. Similarly, Andreadou et al., (2006) noted that treatment with OLE reduced total cholesterol and triglyceride concentrations, and reduced circulating lipids. The mechanism of this hypocholesterolemic action may bedue to the inhibition of the absorption of dietary cholesterol in the intestine or its production by the liver (Bursill and Roach,

2006) or stimulation ofthe biliary secretion of cholesterol and cholesterol excretion in the feces (Krzeminski et al., 2003).However, animal data suggest that the consumption of phenolic components of OLE appears to decrease the activities of key cholesterol-regulatory enzymes,3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (the main target of statins) and acetyl-CoA cholesterol acyltransferase (ACAT), resulting in decreased cholesterol biosynthesis (Lee et al., 2001). Interestingly, a recent paper reporting favourable modification of lipid profiles by OLE, Filipet al., (2015) also observed osteoblast stimulation and hypothesised that as osteoblasts and adipocytes derived from the same mesenchymal stem cells, this may explain the change in lipid profiles.

It has been shown that inflammation biomarkers such as TNF-α, IL-6, and C-reactive protein(CRP), are present at increased concentrations in individuals who are insulin resistant and obese, and these biomarkers portend the development of T2DM. Enhancing of inflammatory pathways in liver cells is sufficient to cause both local (Arkan et al., 2005) as well assystemic insulin resistance (Cai et al., 2005). CRP is primary synthesized in the hepatocytes and regulated by the pro-inflammatory cytokine IL-6 and TNF-α (Gabay and Kushner, 1999) in adiposities. This in part suggests that the associations of CRP concentrations with fasting insulin, fasting glucose, and HOMA-IR could be due to the presence of a chronic systemic inflammation (Nakanishi et al., 2005). The results of the present study demonstrated that consuming HF-HS diet increased serum inflammatory markers (IL-6, TNF-α and C-RP), while serum TAC decreased compared with rats fed on balanced diet. TNF-α was originally identified as a factor that induces hypertriglyceridemia in bacteria infected-animals (Rouzer and Cerami, 1980). In addition, TNF-α directly promotes the overproduction of hepatic apolipoprotein (apo)-B containing VLDL through impairment of hepatic insulin signaling in animals (Qin et al., 2008). Like TNF-α, IL-6 is also associated with hypertriglyceridemia. Subjects with higher serum TGs levels have a higher production capacity of IL-6 as well as TNF-α (Mohrschladt et al., 2000; Jonkers et al., 2001), and increased levels of serum triglycerides are associated with increased levels of IL-6 (Nappo et al., 2002) which is in accordance with the present study. Administration of AOLE or EOLE with HF-HS diet reduced serum inflammatory markers IL-6, TNF-α and C-RP, while serum TAC increased especially in EOLE administered group. Interleukin-6 functions differently depending on its concentration and the tissue it acts upon. A higher increases improve the insulin-regulated glucose metabolism in the muscle (Kim et al., 2009), while chronically mildly elevated levels are associated with a pro-inflammatory insulin resistant condition in the liver. Thus, OLE supplementation may improve insulin sensitivity and glucose uptake through interleukin-6 and possible mechanisms for this effect have been proposed (Carey et al., 2006; Weigert et al., 2006). Liu, (2014) proved that, OLE diminishes liver damage in diabetic rats by inhibiting expression of inflammatory cytokines in liver, such as TNF-α, IL-1β, and IL-6. Also it affects directly on hepatocyte induced hyperinsulinemia that produced by hepatic betatrophin which enhancing insulin production by beta cells of pancreas.

Insulin-like growth factors (IGF) family plays a key role in mammalian growth, development and metabolism by regulating many cell functions as proliferation, differentiation, survival, migration and glucose metabolism(Martin et al., 2013). It is abundant in the blood circulation, where it binds to insulin-like growth factor binding proteins (IGFBPs). IGFBP-3 is the major circulating carrier of IGF-1 and can regulate the biological effects of IGF-1 by segregating IGF-1 into a circulating reservoir, thereby reducing the free fraction of bioactive IGF-1 in the blood (Delafontaine et al., 2004). The negative association between IGF-1 and total cholesterol concentrations and, conversely, the positive association of IGFBP-3 with plasma lipids are consistent with the known role of IGF-1 in lipid metabolism (Pratipanawatr et al., 2002). The present study showed that serum level of GH decreased significantly in rats fed on HF-HS diet, while IGF-I serum level increased compared with rats fed on balanced diet. With respect to binding proteins, IGFBP-1 decreased significantly, while IGFBP-3 increased significantly by consuming HF-HS diet. According to this results the molar ratio between IGF-I and IGFBP-3 increased significantly compared with rats fed on balanced diet. The relationship between obesity and concentrations of IGF-1 is complex. Increased body fat and decreased muscle mass have been associated with hypo-secretion of growth hormone, a known regulator of hepatic IGF-1 production (Jones and Clemmons 1995). GH has been proposed to possess a role in the regulation of metabolic effects on lipolysis and lipogenesis, as well as in differentiation of the preadipocytes (Scavo et al., 2004). GH secretion is decreased in obesity with subsequent loss of its lipolytic activity. In the present study, we observed that OLE supplementation led to increased IGFBP-1 and decreased IGFBP-3 serum concentrations. Increased IGFBP-1 concentrations are associated with lower insulin levels (Heald et al., 2001).

4. Conclusion

The present study produced convincing evidence that olive leaf extract has a protective effect against implications induced by consumption of high fat-high sucrose diet byits hypoglycemic and antiatherogenic effect which improves efficiency of IGF system. Furthermore, consumption of its ethanolic is more effective than aqueous extract.

5. Conflict of Interest

The authors have declared that there is no conflict of interest.

References

[1] AIN (1993), By Reeves, P.G., Nielsen, F.H. and Fahly, G.C. (1993): AIN-93 Purified diets for laboratory rodents: Final report of the American Institute AdHoc writing committee on the reformulation of the AIN-76. A rodent diet, J. Nut., 123: 1939-1951.
[2] Al-Azzawie HF, and Alhamdani MS. (2006): Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sci. 16;78(12):1371-7.
[3] Allain, C.C., (1974) Clin. Chem. 20,470.

[4] Andreadou, I.; Iliodromitis, E.K.; Mikros E.; Constantinou, M.; Kakoulidou, A.; and Kremastinos, D.T. (2006): The olive constituent oleuropein exhibits anti-ischemic, antioxidative and hypolipidemic effects in anesthetized rabbits. J. Nutr. 136 .2219-2213.
[5] Annoni, G., Bottasso, B. M., Ciaci, D., Donato, M.F., Tripoli, A and Iab, J.J.,(1982) Res. Lab. Med. 9,115
[6] Arkan, M.C., Hevener, A.L., Greten, F.R., Maeda, S., Li, Z.W., Long, J.M., Wynshaw-Boris, A., Poli, G., Olefsky, J., and Karin, M. (2005): IKK-_ links inflammation to obesity-induced insulin resistance. Nat. Med. 11: 191–198.
[7] Babu V., Gangadevi T. and Subramoniam A. (2003): Antidiabetic activity of ethanolic extract of Cassia Kleinii leaf in streptozitocin induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. Ind. J. Pharma., 35:290.
[8] Banerjee M, Tripathi LM, Srivastava VM, Puri, A and Shukla R. (2003): Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. Immunopharmacol Immunotoxicol.; 25(2):213-224.
[9] Bursill, C. A.; Roach, P. D. (2006): Modulation of cholesterol metabolism by the green tea polyphenol ( )-epigallocatechin gallate in cultured human liver (HepG2) cells. J. Agric. Food Chem. 54, 1621–1626.
[10] Cai, D., Yuan, M., Frantz, D.F., Melendez, P.A., Hansen, L., Lee, J., and Shoelson, S.E. (2005): Local and systemic insulin resistance resulting from hepatic activation of IKK-_ and NF-_B. Nat. Med. 11: 183–190.
[11] Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG. (2006): Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes 55: 2688–2697.
[12] Chun, M-R Lee, Y J, Kim, K-H., Kim, Y-W., Park, S-Y Lee, L-W, Kim, K-M and Park, Y-K. (2010): Differential Effects of High-carbohydrate and High-fat Diet Composition on Muscle Insulin Resistance in RatsKorean Med Sci ; 25: 1053-1059
[13] Coni, E.; Bendetto, R.; pasquale, M; .Masella, R.; Modesti, D.; Mattei, R.; and Carlini, E.A. (2000): Rotective effect of oleuropein an olive oil biophenol, on low densitylipoprotein oxidizability in rabbits. Lipids 35: 45-54.
[14] D'Haens, G. (2003): Anti- TNF therapy for Crohn's Disease. Current Pharmaceutical Design; 9:289-294.
[15] de Bock M, Derraik JGB, Brennan CM, Biggs JB and Morgan PE. (2013): Olive (Olea uropaea L.) Leaf Polyphenols Improve Insulin Sensitivity in Middle-Aged Overweight Men: A Randomized, Placebo-Controlled, Crossover Trial. PLoS ONE 8(3): e57622.
[16] Delafontaine P, Song YH, Li Y. (2004): Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. Arterioscler Thromb Vasc Biol. 24:435– 444.
[17] Deveci E, Yesil M, Akinci B, Yesil S, Postaci N, Arikan E, Koseoglu M. (2009): Evaluation of insulin resistance in normoglycemic patients with coronary artery disease. Clin Cardiol, 32:32-36.
[18] Eggert ML, Wallaschofski H, Grotevendt A, et al. Cross-sectional and longitudinal relation of IGF1 and IGF-binding protein 3 with lipid metabolism. Eur J Endocrinol. 2014; 171:9–19.
[19] Filip R, Possemiers S, Heyerick A. (2015): Twelve-month consumption of a polyphenol extract from olive (Olea europaea) in a double blind, randomized trial increases serumtotal osteocalcin levels and improves serum lipid profiles inpostmenopausal women with osteopenia. J Nutr Health Aging 19:77–86.
[20] Frosig, C., Rose, A.J., Treebak,J.T., Kiens, B., Richter, E.A and Wojtaszewski, J.F.P. (2007): Effects of endurance exercise training on insulin signaling in human skeletal muscle: interactions at the level of phyphatidylinositol 3-kinase, Akt, and AS 160.Diabetes56:2093-2120.
[21] Gabay C, Kushner I. (1999): Acute-phase proteins and other systemic responses to inflammation.
[22] Garten, A., Schuster,S. and Kiess,W., (2012): The Insulin-Like Growth Factors in Adipogenesis and Obesity. Endocrinol Metab Clin N Am 41: 283–295.
[23] Heald A, Cruickshank J, Riste L, Cade J, Anderson S. (2001): Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance and cardiovascular risk in two populations. Diabetologia 44: 333–339.
[24] Hung, M. Y. Fu, T. Y. Shih, P. H. Lee, C. P and Yen, G. C. (2006): Du-Zhong (Eucommia ulmoides) Olive leaves inhibits CCl4-induced hepatic damage in rats. Food Chem Toxicol 44 1424-31
[25] Janssen JA, Stolk RP, Pols HA, Grobbee DE, de Jong FH, Lamberts SW. (1998): Serum free IGF-1, total IGF-1, IGFBP-1 and IGFBP-3 levels in an elderly population: relation to age and sex steroid levels. Clin Endocrinol (Oxf). 48:471–478.
[26] Jemai H, El Feki A, Sayadi S. (2009): Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats. J Agric Food Chem. 14;57(19):8798-804.
[27] Jones JI, Clemmons DR. (1995)): Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev.16: 3–34.
[28] Jonkers, I.J.; Mohrschladt, M.F.; Westendorp, R.G.; van der Laarse, A.; Smelt, A.H. (2001): Severe hypertriglyceridemia with insulin resistance is associated with systemic inflammation (reversal with bezafibrate therapy in a randomized controlled trial). Am. J. Med. 112, 275–280.
[29] Katz A,Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G and Quon MJ. (2000): Quantitative insulin sensitivity check index: a simple accurate method for assessing insulin sensitivity in humans. J Clin. Endochinol. Metab. 85, 2402-2410.
[30] Kim JH, Bachmann RA, Chen J (2009): Interleukin-6 and Insulin Resistance. Vitam Horm 80: 613–633.
[31] Kinosian B, Glick H, Preiss L & Puder KL. (1995): Cholesterol and coronary heart disease: predicting risks in men by changes in levels and ratios. J. Inves. Med., 43, 443-450.
[32] Kivrak, I., Duru, M. E., Öztürk, M., Mercan, N., Harmandar, M., Topçu, G. (2009): Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of Salvia potentillifolia. Food Chemistry, 116 (2), 470-479.

[33] Koracevic D, Koracevic G, Djordjevic V. (2001): Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 54:356–61.
[34] Krzeminski, R.; Gorinstein, S.; Leontowicz, H.; Leontowicz, M.; Gralak, M.; Czerwinski, J.; Lojek, A.; Ciz, M.; Martin-Belloso, O.; Gligelmo-Miguel, N.; Trakhtenberg, S. (2003): Effect of different olive oils on bile excretion in rats fed cholesterol-containing and cholesterol-free diets. J. Agric. Food Chem. 51, 5774–5779.
[35] Lee J-S, Choi M-S and Jeon S-M. (2001): Lipid-lowering and antioxidative activities of 3,4-di(OH)-cinnamate and 3,4-di(OH)- hydrocinnamate in cholesterol-fed rats. Clin Chim Acta 314:221–229.
[36] Liu YN, Jung JH, Park H and Kin HS. (2014): Olive Leaf Extract Suppresses mRNA Expression of Pro-inflammatory Cytokines and Enhances IRS-1 Expression in the Rats with Streptozotocin and High-Fat Diet induced Diabetes; In press.
[37] Lopes Virella, M. (1977) Clin. Chem. 23,882.
[38] Martin de Bock1, Jose´ G. B. Derraik1, Christine M. Brennan1, Janene B. Biggs1, Philip E. Morgan, Steven C. Hodgkinson1, Paul L. Hofman, Wayne S. Cutfield. (2013): Olive (Olea europaea L.) Leaf Polyphenols Improve Insulin Sensitivity in Middle-Aged Overweight Men: A Randomized, Placebo-Controlled, Crossover Trial; (8): e57622.
[39] Moghaddam M G, Masomi Y, Razavian M, Moradi M (2013): The effect of oral consumption of olive leaves on serum glucose level and lipid profile of diabetic rats. Basic and clinical pathophysiology.1(2): 39-44
[40] Mohrschladt, M.F.; Weverling-Rijnsburger, A.W.; de Man, F.H.; Stoeken, D.J.; Sturk, A.; Smelt, A.H.; Westendorp, R.G. (2000): Hyperlipoproteinemia affects cytokine production in whole blood samples ex vivo. The influence of lipid-lowering therapy. Atherosclerosis. 148, 413–419.
[41] Morimoto LM, Newcomb PA, White E, Bigler J, Potter JD. (2005): Variation in plasma insulin-like growth factor-1 and insulin-like growth factor binding protein-3: personal and lifestyle factors (United States). Cancer Causes Control. 16:917–927.
[42] Nakanishi N, Shiraishi T, Wada M. (2005): Association between C-reactive protein and insulin resistance in a Japanese population: the Minoh Study. Intern Med, 44:542-547.
[43] Nappo, F.; Esposito, K.; Cioffi, M.; Giugliano, G.; Molinari, A.M.; Paolisso, G.; Marfella, R.; Giugliano, D. (2002): Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients (role of fat and carbohydrate meals). J. Am. Coll. Cardiol. 39, 1145–1150.
[44] Ordonez P, Moreno M, Alonso A, Fernandez R, Dıaz F and Gonzalez C. (2007): Insulin sensitivity in alloxan-induced diabetic rats treated with different doses of 17β-oestradiol or progesterone. ExpPhysiol; 92(1): 241–249.
[45] 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. 140:946–953.
[46] Poudyal H, Campbell F, Brown L. (2010): Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed ratsJ Nutr; 140 (5):946-53.
[47] Pradhan AD, Manson JE, Rifai N. (2001): C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 286:327–334.
[48] Pratipanawatr T, Pratipanawatr W, Rosen C, Berria R, Bajaj M, Cusi K, Mandarino L, Kashyap S, Belfort R, DeFronzo RA. (2002): Effect of IGF-I on FFA and glucose metabolism in control and type 2 diabetic subjects. Am J Physiol Endocrinol Metab. 282: E1360–E1368.
[49] Qin, B.; Anderson, R.A.; Adeli, K. (2008): Tumor necrosis factor-alpha directly stimulates the overproduction of hepatic apolipoprotein B100-containing VLDL via impairment of hepatic insulin signaling. Am. J. Physiol. Gastrointest. Liver Physiol. 294, G1120–G1129.
[50] Ravi K, Rajasekaran S and Subramanian S. (2005): Antihyperlipidemia effect of Eugenia Jambolana seed kernel on alloxan induced diabetes in rats. Food chemToxicol; 43: 1433-1439.
[51] Ren J and Anversa P. (2015): The insulin-like growth factor I system: physiological and pathophysiological implication in cardiovascular diseases associated with metabolic syndrome. Biochem Pharmacol. 93:409–417.
[52] Rohrmann S, Linseisen J, Boeing H, Pischon T, Panico S, Agnoli C, Palli D and Tumino R. (2010): Serum levels of IGF-I, IGFBP-3 and colorectal cancer risk: results from the EPIC cohort, plus a meta-analysis of prospective studies. Int J Cancer. 126:1702–15.
[53] Rossetti L, Giaccari A and Defronzo RA. (1990): Glucose toxicity. Diabetes Care; 13: 610–630.
[54] Rouzer, C.A and Cerami, A. (1980): Hypertriglyceridemia associated with Trypanosoma brucei brucei infection in rabbits: Role of defective triglyceride removal. Mol. Biochem. Parasitol. 2, 31–38.
[55] Scavo, L.M., M. Ka ras, M. Murray, D. Leroith. (2004): Insulin-like growth factor-I stimulates both cell growth and lipogenes is during differentiation of human mesenchymal stem cells into adipocytes. J Clin Endocrinol Metab. Jul., 89(7): 3543-53.
[56] Span JP, Pieters GF, Sweep CG, Hermus AR, Smals AG. (2000): Gender difference in insulin-like growth factor I response to growth hormone (GH) treatment in GH-deficient adults: role of sex hormone replacement. J Clin Endocrinol Metab. 85:1121–1125.
[57] Stintzing, F. C., Nerbach, K. M., Mosshammer, M., Carle, R., Yi, W., Sellappan, S., Acoh, C. C., Bunch, R., Felker, P. (2005) Color, betalain pattern, and antioxidant properties of cactus pear (Opuntia spp.) clones. Journal of Agricultural and Food Chemistry, 53 (2), 442-451.
[58] Stumvoll M, Van Haeften T, Fritsche A and Gerich J. (2001): Oral glucose tolerance test indexes for insulin sensitivity and secretion based on various availabilities of sampling times. Diabetes Care. 24, 796-797.
[59] Sumiyoshi, M., Sakanaka, M and Kimuray, Y. (2006): Chronic Intake of High-Fat and High-Sucrose Diets Differentially Affects Glucose Intolerance in Mice J. Nutr. 136: 582–587.
[60] Trinder, P., (1969). Ann.Clin. Biochem. 6, 24.

[61] Wainstein, J., Ganz, T., Boaz, M., Dayan, Y.B., Dolev, E., Kerem, Z. and Madar, Z. (2012): Olive Leaf Extract as a Hypoglycemic Agent in Both Human Diabetic Subjects and in Rats J.Med Food 15 (7) 1–6.
[62] Wang L, Geng C and Jiang L. (2008): The anti-atherosclerotic effect of olive leaf extract is related to suppress inflammatory response in rabbits with experimental atherosclerosis. Eur J Nutr; 47: 235-243.
[63] Wang L, Schulz TC, Sherrer ES. (2007): Self-renewal of human embryonic stem cells requires insulin-like growth factor-1 receptor and ERBB2 receptor signaling. Blood.110:4111–4119.
[64] Weigert C, Hennige AM, Lehmann R, Brodbeck K, Baumgartner F, et al. (2006): Direct cross-talk of interleukin-6 and insulin signal transduction via insulin receptor substrate-1 in skeletal muscle cells. J Biol Chem 281: 7060–7067.
[65] Yang Z., Miyahara H., Takeo J. and Katayama M. (2012): Diet high in fat and sucrose induces rapid onset of obesity-related metabolic syndrome partly through rapid response of genes involved in lipogenesis, insulin signalling and inflammation in mice. Diabetology & Metabolic Syndrome; 4:32.
[66] Yeap, B.B., Chubb, S. A. P., Ho, K. K.Y., Setoh, J.W.S. McCaul, K.A., Norman, P.E., Jamrozik, K. and Flicker, L., (2010): IGF1 and its binding proteins 3 and 1 are differentially associated with metabolic syndrome in older men. European Journal of Endocrinology, 162. 249–257.
[67] Zoair M. A., (2014): a possible antioxidant effect of olive leaf extraction in diabetic rats. GJSR journal. 2(6), pp. 165-170, 31.