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The Protective Effect of Stobadine on Lipid Peroxidation and Paraoxonase-1 Enzyme Activity in the Liver Tissues of Streptozotocin-Induced Diabetic Rats

Streptozotosin ile Diyabet Oluşturulan Rat Karaciğer Dokularında Lipit Peroksidasyonu ve Paraoksonaz-1 Enzim Aktivitesi Üzerine Stobadinin Koruyucu Etkisi

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ABSTRACT

Aim: *Hyperglycemia is known to cause lipid peroxidation due to an increase in free radicals due to glucose auto-oxidation and by suppressing the antioxidant defense system of these radicals. Antioxidant therapy to manage diabetes mellitus and its complications is an emerging trend. Stobadine is a pyridoindole compound and is known to be an effective antioxidant in biological systems. In this study, we aimed to investigate the effects of stobadine on lipid peroxidation and paraoxonase-1 enzyme activities in liver tissues of streptozotocin-induced diabetic rats.*
Methods: *A total of 60 Wistar male rats, each weighing 250 g were randomly distributed into four groups; Control (C), Stobadine (STB), diabetic (D), STB treated diabetes (D+STB). Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg) to animals fasted overnight. Stobadine was administrated to rats as 25 mg/kg/day orally for four months. Rats were sacrificed after anesthesia; Malondialdehyde levels and PON-1 enzyme activities were measured. In homogenized rat liver tissue samples after portioning by manual spectrophotometric method.*
Results: *Malondialdehyde levels were significantly increased and paraoxonase-1 activities were significantly decreased in Group D compared to Groups C and STB ($p<0.001$). Malondialdehyde levels of diabetic rats treated with stobadine decreased while paraoxonase-1 activities were significantly increased ($p<0.001$). No significant difference was found between C and STB groups in terms of MDA levels and paraoxonase-1 activities ($p>0.05$). A negative correlation was detected between MDA levels and paraoxonase-1 enzyme activity ($r=-0.435$, $p<0.001$).*
Conclusion: *Stobadine administration was found to decrease lipid peroxidation and increase paraoxonase-1 enzyme activity in liver tissues of streptozotocin-induced diabetic rats. With further investigations using stobadine and pyridoindole derivatives, it may be possible to use these compounds as potential agents for the prevention of diabetes mellitus and its complications.*

Keywords: *Diabetes mellitus, oxidative stress, paraoxonase, stobadine*

ÖZ

Amaç: *Hipergliseminin, glukoz oto-oksidasyonu sonucu serbest radikallerde bir artışa ve bu radikallerin antioksidan savunma sistemini baskılaması sonucu lipit peroksidasyonuna neden olduğu bilinmektedir. Diyabetes mellitus ve komplikasyonlarının önlenmesi ve tedavisinde antioksidan ajanların kullanımı gelişmekte olan bir trenddir. Stobadin pridoindol yapıda bir bileşiktir ve biyolojik sistemlerde etkili bir antioksidan olduğu bilinmektedir. Bu çalışmada, streptozotosin ile diyabet oluşturulmuş sıçanların karaciğer dokularında, stobadinin lipid peroksidasyonu ve Paraoksonaz-1 enzim aktiviteleri üzerindeki etkilerini araştırmayı amaçladık.*
Yöntem: *Her biri 250 g ağırlığındaki toplam 60 adet Wistar erkek sıçan rastgele; Kontrol (K), Stobadin (STB), Diyabet (D) ve STB ile tedavi edilmiş Diyabet (D+STB) gruplarını oluşturmak üzere dört eşit gruba ayrıldı. Diyabet; periton içi streptozotosin (55 mg/kg) enjeksiyonu ile, Stobadin tedavisi ise 4 ay süre ile 25 mg/kg/gün oral Stobadin verilerek yapıldı. Sıçanlar anestezi sonrası sakrifiye edildi. Porsiyonlandıktan sonra homojenize edilen sıçan karaciğer dokularında, Malondialdehid düzeyleri ve Paraoksonaz-1 enzim aktiviteleri manuel spektrofotometrik yöntemle ölçüldü.*
Bulgular: *K ve STB grubu ile karşılaştırıldığında, D grubunda, Malondialdehid düzeyleri anlamlı artmış, Paraoksonaz-1 aktiviteleri anlamlı azalmış bulundu ($p<0,001$). Stobadin ile tedavi edilen diyabetik ratların ise D grubuna göre Malondialdehid düzeyleri azalırken, Paraoksonaz-1 aktiviteleri anlamlı artmış bulundu ($p<0,001$). Malondialdehid düzeyleri ve Paraoksonaz-1 aktiviteleri açısından K ve STB grupları arasında anlamlı fark bulunamadı ($p>0,05$). Malondialdehid düzeyleri ile Paraoksonaz-1 enzim aktivitesi arasında negatif orta derecede korelasyon saptandı ($r=-0,435$, $p<0,001$).*
Sonuç: *Stobadin verilmesinin, streptozotosin ile diyabet oluşturulmuş ratların karaciğer dokularında lipid peroksidasyonunu azalttığı ve Paraoksonaz-1 enzim aktivitelerini arttırdığı bulunmuştur. Diyabetes mellitus ve komplikasyonlarının önlenmesi ve tedaviye destek için, Stobadin ve pridoindol türevlerinin kullanıldığı yapılacak daha ileri araştırmalarla, bu bileşiklerin potansiyel ajanlar olarak kullanılması olası olabilir.*

Anahtar kelimeler: *Diyabetes mellitus, oksidatif stres, paraoksonaz, stobadin*

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INTRODUCTION

It is known that diabetes mellitus, which is one of the most common metabolic disorders in the world, causes a disruption in the oxidant/antioxidant balance. Oxidative stress may worsen with the increase of free radicals or the decrease of antioxidant defense capacities, or because of the increased free radicals suppressing the antioxidant system. It has been reported in various clinical and experimental studies that hyperglycemia causes an increase in the free radicals as a result of glucose auto-oxidation, thus resulting in lipid peroxidation due to the suppression of the antioxidant defense system[1-5]. Lipid peroxidation is a degenerative process that affects all structures containing lipid in the cells under oxidative stress, which in turn leads to cytopathological results. This process plays an important role in the etiopathology of the development of diabetes complications[6].

The paraoxonase-1 (PON-1) enzyme is a calcium-dependent, high-density lipoprotein (HDL) ester hydrolase, also known as aryldialkylphosphatase, and is synthesized in the liver. It exhibits paraoxonase and arylesterase activities, it is found in the serum as being HDL-dependent, and is otherwise known as lactonase. PON-1 plays an important role in the protection of low-density lipoprotein (LDL) and HDL from oxidizing by hydrolysing the accumulation of lipid peroxide. PON-1 moreover contributes to the antiatherogenic effect of HDL[7,8].

Antioxidant agents with different mechanisms of action are continuously being developed in order to either prevent the formation of reactive oxygen types, or in order to minimize their harmful effects. The use of antioxidant agents in the prevention of diabetes mellitus and its complications, alongside its supportive care, is a newly developing trend[9-11]. Stobadine, which is a good antioxidant in biological systems given its potential as an electron redox, is a synthetic compound with a pyridoindole structure that is, a derivative of heterocyclic indole known for its reactive oxygen scavenger effect. It effectively neutralizes the hydroxyl, peroxyl, and alkoxyl radicals, and inhibits both the amino acid oxidation as well as lipid peroxidation[12].

In this study, we aimed to compare the levels of malondialdehyde, which is the end product of the lipid peroxidation in the liver tissue of streptozotocin (STZ)-induced diabetic rats, and PON-1 enzyme activities with the control group, as well as aimed to evaluate the probable changes in these parameters in the liver tissue of diabetes-induced rats receiving STB treatment.

MATERIALS and METHODS

In this study, all experimental procedures had been conducted in accordance with the rules of the "Guide for the Care and Use of Laboratory Animals". The research protocol had been approved by the Ankara University Ethics Committee of animal studies (18.04.2001, 2001/11). 60 male Wistar rats weighing between 250 to 300 grams were used in the experiments. The rats were fed standard rat food, and allowed free access to drinking water. They were kept in a temperature controlled (18-23°C) environment with a 12-hour light and 12-hour dark cycles. These conditions were kept constant throughout the experiment. The rats were randomly distributed into the following four groups, each comprising of 15 rats: the control group (C), STB group (STB), diabetic group (D), and STB administered diabetic group (D+STB), with each group being respectively housed separately. Diabetes was induced with a single dose of intraperitoneal STZ (55 mg/kg) via injection, and the glucose concentrations in the blood samples taken from the tails of the rats 48 hours after the injections were measured (Accutrend GCT meter Roche Diagnostics, Mannheim, Germany). Rats with blood glucose levels of over 250 mg/dl were accepted as being diabetic. For the STB group, 15 rats, for which the blood glucose levels were known to be within normal limits, were administered orally with 25 mg/kg/day of STB over a period of 16 weeks.In the STB-treated diabetic group, half (n=15) of the diabetes-induced rats (n=30) were administered oral STB (25 mg/kg/day) over 16 weeks[13-15].

Twenty-four hours after the last manipulation, the rats were sacrificed using anesthetics and myorelaxant, whereupon cardiac samples were collected, and liver tissues were removed. The tissues were frozen in liquid nitrogen immediately after being washed with saline solution, and then stored at -80°C until the day of the analysis.

The liver tissues to be used in the experiment were proportioned separately for each parameter to be studied, and homogenized at a 1 : 1 ratio for a half minute in 1/5 saline solution using a Heidolph DIAX 900 homogenizer on the day of the analysis. Homogenates were centrifuged at 5000 g for 20 minutes, whereupon their supernatants were then separated. For each parameter being studied, the homogenization and procedures of centrifugation were repeated invariably.

Malondialdehyde (MDA) analysis

MDA in liver tissue homogenates was analysed based on the principle asserted by Van Ye et al.[16] that these homogenates combine with one mol MDA in thiobarbituric acid (TBA) in acidic environments at temperatures ranging between 85 to 100°C, whereupon they form a purple TBA-MDA complex, and the absorbance of this complex is spectrophotometrically measured at 532 nm. In order to ensure protein sedimentation, the samples were treated with 20% trichloracetic acid and then centrifuged. Some of the supernatant was mixed with the same 0.6% volume of TBA in a separate test tube, and incubated in a bath of boiling water for 30 minutes. After the samples jad cooled, the sample absorbance values were read at 532 nm against the blank. The MDA concentrations were calculated from the standard graphics prepared using 1,1,3,3-tetraetoxipropane. The MDA unit was expressed as nmol/mg protein.

Paraoxonase-1 (PON-1) analysis:

The PON activity was measured with the method based on the p-nitrophenol formation in the presence of PON-1, in which paraoxon was used as a substrate[17]. For the analysis, the p-nitrophenol was measured, and formed with paraoxon (diethyl p-nitrophenyl phosphate, 1 mM) in 50 mM glycine/NaOH (Ph 10.5) containing 2 mM CaCl2 at 25°C and 412 nm. The molar extinction coefficient of p-nitrophenol, e=18.290 M^{-1} cm^{-1} was used in the calculation of the PON-1 enzyme activity. An enzyme unit was defined as the amount of enzyme that catalyses the hydrolysis of 1 milimol substrate, at a temperature of 25°C. The PON-1 activity unit was presented as a mlU/mg protein.

Protein analysis:

The determination of proteins in the liver tissue homogenates were conducted in accordance with the principle dictating that the proteins in the alkali environment formed a complex with Cu^{+2}, which degrades the phosphomolybdate-phosphotungstate reactive[18]. As the protein standard, the standard graphic was drawn using bovine serum albumin (BSA). The tissue protein concentration unit was presented in terms of mg/ml.

Statistical Analysis

All statistical analyses were conducted using SPSS software, (Chicago, IL, ABD) version[21]. The normality controls of the variables were analysed using the Kolmogorov-Smirnov test. The mean values of the groups for the parameters were given as a median (IQR=Inter Quartile Range). The Mann Whitney-U Test and Kruskal Wallis analysis of the variance were used in comparisons of the data. For the pairwise comparisons of the groups, the post-hoc Tukey test was used. The correlation between the parameters was evaluated using the Spearman Correlation Analysis. $P<0.05$ was accepted as being statistically significant.

RESULTS

Since the measurements did not exhibit any normal distribution, the MDA (ngmol/mg protein) levels of the groups and their average values for PON-1 (mlU/mg protein) activities were presented as a median (IQR=Inter Quartile Range). The MDA levels in the D group [13.8 (2.2)] were found to be significantly higher when compared to the Groups C [6.9 (1.7)] and

STB [7 (1.3)]. The PON-1 activities were significantly lower in the Group D [3 (1.4)] than the Group C [4.8 (1.3)] and (p<0.001). The MDA levels of the STB administered diabetic rats [6.8 (1.5)] decreased compared to Group D [13.8 (2.2)], and their PON-1 activities elevated significantly [PON1; Group D: 3 (1.4), and Group D+STB: 4.5 (1.3)] (p<0.001). Any statistically significant difference could not be found for the MDA levels and PON-1 activities between Groups C [MDA: 6.9 (1.7); PON-1: 4.8 (1.3)] and STB [MDA:7 (1.3); PON-1: 4.9 (1.6)] (p>0.05) (Figures 1 and 2). A negative correlation was found between the MDA levels and PON-1 activities (r=-0.435, p<0.001) (Figure 3).

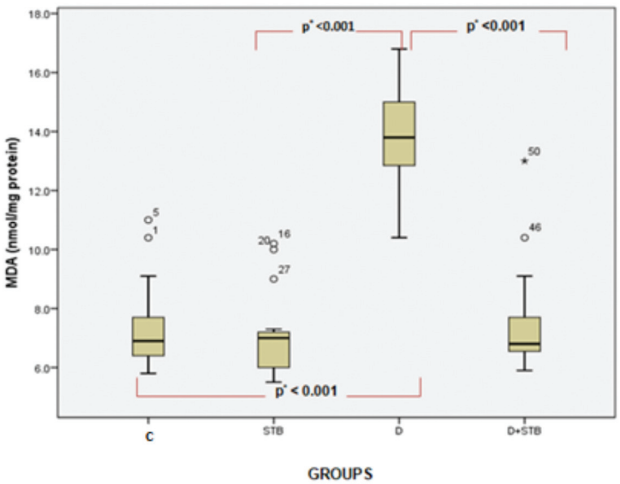

Figure 1. The comparison of MDA (Malondialdehyde) levels between 4 groups: 6.9 (1.7) nmol/mg protein in Control (C) group, 7 (1.3) nmol/mg protein in Stobadine (STB) group, 13.8 (2.2) nmol/mg protein in Diabetes (D) group, 6.8 (1.5) nmol/mg protein in Stobadine treated diabetes (D+STB) group.
**Kruskal Wallis test used*

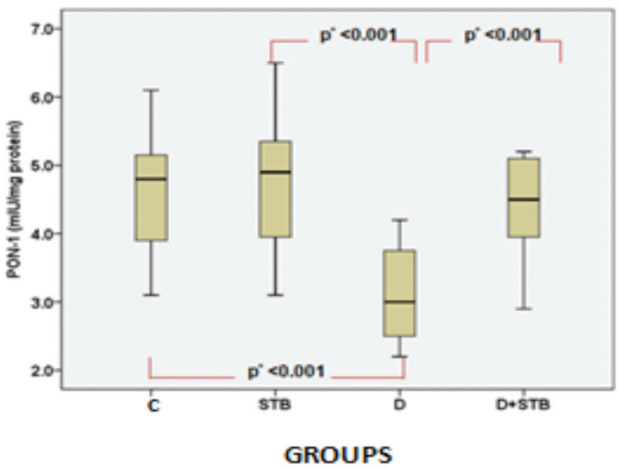

Figure 2. The comparison of PON-1 activities between 4 groups: 4.8 (1.3) mIU/mg protein in Control (C) group, 4.9 (1.6) mIU/mg protein in Stobadine (STB) group, 3 (1.4) mIU/mg protein in Diabetes (D) group, 4.5 (1.3) mIU/mg protein in Stobadine treated diabetes (D+STB) group.
**Kruskal Wallis test used*

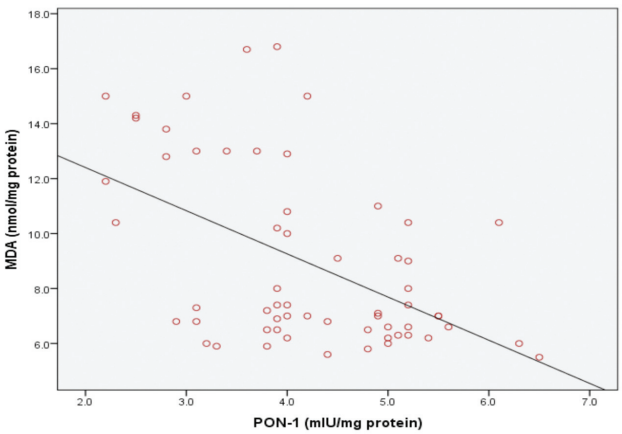

Figure 3. There was a negative correlation between Malondialdehyde (MDA) levels (nmol/mg protein) and Paraoxonase-1 (PON-1) activities (mIU/mg protein); r=-0.435, p<0.001.
Spearman's Correlation test used

DISCUSSION

In the liver tissue homogenates of those rats with streptozotocin-induced diabetes mellitus, the MDA levels were found to be higher, whereas the PON-1 enzyme activities were found to be lower when compared with the control group (p<0.001). Also, according to the findings of this study, the MDA levels of the diabetic rats decreased, and the PON-1 activity elevated following the 16 weeks of STB treatment. According to the overall results of the measurements, a moderate and negative correlation existed between the MDA levels and PON-1 enzyme activities (r=-0.435, p<0.001).

Dose and toxicology studies of STB delivered through various routes (intravenous, intramuscular, intraperitoneal, subcutaneous and oral) and at different doses were performed in mice, rabbits, rats, and dogs. It has been suggested that the oral administration of 10-30 mg/kg/day of stobadine to the rats scavenge free oxygen radicals, and reduces lipid peroxidation[12]. Gajdosikova et al.[19] did not find any adverse hema-

tological, histopathological, or genotoxic effects of stobadine administered orally to the rats at different doses (7.07, 23.6, 70.07 mg/kg/day). On the other hand, Balonova T et al.[20] had evaluated the effects of stobadine on the perinatal and postnatal development of the rat by orally administering different doses (5, 15, 50 mg/kg/day), and had only observed that the given dose of 50 mg/kg/day resulted in mild maternal toxicity. In our study, we applied STB at different doses for various durations in compliance with the STB therapies used in rat tissue studies performed in diabetic rats induced with streptozotocin[13-15].

STZ or alloxane-induced diabetes in rats is a well-designed animal model for Type 1 insulin-dependent-diabetes[1]. It has been reported that hyperglycemia after STZ exposure had decreased the production of reactive oxygen and nitrogen types in various tissues, increased lipid peroxidation and protein carbonylation, and decreased the activity levels of antioxidant enzymes. In addition, the main mechanisms that cause free oxygen radicals in diabetes mellitus originate from glucose auto-oxidation, protein glycation, and the production of advanced glycation end products. During the initial stage of diabetes, the changes in the carbohydrate, lipid, and protein metabolism, and the oxidative stress parameters induce development of great biochemical and functional anomalies in the liver[21-23]. It is argued that a series of mechanisms such as hyperglycemia, the glicosylation of proteins, and oxidative stress all contribute to the pathogenesis of the cellular function disorders causing the cardiovascular, hepatic, and other complications of diabetes[24,25].

MDA is the lipid peroxidation indicator used in the evaluation of liver damage in human and experimental animal studies[26]. The MDA levels, which were found to be high in the C and STB group of the diabetes-induced rats in our study, had decreased after 16 weeks of STB treatment administered to the diabetic rats. This finding is in accordance with other studies on the MDA changes in the liver tissue homogenates of the diabetic rats administered with either STB or other antioxidant treatments[11,13,27]. The reason for the absence of a difference between the C and the STB groups with regards to MDA levels can be explained by the efficiency of STB treatment only in the presence of oxidative stress.

The beta-oxidation of fatty acids increases in diabetic patients due to insulin deficiency. This situation results in hydrogen peroxide accumulation in the tissues, which in turn causes enzyme inactivation via glycation[28]. A decrease in PON enzyme activity has previously been reported in patients with diabetes mellitus[29,30]. PON enzyme activity may arise from the inactivation of PON via glycation, a decrease in the gene expression, or with the inhibition of the HDL synthesis or secretion which are known to be related with serum PON[29,31]. PON, which has been shown to decrease in diabetic patients, may be an indicator of diabetes mellitus complications[32]. The liver is the main organ responsible for PON and arylesterase synthesis. Therefore, the decrease in PON activities in diabetes-induced rats in our study may be related to the aforementioned mechanisms. The elimination of the oxidative damage with the STB supplement can be explained with the statistically significant increase in the enzyme activities of the rat liver homogenates.

STB and other prydoindole derivatives are effective synthetic antioxidants used in the experimental models of many chronic diseases, where oxidative stress plays a role in their etiopathogenesis especially in the case of diabetes. Pekiner et al.[14] showed that 10 weeks of low- dose STB treatment administered to diabetic rats had lowered blood glucose and triacylglycerole levels, and inhibited lipid peroxidation, protein glycosylation, and calcium accumulation in liver and heart tissue, and thus they had argued that STB treatment may prevent or delay the development of the complications of diabetes. Long-term STB treatment in diabetic rats was shown to reduce oxidative damage by decreasing the carbonyl content, conjugated dienes, MDA and oxidation end products in the tissues, as well as to partially increase the enzyme activity[13,15,33,34].

CONCLUSION

STB administration had decreased the MDA levels in the liver tissue of rats with streptozotocin-induced diabetes, and had increased the PON enzyme activities. In future studies, it may be possible to use these compounds safely through studies involving STB and prydoindole derivatives in order to prevent the complications of diabetes mellitus and to support treatment.

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