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Acute hyperglycemia causes oxidative stress which is prevented by vitamin E pretreatment in healthy rabbits

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ABSTRACT: Although there are many studies showing chronic hyperglycemia in diabetes increases oxidative stress, those examining the influence of acute glucose increase are limited. The aim of this study was to investigate the effect of acute hyperglycemia on oxidative stress markers in non-treated and vitamin E pretreated healthy rabbits. Acute hyperglycemia was induced by oral glucose administration (1g/kg). Before and at different time points up to 180 minutes following the administration, blood samples were collected to measure malondialdehyde and glutathione spectrophotometrically as oxidative stress and antioxidant capacity markers respectively. Same markers were also determined in kidney and liver homogenates. There was no difference in glucose tolerance between non-treated and vitamin E pre-treated groups (p=0.10). Plasma malondialdehyde levels were increased after glucose administration in both groups. There was a positive-correlation between blood glucose and plasma malondialdehyde in non-treated (r=+0.447; p=0.008) but not in vitamin E pre-treated group (r=0.076; p=0.361). Plasma glutathione levels were higher in vitamin E pre-treated group at all-time points compared to non-treated group (p<0.05). There was a negative-correlation between blood glucose and plasma glutathione levels in non-treated (r=- 0.357; p=0.033) but not in vitamin E pre-treated group (r=0.007; p=0.485). According to our results acute hyperglycemia provokes oxidative stress which is partially prevented by vitamin E pretreatment.

KEYWORDS: Blood glucose; diabetes; hyperglycemia; malondialdehyde; glutathione.

1. INTRODUCTION

Oxidative stress is one of the known deleterious consequences of hyperglycemia which is a serious complication of both type 1 and type 2 diabetes. Increased oxidative stress and impaired antioxidant mechanisms are shown in diabetes mellitus in various studies [1-3]. Furthermore, amelioration of oxidative stress by pharmacological treatment of diabetes has been attributed to reduction of hyperglycemia [1].

Oxidative stress occurs as a result of disturbed balance between production of reactive oxygen species (ROS) and antioxidant protective mechanisms in favor of the ROS and is now widely believed to be involved in the pathogenesis of major age-related diseases such as neurodegenerative diseases, cancer and a long list of several diseases- ischemia-reperfusion injuries, stroke, hypertension, rheumatic diseases, multiple sclerosis and diabetes. It is also known that the increasing oxidative stress lead to important damages in the vital organs such as kidney and liver [4, 5].

Although there are many studies showing that chronic hyperglycemia in diabetes enhances oxidative stress, those examining the effects of acute glucose increase are limited. In preclinical studies, it was shown that circulating oxidative stress, measured as the concentration of 8-isoprostaglandin and malondialdehyde (MDA), increased in rats due to acute fluctuation of blood glucose concentration [6]. Studies showed that hyperglycemia in rats not only caused hepatic oxidative stress and antioxidant depletion [7] but also enhanced superoxide and MDA accumulation in heart [8]. In another study it was shown that acute hyperglycemia induced by single injection of 20% dextrose increased plasma MDA in mice [9].

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There are few studies in humans confirming that acute hyperglycemia induces oxidative stress. A study conducted in healthy people as well as in diabetics showed that during oral glucose tolerance test (OGTT) some of the most important biological antioxidants in plasma and total plasma radical-trapping activity were significantly decreased [10]. In blood samples collected during hyperglycemia crisis of diabetic patients, superoxide dismutase (SOD) and total antioxidant capacity were found lower while levels of MDA were higher than those of the control patients [11]. After a period of hyperglycemic clamp in type 1 diabetic patients, plasma oxidative stress measured as nitrotyrosine and plasma 8-iso prostaglandin F2 alpha levels were significantly increased compared with basal values [12]. In another study oxidative stress measured as plasma 3-nitrotyrosine concentrations was found increased after 75 g glucose administration in obese participants [13].

There are many clinical studies investigating whether vitamin use has beneficial effects on antioxidant status in diabetes [14]. These comprehensive clinical studies in diabetes have shown useful effects of vitamins B, C, D and E on oxidative stress variables such as glutathione peroxidase, MDA, thiobarbituric acid reactive substances (TBARS), SOD or on total antioxidant capacity [14]. Among them vitamin E was found as the most effective vitamin and its consumption was found beneficial for health by enhancing plasma antioxidant capacity and by reducing MDA and TBARS levels [14].

There are numerous studies showing that chronic hyperglycemia in diabetes enhances oxidative stress which may cause diabetic complications. Studies examining the effects of acute glucose increase are a few and limited to the studies mentioned above. In the present work we aimed to examine the effect of OGTT-induced acute hyperglycemia on oxidative stress. Furthermore, we aimed to reveal whether pretreatment with vitamin E would have any impact on oxidative stress induced by acute hyperglycemia in heathy rabbits.

2. RESULTS

At baseline, blood glucose levels of rabbits were not different in non-treated and vitamin E pre-treated groups (Figure 1A). Blood glucose levels started to increase after oral glucose administration and reached a peak at 60th minute in both groups. There were no differences between non-treated and vitamin E treated groups in the time-blood glucose lines (Figure 1A). Glucose tolerance of the two groups after OGTT as presented by area under curve (AUC; 0-120 minutes) were not different (Figure 1B).

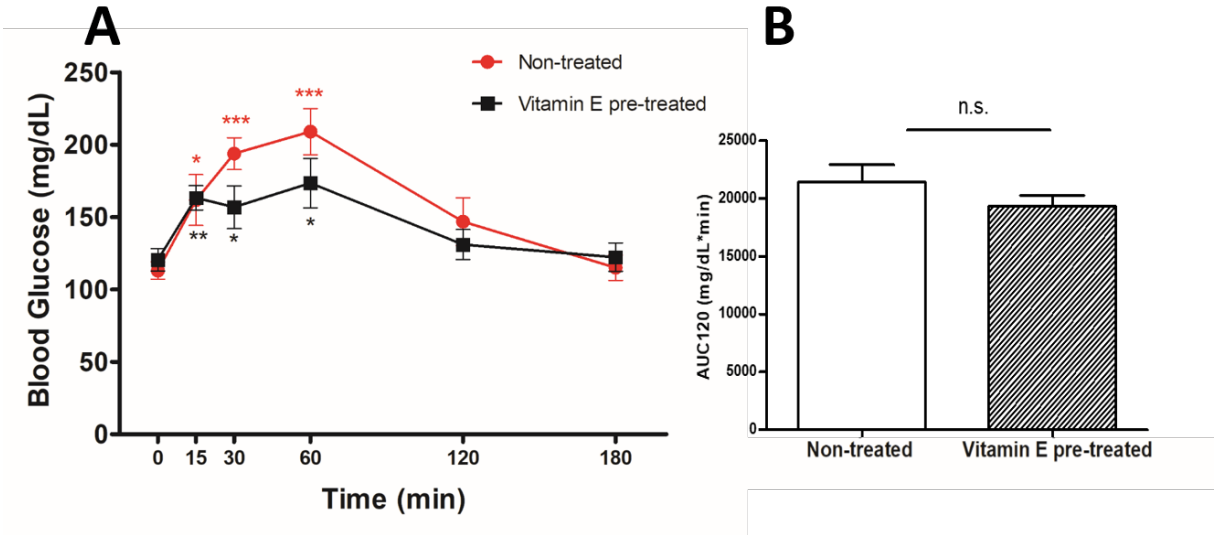

Figure 1. Plasma glucose concentrations following 1 g/kg glucose administration by gavage (A) and area under curve (AUC) of two groups (B). Two-way ANOVA and post hoc Bonferonni test for glucose concentrations and Student's t test for AUC comparison were used and n=6 for each group. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs 0th min of related group. No significance between non-treated vs time matched vitamin E pre-treated group. AUC was calculated by Graphpad Prism5 software (n.s.: not significant).

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Similarly, MDA levels in non-treated group were found increased almost at the same time points (Figure 2A). However, while there was not an apparent increase in MDA levels in vitamin E pre-treated group during the first 30 minutes, a clear rise was seen at 60th minute. Vitamin E pre-treatment provided significant reduction at the 30th and 120th minutes compared to non-treated group. The Pearson correlation analysis showed a significant positive correlation between the MDA and blood glucose levels of non-treated group (r=0.447, P=0.008) but not of vitamin E pre-treated group (r=0.076, P=0.361) (Figure 2B).

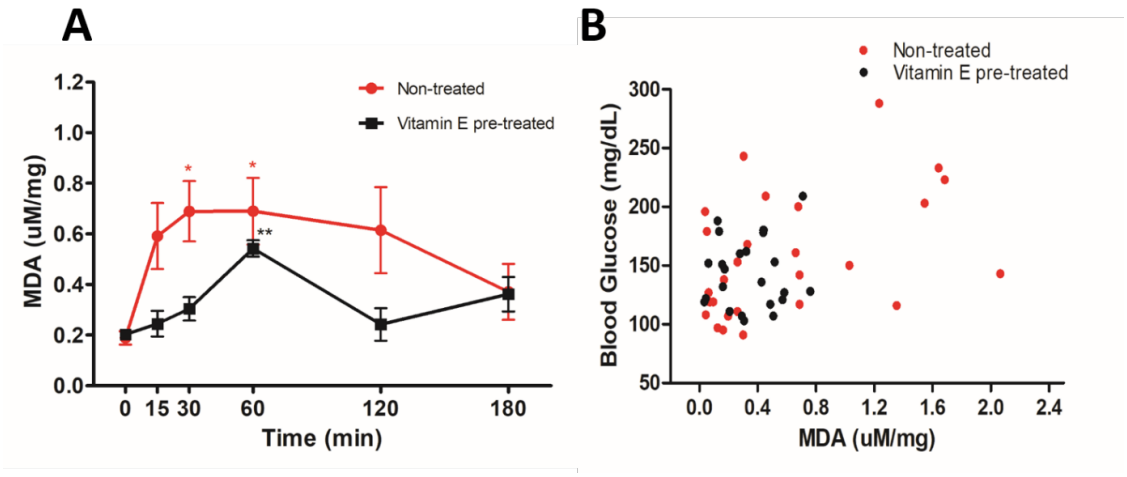

Figure 2. Malondialdehyde (MDA) concentrations (uM/mg) in healthy rabbits following 1 g/kg glucose administration (A). Two-way ANOVA post hoc Bonferroni test was used and n=6 for each group. *; p<0.05; ** p<0.01; vs 0th min of related group, #; p<0.05 vs time matched non-treated group. The correlation between blood glucose levels and MDA concentrations (n=6) (B). For non-treated group Pearson's r=0.447; for vitamin E pre-treatment group r=0.076.

Glutathione (GSH) as an antioxidant marker was measured in plasmas of rabbits at the same time points after OGTT. GSH levels were higher in vitamin E pre-treated group compared to non-treated group after OGTT at every time point (Figure 3A). The Pearson correlation analysis showed a significant negative correlation between the GSH and blood glucose levels of non-treated group (r= -0.357, P= 0.033) but not of vitamin E pre-treated group (r= 0.007, P= 0.485) (Figure 3B).

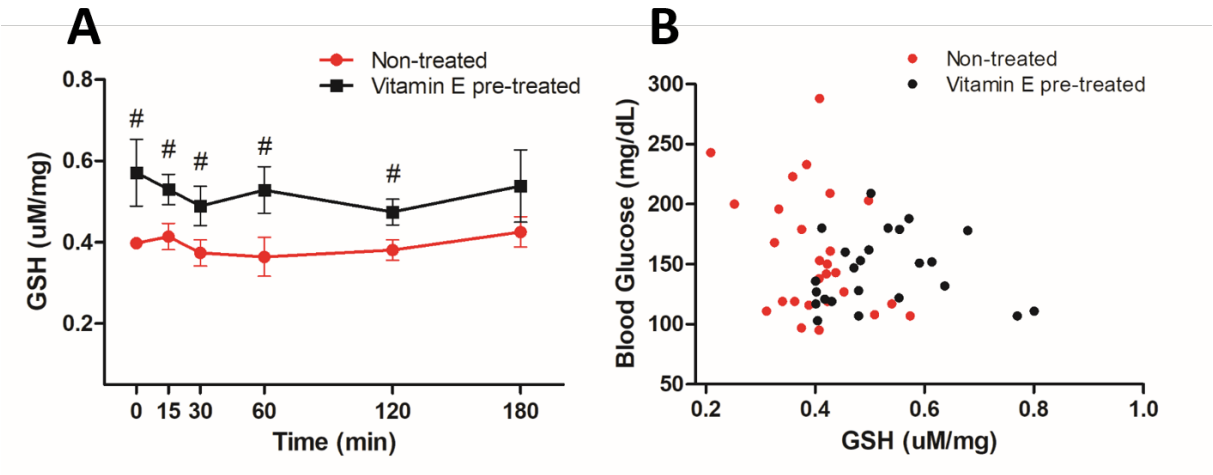

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Figure 3. Glutathion (GSH) concentrations (uM/mg) in healthy rabbits following 1 g/kg glucose administration (A) Two-way ANOVA post hoc Bonferroni test was used and n=6 for each group. # $p<0.05$ vs time matched non-treated group. The correlation between blood glucose levels and GSH concentrations, (n=6) (B) For non-treated group Pearson's r= -0.357; for vitamin E pre-treatment group r= 0.007.

3: Rabbits were sacrificed at the 180th minute after OGTT and livers and kidneys were isolated. MDA levels in liver of non-treated rabbits was found increased compared to control group but this increase was not significantly different. The reduction that was provided by vitamin E pre-treatment was also not statistically significant. On the other side, MDA levels in kidney of non-treated rabbits were found increased compared to control. Although vitamin E pre-treatment slightly decreased the MDA levels, it was not statistically significant (Figure 4A, and 4B).

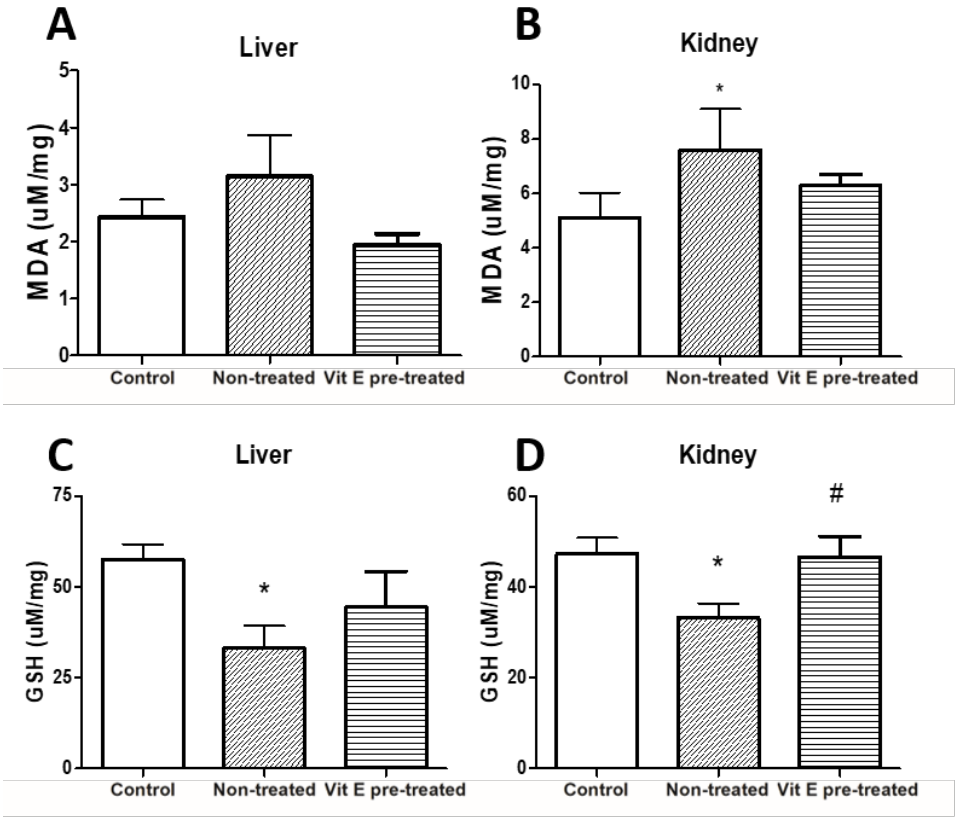

Figure 4. Malondialdehyde (MDA) levels in liver (A) and kidney (B) of rabbits which were sacrificed at the 180th minute of oral glucose tolerance test (n=6 for each group). Glutathione (GSH) levels in liver (C) and kidney (D) of rabbits which were sacrificed at the 180th minute of oral glucose tolerance test. One-way ANOVA post hoc Dunnet's test was used and n=6 for each group. *; $p<0.05$; vs control group, #; $p<0.05$ vs. non-treated group.

The GSH levels were significantly lower in non-treated group compared to control group both in liver and kidney. GSH levels of vitamin E pre-treated group were found significantly higher than non-treated group in kidneys but not in livers of the rabbits (Figure 4C, and 4D)

3. DISCUSSION

Diabetes mellitus is characterized by hyperglycemia which has been proposed to be the reason for increased oxidative stress in diabetic patients. While many studies have demonstrated that chronic hyperglycemia in diabetes results in increased oxidative stress, there are a few examining the effects of acute condition on oxidative stress [6-9]. In the present study we examined the effect of acute hyperglycemia induced by oral glucose administration on oxidative stress and on antioxidant capacity

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measured as MDA and GSH levels respectively in non-treated and vitamin E pre-treated healthy rabbits.

In order to generate acute hyperglycemia, we used oral glucose tolerance test (OGTT). After oral glucose administration, blood glucose levels were measured at different time points up to three hours in non-treated and vitamin E pre-treated rabbits. Although a slight reduction occurred in vitamin E group, it failed to reach to statistical significance at any time point. Glucose tolerance of the two groups as calculated by area under curve were not different showing that vitamin E pre-treatment did not necessarily change the extent of glucose tolerance. In support of this, there are reports demonstrating that vitamin E failed to change insulin and blood glucose levels in diabetic and healthy individuals [15, 16]. On the contrary, in a study performed on diabetic subjects, the ability of chronically administered vitamin E in lowering blood sugar has been demonstrated [17]. This discrepancy could be due to the shorter treatment period with vitamin E and/or to the use of normoglycemic healthy rabbits in our experiments. Of course, a possible effect of vitamin E on secretion or on action of insulin should not be excluded. There are some publications showing that vitamin E alters insulin levels, especially under diabetic conditions [18, 19]. However, studies presenting a lack of relation between insulin level and vitamin E treatment in either diabetic or healthy subjects also exist in the literature [15, 16, 20, 21]. Consistently, failure of vitamin E to change the magnitude of AUC obtained after OGTT, leads us to suggest that influence of vitamin E on insulin resistance is of minor importance under our experimental conditions. Taken together, we think that the change in oxidative stress parameters induced by vitamin E is not because of a reduction in blood glucose level, but rather because of attenuation of oxidative stress status.

After oral glucose administration, blood glucose levels started to increase rapidly with a peak at 60th minute in both groups. Plasma MDA levels in non-treated animals displayed a pattern similar to blood glucose, suggesting that acute hyperglycemia might have led to an increase in oxidative stress. On the other hand, the less increased amount of MDA in vitamin E pre-treated group with respect to non-treated ones may indicate the preventive effect of vitamin E on blood sugar-induced elevation of plasma MDA. A marked increase was seen at 60th minute in vitamin E pre-treated group which was the same time point of the highest blood glucose level. This may be due to the limited protective capacity of vitamin E against a sharp rise in blood glucose. The significant positive correlation between MDA and blood glucose levels seen in non-treated group but not in vitamin E pre-treated group supports the idea that acutely elevated blood glucose levels has the potential to induce oxidative stress whereas vitamin E pre-treatment acts as a protective mechanism against it.

As an antioxidant marker we measured GSH in plasmas of rabbits at the same time points following OGTT. GSH levels did not change at any time compared to starting point both in non-treated and vitamin E pre-treated groups suggesting that plasma GSH levels are not substantially influenced from acute blood glucose increments. However, the insignificant decreasing trend in GSH levels which exhibited a weak inverse correlation with the increase in blood glucose that was not observed in vitamin E pre-treated group, may indicate to the protective action of vitamin E. Interestingly, in OGTT; GSH levels in vitamin E pre-treated group were higher compared to non-treated group at each time point. Assuming that GSH levels measured at 0 min represent the effect of vitamin E by itself (prior to glucose administration), it seems likely that vitamin E pre-treatment might have set the basal levels of GSH up to a higher degree. This supports the view that vitamin E is an important agent for strengthening the antioxidant capacity of the body [22].

At the 180th minute following oral glucose administration liver and kidney tissues of OGTT groups and also control group were isolated to measure MDA and GSH levels. The changes of MDA in the liver of rabbits were not different between the groups. However, the MDA levels were significantly higher in the kidneys of glucose treated group. The vitamin E pre-treatment reduced the MDA even though it was not significant. It is known that kidney is among the most intensively affected organs from hyperglycemia and it was suggested that this damage is associated with increased oxidative stress [23]. Even if blood MDA level returns to normal, the damage may take longer time in the kidney to decline. Hence, acute renal failure was found to be associated with increased MDA levels in the kidney [24] more dramatically [25]. GSH levels in liver and kidney of non-treated group were found significantly lower than control group. However, GSH levels in vitamin E pre-treated rabbits were higher especially in kidney compared to non-treated rabbits which may be attributed to protective effects of vitamin E pre-treatment in tissues as well.

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Oxidative stress induced by hyperglycemia has an important role in the development of diabetic complications. To date, the studies of chronic hyperglycemia on oxidative stress have been underlined whereas the damage of acute hyperglycemia is neglected. To our knowledge, our study provides the first in vivo evidence that even acute hyperglycemia induces oxidative stress measured as plasma MDA that is prevented by vitamin E pretreatment in healthy rabbits.

Even though some limitations such as the small sample size, our study points out a significant positive correlation between blood glucose levels and oxidative stress. Therefore, a suggestion for diabetic patients to avoid acute hyperglycemia by controlling their diet and by adhering to their treatment regimen to minimize glucose rises would be useful.

The oxidative stress may develop in those individuals with impaired antioxidant defence. Supporting, our study demonstrated the protective effect of vitamin E on oxidative stress. In order to strengthen antioxidant defense; adding vitamins -especially vitamin E- to diabetes treatment regimen can be considered. A part from the diabetic patients, vitamin E pretreatment may also be considered for the healthy individuals who will be subjected to OGTT. It should be kept in mind that it is important to avoid acute hyperglycemia as well as chronic in order to avoid oxidative stress.

4. CONCLUSION

Our study provides the first in vivo evidence that acute hyperglycemia induces oxidative stress that is prevented by vitamin E pretreatment in healthy condition. The positive correlation between increased glucose level and MDA; negative correlation with GSH supported our hypothesis that hyperglycemia increases oxidative stress. Vitamin E pretreatment has protective impact on this acute hyperglycemia induced oxidative stress.

5. MATERIALS AND METHODS

5.1 Animals

New Zealand adult white rabbits (3.00-3.50 kg) from both sexes were used. The rabbits were housed in a constant temperature-room (22 ± 1 °C) with 12h/12h light/dark cycle and fed with standart pellet diet. Animals were acclimatized for a week before experiments. Eighteen rabbits were used for the experiments. All experiments were approved by Kobay D.H.A A. Ş. Animal Experimentations Local Ethics Board (2019-249). All the procedures with animals were performed according the rules of "Guide for the Care and Use of Laboratory Animals".

5.2 Plasma and Tissue Collection

Approximately 2 ml volume of blood from ear artery of rabbits was taken into heparinized tubes before (zero point) and at 15th, 30th, 60th, 120th and 180th minutes after oral administrations of glucose. The tubes were centrifuged for 10 min at 2000 g. Plasmas were separated, aliquoted and stored at -80 ° C for the analysis of MDA and GSH as oxidative stress and antioxidant capacity markers respectively. There are various parameters to measure oxidative stress and antioxidant situation. MDA and GSH parameters were chosen considering that they are the most measured markers in literature which makes them comparable, also they were suggested as standard measures before [14]. The same markers were also assessed in livers and kidneys of the rabbits. Control group was administered distilled water orally and sacrificed at the same time point (180th minute) with OGTT groups. For the assessments in tissues, control group was created in order to clarify the basal levels of MDA and GSH without any hyperglycemia induction. These basal levels of MDA and GSH in plasma were obtained by measurements at the 0th hour of OGTT. The tissues were isolated immediately after euthanasia in all groups. Euthanasia was done with intramuscularly ketamine-xylazine (70-10 mg/kg) injection). Each tissue was washed in saline and deionized water and was frozen immediately in liquid nitrogen, then separated into pieces and kept at -80°C. For the measurement of oxidative stress markers, tissues were homogenized with buffer (10 mM Tris/1 mM DTPA/1 mM PMSF) by using an ultrasonic homogenizer (Bandelin Sonopuls, Germany) and were centrifuged at 2000 g at 4°C for 10 minutes and the supernatants were stored at -80°C. Protein content of the samples was determined with using bovine serum albumin standards according to the protocol in the Pierce BCA Protein Assay Kit

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(ThermoScientific, USA). The absorbance values of the samples were measured at 562 nm, and the results expressed as mg/mL.

5.3 Treatment and Oral Glucose Tolerance Test (OGTT)

In treatment group, animals were treated with 10 mg/kg vitamin E (DL-alpha tocopherol acetate, Evigen® amp, Aksu Farma, Istanbul, Turkey) for 4 days consecutively [26] and OGTT was performed 2 hours after the last administration. Dose and treatment duration of vitamin E was chosen according to the study [26] which showed that vitamin E prophylactic treatment prevents the elevation of oxidative stress markers induced by lipopolysaccharide injection.

In order to mimic the action of OGTT in humans, we administered glucose orally to rabbits. Following 18 hours fasting period, glucose solution (1 g/kg) was given to the rabbits with oral gavage. The blood glucose concentrations were measured with a glucose meter before (zero point) and at 15th, 30th, 60th, 120th and 180th minutes after oral administrations (FreeStyle, Abbott, USA).

5.4 Measurements of Malondialdyde (MDA) Levels

The MDA levels were measured in plasmas at the time point given above and in livers and kidneys of the rabbits. Lipid peroxidation was assessed by a commercial TBARS assay kit (Cayman, USA). This kit measures the concentration of MDA, the naturally occurring product of lipid peroxidation. MDA forms a complex with thiobarbituric acid (TBA) under optimal conditions and the color intensity of MDA-TBA complex was measured at 530 nm spectrophotometrically. The amount of MDA was calculated by using MDA standards and the results were given as µM/mg protein.

5.5 Measurements of Glutathion (GSH) Levels

The GSH levels were also measured in plasmas at the same time points and in the liver and kidney of the rabbits by a spectrophotometric assay as described before [27, 28]. This assay is based on reducing Ellman's Reagent with sulfhydryl groups so as to give 1 mol of 2-nitro-5-mercaptobenzoic acid for 1 mol of sulfhydryl group. The nitromercaptobenzoic acid formed gave an intense yellow color that was measured spectrophotometrically at 412 nm. The results were expressed as µM/mg protein.

5.6 Statistical Evaluation

All data were presented as means ± standard error of means (SEM). Two-way ANOVA post hoc Bonferroni test was performed in the comparisons that have two variables. AUC's of the groups were analysed with Student's t test. One-way ANOVA post hoc Dunnet's test was performed for group analysis with one variable. Pearson correlation was used to analyze the correlation between blood glucose and MDA or GSH levels (GraphPad Prism 5 software). Statistical significance was defined as $P < 0.05$.

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