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Research Article

The changes of oxidative stress and Δ^9-tetrahydrocannabinol accumulation in liver of type-2 diabetic rats

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Abstract

In this study, we aimed to explore the changes of oxidative stress, Δ^9- tetrahydrocannabinol (Δ^9-THC) and its metabolites accumulation in the liver of type-2 diabetic rats treated with Δ^9-THC. 8-10 week-old Sprague-Dawley rats were divided into four groups. Group I: Physiological saline was administered intraperitoneally(i.p) (n=7). Group II: Rats that was given Δ^9-THC for 7 days (3 mg/kg/day) (n=6) (i.p). Group III: Streptozotocin(STZ, 65 mg/kg)+Nicotinamide(NAD, 85 mg/kg) (n=7) (i.p). Group IV: Diabetic rats that were given Δ^9-THC(3 mg/kg/day) for 7 days (n=7) (i.p). The biochemical investigation was carried out on the serum and liver tissue. Δ^9-THC and its metabolites were analyzed by using GC-MS in the liver of rats. Liver glutathione level in diabetes+ Δ^9-THC increased as compared to diabetic rats. The increased lipid peroxidation and protein carbonyl levels in diabetes showed a low reduction with Δ^9-THC. Catalase and superoxide dismutase activities in liver of diabetic rats increased with Δ^9-THC treatment, non-statistically. Serum uric acid level, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities slightly increased in diabetic group as compared to control group. While the AST level reduced in the liver, ALT level did not be affected in Δ^9-THC treated with diabetic rats as compared with the diabetic group. Δ^9-THC level in liver was negative in given Δ^9-THC groups. But the liver THC-COOH metabolite accumulation in Δ^9-THC group is higher than Diabetes + Δ^9-THC group. We could say that Δ^9-THC can reduce diabetes-induced oxidative damage in the liver of type-2 diabetic rats. The metabolization of Δ^9-THC in the liver can be accelerated with diabetes.

Keywords: Type-2 diabetes, Δ^9-tetrahydrocannabinol, liver, oxidative stress, rat
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Tip-2 diyabetik sıçanların karaciğerinde oksidatif stres ve Δ^9- tetrahidrokannabinol birikim değişiklikleri

Özet

Çalışmamızda, Δ^9-THC uygulanan tip-2 diyabetik sıçanların karaciğerlerinde oksidatif stres ve Δ^9-THC birikim değişikliklerinin incelenmesi amaçlanmıştır. 8-10 haftalık, erkek Sprague-Dawley sıçanlar dört gruba ayrıldı. Grup I: Serum fizyolojik intraperitoneal (i.p.) olarak uygulandı (n=7). Grup II: Sıçanlara 7 gün süresince Δ^9-THC (3 mg/kg/gün) (i.p.) verildi (n=6). Grup III: Streptozotosin (STZ, 65 mg/kg) + Nikotinamid (NAD, 85 mg/kg) (n=7) (i.p.).Grup IV: Diyabetik sıçanlara 7 gün Δ^9-THC (3 mg/kg/gün) (i.p.) (n=7) verildi. Serum ve karaciğer dokularında biyokimyasal incelemeler yapıldı. Karaciğerde Δ^9-THC ve metabolitleri GC-MS kullanılarak analiz edildi. Diyabet + Δ^9-THC grubunda karaciğer glutatyon seviyesi diyabetik sıçanlara göre arttı. Diyabette artmış olan lipid peroksidasyon ve protein karbonil seviyeleri Δ^9-THC uygulamasıyla düşük seviyede bir azalma gösterdi. Diyabetik sıçanların karaciğerlerinde katalaz ve superoksid dismutaz aktiviteleri Δ^9-THC uygulamasıyla istatiksel olarak anlamsız olarak arttı. Kontrol grup ile kıyaslandığında, serum ürik asit seviyeleri alanin aminotransaminaz (ALT) ve aspartat aminotransaminaz (AST) aktiviteleri, diyabetik grupta çok az arttı. Diyabetik grup ile kıyaslandığında, diyabetiklere Δ^9-THC uygulaması karaciğerde AST'yi düşürüken ALT'yi etkilemedi. Δ^9-THC verilen grupta karaciğer Δ^9-THC seviyesi negatifti. Fakat Δ^9-THC grubunda karaciğer THC-COOH metabolit birikimi diyabet + Δ^9-THC grup ile kıyaslandığında daha yüksekti. Tip 2 diyabetik sıçanlara Δ^9-THC uygulanmasının, karaciğerde diyabetin neden olduğu oksidatif hasarı azaltabileceğini söyleyebiliriz. Δ^9-THC'nin karaciğerde metabolizasyonu diyabet ile hızlanabilir.

Anahtar kelimeler: Tip-2 diyabet, Δ^9-tetrahidrokannabinol, karaciğer, oksidatif stres, sıçan

Introduction

It has been known that cannabinoids were isolated from the cannabis plant (*Cannabis sativa* L.) and Δ^9-tetrahydrocannabinol (Δ^9 -THC) is the main active psychoactive constituent of *C. sativa* (Kochanowski and Kala 2005; Ahmed et al. 2015). Δ^9-THC turn into its active metabolite 11-hydroxy-Δ^9-THC (11-OH-THC), and then converted to an inactive metabolite 11-nor-9-carboxy- Δ^9-THC (THC-COOH) (Kochanowski and Kala 2005; Gieringer et al. 2008). Δ^9-THC and its metabolites may be determined in blood, liver and kidney tissues (Kemp et al. 2015). It is reported that *C. sativa* and its products have been used as medicinal agents (Elsohly and Slade 2005). According to Fasinu et al. (2016), sixteen states in the United States have legalized cannabidiol (CBD) and Δ^9-THC contenting products due to using in medicine. Pinto et al. (2010) reported that Δ^9-THC administration has not any harmful effects on healthy mice liver. Furthermore, according to Chen et al. (2005), Δ^9-THC can block reactive oxygen radicals (ROS) generation.

Type 2 diabetes is the most common type of *Diabetes mellitus* which is a multifactorial disease and characterized by hyperglycemia and insulin resistance (Verspohl 2009). Oxidative stress is based on an imbalance between antioxidants and the generation of ROS (Kassab and Piwowar 2012). The studies have shown that oxidative stress plays an important role in diabetes (Baburao and Anand 2012; Stadler, 2012; Ávila Dde et al. 2013).

The aims of the present study; (i) to explain the effects of Δ^9-THC on oxidative stress in liver of type 2 diabetes, and (ii) to detect the level of Δ^9-THC metabolites in the liver of diabetic and control animals treated with Δ^9-THC.

Materials and methods

Animals and ethics

8–10 weeks-old male Sprague-Dawley rats were housed in a temperature-controlled clean room with a 12 h light/dark cycle and fed with tap water and standard chow *ad libitum*. All experimental procedures were performed according to the guidelines of the Local Ethic Committee of Animal Research (Istanbul University, HADYEK 2015/06).

Experimental design

The animals were selected randomly and arranged into four groups;

Group 1: (n = 7) Control rats that received serum physiologic intraperitoneally (i.p.).

Group 2: (n = 6) Δ^9-THC (3 mg/kg/day, i.p. Lipomed THC-135-100LE) was administered to the rats for seven days.

Group 3: (n = 7) For diabetes, the rats were injected with a single dose of streptozotocin (STZ) (65 mg/kg, Sigma-Aldrich S0130) dissolved in serum physiologic 15 min after the injection of nicotinamide (NAD) (85 mg/kg, Sigma-Aldrich N3376) in serum physiologic (i.p.) (Masiello et al. 1998).

Blood glucose levels of the rats were measured 72 h after the STZ + NAD injection. The animals with blood glucose concentrations more than 200 mg/dL were accepted as diabetic.

Group 4: (n = 7) diabetic animals were treated (i.p.) with Δ^9-THC (3 mg/kg/day) during 7 days.

The experiment was terminated on day 15th after the Δ^9-THC injections, the animals were anesthetized i.p. with ketamine-HCl (50 mg/kg, Pfizer) and xylazine-HCl (10 mg/kg, Bayer). Immediately, the blood and liver tissue samples were collected from rats.

Analysis of serum and liver tissue

The blood samples were centrifuged, and serums were separated. The liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and uric acid (UA) level were measured in all serum samples using the Siemens Advia 1800 chemistry system. The liver tissue samples were homogenized in cold 0.9 % NaCl and made up to 10 % homogenate. The homogenates were centrifuged. The clear supernatant fractions were removed for the biochemical analysis. The protein content was determined by Lowry's method using bovine serum albumin as standard (Lowry et al. 1951). The glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PCO) levels in liver homogenates were estimated according to Beutler's, Ledwozyw's and Reznick and Packer's methods, respectively (Beutler 1963; Ledwozyw et al. 1986; Reznick and Packer 1994). Liver catalase (CAT) activity was determined by using Aebi's method (Aebi 1984). The superoxide dismutase (SOD) activity was assayed in liver tissues by using the method of Sun (Sun et al. 1988). Δ^9-THC and its metabolites were analyzed by using GC-MS in the liver of rats.

Statistical analysis

The statistical analysis was conducted using the SPSS 21.0 software program. The descriptive statistics of the data were expressed using the mean ± standard error of the mean (SEM). The data were evaluated for statistical significance using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant.

Results

The levels of alanine aminotransferase (ALT) aspartate aminotransferase (AST) and uric acid (UA) are seen in Fig.1. ALT and AST levels showed an insignificant increase in type 2 diabetic rats as compared to control group. It was determined that while serum ALT level did not show any change in Δ^9-THC treated with diabetic rats as compared to the diabetic group, AST level decreased, non-significantly. A slight increase in serum UA level was observed in STZ/NAD-induced rats when compared to control group. The increased UA level in diabetic was reversed with Δ^9-THC treatment, insignificantly. In Table 1, Liver glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PCO) levels are presented. The liver GSH levels insignificantly decreased in diabetic group as compared with control rats. GSH levels increased by treatment with Δ^9-THC in diabetic rats compared to the diabetic group.

Δ⁹-THC administration to diabetic reduced LPO and PCO levels in the liver as compared to non-treated diabetic rats. The liver catalase (CAT) and superoxide dismutase (SOD) activities in diabetic rats reduced when compared with control rats. Both of enzyme activities showed an insignificant increase in STZ/NAD-induced diabetic animals with Δ⁹-THC treatment (Table 2). We examined the accumulation of Δ⁹-THC and its metabolites in liver tissues of Δ⁹-THC and diabetes + Δ⁹-THC groups. Δ⁹-THC level in liver was negative in given Δ⁹-THC groups. But the liver THC-COOH metabolite accumulation in Δ⁹-THC group is higher than STZ/NAD-induced diabetes + Δ⁹-THC group, non-significantly (Fig. 2).

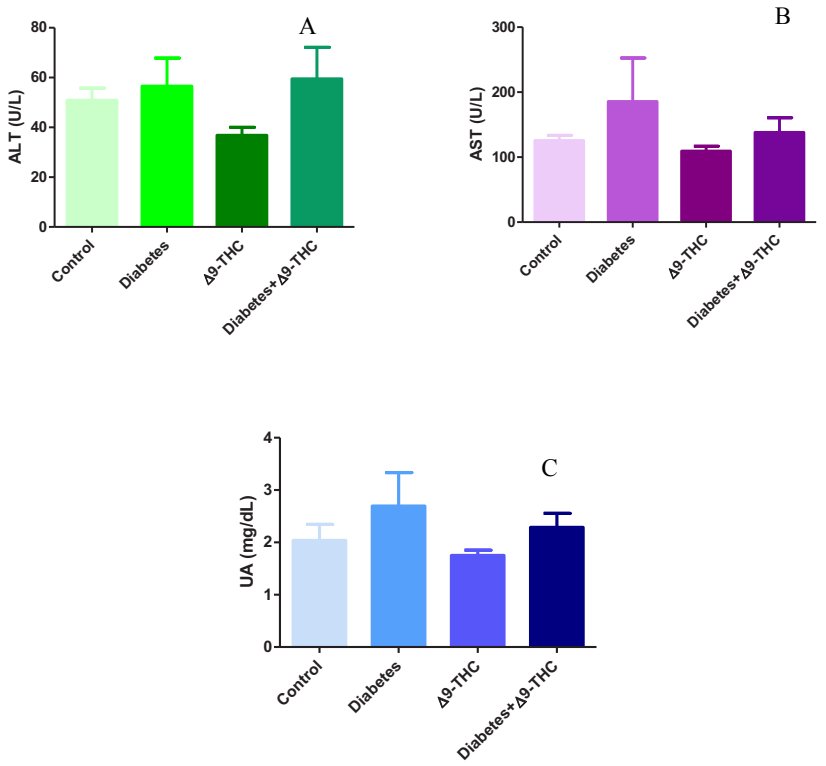

Figure 1. The levels of liver enzymes and uric acid in serum. A: alanine aminotransferase (ALT); B: aspartate aminotransferase (AST) and uric acid (UA). Data are expressed as mean ± SEM.

Table 1. Liver glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PCO) levels in all groups.

	GSH (nmol/mg protein)*	LPO (nmol/mg protein)*	PCO (nmol/mg protein)*
Control	7.36 ± 0.61	0.14 ± 0.03	0.36 ± 0.07
Diabetes	6.05 ± 0.31	0.21 ± 0.02	0.56 ± 0.17
Δ⁹-THC	6.53 ± 0.29	0.18 ± 0.02	0.32 ± 0.06
Diabetes + Δ⁹-THC	6.88 ± 1.25	0.19 ± 0.01	0.44 ± 0.11
P_{ANOVA}	> 0.05	> 0.05	> 0.05

*Mean ± standard error (SEM)

Table 2. The catalase (CAT) and superoxide dismutase (SOD) activities in liver of rats.

	CAT (U/mg protein)*	SOD (U/mg protein)*
Control	92.62 ± 16.7	1.30 ± 0.11
Diabetes	5.70 ± 2.67	1.19 ± 0.03
Δ^9-THC	54.71 ± 24.56	1.04 ± 0.02
Diabetes + Δ^9-THC	67.34 ± 31.79	1.94 ± 0.63
P_{ANOVA}	> 0.05	> 0.05

*Mean ± standard error (SEM).

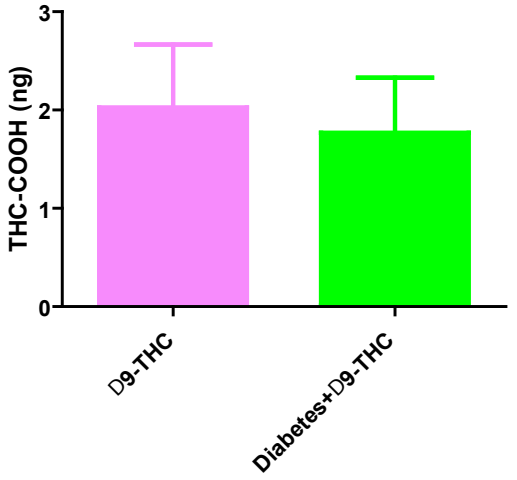

Figure 2. The levels of THC-COOH (ng) in liver of Δ^9-THC and STZ/NAD-induced diabetes + Δ^9-THC groups on day 15 after the Δ^9-THC injections. Data are expressed as mean ± SEM.

Discussion

The present study shows that STZ/NAD-induced type 2 diabetes caused the rise of oxidative damage in the liver of rats. Furthermore, treatment with Δ^9-THC modulated STZ/NAD-induced biochemical changes. The accumulation of Δ^9-THC was detected in the liver of rats.

Type 2 diabetes causes insulin resistance and decreased insulin secretion. The disease progression induced tissue damages shows various symptoms of complications (Malenica et al. 2016). The liver is an important organ in the regulation of glucose level. The clinical and experimental studies reported that liver cell damage occurs in type 2 diabetes (Parveen et al. 2010; Ramljak et al. 2015). There are strong evidence that increased oxidative stress is related with the pathogenesis of many diseases. Moreover, oxidative stress plays also a major role in the development of diabetes complications (Oliveira et al. 2016; Salmon 2016). In the previous study, LPO and PCO levels increased in liver, small intestine and stomach of type 2 diabetic neonatal rats and GSH level decreased (Karatug et al. 2012; Coskun et al. 2013; Koyuturk et al. 2015). Likely, we observed similar changes in LPO, PCO and GSH levels in the liver of diabetic adult rats. Individuals with type 2 diabetes and experimental diabetic rats have rising liver enzymes levels in serum as compared to controls (Harris 2005; Koyuturk et al. 2015). Similar to previous studies, we found the serum ALT and AST liver enzymes raised in STZ/NAD- induced diabetes group when compared with healthy rats.

Investigators noticed medical plants can use against oxidative damage in experimental diabetic rats (Oliveira et al. 2016). Recently, pharmacological studies have been carried out with the therapeutic properties of Δ^9-THC obtained from *C. sativa* plant. Chen et al. suggested that Δ^9-THC should play a role as an antioxidant substance (Chen et al. 2005; Mcpartland et al. 2015). In the present study, we applied non-synthetic Δ^9-THC to STZ/NAD-induced rats. Δ^9-THC treatment changed the oxidative stress parameters as non-significantly.

A modulation was observed oxidative stress state in diabetic rats treated with Δ^9-THC. The reduction in activities of enzymatic antioxidants such CAT and SOD is caused the elevation of ROS. Arulselvan and Subramanian (2007) reported that the increased oxidative stress in diabetes might be induced a decrease of CAT enzyme activity in tissue. Furthermore, it was observed that SOD enzyme activity in diabetic rats reduced (Sozmen et al. 2001). In this study, both enzymes showed a reduction in diabetics, but Δ^9-THC administration elevated the antioxidant enzymes to some extent on diabetic animals. Δ^9-THC may decrease oxidative stress in diabetes by raised antioxidant enzymes.

Δ^9-THC accumulates been observed in adipose and brain tissues (Kreuz and Axelrod 1973). Addition to, profiles of Δ^9 -THC and its metabolite can be detected in plasma samples (Brenneisen et al. 2010). The excretion of Δ^9 -THC and its metabolite 11-OH-THC were measured in urine of chronic cannabis users for -4, -7 and -24 days after the cannabis cessation. Its metabolites were detectable in urine at lowest levels in 24 days (Lowe and Axelrod 2009).

According to our previous study, the Δ^9-THC metabolite level in urine of non-diabetic rats was lower than that of the diabetic animals. Otherwise, the accumulation of THC-COOH in the liver of non-diabetics was higher than of the diabetic rats. It pointed out that liver may lay up Δ^9-THC and its metabolite, their excretion with urine notwithstanding.

Conclusions

Our findings indicate that Δ^9-THC administration may serve a protective role to some extent for liver in diabetes by blocking oxidative stress state. The metabolization of Δ^9-THC in the liver can probably be accelerated with diabetes. However, further investigation is necessary to clarify the effects of Δ^9-THC supplementation on type 2 diabetes.

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References

Aebi H. (1984) Catalase in vitro. *Methods Enzymol*, 105: 121–6.

Ahmed S.A., Ross S.A., Slade D., Radwan M.M., Khan I.A. and ElSohly M.A. (2015) Minor oxygenated cannabinoids from high potency *Cannabis sativa* L. *Phytochemistry,* 117: 194-9.

Arulselvan P. and Subramanian S.P. (2007) Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultrastructural changes of pancreatic β cells in experimental diabetes in rats. *Chem Biol Interact*, 165: 155–64.

Ávila Dde L., Araújo G.R., Silva M., Miranda P.H., Diniz M.F., Pedrosa M.L., Silva M.E.,de Lima W.G. and Costa D.C. (2013) Vildagliptin ameliorates oxidative stress and pancreatic beta cell destruction in type 1 diabetic rats. *Arch Med Res*, 44: 194–202.

Baburao Jain A. and Anand J.V. (2012) Vitamin E, its beneficial role in diabetes ellitus (DM) and its complications. *J Clin Diagn Res*, 6: 1624–8.

Beutler E., Duron O. and Kelly B.M. (1963) Improved method for the determination of blood glutathione. *J Lab Clin Med*, 51: 882-8.

Brenneisen R., Meyer P., Chtioui H., Saugy M. and Kamber M. (2010) Plasma and urine profiles of Delta9-tetrahydrocannabinol and its metabolites 11-hydroxy-Delta9 tetrahydrocannabinol and 11-nor-9-carboxy-Delta9-tetrahydrocannabinol after cannabis smoking by male volunteers to estimate recent consumption by athletes. *Anal Bioanal Chem*, 396(7): 2493-502.

Chen J., Lee C.T., Errico S., Deng X., Cadet J.L. and Freed W.J. (2005) Protective effects of Delta(9)-tetrahydrocannabinol against N-methyl-d-aspartate-induced AF5 cell death. *Brain Res Mol Brain Res*, 134(2): 215-25.

Coskun Z.M., Sacan O., Karatug A., Turk N., Yanardag R., Bolkent S. and Bolkent S. (2013) Regulation of oxidative stress and

somatostatin, cholecystokinin, apelin gene expressions by ghrelin in the stomach of newborn diabetic rats. *Acta Histochem*, 115(7): 740-7.

ElSohly M.A. and Slade D. (2005) Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sci*, 78: 539-48.

Fasinu P.S., Phillips S., ElSohly M.A. and Walker L.A. (2016) Current status and prospects for cannabidiol preparations as new therapeutic agents. *Pharmacotherapy*, 36(7): 781-96.

Gieringer D., Rosenthal E. and Carter G.T. (2008) *Marijuana medical handbook: A practical guide to the therapeutic uses of marijuana*. Berkley, CA, USA.

Harris E.H. (2005) Elevated liver function tests in type 2 diabetes. *Clin Diabetes*, 3: 115–9.

Kassab A. and Piwowar A. (2012) Cell oxidant stress delivery and cell dysfunction onset in type 2 diabetes. *Biochimie*, 94: 1837–48.

Karatug A., Sacan O., Coskun Z.M., Bolkent S., Yanardag R., Turk N. and Bolkent S. (2012) Regulation of gene expression and biochemical changes in small intestine of newborn diabetic rats by exogenous ghrelin. *Peptides*, 33(1): 101-8.

Kemp P.M., Cardona P.S., Chaturvedi A.K. and Soper J.W. (2015) Distribution of Δ(9)-tetrahydrocannabinol and 11-Nor-9-Carboxy-Δ(9)-tetrahydrocannabinol acid in postmortem biological fluids and tissues from pilots fatally injured in aviation accidents. *J Forensic Sci*, 60(4): 942-9.

Kochanowski M. and Kała M. (2005) Tetrahydrocannabinols in clinical and forensic toxicology. *Przegl Lek*, 62: 576-80.

Koyuturk M., Sacan O., Karabulut S., Turk N., Bolkent S., Yanardag R. and Bolkent S. (2015) The role of ghrelin on apoptosis, cell proliferation and oxidant-antioxidant system in the liver of neonatal diabetic rats. *Cell Biol Int*, 39(7): 834-41.

Kreuz D.S. and Axelrod J. (1973) Delta-9-tetrahydrocannabinol: localization in body at. *Science*, 179: 391-3.

Ledwozyw A., Michalak J., Stepień A and Kadziolka A. (1986) The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. *Clinica Chim Acta*, 155: 275-83.

Lowe R.H., Abraham T.T., Darwin W.D., Herning R., Cadet J.L. and Huestis M.A. (2009) Extended urinary Delta9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure. *Drug Alcohol Depend*, 105: 24-32.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall R.J. (1951) Protein measurement with the folin phenol reagent. *J Biol Chem*, 193: 265-75.

Malenica M., Prnjavorac B., Causevic A., Dujic T., Bego T. and Semiz S. (2016) Use of databases for early recognition of risk of diabetic complication by analysis of liver enzymes in type 2 diabetes mellitus. *Acta Inform Med*, 24(2): 90-3.

Masiello P., Broca C., Gross R., Roye M., Manteghetti M., Hillaire-Buys D., Novelli M. and Ribes G. (1998) Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes*, 47(2): 224-9.

McPartland J.M., Duncan M., Di Marzo V. and Pertwee R.G. (2015) Are cannabidiol and Δ(9)-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. *Br J Pharmacol*, 172(3): 737-53.

Oliveira J.S., Silva A.A. and Silva V.A. Junior. (2016) Phytotherapy in reducing glycemic index and testicular oxidative stress resulting from induced diabetes: a review. *Braz J Biol*, doi: 10.1590/1519-6984.09915.

Parveen K., Khan M.R., Mujeeb M. and Siddiqui W.A. (2010) Protective effects of pycnogenol on hyperglycemia-induced oxidative damage in the liver of type 2 diabetic rats. *Chem Biol Interact*, 186(2): 219-27.

Pinto C.E., Moura E., Serrão M.P., Martins M.J. and Vieira-Coelho M.A. (2010) Effect of (-)-Delta(9)-tetrahydrocannabinoid on the

hepatic redox state of mice. *Braz J Med Biol Res*, 43(4): 325-9.

Ramljak S., Hermanns I., Demircik F. and Pfützner A. (2015) Assessment of hepatic disorders in patients with type 2 diabetes by means of a panel of specific biomarkers for liver ınjury. *Clin Lab*, 61(11): 1687-93.

Reznick A.Z. and Packer L. (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods Enzymol*, 233: 357-63.

Salmon A.B. (2016) Beyond diabetes: does obesity-ınduced oxidative stress drive the aging process? *Antioxidants (Basel)*,18; 5(3).

Sozmen E.Y., Sozmen B., Delen Y. and Onat T. (2001) Catalase/superoxide dismutase (SOD) and catlase/paraoxonase (PON) ratios may implicate poor glycemic control. *Arch Med Res*, 32: 283–7.

Stadler K. (2012) Oxidative stress in diabetes. *Adv Exp Med Biol*, 771: 272–87.

Sun Y., Oberley L.W. and Li Y. (1988) A simple method for clinical assay of superoxide dismutase. *Clin Chem*, 34: 497–500.

Verspohl E.J. (2009) Novel therapeutics for type 2 diabetes: incretin hormone mimetics (glucagon-like peptide-1 receptor agonists) and dipeptidyl peptidase-4 inhibitors. *Pharmacol Ther*, 124: 113-38.