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A POSSIBLE ANTIOXIDANTEFFECT OF OLIVE LEAF EXTRACTION IN DIABETIC RATS

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ABSTRACT

Nowadays, herbal drugs are gaining popularity in the treatment of diabetes and its complications. The current study aims to evaluate the significance of supplementation of Olive leaves extract (OLE) of *Oleaeuropaea*plant as an antioxidant in reducing the metabolic abnormalities in diabetic male albino rats. Thirty male albino rats were divided equally into three groups including control, diabetic and diabetic under OLE treatment. Diabetic rats were administered OLE orally twice daily for 30 days. At the end of the experimental period, levels of serum insulin and glucose in addition to lipids pattern such as total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins(VLDL) and renal markers were determined biochemically in sera of control and experimental groups. Also, values of homeostasis model assessment of insulin resistance (HOMA-IR) were calculated for each group. Diabetic rats showed marked decline in levels of serum insulin accompanied with marked elevation in levels of fasting blood glucose and values of HOMA_IR as compared to the corresponding controls. All the estimated parameters of renal function were elevated in a significant manner ($P<0.01$) in diabetic rats relative to the corresponding controls.In addition, diabetic rats showed marked increase in serumTC, TG, LDL, VLDL accompanied with marked decline in HDL relative to the corresponding controls. Supplementation of diabetic rats with OLE significantly ameliorated most the estimated biochemical parameters.These results demonstrate that OLE have an important role in inhibiting hyperglycemia and ameliorating metabolic abnormalities induced by diabetes through its antioxidant advantage.

Keywords: *Diabetes, lipids, insulin resistance, olive leaves extract, antioxidant.*

INTRODUCTION

Diabetesisthe most common endocrine disorder affecting millions of people worldwide.More than346 million of people worldwide suffer from this disease (*Danaei*, 2011).Pronounced changes in the life style, consumption of energy-rich diet and obesity are important causes for the rise of diabetes at an alarming rate (Sikarwar and Patil, 2010).

Diabetes is caused by an absolute or relative lack of insulin and/or malfunction of insulin action (Balkau, 2000; Rasineni, 2010). It is characterized by hyperglycemia,disturbances in carbohydrate, protein, and fat metabolisms, in addition to long-termcomplications affecting the eyes, kidneys, nerves, heart and bloodvessels(Hung, 2005, Thripathi ; Sivastava, 2006 ; Gupta, 2008).

In spite of the great progress in management of diabetes by synthetic drugs, herbal drugs are gaining popularity in the treatment of diabetes and its complications. The search for improved, safe and natural antidiabetic agents has been recommended by World Health Organization (WHO, 2002).

Olive leaf from *Oleaeuropaea*, the olive tree, is native to the Mediterranean and has been claimed to have medicinal values including antidiabeticand antioxidant activities(Gonzalez, 1992, Wojcikowski, 2007, Eidi, 2009).The current study was designed to evaluate the antioxidant efficacy of olive leaf extraction (OLE)in reducing the metabolic abnormalities accompanied to alloxan-induced diabetes in male albino rats.

MATERIALS AND METHODS

Thirty male albino rats *(Rattusnorvegicus)* weighing approximately 160-180g were housed in clear plastic cages (2 animals/cage) with wood chips as bedding and given a standard pellet rodent diet, in addition of water adlibitum. The rats were maintained under standard laboratory conditions at 25±2ºC, relative humidity 50±15% and normal photoperiod (12h light/dark cycle).

Alloxan was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Biochemical kits were obtained from normal commercial sources.

Induction of diabetes

The animals were rendered diabetes by a single intraperitoneal injection of alloxan (120 mg/kg body weight) in freshly prepared physiological saline. Diabetic state of animals was monitored for its stability for seven successive days after alloxan treatment. On day 8 of alloxan injection, only animals with fasting blood glucose levels ≥300 mg/dl were selected as diabetic rats for the current experiment. The control rats were injected with physiological saline alone as placebo.

Preparation of Aqueous Extract of olive leaves:

Freshly harvested leaves of *Oleaeuropaea*plant were dried in the shade, and then powdered with a blender. Crude hot water extract of the plant leaves was prepared by boiling the plant powder (2g) up till200ml distilled water for 15 min. The obtained extract was allowed to cool at room temperature then filtered through Whatman No.2 filterPaper.

Experimental protocol

The current study was performed in accordance of the International Guidelines regarding animal experiment. Three experimental groups,ten rats for each, were used. The third group was treated intragastricallywith olive leaves extracttwicedaily at 8 am and 8 pm for 30days as follows:

- Group I (Control group): Non-diabetic control rats .
- Group II (Diabetic group): Diabetic rats.
- Group III (Diabetic group + OLE): Diabetic rats supplemented with OLE (7.5mg/kg body weight).

Collection of blood and estimation of biochemical parameters:

One month after treatment, blood samples were collected from overnight fasted rats in centrifuge tubes by cardiac puncture under mild ether anesthesia.Blood samples were used in separation of sera by centrifugation at 4000 rpm for 10 min at 4°C and immediately stored at -20°C for further analysis of biochemical parameters.

Serum glucose was estimated using a commercially available kit according to the method of Trinder(Trinder, 1969).Serum insulin level was measured by an enzyme immunoassay kit (SPI-Bio société de pharmacologieetd'lmmunoloie-Bio, France); while values of HOMA-IR were calculated using the following equation:

HOMA-IR= fasting serum glucose (mg/dl) x fasting serum insulin (µU/ml)/450.

Levels of serum urea, creatinine and uric acid were estimated according to Patton and Crouch (1977); Tietz(1987) ; Aoki .(1992), respectively. Serumtotalcholesterol(Henry ., 1997); triglycerides(Fossati ; Principe, 1982) and HDL(Burstein ., 1972)were estimated colorimetrically using high quality kits according to manufacturer's protocol;while VLDL was calculated as triglyceride/5andLDL was calculated applying the Friedwald's equation (Friedewald. 1972).

Friedewald's equation: LDL (mg/dl) = TC-HDL - [TG/5].

VLDL = TG/5

Risk 1 = TC / HDL

Risk 2 = LDL / HDL

STATISTICAL ANALYSIS

The results were expressed as Mean ± SE of 10 rats per group and the statistical significance was evaluated by one way analysis of variance (ANOVA) followed by Duncan post Hoc testusing the SPSS/17.0 software. Values were considered statistically significant at $P< 0.05$.

RESULTS AND DISCUSSION

RESULTS

Table 1. Effect of OLE on levels of insulin and glucose in diabetic male albino rats

Groups	Insulin (µIU/ml)	Glucose (mg/dl)
Control	41.36±0.45[a]	87.44±0.77[a]
Diabetic	24.54±0.36[b]	292.20±0.84[b]
Diabetic+OLE	37.88±0.45[c]	92.62±0.93[c]

Values represent mean ±S.E. (n=10 rats).
Values with different superscripts differ from each other significantly (P<0.01).

Asignificant decrease (p<0.01)inlevels of serum insulin (-40.67%) accompanied with marked elevation (p<0.01)in levels of blood glucose (234.17%)wererecorded in diabetic rats (Group II) when comparedto the control rats (Group I). Marked recovery (P<0.01) in insulin and glucose levels was recorded in diabetic animals post consumption of OLE for one month.

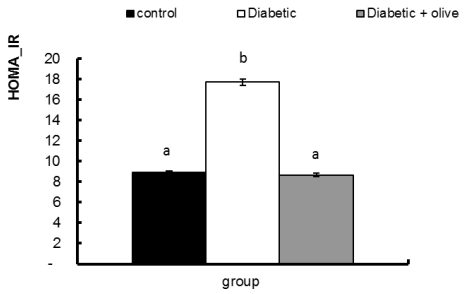

Figure 1. Effect of OLE on values of HOMA_IR of diabetic male albino rats

The results are expressed as Means±SE (n=10). Bars with different letters are different significantly (P<0.05).

HOMA_IR values were significantly higher (P<0.05) in diabetic rats when compared to the corresponding controls (98.30%), while treatment of diabetic rats with OLE returned HOMA_IR values to normal.

Table 2. Effect of OLE on indices of lipid profilein diabetic male albino rats

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TC/HDL	LDL/HDL
Control	141.98±0.82[a]	132.90±0.80[a]	42.10±0.68[a]	77.20±0.93[a]	26.60±0.16[a]	3.38±0.07[a]	1.84±0.05[a]
Diabetic	231.52±0.52[b]	283.96±0.86[b]	38.08±0.38[b]	116.40±0.68[b]	56.80±0.16[b]	6.08±0.05[b]	3.06±0.03[B]
Diabetic+OLE	154.06±0.67[c]	138.16±0.48[c]	58.36±0.52[c]	84.36±0.11[c]	27.68±0.12[c]	2.64±0.01[C]	1.45±0.01[C]

Values represent Mean ±S.E. (n=10 rats).
Values with different superscripts differ from each other significantly (P<0.01)

Diabetic animals showed marked elevation (P<0.01) in TC (63.06%), TG (113.66%), LDL (50.78%), VLDL (113.53%) and ratios of TC/HDL (79.9 %) and LDL/HDL (66.30) accompanied with marked decline in HDL (9.55 %) relative to the corresponding controls. Treatment of diabetic rats with OLE improved the sera lipid profiles as shown by significant (p<0.01) reduction in the values of TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDL with marked elevation of HDL.

Table 3. Effect of OLE on levels of creatinine, urea and uric acidin diabetic male albino rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)	uric acid (mg/dl)
Control	0.91±0.01[a]	32.82±0.74[a]	2.62±0.06[a]
Diabetic	1.66±0.08[b]	63.44±0.58[b]	7.24±0.09[b]
Diabetic+ OLE	0.82±0.008[a]	32.74±0.75[a]	2.64±0.08[a]

Values represent Mean ±S.E. (n=10 rats).
Values with different superscripts differ from each other significantly (P<0.01)

The recorded renal markers elevated markedly (P<0.01) in sera of diabetic rats with percentage of change 82.42%, 93.30% and 176.34% higher than those of control rats for creatinine, urea and uric acid, respectively. Treatment of diabetic rats with OLE returned these parameters towards normal.

DISCUSSION

In recent years, much attention has been focused on using natural products as an alternative therapyfortreatment of many diseases including diabetes mellitus.In the present study, we aimed to examinethe efficacy of OLE in reducing the metabolic abnormalities accompanied to alloxan-induced diabetes in male albino rats.

The reduction in serum insulin levelsandelevationof glucose levelsrecorded in the alloxan-treated rats is attributed to the hypo-secretion of insulin by pancreatic β-cells.Alloxan selectively destroys the pancreatic insulin secreting β-cells and induces hyperglycemia (Kurup and Bhonde, 2000; Szkudelski, 2001).These results are consistent with previous findings recorded byShah. (2007) ; Sivaraj. (2009).HOMA-IR has proved to be a robust tool for the surrogate assessment of insulin resistance (Lann and LeRoith, 2007; Antuna-Puente ., 2011).

In the current study, diabetic rats showed high values of HOMA_IR. This finding is in accordance with Rossetti .(1990) who confirmed that high glucose concentrations cause 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 hyperglycaemia is still unclear. It may be attributed to modifications in structure of insulin receptors and the glucose transport system, resulting in impaired signal transmission (Burantetal.,1986 ;Ordonez, 2007).

OLE exhibited significant anti-hyperglycemic and anti-hypoinsulinemia activity in diabetic animals as compared to untreated diabetic rats.OLE has been reported with an effective hypoglycemic action in diabetic animals (Gonzalez ., 1992,Jouadetal, 2001; Al-AzzawieandAlhamdani, 2006).The acute hypoglycemic effect of OLE may result from a potentiation of glucose-induced insulin release from the residual β-cells and increased peripheral uptake of glucose (Gonzalez etal., 1992).In addition, a novel hormone called betatrophin found to be secreted by liver and adipose tissues. This hormone prompts beta cells in the pancreas to multiply and produce more insulin(Yi ., 2013). Liu .(2014)proved that, OLE attenuates liver damage in diabetic rats by inhibiting expression of inflammatory cytokines in liver, such as TNF-α, IL-1β, and IL-6. The anti-hypo or hyper insulinemiceffect of OLE may be attributed to its protective effect against hepatocyte damage. These hepatocytes produce more betatrophin enhancing insulin production by beta cells of pancreas.this was in harmony with the present results where treatment with OLE ameliorate the functions of the liver and the reduction of body weight.Also, OLE was shown to have a modulatory effect on values of HOMA_IR, which may be attributed to theenhanced peripheral uptake of glucose. Indeed, OLE has a potent antioxidant action(Visiolietal., 2002), so the hypoglycemic effect of OLE through its action as an antioxidant cannot be ruled out (Al-Azzawie; Alhamdani, 2006).

The current study reveals high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL and low HDL levels in diabetic rats 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.

In the present investigation, diabetic animals showed marked elevation in the ratios of TC/HDL and LDL/HDL that increases the risk of coronary heart disease.The current observations are in analogy to earlier results obtained by many investigators (Jarald., 2008; Sivaraj ., 2009 ; Dineshkumar., 2010). Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (Coppack ., 1994 ; Ohno ., 2000). This stimulates the hepatic triglyceride synthesis leading to hypertriglyceridemia as well as over production of LDL and VLDL by the liver (Coppack ., 1994).

Results of the present work showed that,OLE significantly amelioratedsera lipid profiles by reducing the values of TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDLand elevating HDL levels. This indicates that, OLE has a potential role in preventing formation of atherosclerosis and coronary heart disease in diabetic patients.OLE contains biofunctionalcomponents,such as oleuropein,which may play as a regulatory lipid agent and have anti-atherosclerotic effect. On the same context, the anti-atherosclerotic effect of OLE was also demonstrated in rabbits on a high-lipid diet (Wang ., 2008).

Removal of metabolite wastes such as urea, uric acid and creatinine by the kidneys maintains optimum chemical composition of body fluids. In the current study, the increased levels of creatinine, urea and uric acid indicate kidney dysfunction in diabetic rats.Renal dysfunction indicated by elevation of renal markers in diabetic rats has been proved through previous studies (Murugan and Pari, 2007; Jaraldetal., 2008; Chandramohanetal., 2009). Elevation of the renal markersmay be due to metabolic disturbance in diabetic animals reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels (Madinovetal., 2000).Treatment of diabetic rats with OLE reversed these parameters towards normalcy which could be due to decreased metabolic disturbances of other pathways such as protein and nucleic acid metabolism as evidenced by improved glucoselevel.These findings are in consistence with the results obtained by Jarald, (2008) on treatment of diabetic rats with aqueous extract of the plant Cynodondactylon. Also, Chandramohanetal. (2009)obtained similar results after treatment of diabetic rats with 3-hydroxymethyl xylitolfor 45 days.

In conclusion, the results of the present study suggest that OLE has an important role through its antioxidant advantage and effect in an inhibiting hyperglycemia and ameliorating metabolic abnormalities induced by diabetes.

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