Effects of Oleuropein on Hyperglycemia, Total Oxidant and Antioxidant Capacity in Streptozotosin-Diabetic Rats Treated with Metformin and Insulin

Nadide Nabil KAMİLOĞLU[*1],Hülya DAĞDELEN HASTÜRK[2]

[1]Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, 36300, Kars, Turkey.
[2]Department of Physiology, Health Sciences Institute, Kafkas University, 36300, Kars, Turkey.

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Abstract: In this study, it was aimed to investigate the effects of oleuropein on hyperglycemia and oxidant-antioxidant levels in metformin-insulin treated streptozotocin-diabetic rats. For this purpose, 40 Sprague-Dawley rats were used. Animals were divided into 5 groups as 4 experimental and 1 Control each containing 8 rats. 1 ml isotonic NaCl solution was injected intraperitoneally (ip) to the Control group while 50 mg/kg STZ ip was given to the G1, 50 mg/kg STZ + 100 mg/kg Metformin + 4 IU/kg insulin ip was given to the G2, 50 mg/kg STZ + 100 mg/kg Metformin + 4 IU/kg insulin ip and 30 mg/kg orally oleuropein was given to the G3 and 50 mg/kg STZ ip and 30 mg/kg oleuropein orally was given to the G4. Blood glucose values were statistically higher in the experimental groups than the control after streptozotocin administration (P<0.001, P<0.01, P<0.01 respectively). Plasma Total Antioxidant Capacity (TAC) of G2 and G3 were statistically higher than G1 (P<0.001). It was found that plasma TAC levels of G3 were statistically higher than the control (P<0.05). It was determined that the G1 (P<0.001), G2 (P<0.01) and G3 (P<0.05) group had significantly higher plasma Total Oxidant Capacity (TOC) than the control group. TAC levels in the liver of the experimental groups were determined to be statistically insignificant according to the control group. The liver TOC levels of the G2 and G4 group were significantly higher than the control group (P<0.01, P<0.001 respectively). In conclusion, the use of oleuropein alone did not affect the blood glucose level, the live weight, the plasma and the liver TAC and TOC levels, but the use of metformin and insulin with oleuropein as an antioxidant and free radical scavenger following to the rising oxidation in diabetes were supported and strengthen the system.

Keywords: *Diabet, Insulin, Metformine, Oleuropein, TAC, TOC.*

Streptozotosin ile Diyabet Oluşturulmuş ve Metformin-İnsülin ile Diyabet Tedavisi Gören Ratlarda, Oleuropeinin Hiperglisemi, Total Oksidan ve Total Antioksidan Düzeyleri Üzerine Etkileri

Özet: Bu çalışmada, streptozotosin ile diyabet oluşturulmuş ve metformin ve insülin ile diyabet tedavisi gören ratlarda, oleuropeinin hiperglisemi, oksidan-antioksidan düzeyleri üzerine etkilerinin araştırılması amaçlandı. Bu maksatla, 40 adet erkek Sprague Dawley rat kullanıldı. Ratlar her grupta 8 hayvan bulunacak şekilde 4 deney ve 1 kontrol grubu olmak üzere 5 gruba ayrıldı. Kontrol grubuna %0.09 NaCl intraperitoneal (ip) uygulandı. G1'e 50 mg/kg STZ ip, G2'ye 50 mg/kg STZ + 100 mg/kg metformin + 4 İU/kg insülin ip, G3'e 50 mg/kg STZ + 100 mg/kg metformin + 4 İU/kg insülin ip ve 30 mg/kg oleuropein oral olarak ve G4'e 50 mg/kg STZ ip olarak ve 30 mg/kg oleuropein oral olarak uygulandı. Streptozotosin uygulandıktan sonraki 3, 10 ve 21. günlerde deneme gruplarının kan glukoz değerleri kontrol grubuna göre istatistiksel olarak önemli düzeyde yüksek bulunmuştur (sırasıyla P<0.001, P<0.01, P<0.01). Plazma total antioksidan kapasiteleri gruplar arasında kıyaslandığında, G2 ve G3 grubunun Total Antioksidan Kapasite (TAC) düzeyleri, G1 grubuna göre istatistiksel olarak daha yüksek belirlenmiştir (P<0.001). Kontrol grubu ile deneme gurupları arasında kıyaslama yapıldığında G3 grubunun TAC düzeylerinin, kontrol grubuna göre istatistiksel olarak yüksek olduğu belirlenmiştir (P<0.05). G1 (P<0.001), G2 (P<0.01) ve G3 (P<0.05) grubunun plazma Total Oksidan Kapasitelerinin (TOC) kontrol grubuna göre istatistiksel olarak yüksek olduğu belirlenmiştir. Deneme gruplarının karaciğer TAC düzeylerinin kontrol grubuna göre istatistiksel olarak önemsiz olduğu tespit edildi. Karaciğer total oksidan kapasiteleri gruplar arasında kıyaslandığında G2 grubu ile G4 gurubunun TOC düzeylerinin kontrol grubuna göre istatistiksel olarak yüksek olduğu (sırasıyla P<0.01, P<0.001) belirlenmiştir. Sonuç olarak oleuropeinin tek başına kullanımının kan glukoz düzeyini, canlı ağırlığı ve plazma ile karaciğer TAC ve TOC kapasitelerini etkilemediği, ancak diyabette yükselen oksidasyona karşılık, metformin ve insülinle birlikte kullanımının antioksidan sisteme destek olduğu ve serbest radikal tutucu etkiyi kuvvetlendirdiği düşünülmektedir.

Anahtar Kelimeler: *Diabet, İnsulin, Metformine, Oleuropein, TAC, TOC.*

Introduction

Type 2 diabetes mellitus (DM) is a heterogeneous group of diseases often includes varying levels of insulin resistance, disorders characterized by increased glucose production and decreased insulin secretion. This metabolic disease constitute more than 90% of the diabetes cases.

Different genetic and metabolic disorders associated with insulin action or secretion leads to Type 2 DM of which common feature is hyperglycemia. While the main feature is hyperglycemia in DM, pathophysiological mechanisms causing hyperglycemia is different. Impaired insulin secretion or insulin resistance is a key feature of hyperglycemia in Type 2 DM (Emanuelli et al., 2004; Özata and Yöntem, 2006; Pirie et al., 1996). Also reasarchers indicated that excessive production of free radicals is the primary cause of chronic hyperglycemia. High levels of free radicals and deterioration of antioxidant defense mechanisms can lead to the development of insulin resistance, the increase of lipid peroxidation and damage of cellular organelles. At the same time, diabetic complications are considered to occur as a result of hyperglycemia, excessive free radical production and increased oxidative stress (Novelli et al., 2001; Novelli et al., 2004). Chronic hyperglycemia produces dysregulation of cellular metabolism, interrupts cell integrity, induce reactive oxygen radicals and promotes apoptosis. Therefore oxidative stress mediates the deleterious effects of diabetes on many tissue function.

Antioxidants neutralize harmful substances called free radicals and are extremely important substances for preventing the destruction of the cells. The suppression of oxidative stress by modulating anti-oxidant capacity may also alleviates high glucose-mediated oxidative stres. In fact, organisms, are able to defend themselves against oxidative stres due to the biological antioxidant enzymes. However, this wealth is not inexhaustible. Therefore, organisms should be supplemented with nutrients containing antioxidant properties. The importance of polyphenols as antioxidant utilized for this purpose have been reported by researchers. Polyphenols present in olive, olive oil and olive leaf extract has proven to prevent oxidation of the cells and to retard aging (Galli and Visioli, 1999; Tsimidou, 1998; Vlahov, 1992). Due to side effects of therapeutic agents such as oral hypoglycemic drugs, there is increasing interest in herbal medicine for treating diabetes recently. Many traditional medicinal plant extracts are used in the treatment of diabetes. In recent years, reducing the negative effects of oxidative stress and free radicals in diabetic patients by using non-vitamin antioxidants such as flavonoids and polyphenols is recommended by the researchers (Asgary et al., 2002; Lean et al., 1999).

Oleuropein which is a phenolic compound extracted from the olive leaf is still being investigated for its effects on the hyperglycemia and oxidant-antioxidant system in diabetes (Tripoli et al., 2005). This compound is a hydroxylated and glycosylated elenolik acid tyrosol ester. Oleuropein and its metabolic hydroxytrotrozils are known to have strong antioxidant abilities (Tsimidou, 1998). Olive leaves and their products are defined as healthy diet elements due to its phenolic compounds (Visioli and Galli, 1994; Visioli and Galli, 1995). Meanwhile, it is reported in studies (Galli and Visioli, 1999; Garrido-Fernandez et al., 1997; Vhalov, 1992) that oleuropein allows to decrease high blood sugar level and shows hypoglycemic effect. Also, (Tsimidou, 1998; Vhalov, 1992) the use of oleuropein derivatives is recommended for the protection, prevention and treatment of disease due to its immune system supporting effect and antioxidant action.

In this study, we aimed to investigate the effects of oleuropein on hyperglycemia and on oxidant-antioxidant balance in streptozotocin induced diabetic rats and diabetic rats treated with metformin and insulin.

Materials and Methods

Experimental Protocols and Treatment of Animals: This study was approved by the Institutional Animal Care and Use Committee of Kafkas University with ethics decision number of 2010-44. In this study 40 male, eight-months old-Sprague–Dawley rats of 250±50 g body weight of age were used. Rats were housed at room temperature with a lighting schedule of 12 h light-dark cycle and humidity of 55%. Animals had free access to a standard rodent pellet diet and tap water *ad libitum*. The rats were divided into 5 groups comprising 4 experimental and a Control groups each containing 8 animals. Diabetes was induced by a single intraperitoneal injection of 50 mg/kg bodyweight streptozotocin (STZ, S0130, Sigma Chemical Inc., St Louis, MO, USA). To the Control rats 1 ml isotonic saline was applied intraperitoneally. Glucose concentrations of Control and experimental groups were measured by a glucometer (On-Call Plus, Acon Lab., San Diego, USA) before and 72th h after STZ injection. To monitor blood glucose concentrations, blood samples were collected from the tail vein of the rats. Rats showing blood glucose concentrations over 180 to 200 mg/dL were considered diabetic. A total of 32 rats with diabetes were randomly divided into four groups. G1 was used as diabetic control, to the rats of G2 100 mg/kg metformine and 4 IU/kg insülin was administered intraperitoneally, to G3 100 mg/kg metformine, 4 IU/kg insülin was administered intraperitoneally and 30 mg/kg oleuropein orally, to G4 only 30

mg/kg oleuropein was administered orally. This applications continued for 21 consecutive days.

Collection of Samples: Blood samples were harvested from each group of rats by intracardiac needle. The plasma was separated by centrifugation at 3000 rpm for 10 min. Livers were removed, blotted, weighed, and homogenized. Plasma samples and supernatants of liver homogenates were used for analysis of total oxidant capacity (TOC) and total antioxidant capacity (TAC).

Measurement of TAC and TOC: TAC and TOC of serum and liver were determined using a Rel Assay Diagnostic Kit (Gaziantep, Turkey).

Statistical Analysis: Statistical analyzes were assessed by the one-way ANOVA test using the SPSS 16.0 program (SPSS for Windows, Chicago, IL, USA) fallowed post hoc analyses of groups were made according to the Tukey Test. Variables were expressed as mean±standard deviation (SD) and P<0.05 was considered statistically significant.

Results

Plasma glucose levels: Changes in plasma glucose levels are shown in Figure 1. Blood glucose values were significantly different between experimental groups and control (P<0.001) on 3 days after the streptozotocin administration. Glucose levels of group G1 and G2 on 10th day (P<0.01, P<0.001) and group G1, G2 and G4 on 21th day (P<0.001, P<0.05 and P<0.01, respectively) were significantly higher than the control group.

Plasma total antioxidant capacity: Plasma total antioxidant capacity of the groups are shown in Figure 2. When Total antioxidant capacity (TAC) in the experimental group compared with the G1, TAC levels of G2 and G3 were determined to be significantly higher (P<0.001). However, no statistically significant difference was detected between the G1 and G4. In comparison between the experimental group and the Control, TAC level of the G3 was determined to be statistically higher than the Control (P<0.05). TAC levels of G4 were determined to be lower than the control.

Liver total antioxidant capacity: Liver total antioxidant capacity (TAC) of the groups are shown in Figure 3. No statistically significant change was found in TAC levels of the experimental group as compared with the Control.

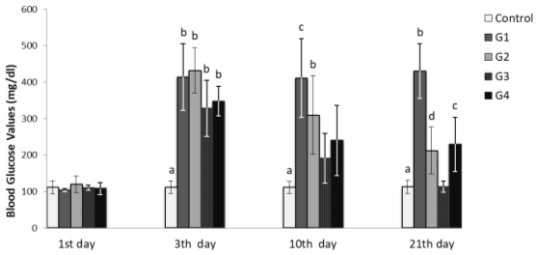

Figure 1. Changes in plasma glucose levels of groups (mg/dl). $^{a-b}$: P<0.001, $^{a-c}$: P<0.01, $^{a-d}$: P<0.05.

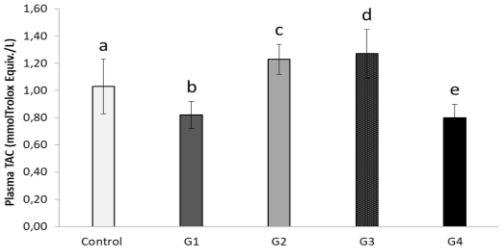

Figure 2. Plasma TAC of the groups (mmolTrolox Equiv./L). $^{a-d, a-e}$: P<0.05, $^{b-c, b-d, c-e, d-e}$: P<0.001.

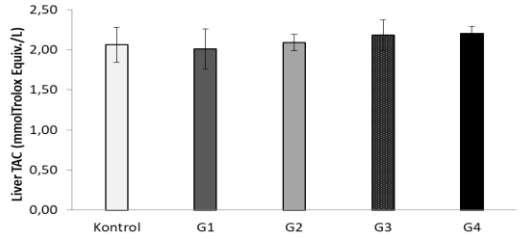

Figure 3. Liver TAC of the groups (mmolTrolox Equiv./L).

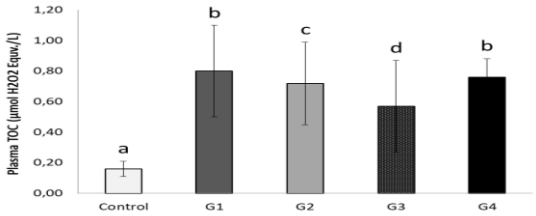

Figure 4. Changes in the plasma TOC (µmol H2O2 Equv./L). $^{a-b}$: P<0.001, $^{a-c}$: P<0.01, $^{a-d}$: P<0.05.

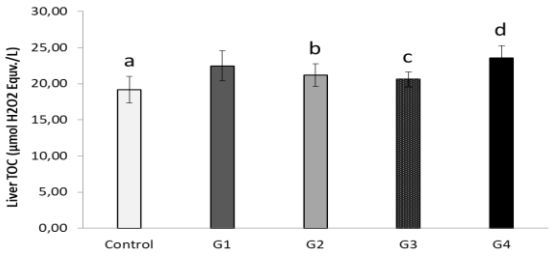

Figure 5. Changes in the liver TOC (µmol H2O2 Equv./L). $^{a-b}$: P<0.01, $^{a-d}$: P<0.001, $^{c-d}$: P<0.05.

Plasma total oxidant capacity: Changes in the plasma total oxidant capacity (TOC) of the groups are shown in Figure 4. TOC levels of G1-G4 (P<0.001), G2 (P<0.01) and G3 (P<0.05) were determined to be significantly higher as compared

to the Control. No statistically significant difference was detected between groups when the experimental groups compared with the G1.

Liver total oxidant capacity: Changes in the liver total oxidant capacity of the groups are shown in Figure 5. Liver total oxidant capacity (TOC) of G2 and G4 were statistically higher than the Control ($P<0.01$, $P<0.001$, respectively). There were no statistically significant change between Liver TOC levels of G1 and treatment groups.

Discussion

Complications like micro- and macrovascular diseases, chronic renal failure and diabetic nephropathy due to diabetes is still the most important causes of morbidity and mortality among DM patients in the world (Yenigün, 2001, Üstüner, 1999). In terms of glucose balance, insulin serves a key role and the lack of insulin leads to increased blood glucose levels and a variety of complications in diabetics. In order to avoid these complications insulin support should be provided to keep blood sugar level in normal glycemic values. In recent years, insulin therapy in combination with oral antidiabetic drugs is maintained in Type 2 diabetes. In all of the current diabetes treatment, metformin is a safe agent that increases insulin sensitivity and lowers fasting blood glucose levels down to 78 mg/dL (Schneider, 1991). Because of the reduction in total insulin dose by 36%, metformin is added to diabetes treatment in combination with insulin. On the other hand, it has been proven by many studies that monounsaturated fatty acids found in the olive leaf extract lower blood sugar in diabetes patients (Kumar et al., 2001). Also, olive oil was demonstrated to reduce blood glucose levels by 12% in diabetics (Visioli and Galli, 1995). It is reported that oleuropein facilitates the reduction of blood sugar and stimulate insulin secretion by the pancreas (Visioli and Galli, 1994). In our study, oleuropein alone did not affect blood glucose levels, but the effect of lowering blood glucose level was determined to be higher when used in combination with metformin and insulin. In addition this combination the live weight was kept unchanged.

High levels of glycoproteins, defective glucose auto-oxidation and damaged antioxidant balance have been found in diabetic patients. The variety of lipid-dependent gene expression and lipid peroxidation due to oxidative stress induced by free radicals are found to be responsible for the various diabetic complications. Reports showed that low-grade chronic inflammation and abnormal immune response plays a key role in the development of Type 2 diabetes mellitus and in the pathogenesis of insulin resistance (Niwa et al., 2011). It has been reported that reactive molecules formed by chronic inflammation that cause β-cell destruction due to increased oxidative stres and insulin reduction in the pancreas and also potentiate diabetes along with DNA damage (Niwa et al., 2011). Reasarchers has determined that gene expression caused by oxidative stress induced by free radical peroxidation is responsible for the development of micro and macro vascular complications in diabetics (Aslan et al., 2007; Sheetz and King, 2002). The changes in the plasma lipid profile leads to the development of atherosclerosis abnormalities in diabetic patients. On the other hand, many enzymatic and non-enzymatic antioxidant defense systems play a protective role against the harmful effects of reactive oxygen species. However, the increased production of free radicals and decreased antioxidant level is responsible for the formation of oxidative stress in diabetes. It has been shown that hyperglycemic conditions change oxidant-antioxidant balance towards the increase of the oxidants in diabetes mellitus (Wolff, 1993). Researchers (Pan et al., 2008, Abou-Seif and Youssef, 2004) reported that oxidative DNA damage, protein glycation and oxidation products are responsible for the pathogenesis of pancreatic and vascular complications in diabetes. In our study, we have detected no difference in TAC of plasma and liver, but significantly higher capacity of plasma and liver TOC in streptozotocin-diabetic rats.

Most patients with Type 2 diabetes require multiple antihyperglycemic medications to ensure optimal glycemic control. There are various drug combinations used for this purpose. Use of metformin with insulin is stated to be frequently used method for reducing the dose of both drugs in treatment options today (ADA, 2005; Cusi and DeFronzo, 1998). Metformin has been reported to reduce hyperglycemia and prevent hyperlipidemia in Type 2 diabetic patients (ADA, 2005; Cusi and DeFronzo, 1998, Lee and Kwon 2004, Stepensky et al. 2002). Also, Li et al. (2011) showed that the use of metformin reduces obesity-related insulin resistance and hyperinsulinemia and stabilize β-cell function. The administration of metformin have a significant impact on reducing diabetic protein oxidation products and eliminating the the formation of free radicals that have inflammatory effects and DNA oxidation (Farah et al. 2008, Xia et al. 2008). Meanwhile reports (Lhommeau et al., 2011, Montes-Cortes et al., 2010) showed that insulin have an important influence on suppressing the formation of free radicals, deactivation of

singlet oxygen and play an important role in supporting the antioxidant system. The researchs conducted in recent years indicate that the use of insulin with metformin provides superior treatment option from its use alone (Sliwinska et al., 2006). The use of some combinations of insulin with metformin is reported to correct the decreased activity of certain organs such as gastro-intestinal tract, liver and kidney tissue (Lee et al., 2011). In our study, antioxidant capacity of plasma and liver was determined to remain the same with the control group and the use of metformin in combination with insulin as a good anti-oxidant and anti-stress agent. A variety of antioxidant compounds used in diabetes are thought to be protective against tissue damage due to free radical scavenger properties and working as glycation inhibitors (Davie et al., 1992; Sinclair et al., 1992). Oleuropein that active phenolic compound in olive leaf extract is quite a good H_2O_2 holder (Tetik, 2005). Also, it is showed that oleuropein suppresses hyperglycemia, oxidative metabolism of H_2O_2 and diabetes-induced oxidative stres and assist in preventing diabetic complications (Al-Azzawie and Alhamdani, 2006, Singh et al., 2008). In our study, it was observed that the use of oleuropein alone did not cause the expected change in the plasma and liver TAC capacity. However, we detected that combined use of metformin, insulin, and oleuropein significantly increased plasma antioxidant capacity. These results showed that single use of oleuropein as an antioxidant did not create adequate effect in diabetes.

Conclusions

In conclusion, the use of oleuropein alone did not affect blood glucose level, the live weight, the plasma and the liver TAC and TOC levels in SZT induced diabetic rats, but the use of metformin and insulin as antidiyabetic agents with oleuropein as an anti-oxidant and free radical scavenger according to the rising oxidation in diabetes were supported and strengthen the system. It was said that administration of the metformine, insulin and oleuropein on streptozotocin-diabetic rats have protective role on hyperglycemia and oxidant-antioxidant levels.

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***Corresponding author:**
Prof. Dr.Nadide Nabil Kamiloğlu,
Kafkas Üniversitesi, Veteriner Fakültesi, Fizyoloji Anabilim Dalı, KARS.
E-mail: nkamiloglu@hotmail.com