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Effects of Oleuropein on Nitric Oxide, Glutathione, Malondialdehyde Levels and Glutathione Peroxidase Activities in Various Tissues of Streptozotocin-Induced Diabetic Rats Treated with Metformin and Insulin

Streptozotosin ile Diyabet Oluşturulmuş ve Metformin ile İnsülin Uygulanan Ratlarda Oleuropein'in Çeşitli Dokularda Nitrik Oksit, Glutatyon, Malondialdehit Düzeyleri ve Glutatyon Peroksidaz Aktivitesi Üzerine Etkileri

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ABSTRACT Objective: The aim of the present study was to investigate the effects of oleuropein on nitric oxide (NO) and oxidant-antioxidant levels in tissues of streptozotocin-induced diabetic rats treated with metformin and insulin. **Material and Methods:** A total of 40 male Sprague-Dawley rats were used in this study. Animals were divided into 5 groups (n=8) including 3 experiment and 2 control groups. Saline solution was injected intraperitoneally (IP) to the rats in control group. 50 mg/kg STZ was given IP to the Diabetic Control, 50 mg/kg streptozotocin (STZ)+100 mg/kg Metformin+4 IU/kg insulin was given IP to Group 1, 50 mg/kg STZ+100 mg/kg Metformin+4 IU/kg insulin was given IP and 30 mg/kg oleuropein was given orally to Group 2 and 50 mg/kg STZ IP and 30 mg/kg oleuropein was given orally to Group 3. **Results:** Metformin and insulin with oleuropein have a synergistic effect on regulatory activity of the GSH, MDA, NO levels and GSHPx activities of the kidney, brain and heart tissues. Using metformin and insulin with oleuropein increased the GSH levels of the kidney ($p<0.001$), brain ($p<0.001$) and heart tissues ($p<0.05$); also increased the GSHPx activity of the brain ($p<0.01$) and the heart tissues ($p<0.001$) and reduced MDA and NO levels of the kidney, brain and heart tissues ($p<0.001$). There were no statistically significant differences in GSHPx activity of kidney tissues of experimental groups. **Conclusion:** Using oleuropein, on streptozotocin-diabetics, in combination with metformin and insulin seems to have synergistic effect on the antioxidants in kidney, brain and heart tissues and effective in regulating the NO level on tissues.

Keywords: Experimental diabetes mellitus; glutathione; glutathione peroxidase; insulin; malondialdehyde; metformin; nitric oxide; oleuropein

ÖZET Amaç: Bu çalışmada, streptozotosin ile diyabet oluşturulmuş ve metformin ve insülin tedavisi gören ratlarda, oleuropein'in dokularda nitrik oksit (NO) ve oksidan-antioksidan düzeyleri üzerine etkilerinin araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmada, 40 Sprague-Dawley cinsi erkek rat 3 deney ve 2 kontrol olmak üzere, 5 gruba ayrıldı (n=8). Kontrol grubuna intraperitoneal (IP) olarak izotonik sodyum klorür çözeltisi uygulandı. Diyabetik Kontrol Grubu'na IP olarak 50 mg/kg STZ, Grup 1'e IP olarak 50 mg/kg Streptozotosin (STZ)+100 mg/kg Metformin+4 IU/kg insülin, Grup 2'ye IP olarak 50 mg/kg STZ+100 mg/kg Metformin+4 IU/kg insülin ve oral olarak 30 mg/kg oleuropein ve Grup 3'e IP olarak 50 mg/kg STZ ve oral olarak 30 mg/kg oleuropein verildi. **Bulgular:** Oleuropein ile birlikte metformin ve insülin kullanımının, böbrek, beyin ve kalp dokularının glutatyon (GSH), malondialdehit (MDA) ve NO düzeylerinin ve GSHPx aktivitelerinin düzenlenmesinde sinerjik bir etkiye sahip olduğu belirlendi. Oleuropein ile birlikte metformin ve insülin uygulanmasının böbrek ($p<0,001$), beyin ($p<0,001$) ve kalp dokusu ($p<0,05$) GSH düzeyleri ile beyin ($p<0,01$) ve kalp dokusu ($p<0,001$) GSHPx aktivitelerini arttırırken, böbrek, beyin ve kalp dokularında ($p<0,001$) MDA ve NO seviyelerini azalttığı belirlendi. Grupların, böbrek dokusu glutatyon peroksidaz (GPHPx) aktivitelerinde ise istatistiksel bir farklılığa rastlanmadı. **Sonuç:** Elde ettiğimiz veriler, streptozotosin ile diyabet oluşturulmuş ratlarda insülin ve metformin ile birlikte oleuropein uygulanmasının böbrek, beyin ve kalp dokularında antioksidan denge ve NO düzeyleri üzerinde düzenleyici aktivite gösterdiğini ortaya koymaktadır.

Anahtar Kelimeler: Deneysel diabetes mellitus; glutatyon; glutatyon peroksidaz; insülin; malondialdehid; metformin; nitrik oksid; oleuropein

Diabetes mellitus is characterized with malfunctions in insulin secretion and the instability of glucose and disorders of protein, lipid, and carbohydrate metabolism which may lead to various complications that affect many organs. Researchers have reported that reactive oxygen species (ROS) are associated with the pathogenesis of diabetes mellitus.[1,2] As a result of the oxygen free radicals (OFRs) overproduction, oxidative stress increases in bloodstream and dramatically contributes to background of many diseases.[3] Several biochemical pathways including glucose autooxidation, nonenzymatic protein glycation, mitochondrial ROS overproduction associated with hyperglycemia increase ROS generation.[3] However, physiologically, a balance condition presents between the radical production and protection in the cells. Additionally, imbalance conditions, such as decreased or reduced cellular antioxidant and antioxidant enzyme levels, lead to oxidative damage. These damages may effect protein, lipid, and nucleic acid structures and it is known that, both insulin dependent (type 1) and non-insulin-dependent diabetes (type 2) causes these damages.[4] Recent research has focused on the effects of exogenous antioxidants that may be effective in reducing the oxidative stress responsible for the formation of diabetes and diabetic complications.[5,6] Researchers also showed that the maintenance of adequate exogenous antioxidant levels prevent or even manage a great number of diabetic complications.[7-9] For this purpose, many antioxidants like coenzyme Q_{10}, vitamin E, beta-catotene, vitamin A, phytoestrogens, polyphenols and oleuropein have been used.[4,10-14] In addition, oleuropein that is the principal active component of olive leaf extract acts as an antioxidant through its ability to scavenge the superoxide anions and hydroxyl radicals by providing hydroxyl group to directly neutralize and quench free radicals.[15,16] Oleuropein that a natural product of the secoiridoid group can also produce bioactive substances by hydrolysis, namely elenolic acid and 3, 4-dihydroxy-phenylethanol.[15] These compounds are responsible for the antioxidant properties of oleuropein. In addition, antihyperglycaemic effect of oleuropein have been reported in diabetic rats by Gonzalez et al. Also, it showed that olive leaf those with high levels of oleuropein have a significant role in eliminating effects of diabetes mellitus that occurs due to intensive oxidative stress.[17,18]

The high blood glucose levels observed in diabetes may contribute to the progression of the disease through negative effects on β-cells and insulin sensitivity. Both problems can be corrected by therapeutically reducing the glucose level. A biguanide antihyperglycemic agent, metformin and/or insulin therapy is used for the management of the diabetes that reduced the risk of complications in patients requiring compact care. Reduced cardiovascular, nephrotoxic and norotoxic problem rates have shown in studies conducted with metformin users.[19-21] Researchers also believe that, metformin may have an additional mechanism of action in addition to its antihyperglycemic properties.[20] Studies reported that metformin supports the antioxidant enzyme activities and glutathione levels, and decreases lipid peroxidation markers in type 2 diabetes.[4] Moreover, metformin has free radical scavenging properties, thus it plays a role in decreasing ROS levels in glucose-mediate stimulated cells.[22] In addition, insulin is used for glycemic control in diabetes and it prevents and reduces the progression of diabetes-related complications. Aside from optimization of hemodynamic status, insulin treatment also prevented acute renal failure and the neurodegeneration.[4]

Nitric oxide (NO) is an endogenous mediator involved in various physiological and pathogenic processes.[23] Overproduction of NO has been associated with progression of the severity of diseases, including diabetes.[24] Endothelial cells regulate vascular tone function via producing NO. Preservation of vascular tone by NO is insufficient in heart failure, diabetes and hypertensive conditions. It is known that, there is a malfunction of endothelial production and/or function of NO in almost all of these disorders.[25] Nitric oxide also plays many different and important roles in the brain. It is a neurotransmitter and a free radical, and also an important component for cerebral

blood flow and inflammation.[26] It is also thought to be an important factor for pathophysiological mechanisms in the brain at the same time.[27,28] Therefore, prolonged inhibition of NO synthesis leads to many systemic problems. However, potent inhibition of oxidative stress with certain antioxidants or/and with therapeutic treatment options under experimental diabetic conditions has been implicated in many organ protection. In the present study, we aimed to investigate the effects of oleuropein on NO and oxidant-antioxidant balance in streptozotocin induced diabetic rats and diabetic rats treated with metformin and insulin.

MATERIAL AND METHODS

ANIMALS AND EXPERIMENTAL PROTOCOLS

This study was approved by the Institutional Animal Care and Use Committee of Kafkas University (Ethics Decision Number: 2018-104). All studies in this article were carried out in line with Guide for the Care and Use of the Laboratory Animals principles and animal rights were protected. All animals were kept and maintained under the standard guidelines and housed in The Experimental Animal Implementation and Research Centre of Kafkas Üniversity, Kars, Turkey. Eight-month-old, 40 male Sprague-Dawley rats weighing 250±50 g were used. All 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 (4 experimental and 1 control) with 8 in each group. Diabetes was induced by a single intraperitoneal (IP) injection of 50 mg/kg bodyweight streptozotocin (STZ, S0130, Sigma Chemical Inc., St Louis, MO, USA). Control rats were applied isotonic saline intraperitoneally. Glucose concentrations of control and study groups were measured by a glucometer (On-Call Plus, Acon Lab., San Diego, USA) before and at 72th h after STZ injection. To monitore blood glucose concentrations, blood was collected from the tail vein of the rats. As a result of the measurement, over 180 to 200 mg/dL blood glucose concentrations were considered diabetic.

A total of 32 rats with diabetes were randomly divided into four groups. Rats without STZ injection were used as the Control (n=8) and saline solution administered intraperitoneally. Only STZ was injected to Diabetic Control (n=8), 100 mg/kg metformin+4 IU/kg insulin and STZ were administered IP to Group 1 (n=8), 100 mg/kg metformin+4 IU/kg insulin+STZ IP and 30 mg/kg oleuropein orally were administered to Group 2 (n=8), IP STZ and 30 mg/kg oleuropein was administered orally to Group 3 (n=8). This procedure continued for 21 consecutive days.

COLLECTION AND PREPARATION OF SAMPLES

Brain, heart and kidney tissue samples were harvested from each group of rats. Tissue samples were used for colorimetric analysis of Glutathione peroxidase (GSHPx) activities, Glutathione (GSH), Malondialdehyde (MDA) and NO levels.

The tissues were homogenized with a coolant homogenizer (**A**: 50 mM, H_2PO_4 and **B**: 50 mM $Na_2HPO_4.2H_2O$, A:B (v/v)=1:1.5) at 290 g for 3 minutes, immediately after fixation with 0.15 M KCl at 4 °C. The homogenates were centrifuged for 15 minutes at 2400 g at 4 °C, and the resulting supernatants were stored at -25 °C until analyzed.

ANALYTICAL PROCEDURES

The levels of NO, GSH, GSHPx and MDA were measured spectrophotometrically (UV-1201, Shimadzu, Japan).[24,29-31] The protein content in the tissue homogenate was measured by the method of Peterson with bovine serum albumin as the standard.[32]

STATISTICAL ANALYSIS

Statistical analysis was done with one-way Anova test. The differences between the two groups were compared with Mann-Whitney U test. Variables were expressed as mean±standard deviation (SD) and $p<0.05$ was considered statistically significant. Statistical analyses were performed by using SPSS 16.0 (SPSS for Windows, Chicago, IL, USA).

RESULTS

TISSUE MDA LEVELS

Changes in tissue MDA levels of groups are shown in Figure 1 and Table 1. There was a significant increase in kidney tissue MDA levels of Diabetic Control ($p<0.001$), Group 1 ($p<0.01$) and Group 3 ($p<0.001$) in comparison with Control. On the other hand, there was a significant decrease in kidney tissue MDA levels of Group 1 ($p<0.001$), Group 2 ($p<0.001$) and Group 3 ($p<0.001$) compared

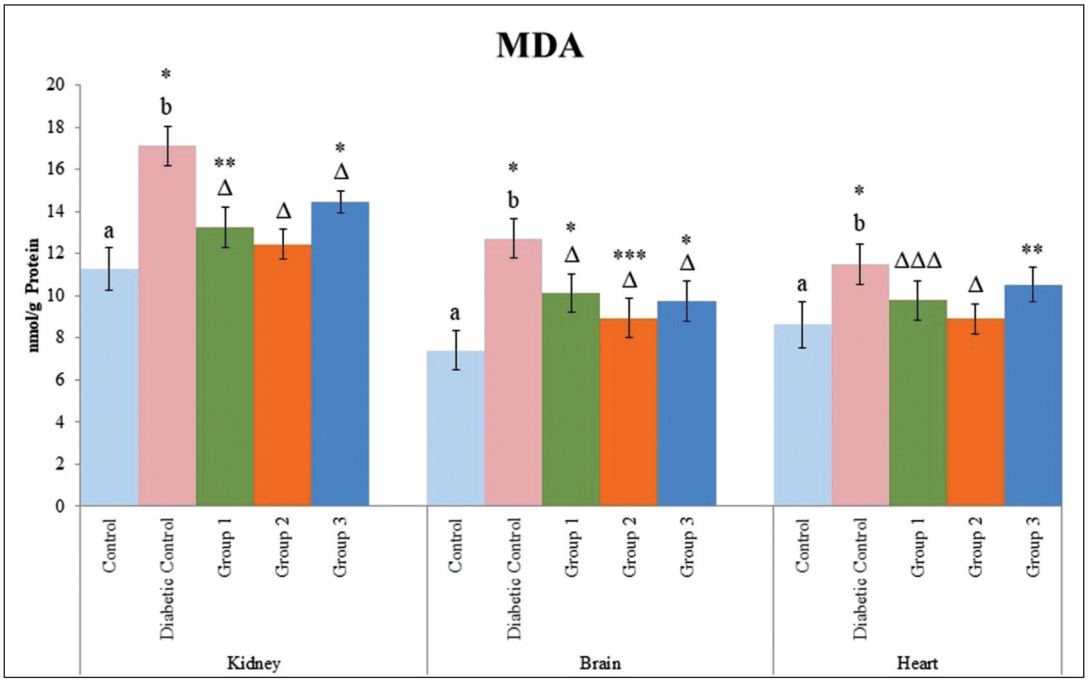

FIGURE 1: Tissue MDA levels of groups. *Indicate significant differences between control and experimental groups, different laters indicate significant differences between groups. **Control:** %0,9 NaCl; **Diabetic Control:** 50 mg/kg Streptozotocin; **Group 1:** 50 mg/kg Streptozotocin+100 mg/kg Metformin+4 IU/kg Insulin; **Group 2:** 50 mg/kg Streptozotocin +100 mg/kg Metformin+4 IU/kg Insulin+30 mg/kg Oleuropein; **Group 3:** 50 mg/kg Streptozotocin+30 mg/kg Oleuropein (a-*: $p<0.001$, a-**: $p<0.01$, a-***: $p<0.05$, b-$^{\Delta}$: $p<0.001$, b-$^{\Delta\Delta}$: $p<0.01$, b-$^{\Delta\Delta\Delta}$: $p<0.05$).

TABLE 1: Alterations of the MDA, GSH, GSHPx and NO levels in the kidney, brain and heart tissues.

Parameters	Tissues	Control	Diabetic Control	Group 1	Group 2	Group 3
MDA (nmol/g Protein)	Kidney	11.28 ± 1.02^{a}	$17.1\pm0.91^{b,*}$	$13.24\pm0.95^{\Delta,**}$	$12.44\pm0.7^{\Delta}$	$14.45\pm0.53^{\Delta,**}$
	Brain	7.41 ± 0.95^{a}	$12.71\pm0.93^{b,*}$	$10.1\pm0.91^{\Delta,*}$	$8.93\pm0.92^{\Delta,***}$	$9.74\pm09.6^{\Delta,*}$
	Heart	11.49 ± 1.09^{a}	$11.49\pm0.98^{b,*}$	$9.77\pm0.91^{\Delta\Delta\Delta}$	$8.9\pm0.72^{\Delta}$	$10.53\pm0.83^{**}$
GSH (µg/g Protein)	Kidney	10.26 ± 0.86^{a}	$7.85\pm0.48^{b,*}$	$9.39\pm0.52^{\Delta\Delta}$	$9.65\pm0.82^{\Delta}$	$8.44\pm0.67^{*}$
	Brain	12.05 ± 0.84^{a}	$8.04\pm0.72^{b,*}$	$9.62\pm0.74^{\Delta\Delta,*}$	$10.98\pm0.56^{\Delta,***}$	$8.9\pm0.44^{*}$
	Heart	7.74 ± 0.73^{a}	$5.3\pm0.43^{b,*}$	$6.02\pm0.7^{*}$	$6.38\pm0.76^{\Delta\Delta\Delta,**}$	$5.62\pm0.53^{*}$
GSHPx (IU/ml Protein)	Kidney	0.016 ± 0.0019	0.0154 ± 0.0013	0.0159 ± 0.0021	0.016 ± 0.0018	0.0155 ± 0.0007
	Brain	0.0583 ± 0.0043^{a}	$0.033\pm0.0025^{b,*}$	$0.0379\pm0.0025^{\Delta\Delta\Delta,*}$	$0.0385\pm0.0022^{\Delta\Delta,*}$	$0.036\pm0.001^{*}$
	Heart	0.0717 ± 0.0052^{a}	$0.0613\pm0.004^{b,*}$	$0.0713\pm0.0045^{\Delta\Delta}$	$0.0724\pm0.004^{\Delta}$	$0.0714\pm0.0041^{\Delta\Delta}$
NO (nmol/g Protein)	Kidney	253.25 ± 14^{a}	$543.57\pm27.69^{b,*}$	$452.43\pm17.3^{\Delta,*}$	$348.58\pm17.52^{\Delta,*}$	$510.74\pm29.38^{*}$
	Brain	269.39 ± 17.39^{a}	$513.88\pm35.92^{b,*}$	$457.70\pm11.77^{\Delta,*}$	$414.80\pm14.84^{\Delta,*}$	$488.99\pm15.94^{*}$
	Heart	258.49 ± 16.94^{a}	$538.42\pm20.82^{b,*}$	$481.43\pm30.89^{\Delta\Delta,*}$	$403.51\pm23.34^{\Delta,*}$	$502.80\pm27.98^{*}$

Control: % 0,9 NaCl, **Diabetic Control:** 50 mg/kg Streptozotocin, **Group 1:** 50 mg/kg Streptozotocin +100 mg/kg Metformin + 4 IU/kg Insulin, **Group 2:** 50 mg/kg Streptozotocin +100 mg/kg Metformin + 4 IU/kg Insulin + 30 mg/kg Oleuropein, **Group 3:** 50 mg/kg Streptozotocin+30 mg/kg Oleuropein (a-*: $p<0.001$, a-**: $p<0.01$, a-***: $p<0.05$, b-$^{\Delta}$: $p<0.001$, b-$^{\Delta\Delta}$: $p<0.01$, b-$^{\Delta\Delta\Delta}$: $p<0.05$).

with the Diabetic Control. There was a significant increase in MDA levels of the brain tissue in the Diabetic Control ($p<0.001$), Group 1 ($p<0.001$), Group 2 ($p<0.05$) and Group 3 ($p<0.001$) compared to the Control. There was a significant decrease in MDA levels of the brain tissue in Group 1, Group 2 and Group 3 compared with the Diabetic Control ($p<0.001$). There was a significant increase in heart tissue MDA levels of Diabetic Control ($p<0.001$) and Group 3 ($p<0.01$) compared to the Control. While a decrease was consisted heart tissue MDA levels of Group 1 ($p<0.05$) and Group 2 ($p<0.001$), no statistically significant difference was found in Group 3 compared to the Diabetic Control.

TISSUE GSH LEVELS

Alteration in tissue GSH levels are shown in Figure 2 and Table 1. Diabetic Control and Group 3 ($p<0.001$) showed a significant decrease in kidney tissue GSH levels compared to the Control, the kidney tissue GSH levels were found to increase in Group 1 ($p<0.01$) and Group 2 ($p<0.001$) in comparison with the Diabetic Control. No statistical difference was determined in the kidney tissue GSH levels of Group 3. Brain tissue GSH levels of Diabetic Control ($p<0.001$), Group 1 ($p<0.001$), Group 2 ($p<0.05$) and Group 3 ($p<0.001$) showed a significant decrease in comparison with the Control. On the other hand, there was a significant increase in brain tissue GSH levels of Group 1 ($p<0.01$) and Group 2 ($p<0.001$) in comparison with the Diabetic Control, whereas there was no statistically significant change in Group 3. There was a significant decrease in heart tissue GSH levels of Diabetic Control ($p<0.001$), Group 1 ($p<0.001$), Group 2 ($p<0.01$) and Group 3 ($p<0.001$) compared with Control. But in comparison with the Diabetic Control, only Group 2 ($p<0.05$) showed a statistically significant increase.

TISSUE GSHPx ACTIVITIES

Alteration in tissue GSHPx activities are shown in Figure 3 and Table 1. No statistically significant differences were found in kidney tissue GSHPx activities between the groups. A statistically

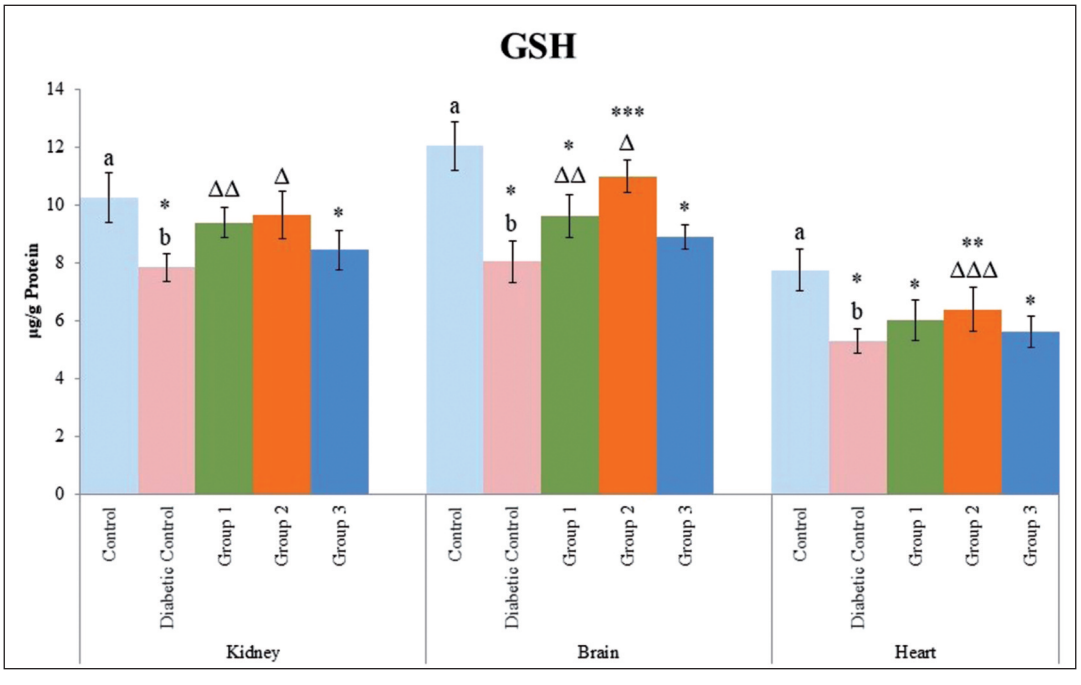

FIGURE 2: Tissue GSH levels of groups. *Indicate significant differences between control and experimental groups, different laters indicate significant differences between groups. **Control:** %0,9 NaCl; **Diabetic Control:** 50 mg/kg Streptozotocin; **Group 1:** 50 mg/kg Streptozotocin +100 mg/kg Metformin+4 IU/kg Insulin; **Group 2:** 50 mg/kg Streptozotocin+100 mg/kg Metformin+4 IU/kg Insulin+30 mg/kg Oleuropein; **Group 3:** 50 mg/kg Streptozotocin+30 mg/kg Oleuropein (a-*: $p<0.001$, a-**: $p<0.01$, a-***: $p<0.05$, b-$^{\Delta}$: $p<0.001$, b-$^{\Delta\Delta}$: $p<0.01$, b-$^{\Delta\Delta\Delta}$: $p<0,05$).

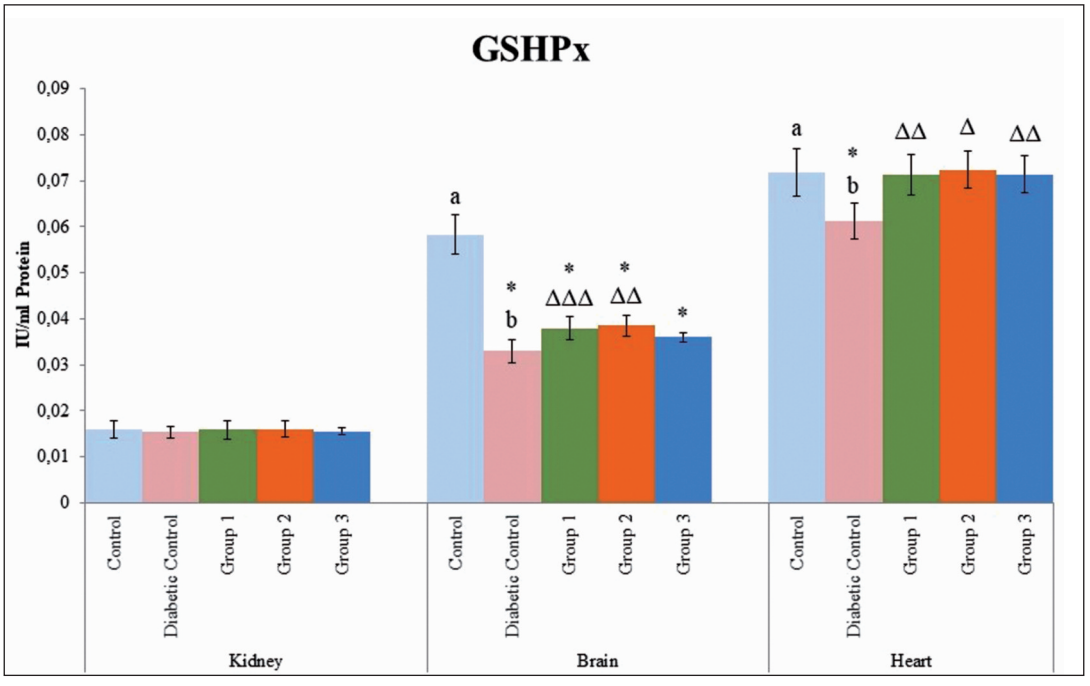

FIGURE 3: Tissue GSHPx activities of groups. *Indicate significant differences between control and experimental groups, different laters indicate significant differences between groups. **Control:** %0,9 NaCl; **Diabetic Control:** 50 mg/kg Streptozotocin; **Group 1:** 50 mg/kg Streptozotocin+100 mg/kg Metformin+4 IU/kg Insulin; **Group 2:** 50 mg/kg Streptozotocin +100 mg/kg Metformin + 4 IU/kg Insulin + 30 mg/kg Oleuropein; **Group 3:** 50 mg/kg Streptozotocin+30 mg/kg Oleuropein (a-*: p<0.001, a-**: p<0.01, a-***: p<0.05, b-$^{\Delta}$: p<0.001, b-$^{\Delta\Delta}$: p<0,01, b-$^{\Delta\Delta\Delta}$: p<0.05).

significant decrease was detected in brain tissue GSHPx activities of all groups (p<0.001) compared to the Control. A statistically significant increase was found in Group 1 (p<0.05) and Group 2 (p<0.01) compared to the Diabetic Control, but no difference was found in Group 3. There was no statistical difference in GSHPx activities of the hearth tissue between the Control and the experimental groups. Hearth tissue GSHPx activities of Group 1 (p<0.01), Group 2 (p<0.001) and Group 3 (p<0.01) were found to be high compared to the Diabetic Control.

TISSUE NO LEVELS

Changes in tissue NO levels are shown in Figure 4 and Table 1. It was observed that the NO levels of the kidney, brain and heart tissues were significantly increased in all groups when compared to the Control (p<0.001). The NO levels of Group 1 and 2 significantly decreased (p<0.001) in the kidney, brain and heart tissues but no change was detected in Group 3 as compared with the Diabetic Control.

DISCUSSION

It is known that oxidative stress plays a role in the pathogenesis and complications of diabetes. Increased oxidative stress parameters in the plasma cause chemical changes and insulin malformations. Therefore, administration of insulin replacement is essential for regulating blood glucose levels in type 2 diabetes, which has progressive beta cell dysfunction.[3] In addition, metformin contributes to normalization of diabetes-mediated damages. Moreover, phytochemical antioxidants help to protect from diabetic complications by decreasing oxidative stress. Phytochemicals with antioxidant effects have an important role in improvement of diabetes mellitus. Many studies show that antioxidant plants and their antioxidant components have positive effects on complications of diabetes. Al-Azzawie and Alhamdani demonstrate that oleuropein has a protective effect on diabetic complications associated with oxidative stress.[18] Besides, Huang et al. reported that oleuropein administration significantly decreased

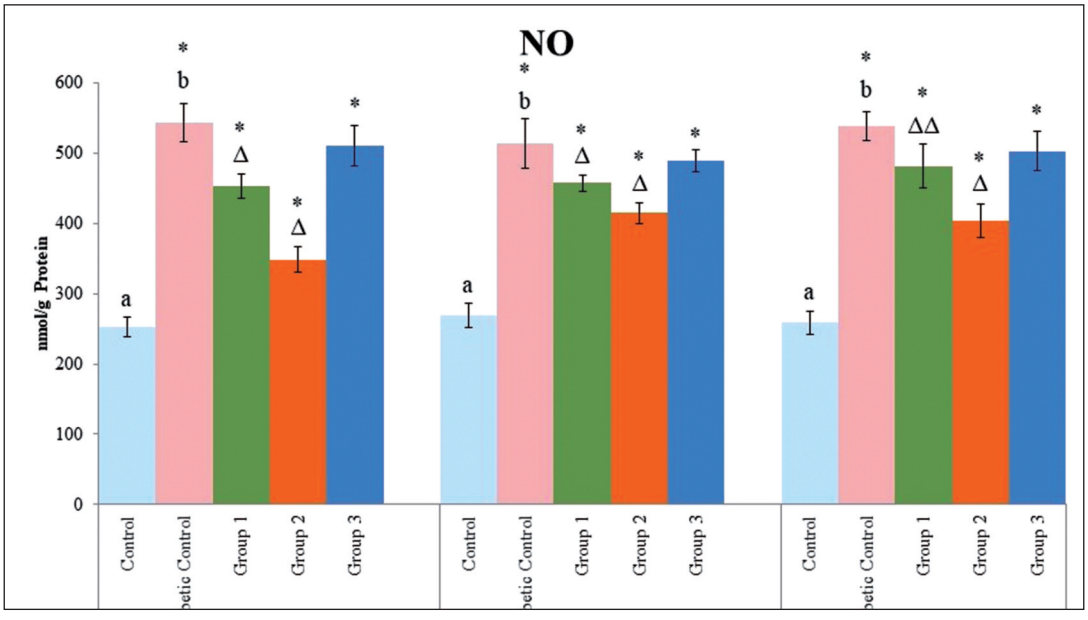

FIGURE 4: Tissue NO levels of groups. *Indicate significant differences between control and experimental groups, different laters indicate significant differences between groups. *:p<0,001, **Control:** %0,9 NaCl; **Diabetic Control:** 50 mg/kg Streptozotocin; **Group 1:** 50 mg/kg Streptozotocin+100 mg/kg Metformin+4 IU/kg Insulin; **Group 2:** 50 mg/kg Streptozotocin+100 mg/kg Metformin + 4 IU/kg Insulin + 30 mg/kg Oleuropein; **Group 3:** 50 mg/kg Streptozotocin+30 mg/kg Oleuropein (a-*: p<0.001, a-**: p<0.01, a-***: p<0.05, b-$^{\Delta}$: p<0.001, b-$^{\Delta\Delta}$: p<0.01, b-$^{\Delta\Delta\Delta}$: p<0.05).

the extent of diet-induced atherosclerosis in apo-E knockout mice.[12] Similarly, polyphenols of olive leaf inhibit in vitro platelet activation in non-smoking males.[33] Same researchers offer that the antiplatelet effects of olive leaves extract may be beneficial to protect from thrombosis and other cardiovascular diseases. In addition, several studies have showed that cardiovascular mortality rates decrease in metformin users through inhibition of glukoneogenetic patway in Type 2 diabetes.[34-36]

During diabetes, characteristic changes were shown mainly in the kidney leading to renal insufficiency or complete kidney failure. Increased blood glucose level can also alter renal structure and function. Data of Kakkar et al. indicate that oxidative stress may contribute to development of diabetic nephropathy.[2] Metformin has some renal benefits due to reducing glucose production in liver and enhances glucose uptake in tissues.[22] Besides, studies reported that metformin increases glutathione levels and the antioxidant enzyme activities in red blood cells and liver tissues and reduces some oxidative stress parameters in type 2 diabetes.[22] Also, oleuropein acts as a nephro-protective agent by reducing serum blood urea nitrogen (BUN) and creatinine levels, thereby reduces renal damage and protects kidney function.[9] Additionally, Geyikoğlu et al. demonstrated that oleuropein protects renal tissues against oxidative stress-mediated pathological findings, and suggested that oleuropein is a protector for serious renal damage.[37] In the present study, decreased glucose levels by administration of insulin and metformin indirectly decreased tissue oxidative stress, while oleuropein supplementation increased this effect.

The brain is a major organ in terms of high oxygen consumption, polyunsaturated fatty acids and transition metals content, and poor antioxidant defences. Because of these charecteristics, it is one of the most favorable targets for ROS, and mitochondrial dysfunction has been shown to be a typical phenomenon in the diabetic rat brain.[20] Correia et al. indicate that metformin may be an effective neuroprotective agent due to its oxidative stress suppressor role in the brain.[20] Olive phenols have also important protective effects against brain cerebral ischemia, brain damage after hypoxia-reoxygenation in diabetic rats.[6,38] Another study also indicated that polyphenolic compounds of

oleuropein have neuroprotective effect on healthy rat brain slices.[39] However, although the neuroprotective mechanism of olive oil is still unknown, it is thought to be due to its antioxidant properties and anti-inflammatory effects. In the present research, the antioxidant activity obtained by the use of only oleropein did not reduce the diabetic oxidative stress in tissues. Furthermore, use of only oleuropein in type 2 diabetics did not significantly alter antioxidant enzyme activities. However, the use of metformin and insulin in combination with oleuropein has been shown to have a stronger effect in suppressing lipid peroxidation and the protected antioxidants of the brain, hearth and kidney tissues.

Oxidative stress in diabetes mellitus is characterized with high production of nitric oxide (NO). This stuation also leads to irreversible endotelial, renal and neuronal damage via altered intracellular prostaglandin production.[40,41] Hence, there are observations that NO acts as a cause of cytotoxicity. Especially interactions between NO and oxygen-derived radicals and vascular oxidant stress is represented as common pathological mechanism for atherosclerosis, neuronal and renal failure.[25] However, a study by Mugge et al. has shown that superoxide dismutase (SOD) treatment partially restores impaired endothelium-dependent relaxation of aorta.[42] In addition, while endothelium-dependent vasodilation is substantially impaired in diabetics, pre-treatment with an antioxidant was shown to improve endothelial dysfunction in diabetic rats. Besides, NO plays a multifaceted and important role in the brain. Nitric oxide is also a neurotransmitter, a free radical and regulator of cerebral blood flow and inflammation. Alterations in cerebral NO levels have involved in pathophysiological events in brain. In addition, NO have been shown to mediate hypoxia-reperfusion injury in kidney. In our study, elevated tissue NO levels in rats with type 2 diabetes showed a marked improvement when oleuropein was added to treatment. In addition, it was observed that oleuropein alone could not change NO levels in tissues of type 2 diabetics.

In conclusion, the use of oleuropein alone does not influence oxidant-antioxidant balance of tissues, but the use of metformin and insulin as antidiabetic with oleuropein as an anti-oxidant and free radical scavenger following the rising oxidation in diabetes was supported and strengthened the tissue antioxidant system. It may be concluded that administration of metformin, insulin and oleuropein has a role to protect the tissues from oxidative stress in streptozotocin-diabetic rats.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

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