Effects of Estrogen Replacement Therapy with Vitamin E on Oxidative Stress in Hepatic and Pancreatic Tissues of Ovariectomized Diabetic Rats

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Abstract: We investigate the effects of 17β-estradiol (E_2) and vitamin E on oxidative stress of hepatic and pancreatic tissues in Ovariectomized (OVX) diabetic rats. Fortynine rats were equally divided into seven groups: Control; OVX; OVX+E_2; OVX+E_2+Vitamin E; OVX+Diabetic; OVX+Diabetic+E_2 and OVX+Diabetic+E_2+Vitamin E. The Glutathione Peroxidase (GSH-Px), Superoxide Dismutase (SOD), Catalase (CAT), Glutathione (GSH) and vitamin A and β-carotene in liver and pancreas were reduced when compared with control but Malondialdehide (MDA) was raised. E_2 and E_2+vitamin E supplementations to OVX rats increased GSH-Px, SOD, CAT, GSH, vitamin A and β-carotene in liver and pancreas but decreased MDA. MDA in liver and pancreas, Aspartate amino Transferase (AST), Alanine amino Transferase (ALT), Lactate Dehydrogenase (LDH), β-glutamyl Transferase (GGT) and Alkaline Phosphatase (ALP) in the serum of diabetic OVX rats were raised when compared with OVX but the activities antioxidant enzyme, GSH, vitamin A and β-carotene in liver and pancreas were lowered. The GSH-Px, SOD, CAT, GSH, vitamin A and β-carotene in liver and pancreas increased, although MDA in liver and pancreas and AST, ALT, LDH, GGT and ALP in serum decreased in diabetic OVX after E_2 and E_2+vitamin E supplementation.

Key words: Ovariectomy, estrogen, vitamin E, MDA, AST, ALT

INTRODUCTION

Diabetes during menopause involve an increased risk of various tissue complications (Saengsirisuwan et al., 2009; Yong et al., 2005) yet the benefit of estrogen treatment for various tissue complications is controversial (Moorty et al., 2005). Laboratory evidence supports the protective actions of E_2. E_2 and its derivates are strong endogenous anti-oxidants that reduce lipid peroxidation levels in brain and serum (Ulas and Cay, 2010a, b). The degree of lipid peroxidation is assessed in accordance to the MDA formation, a routinely used index of lipid peroxidation.

The level of MDA an end product of lipid peroxidation is found to significantly increase in the various tissues of diabetic (Hamden et al., 2008) and OVX rats (Yalin et al., 2006). Also, some researchers have demonstrated that the hyperglycemia-evoked oxidative stress plays a crucial role in the development of diabetic complications including hepatotoxicity which is thought to stem from augmented Reactive Oxygen Species (ROS) due probably to excessive generation of ROS and decreased antioxidant defences (Hamden et al., 2008). Oxidative stress may be increased in menopausal and diabetes owing to a hyperproduction of ROS such as O_2^-, $OH^·$ and H_2O_2 or a defiency in the antioxidant defence system. Impaired radical scavenger function has been linked to the decreased activity of enzymatic and non enzymatic scavengers (Ulas and Cay, 2010b). In particular, the action of SOD which converts O_2^- into H_2O_2 has been found to be decreased in the various tissues of diabetic (Hamden et al., 2009) and OVX rats (Yalin et al., 2006). Similarly, a reduction in the action of GSH-Px and CAT, enzymes involved in the detoxification of H_2O_2 has been observed (Yalin et al., 2006; Hamden et al., 2009; Ulas and Cay, 2010b). On the other hand, decreased GSH as the main component of endogeneous nonprotein sulfydryl pool is known to be a major low molecular weight scavenger of free radicals in the cytoplasm. Among the non-enzymatic scavengers, GSH, vitamins E and C, the main intra cellular antioxidant has been found to be decreased in diabetic (Nwose, 2009) and OVX rats (Vandana et al., 2006).

Vitamins such as vitamins E, A, C and β-carotene have major roles in lipid membrane protection. Determination of the numerous individual antioxidants is almost impossible. Recent studies from the laboratory also demonstrated that vitamins E, A, C and β-carotene can

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prevent ROS level in various cells (Vandana *et al.*, 2006). Strogens, like vitamin E possess ROS-scavenging chain-breaking antioxidant activity as hydrogen donors from their phenol-hydroxyl ring. Additionally, estrogens can induce antioxidant enzyme expression by stimulating the antioxidant defence system and inhibited the formation of lipid peroxides in plasma and tissues *in vitro* (Ulas and Cay, 2010a, b). In a recent study, Noh *et al.* (1999) observed that estrogen replacement in OVX rats produces a marked increase in the serum level vitamin E and a significant increase in the liver concentration of vitamin E. Against this background, we decided to use the lipophilic antioxidant vitamin E and the E_2 together as antioxidant protection for STZ-induced radical production in diabetic and OVX rats.

Given its importance, the specific research on MDA and enzymatic and non-enzymatic antioxidant levels in tissues of OVX diabetic rats are very limited. Furthermore, since the incidence of diabetes is higher in men than in female until female reaches menopausa (Naziroglu *et al.*, 2004), a potential protective role of E_2 and E_2+vitamin E against the development of liver complications in OVX diabetic rats offers an area of great research interest. Therefore, the purpose of the present study is to examine the influence of E_2 and E_2+vitamin E on parameters of oxidative stress and levels of enzymatic and non-enzymatic antioxidants in the pancreas and liver tissues of OVX diabetic rats.

MATERIALS AND METHODS

All chemicals were obtained from Sigma Chemical Inc. (St. Louis, MO) and all organic solvents from Merck Chemical Inc. (Germany) except vitamin E and E_2 (17β-estradiol). The injectable form vitamin E (Ephynal, β-tocopheryl acetate) was obtained from F. Hoffman La Roche (Istanbul, Turkey). The subcutan form E_2 was obtained from Sigma, St. Louis, MO). All reagents were analytical grade. All reagents except the phosphate buffers were prepared daily and stored at +4°C. The reagents were equilibrated at room temperature for half an hour before the analysis was initiated or reagents containers were refilled. Phosphate buffers are stable at +4°C for 1 month.

Animal model: Adult female Wistar-albino rats (10-12 weeks, weighing 180-250 g) purchased from the Firat University Animal Experiment Central. The rats were kept in an enveriomentally breeding room (temperature: 20-23°C, humidity: 55-60% and photoperiod: 12 h light dark cycles). All rats had free access to tap water and fasted overnight before blood and tissue collection. The

handling of the rats was approved by the local ethical committee for the care and use of laboratory animals.

Surgical procedures: Bilateral ovariectomy was performed to all groups except group 1. The rats were anaesthetized with ketamine (ketalar, Eczacibasi Pharmaceutical Co.) via intraperitoneal. A 10 mm incision was made in the lower part of the abdomen. The skin was separated from the underlying muscles, muscle fibers incised and a forceps placed at the boundary between the oviduct and the uterus. After removing the ovary and oviduct, the uterus was put back into the abdominal cavity and the incision was closed with sutures (Robertson *et al.*, 1984).

Induction of diabetes: Experimental diabetes was induced with a single intraperitoneal injection of 45 mg kg^{-1} Streptozotocin (STZ) dissolved in sodium citrate buffer (pH 4.5). Blood glucose levels were measured with a glucometer (Roche Diagnostic, Manheim, Germany) in all rats after 3 days of STZ treatment. The animals with a blood glucose level <200 mg dL^{-1} were excluded from the study.

Experimental design: About 49 rats were equally divided into seven groups (each animal placed separetely in the stainless-steel cage). The groups were formed as follows: Group 1 (control) consisted of normal rats; group 2 (OVX) Ovariectomized rats; group 3 (OVX+E_2); group 4 (OVX+E_2+vitamin E); group 5 (OVX+Diabetic); group 6 (OVX+Diabetic+E_2); group 7 (OVX+Diabetic+E_2+vitamin E). Thereafter, E_2 (subcutan at a dose of 40 µg kg^{-1}) and vitamin E (intraperitoneal at a dose of 100 mg kg^{-1}) was administered 15 days after ovariectomy and continued for 4 weeks. At the end of the treatment period (4 weeks), the rats were anaesthetized with ether for 5 min and blood samples were obtained by cardiac puncture into tubes without anticoagulation. The serum was prepared by centrifugation (1000 xg, 10 min +4°C). Pancreas and liver were removed, cleaned of fat and weighed; all these samples were harvested and stored at -20 until biochemical assays.

Biochemical analysis: The levels of AST, ALT, LDH, GGT and ALP in serum were determined by routine kits using an autoanalyzer (Olympus AU 600, Tokyo, Japan). The activities of GSH-Px, SOD, CAT and the levels of GSH, vitamin A, β-carotene, MDA in the liver and pancreas tissues were determined. MDA levels were measured with the thiobarbituric-acid reaction by the method of Placer *et al.* (1966). The quantification of TBARS was determined by comparing the absorption to

the standard curve of malondialdehyde equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3 tetra-methoxypropane. The values of TBARS were expressed as nmol g^{-1} tissue. Every sample was assayed in duplicate and the assay coefficients of variation for TBARS were <3%. CAT activities were determined by Goth which is based on the disappearance of H_2O_2 at 240 nm (Goth, 1991). SOD activities were determined according to the method of Sun *et al.* (1988). GSH-Px activities were measured spectrophotometrically at 37°C and 412 nm according to Lawrence and Burk (1976).

The GSH levels were measured at 412 nm using the method of Sedlak and Linsay (1968). The protein content in the liver and pancreas was measured by method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Vitamin A and β-carotene levels were determined by applying the method of Suziki and Katoh (1990).

Statistical analysis: Statistical analyses were performed on a personal computer with the use of SPSS software 15.0 (SPSS Inc. Chicago IL, USA). The results are expressed as mean±Standard Deviation (SD). Data were Analyzed using one-way Analysis of Variance (ANOVA) followed by LSD test, p<0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The activities of GSH-Px (p<0.01 and 0.05), SOD and CAT and the levels of GSH, vitamin A and β-carotene

in the liver and pancreas were significantly decreased (p<0.05) in OVX group compared to those in the control group but MDA level in liver and pancreas was elevated via ovariectomy (p<0.05) (Table 1 and 2). However, the activities of AST, ALT, LDH, GGT and ALP in serum were not changed significantly (Fig. 1-5). Furthermore, E_2 and E_2+Vitamin E supplementations to OVX rats have caused a significant increase (p<0.05) in the activities of GSH-Px, SOD and CAT and levels of GSH, vitamin A and β-carotene in liver and pancreas but a decrease (p<0.05) MDA levels in liver and pancreas (Table 1 and 2). Nonetheless, activities of AST, ALT, LDH, GGT and ALP in serum were not changed significantly (Fig. 1-5).

The MDA (p<0.05) levels of liver and pancreas and the activities of AST, ALT, LDH, GGT and ALP in the serum of OVX diabetic rats were found significantly higher (p<0.001) than those in OVX group (Table 1 and 2, Fig. 1-5). However, the activities of GSH-Px, SOD, CAT and the levels of GSH, vitamin A and β-carotene in liver and pancreas were significantly lower (p<0.05) in OVX diabetic rats when compared to OVX group. Furthermore, E_2 and E_2+vitamin E supplementations to OVX diabetic rats have caused a significant increase in the activities of GSH-Px (p<0.05), SOD (p<0.05) and CAT (p<0.05) and the levels of GSH (p<0.05), vitamin A (p<0.05 and 0.01) and β-carotene (p<0.05 and 0.01) in liver but a decrease in MDA levels (p<0.05 and p<0.001) in liver, respectively (Table 1). Similarly, there were significant increases in the activities of GSH-Px (p<0.05 and 0.001), SOD (p<0.01) and CAT (p<0.001) and the levels of GSH (p<0.05 and 0.001), vitamin A (p<0.05 and 0.001) and β-carotene

Table 1: Enzyme activities of GSH-Px (IU g^{-1} protein), SOD (U mg^{-1} protein) and CAT (U mg^{-1} protein) and GSH (nmol g^{-1} tissue), vitamin A (nmol g^{-1} tissue), β-carotene (nmol g^{-1} tissue) and MDA (nmol g^{-1} tissue) levels in the liver after 4 weeks of 17β-estradiol (E_2) and vitamin E (vit. E) administration in Ovariectomized (OVX) and Diabetic (DM) rats (mean±SD, n = 7)

Groups	GSH-Px	SOD	CAT	GSH	Vitamin A	β-carotene	MDA
Control	11.23±0.61	7.70±0.12	68.70±3.50	7.54±0.28	28.60±0.70	20.06±0.01	0.48±0.13
OVX	7.63±0.38[b]	6.56±0.15[a]	57.03±2.60[a]	6.28±0.04[a]	19.40±0.50[a]	12.20±0.02[a]	1.33±0.12[a]
OVX+E_2	9.43±0.19[c]	7.69±0.11[c]	67.01±1.20[c]	7.55±0.01[c]	25.00±0.10[c]	19.07±0.03[c]	0.45±0.11[c]
OVX+E_2+Vit. E	9.89±0.30[c]	7.71±0.13[c]	68.30±5.30[c]	7.53±0.13[c]	25.01±0.20[c]	19.10±0.04[c]	0.44±0.21[c]
OVX+DM	2.03±0.21[d]	2.14±0.21[d]	37.80±2.31[d]	2.44±0.01[d]	8.27±0.40[d]	7.07±0.04[d]	3.09±0.12[d]
OVX+DM+E_2	3.89±0.67[e]	3.06±0.17[e]	48.70±1.40[e]	3.78±0.17[e]	16.36±0.20[e]	18.16±0.04[e]	2.04±0.17[e]
OVX+DM+E_2+Vit. E	4.01±0.27[e]	3.55±0.23[e]	49.50±1.50[e]	3.98±0.04[e]	22.13±0.10[f]	20.80±0.08[f]	0.45±0.16[g]

[a]p<0.05, [b]p<0.01 as compared with control group; [c]p<0.05, [d]p<0.001 as compared with OVX group; [e]p<0.05, [f]p<0.01 and [g]p<0.001 as compared with OVX+DM group

Table 2: Enzyme activities of GSH-Px (IU g^{-1} protein), SOD (U mg^{-1} protein) and CAT (U mg^{-1} protein) and GSH (nmol g^{-1} tissue), vitamin A (vit. A, nmol g^{-1} tissue), β-carotene (nmol g^{-1} tissue) and MDA (nmol g^{-1} tissue) levels in the pancreas after 4 weeks of 17β-estradiol (E_2) and Vitamin E (Vit. E) administration in Ovariectomized (OVX) and Diabetic (DM) rats (mean±SD, n = 7)

Groups	GSH-Px	SOD	CAT	GSH	Vitamin A	β-carotene	MDA
Control	3.06±0.76	3.44±0.10	19.80±2.10	3.65±0.03	16.17±0.21	18.01±0.02	0.18±0.06
OVX	2.70±0.18[a]	2.14±0.12[a]	12.50±2.00[a]	2.98±0.02[a]	9.15±0.25[a]	10.90±0.01[a]	0.94±0.13[a]
OVX+E_2	3.01±0.10[b]	3.41±0.32[b]	18.20±4.30[b]	3.63±0.03[b]	16.00±0.20[b]	18.00±0.03[b]	0.17±0.04[b]
OVX+E_2+Vit.E	3.08±0.19[b]	3.44±0.21[b]	19.90±1.20[b]	3.66±0.12[b]	16.11±0.23[b]	18.10±0.04[b]	0.15±0.11[b]
OVX+DM	0.76±0.21[c]	0.65±0.28[c]	6.70±2.30[c]	0.86±0.01[c]	5.27±0.40[c]	6.07±0.04[c]	1.39±0.10[c]
OVX+DM+E_2	1.12±0.26[d]	1.98±0.11[e]	12.80±1.20[f]	1.52±0.21[d]	12.36±0.20[d]	14.16±0.04[d]	0.98±0.31[d]
OVX+DM+E_2+Vit.E	3.07±0.21[f]	2.01±0.13[e]	13.40±4.70[f]	3.60±0.02[f]	16.13±0.10[f]	15.80±0.08[e]	0.10±0.15[f]

[a]p<0.05 as compared with control; [b]p<0.05, [c]p<0.001 as compared with OVX group; [d]p<0.05, [e]p<0.01, [f]p<0.001 as compared with OVX+DM group
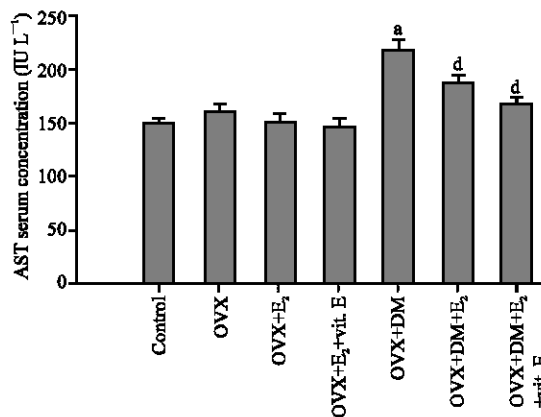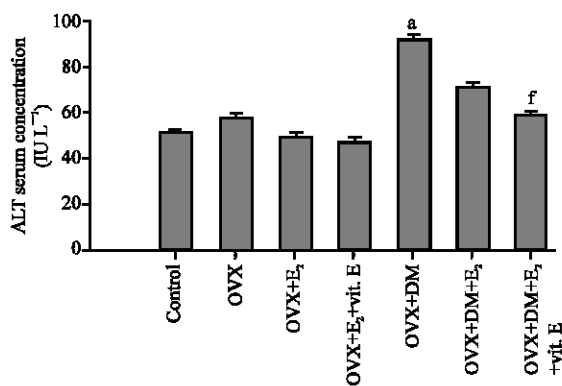
Fig. 1: Changes in serum AST concentrations in OVX Diabetic (DM) rats treated 17β-estradiol (E₂) alone and with the addition of vitamin E for 4 weeks (mean±SD, n = 7), ᵃp<0.001 as compared with group OVX, ᵈp<0.05 as compared with group OVX+DM

Fig. 2: Changes in serum ALT concentrations in OVX Diabetic (DM) rats treated 17β-estradiol (E₂) alone and with the addition of vitamins E for 4 weeks (mean±SD, n = 7), ᵃp<0.001 as compared with group OVX, ᵈp<0.05 and ᶠp<0.01 as compared with group OVX+DM

Fig. 3: Changes in serum LDH concentrations in OVX Diabetic (DM) rats treated 17β-estradiol (E₂) alone and with the addition of vitamins E for 4 weeks (mean±SD, n = 7), ᵃp<0.001 as compared with group OVX, ᵈp<0.05 and ᵉp<0.001 as compared with group OVX+DM

Fig. 4: Changes in serum GGT concentrations in OVX Diabetic (DM) rats treated 17β-estradiol (E₂) alone and with the addition of vitamins E for 4 weeks (mean±SD, n = 7), ᵃp<0.001 as compared with group OVX, ᵈp<0.05 as compared with group OVX+DM

($p<0.05$, $p<0.01$) but a decrease in MDA levels in pancreases (Table 2) and decreases in the levels of AST ($p<0.05$), ALT ($p<0.05$ and 0.001), LDH ($p<0.05$ and $p<0.001$), GGT ($p<0.05$) and ALP ($p<0.05$) in the serum of E_2 and E_2+Vitamin E supplemented OVX diabetic rats (Fig. 1-5).

Peroxidation of lipids can dramatically change the properties of biological membranes and may lead to severe cell damage. Especially, free radicals, generated mainly by liver and pancreas cells are thought to cause tissue injury by initiating lipid peroxidation and irreversibly modifying membrane structure (Xu *et al.*, 2002; Yalin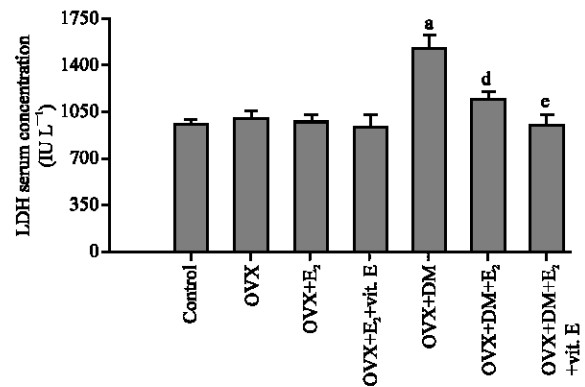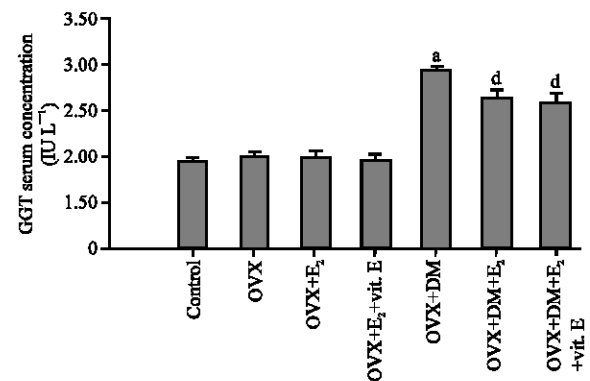 *et al.*, 2006). Menopause is associated with enhanced generation of free radicals which lead to multiple organ dysfunctions (Moorty *et al.*, 2005). Additionally, in menopause, some special features emerge including an increase in level of MDA and a decrease in the content of antioxidants vitamins concentrations and enzyme activities. Some studies indicate that the natural or surgical loss of estrogen may contribute to an increased risk of metabolic disease after menopause (Unfer *et al.*, 2006). The antioxidant effect of estrogens has been regarded as the main mechanism in protecting liver and pancreas tissues from oxidative damage (Hamden *et al.*, 2008, 2009). Furthermore, estrogens are shown to induce antioxidant enzyme expression by stimulating the antioxidant defence system in these
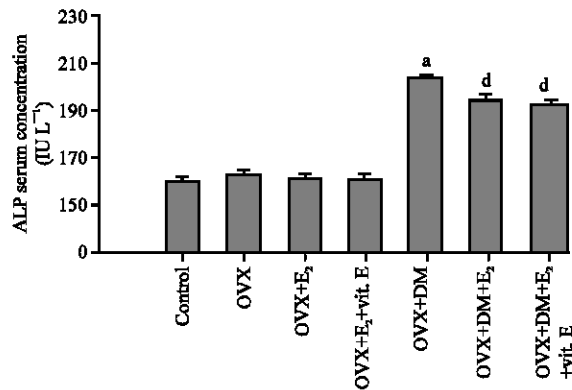
Fig. 5: Changes in serum ALP concentrations in OVX Diabetic (DM) rats treated 17β-estradiol (E$_2$) alone and with the addition of vitamins E for 4 weeks (mean±SD, n = 7), [a]p<0.001 as compared with group OVX, [d]p<0.05 as compared with group OVX+DM

tissues (Hamden *et al.*, 2008). From the findings of the present study, it is observed that the level of MDA as a marker oxidative damage, increased in liver and pancreas tissues due to ovariectomy.

These increases were much greater after diabetes in addition to ovariectomy. Administration of E$_2$ and E$_2$+vitamin E after ovariectomy led to a prevention of oxidative damage. Removing estrogen from the body caused reduction in the activities of GSH-Px, SOD, CAT and GSH, vitamin A and β-carotene levels (Table 1 and 2). However, other cell damage markers (AST, ALT, LDH, GGT and ALP activities) did not show any change (Fig. 1-5). On the other hand, treatment of ovariectomy rats with E$_2$ and E$_2$+vitamin E for 28 consecutive days could restore the activities of the above enzymes to their normal levels.

These results are consistent with the previous studies that have been directed towards the influence ovariectomy on the levels of MDA (Ulas and Cay, 2010a, b). However, Ulas and Cay (2010b) reported that E$_2$ or vitamin E were effective in preventing or ameliorating of MDA levels in various tissues of ovariectomized rats. Also, Naziroglu *et al.* (2004) found that reduction of MDA levels in plasma of menopausal women is due to hormone replacement therapy and vitamins E and C treatments. Ovariectomy-specific decreases in certain antioxidants in liver were reported, e.g., GSH level by Borras *et al.* (2003), GSH-Px activity by Cay *et al.* (2001) and SOD and CAT activities by Yalin *et al.* (2006). Diabetes is widely accepted to induce metabolic derangement leading to oxidant-antioxidant imbalance (Cay *et al.*, 2001). The hyperglicemia produced by STZ leads to the over-

production of free radicals and the inactivation of the antioxidant enzymes by the non-enzymatic glycation of proteins and exerts deleterious effects on the function of pancreas and liver cells (Jin *et al.*, 2008). This hyperglycemia is accompanied with the increase in marked oxidative impact as evidenced by the significant increase in pancreatic and hepatic MDA levels and liver damage markers (AST, ALT, LDH, GGT and ALP) (Duzguner and Kaya, 2007; Inoue *et al.*, 2008) and decrease in pancreatic and hepatic antioxidants including GSH-Px, SOD, CAT activities and GSH, vitamin A and β-carotene levels (El-Demerdash *et al.*, 2005; Ulas and Cay, 2010b).

In this study, AST, ALT, LDH, GGT and ALP activities were increased in serum of OVX diabetic rats compared to the OVX rats (Fig. 1-5). However, GSH-Px, SOD, CAT activities and GSH, vitamin A and β-carotene levels were decreased in liver and pancreas of OVX diabetic rats compared to the OVX rats (Table 1 and 2). Supporting our finding, it has been found that liver was necrotized in diabetic rats Duzguner and Kaya (2007). Thus, increment of the activities of AST, ALT, LDH, GGT and ALP in serum is mainly due to leakage of these enzymes from the liver cystocele into the blood stream (Mansour *et al.*, 2002). These results indicated that diabetes could lead to pancreatic and hepatic dysfunctions.

However, treatment of OVX diabetic rats with any of the E$_2$ and E$_2$+vitamin E caused reduction in the activity of these enzymes in serum but were increased enzymatic and non enzymatic antioxidant enzymes in liver and pancreas compared to mean values of both OVX and OVX+diabetic groups. These results are in agreement with the finding of Al-Shamsi *et al.* (2006), Cay and Naziroglu (1999) and Hamden *et al.* (2009), suggesting that treatment of the diabetic rats with either vitamin E or E$_2$ lowers the liver and pancreas damage markers in serum.

Steroid hormones, especially estriol and E$_2$ are natural antioxidants. E$_2$ is a fat-soluble that can contribute to membrane fluidity by direct interactions with phospholipids. It has been suggested that E$_2$, due to the presence of the hydroxyl group on the phenolic, a ring as in vitamin E can suppress free radical induced peroxidation chain reactions (Ulas and Cay, 2010a, b). E$_2$ was also shown to attenuate the peroxidation of vascular smooth muscle cells and peroxidation induced growth and migration. Thus, it appears that E$_2$ help to prevent or stop free radical damaging cascades to aid maintaining cellular integrity in variety of tissues including the liver and pancreas (Liu *et al.*, 2002; Lio *et al.*, 2004; Hamden *et al.*, 2009). Moreover, diabetes or ovariectomy induced reduction in antioxidant levels in both tissues is amplified by the administration of E$_2$ (Hamden *et al.*, 2008; Ulas and

Cay, 2010a, b). The results show that administration of E_2 significantly inhibits MDA production and cellular injury which protects the pancreas and the liver against free radical induced oxidative damage. In support of this finding, it was recently shown that E_2 suppresses lipid peroxidation, apoptosis, necrosis and fibrosis and induces antioxidant enzyme production in pancreatic and hepatic tissues of rats (Liu *et al.*, 2002).

Vitamins E, A, C and β-carotene are seem to protect the body cells against hepatic and pancreatic diseases, perhaps by their antioxidant properties (Wenger *et al.*, 2001; Seven *et al.*, 2004; Hamden *et al.*, 2009). One of the most important natural lipophilic antioxidants is α-tocopherol. α-Tocopherol has the highest biological activity and is the most widely available form of vitamin E in food. The other isomers (β, γ, δ) some of which are more abundant in a typical western diet are less biologically active than α-tocopherol (Muis *et al.*, 2004). Administration of vitamin E increases the plasma levels of vitamin A and β-carotene by their synergetic interaction. The available evidence indicates that vitamin A and β-carotene metabolism have been linked to vitamin E as an antioxidant in the stability of biological membranes their protective role against some diseases in humans and animals is not completely understood though (Kokcam and Naziroglu, 2002).

In addition, there is a relationship between oestrogens and vitamin E as a natural antioxidant *in vivo*. On the other hand, the antioxidant effect of E_2 on liver microsomal membranes has been well demonstrated. Under vitro conditions, estradiol regenerates oxidized tocopherol radicals by donating a hydrogen atom of the OH group at aromatic ring (Ulas and Cay, 2010a, b). The present study also shows that enzymatic antioxidants and β-carotene and vitamin A levels both in OVX diabetic and OVX rats might be increased by vitamin $E+E_2$ administration as result of its role action radical inhibition. This result may be a good indicator of the protective role of vitamin E in the menopausal diabetic female. One study has reported increased oxidative stress and lower concentrations of antioxidants in the patients with type I diabetes (Marra *et al.*, 2002). In the study, the enzymatic and non enzymatic antioxidants concentrations (GSH-Px, SOD and CAT activities and GSH, vitamin A and beta-carotene levels) in liver were found lower in the diabetic OVX rats with diabetic hepatopathy than in the OVX rats enhancement of oxidative stress (Table 1 and 2). The antioxidant enzymes GSH-Px, SOD and CAT activities are known to be inhibited in diabetes mellitus because of non-enzymatic glicosylation and oxidation. Additionally, the positive effect of administration of vitamin E on these enzymes observed in the present study could be explained by two possible mechanisms. First, the antioxidative effect of vitamin E may prevent further glycosylation and peroxidation of proteins by interacting with free radicals and therefore, minimizing their harmful effects. Second, vitamin E may induce the protein synthesis of antioxidant enzymes which explains the increased activity after administration of the vitamin E. In support of this phenomenon are the observations by Pawlaska-Goral *et al.* (2002) and Borras *et al.* (2005) who found that polyphenolic substances such as estrogens, flavonoids and vitamins increased the activities of GSH-Px and SOD enzymes. From the administration of the E_2 and E_2+vitamin E in diabetic OVX rats, we observed a significant restoration in the levels GSH-Px, SOD and CAT activities (Table 1 and 2).

CONCLUSION

In this study, we have determined that both enzymatic and non enzymatic antioxidants in the liver and pancreas tissues were decreased both in OVX and diabetic OVX rats. These alterations may contribute to a lower cellular scavenging capacity and hence, an increase of MDA levels as shown by the increase of lipid peroxidation. However, administration of E_2 and E_2+vitamin E, both in OVX and diabetic OVX rats, appears to have led to significant improvement on antioxidant concentrations. Hence, the findings clearly show the modulator role of E_2 and E_2+vitamin E in the control of enzymes responsible for the protection of rat liver and pancreas cells against oxidative damage caused by lipid peroxidation.

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