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Taurine Is More Effective than Melatonin on Cytochrome P450 2E1 and Some Oxidative Stress Markers in Streptozotocin-Induced Diabetic Rats

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ABSTRACT: Melatonin and taurine have alleviative effects in streptozotocin (STZ)-induced diabetic rats. Male Wistar rats were divided into nondiabetic, diabetic, diabetic melatonin supplemented and diabetic taurine supplemented groups. At the end of the study, both blood and liver were collected for determination of some oxidative stress parameters, and hepatic cytochrome P450 2E1 (CYP2E1) enzyme activity and gene expression. An increased CYP2E1 activity and expression level with a concomitant significant change in oxidative stress parameters were found in STZ-induced diabetic rats. Taurine or melatonin supplementation to the diabetic rats alleviated these experimental parameters with a more significant effect for taurine than that of melatonin. Suppression of β-hydroxybutyrate (β-HB) production by taurine can be one of the mechanisms of a reduction in CYP2E1. Taurine was effective more than melatonin in reducing CYP2E1 activity and expression; therefore antioxidants might prove beneficial in type 1 diabetes associated with manifestations of liver injury.

KEYWORDS: cytochrome P450 2E1, hepatic injury, melatonin, taurine, type 1 diabetes

INTRODUCTION

Increased oxidative stress, as measured by indices of elevated lipid peroxidation, oxidative modification products of proteins and depletion of endogenous antioxidant, is commonly found in streptozotocin (STZ)-induced diabetic rats, and these alterations may cause tissues to be more susceptible to oxidative damage. The vulnerability of each tissue to oxidative stress can vary depending upon their expressed antioxidant enzymes.[1] Liver is the focal organ of oxidative and detoxifying processes; in addition, free radical reactions and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including diabetes mellitus.[2] Cytochrome P450 2E1 (CYP2E1) has been implicated in the generation of tissue damaging hydroxyl radicals in patients suffering from diabetes and liver diseases.[3,4] Therefore, any agent with an inhibitory effect on CYP2E1 activity may be able to alleviate such oxidative stress effects. Several studies have recently dealt with either maintenance of antioxidant defense of diabetic liver or reduction of peroxidative stress induced hepatic damage in experimental models.[3,5,6] Hence, it is recommended that therapy with antioxidants may signify a useful pharmacologic overture to the management of diabetes. The antioxidant role of taurine (2-aminoethanesulfonic acid) has been attributed to its ability to scavenge reactive oxygen species (ROS), to reduce the production of lipid peroxidation end products, and to stabilize biomembranes.[7] Melatonin (*N*-acetyl-5-methoxytryptamine), a secretory product of the pineal gland, is a potent scavenger of hydroxyl and peroxyl radical that regulates the activity of antioxidant enzymes.[8]

So, the aim of the present study was to compare between the effect of either taurine or melatonin on CYP2E1 activity, gene expression and some oxidative stress parameters in STZ-induced diabetes in rats, in order to elucidate their possible mechanisms on reducing hepatic insult in type 1 diabetes.

MATERIALS AND METHODS

1. Chemicals. Biochemicals and substrates were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). STZ (trade name Zanosar) was in the form of 1 g vials. The treatment dose of taurine and melatonin was chosen according to Yao et al.[9] and Montilla et al.,[10] respectively.

2. Animals. *2.1. Experimental Animals and Diets.* Forty male Wistar rats weighing approximately between 150 and 170 g were acclimatized under laboratory conditions for 2 weeks prior to the experiments. They were maintained under standard conditions of temperature (23 ± 2 °C) with alternating 12 h light/dark cycles and were allowed free access to food and water all times.

2.2. Induction of Diabetes. Induction of diabetes was made by a single intraperitoneal injection of STZ at a dose of 70 mg/kg body weight in freshly prepared ice-cold citrate buffer (0.1 M pH 4.0) for 3 consecutive days. STZ used to induce damage to pancreatic beta cells, which exhibited massive glycosuria and hyperglycemia within 3 days. Diabetes was defined as a blood glucose level greater than 250 mg/dL[3] using a drop of blood obtained by a tail-vein puncture monitored at different time intervals throughout the study using ACCUTREND reagent strips (Roche Diagnostics GmbH, Germany).

2.3. Animal Treatment. In addition to 10 rats receiving an intraperitoneal injection of a single dose of 0.1 mol/L sodium citrate buffer (pH 4.5) (normal control group; G-I), diabetic rats (after induction of diabetic state within 72 h after STZ injection) were divided into three groups with 10 rats in each group. One group was maintained on a standard diet (untreated-diabetes group; G-II), another one was injected by melatonin daily intraperitoneally three days prior to induction of diabetes in a dose of 200 μg/kg/day dissolved in 0.5 mL of normal saline (melatonin-diabetic treated group; G-III), and the last group treated

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with 2% (w/v) taurine in the drinking water which was made fresh daily (taurine-diabetic treated; G-IV). The body weight, food, and water intake were monitored daily throughout the study period (4 weeks). Dead rats were excluded from the study.

At the end of the experiment, after an overnight fast, the rats were decapitated. Then (1) the blood was collected partly in a plain tube to obtain serum and partly on K_3EDTA (tripotassium ethylenediaminetetraacetic acid) coated tubes to obtain plasma, centrifuged at 1300*g* for 20 min at 4 °C, and used for determination of some biochemical analysis, mentioned later on; (2) the liver was excised, washed with ice cold saline to remove extraneous materials, cut into four parts and used as follows.

3. Liver Homogenates: Preparation of Liver Tissue Homogenates. The first part of the liver (3 g) was minced and homogenized (Potter-Elvenhjem tissue homogenizer) in 10 mM potassium phosphate containing 1 mM EDTA, pH 7.4, and centrifuged at 12000*g* for 30 min at 4 °C. The resultant supernatant (free of insoluble materials), was collected, stored at −80 °C and used for determination of malondialdehyde (MDA) level, glutathione peroxidase (GPx) enzyme activity and protein content.

The second part of the liver was used for microsome preparation as described elsewhere.[11] It was homogenized in 4 vol (w/v) of a 20 mM Tris/HCl, 150 mM KCl and 1 mM EDTA solution (pH 7.6), with a Teflon pestle (Potter-Elvehjem, Inframo, Wayne, NJ), and centrifuged at 9000*g* for 20 min. The postmitochondrial supernatant obtained was centrifuged at 105000*g* for 60 min. The pellet obtained was resuspended in a 20 mM Tris/HCl, 150 mM KCl and 3 mM $MgCl_2$ solution (pH 7.6) and centrifuged again at 105000*g* for 60 min to obtain the final microsomal fraction. The microsomes were finally resuspended in the Tris/HCl/KCl/$MgCl_2$ solution, containing 15% (v/v) glycerol in water (pH 7.6), immediately stored at −70 °C until used for determination of CYP2E1 activity. Centrifugation was performed using TL-100 Beckmann ultracentrifuge.

The third part of the liver was used for determination of CYP2E1 expression as mentioned below.

The fourth part was used for histopathological examination.

4. Biochemical Analysis. *4.1. Fasting Plasma Glucose Level.* This was determined by the glucose oxidase method using a commercial kit (Elitech Diagnostics Company, France) according to the manufacturer's instructions.

4.2. Total Serum Cholesterol and Triglyceride Levels. These were estimated using the appropriate kits (Boehringer Mannheim, Germany).

4.3. Serum Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Activities. These were measured using Randox kits according to manufacturer's instructions.

4.4. Plasma β-Hydroxybutyrate (β-HB) Concentration. This was determined using the kits purchased from Randox Laboratories (Antrum, U.K.) according to McGarry et al.[12]

4.5. Plasma Advanced Oxidation Protein Product (AOPP) Level. This was determined according to Witko-Sarsat et al.[13] Briefly, AOPP were measured by spectrophotometry on a microplate reader and were calibrated with chloramine-T (Sigma, St. Louis, MO) solutions that in the presence of potassium iodide absorb at 340 nm. In test wells, 200 μL of plasma diluted 1/5 in phosphate buffer saline (PBS) was placed on a 96-well microtiter plate, and 20 μL of acetic acid was added. In standard wells, 10 μL of 1.16 M potassium iodide (Sigma) was added to 200 μL of chloramine-T solution (0−100 μmol/L) followed by 20 μL of acetic acid. The absorbance of the reaction mixture is immediately read at 340 nm on the microplate reader against a blank containing 200 μL of PBS, 10 μL of potassium iodide, and 20 μL of acetic acid. AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents (μmol/L).

4.6. Serum Paraoxonase-1 (PON1) Enzyme Activity. This was measured by adding serum to Tris buffer (100 mmol/L, pH 8.0) containing 2 mmol/L $CaCl_2$ and 1 mmol/L paraoxon (*O*,*O*-diethyl-*O*-nitrophenylphosphate . The rate of generation of *p*-nitrophenol was determined at 405 nm, 25 °C with the use of an Ultro spec 1000 (Pharmacia Biotech) spectrophotometer as described previously.[14]

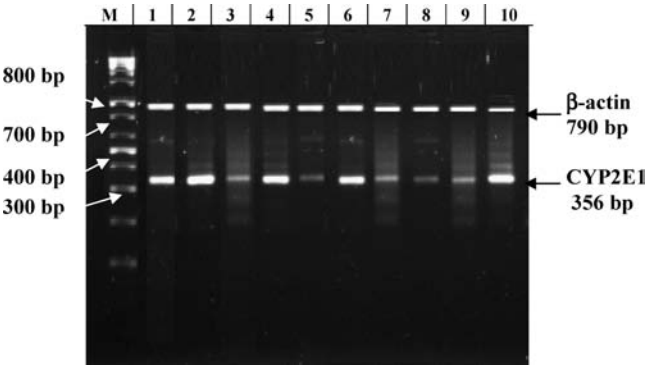

Figure 1. RT-PCR of CYP2E1 expression. Lane M: 100 bp size DNA marker. Lanes 1−10 show CYP2E1 bands (356bp) with different intensity and β-actin bands (790) which served as internal control. Lanes: 1, 2, 4, 6 and 10 represent group II. Lanes 3 and 9: group III. Lane 5: group IV. Lane 8: group I.

4.7. Liver Tissue Malondialdehyde (MDA). This was measured according to Halliwell and Chirico[15] and depends on MDA reaction with thiobarbituric acid (TBA) producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm. MDA was expressed as nmol/g tissue and was calculated using 1.65 × 105 M^{-1} cm^{-1} as molar absorption coefficient.

4.8. Liver Tissue GPx Activity. This was measured according to Flohe and Gunzler[16] based on the coupling reaction with glutathione reductase (GR). The oxidized glutathione (GSSG) formed after reduction of hydroperoxide by GPx is recycled to its reduced state by GR in the presence of NADPH. The oxidation of NADPH is accompanied by a decrease in absorbance at 340 nm. One unit of GPx was defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of NADPH per minute at 25 °C. The enzyme activity was calculated using a molar extinction coefficient for NADPH of 6.220 M^{-1} cm^{-1} and normalized to protein concentration, and it was expressed as nanomoles of NADPH consumed per minute per milligram of protein.

4.9. Hepatic Microsomal CYP2E1 Activity. This was estimated spectrophotometrically by measuring the hydroxylation of *p*-nitrophenol to produce *p*-nitrocatechol according to Reinke and Moyer.[17] Briefly, 200 μL of microsomes (equivalent to 1−2 mg of protein) was mixed with 0.2 mM *p*-nitrophenol, 50 mM Tris- HCl and 5 mM $MgCl_2$ (pH 7.4). The reaction was performed at 37 °C and was started by the addition of 1 mM NADPH. After 10 min, the reaction was stopped with 0.5 mL of 0.6 N perchloric acid. The precipitated proteins were removed by centrifugation at 3000*g* for 5 min, 1 mL of the supernatants was mixed with 100 μL of 10 N NaOH, and absorbance was read at 510 nm. An extinction coefficient of 9.53 mM^{-1} cm^{-1} for *p*-nitrocatechol was used, and the result was expressed as nanomoles of *p*-nitrocatechol formed/min/mg protein.

4.10. Protein Content in Tissue Homogenate. This was determined by the Lowry method using bovine serum albumin as a standard.[18]

4.11. Gene Expression of Cytochrome P450 2E1

4.11.1. Preparation of Total Liver RNA. Total RNA was prepared from liver tissue according to the manufacturer's instructions (Promega). Approximately 100−200 mg of liver tissue was homogenized and subjected to RNA extraction to disrupt cells followed by denaturation of nucleoprotein complexes, inactivation of endogenous ribonuclease (RNase) activity, and finally removal of proteins and DNA. The resulting RNA was reconstituted in diethyl pyrocarbonate-treated water (DEPC-H_2O), checked for purity and integrity spectrophotometrically at 260 nm and by gel electrophoresis and used for RT-PCR.

Table 1. Comparison between the Studied Groups Regarding Body Weight, Fasting Plasma Glucose, Lipid Profile, Hepatic Enzyme Activities and Level of β-Hydroxybutyrate[a]

parameter	G-I	G-II	G-III	G-IV	p
final body weight (g)	152.0 ± 18	95.0 ± 21[b]	116.3 ± 18. 2[b]	115.5 ± 17[b]	<0.001; all are sign. except II vs III, II vs IV, III vs IV
final fasting plasma glucose level (mg/dL)	101.0 ± 11	300.0 ± 20[b]	285.4 ± 10.5[b,]	180.7 ± 15.2[b,c]	<0.001; all are sign. except III vs II
triglyceride (mg/dL)	123.2 ± 19.7	145.8 ± 15.8[b]	122.6 ± 21.1[c]	118.8 ± 17.9[c]	<0.001; all are sign. except I vs III, I vs IV, III vs IV
total cholesterol (mg/dL)	79.4 ± 12.8	122.7 ± 24.8[b]	92.7 ± 14.9[c]	82.3 ± 6.1[c]	<0.001; all are sign. except I vs III, I vs IV, III vs IV
ALT (U/L)	30 ± 2	40 ± 3[b]	34 ± 5[c]	31 ± 3[c]	<0.001; all are sign. except I vs III, I vs IV
AST (U/L)	35 ± 3	50 ± 4[b]	39 ± 4[c]	38 ± 3[c]	<0.001; all are sign. except I vs III, I vs IV, III vs IV
β-hydroxybutyrate (mmol/L)	0.7 ± 0.2	4.5 ± 0.3[b]	2.9 ± 0.3[b,c]	1.8 ± 0.4[b,c]	<0.001; all groups are sign.

[a] Values are expressed as mean ± SD, for ten animals in each group; G-I = control group; G-II = untreated STZ induced diabetic group; G-III = melatonin treated diabetic group; G-VI = taurine treated diabetic group. [b] Significantly different from the control group ($p < 0.05$). [c] Significantly different from the STZ treated group ($p < 0.05$).

Table 2. Comparison between the Studied Groups Regarding Oxidative Stress Parameters and Hepatic CYP2E1 Activities[a]

parameter	G-I	G-II	G-III	G-IV	p
plasma AOPP (μmol/L)	24.4 ± 4.2	30.3 ± 6.2[b]	24.7 ± 4.4[c]	24.9 ± 2.1[c]	<0.01; all are sign. except I vs III, I vs IV, III vs IV
serum PON 1 activity (nmol/min/mL)	13.6 ± 3.1	6.9 ± 2.5[b]	14.2 ± 2.8[c]	15.1 ± 2.5[c]	<0.001; all are sign. except I vs III, I vs IV, III vs IV
liver MDA (nmol/g tissue)	23.6 ± 3.1	31.6 ± 5. 5[b]	24.3 ± 3.8[c]	23.9 ± 2.8[c]	<0.001; all are sign. except I vs III, I vs IV, III vs IV
liver GPx activity (nmol of NADPH oxidized/min/mg)	7.9 ± 1.1	4.8 ± 1.1[b]	5.8 ± 1.4[b]	6.5 ± 1.5[c]	<0.001; all are sign. except I vs IV, II vs III, III vs IV
hepatic CYP2E1 activities (nmol/min/mg protein)	2.1 ± 0.6	5.2 ± 1.6[b]	3.5 ± 0.7[b,c]	2.7 ± 0.8[c]	<0.001; all are sign. except I vs IV, III vs IV

[a] Values are expressed as mean ± SD, for ten animals in each group; G-I = control group, G-II = untreated STZ induced diabetic group; G-III = melatonin treated diabetic group; G-VI = taurine treated diabetic group. [b] Significantly different from the control group ($p < 0.05$). [c] Significantly different from the STZ treated group ($p < 0.05$).

4.11.2. RT-PCR for CYP2E1. Reverse transcription of 10 μg of RNA to the corresponding amount of cDNA was carried out according to the manufacturer's instructions (Promega, USA), and the material was stored at −20 °C until use. Amplification of cDNA and the selection of the primer sequences were done according to Yang and Raner.[19] The PCR reactions of cDNA were performed in a final volume of 50 μL consisting of 5 μL of cDNA, 5 μL of 10X PCR buffer, 200 μM of each deoxynucleotide triphosphate, 2.5 mM $MgCl_2$, 1.5U AmpliTaq DNA polymerase (Promega,USA) and 50 pmol each of the following CYP2E1 specific forward and reverse primers (Life Technologies, USA): forward, 5′-TGCCATCAAGGATAGGCAAG-3′; reverse, 5′-AATGCTGCAAAATGGCACAC-3′. PCR reactions were carried out in a Perkin-Elmer 9600 thermal cycler starting with heating at 95 °C for 4 min and then using melting, annealing, and extension cycling conditions of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 2 min respectively . All amplifications were carried out for 30 cycles followed by final extension time at 75 °C for 4 min. All reactions were conducted with β-actin primers (790 bp) as internal controls (5′primer, 5′TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA 3′; 3′primer, 3′CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG 5′, Stratagene).

An aliquot of 20 μL from each amplified cDNA product (356 bp) was separated on 2% agarose gel (Sigma), and visualized by UV light illuminator using ethidium bromide staining and photographed by Polaroid film. The intensity of bands was quantified by gel proanalyzer image analyzer version III where the area of a selected band was blotted against calibration curve of a single image using multiple standard bands to give the band amount in nanograms (ng), and the results were analyzed by this computer software (Biorad, U.K.) (Figure 1).

5. Statistical Analysis. All the values are expressed as mean ± SD. Statistical analyses were performed using Student's *t* test for comparing two groups. When comparing more than 2 groups, one-way analysis of variance followed by Tukey's post hoc test (multiple comparisons) was performed. A Spearman rank correlation was performed to determine the correlation between the studied parameters. A *p*-value <0.05 was considered significant. SPSS version 10.0 was used for all statistical analysis.

RESULTS

Body Weight, Plasma Glucose Levels, Lipid Profile, Hepatic Enzyme Activities and β-Hydroxybutyrate. Table (1) summarizes the mean changes in body weights, plasma glucose levels, lipid profile, hepatic enzyme activities and β-HB in all the studied groups. Before the commencement of this study, all the groups had similar body weight, comparable levels of plasma glucose and lipid profiles (data not shown).

After STZ administration, rats demonstrated the typical characteristics of type 1 diabetes such as increase in food and water intake and polyuria (data not shown); the body weights were significantly decreased and the plasma glucose level and serum cholesterol and triglyceride concentrations were significantly increased in diabetic rats (G-II), when compared to control rats (G-I).

Administration of melatonin (G-III), or taurine (G-IV) significantly alleviated the hyperlipidemia, and restored the body weight slightly nonsignificantly as compared to G-I (but higher body weight than that of diabetes group (G-II)), but regarding plasma glucose, melatonin induced insignificant changes, however taurine induced a significant decrease in plasma glucose level as compared to G-II with still significant differences with G-I.

Of note, STZ-induced diabetes caused an increase in the ALT and AST activities in the serum, and in plasma β-HB level. However, melatonin or taurine supplementation not only reduced the ALT and AST serum levels but also significantly reduced plasma β-HB (with marked decrease in G-IV compared to G-III) (Table 1).

Table 3. Correlation between Hepatic CYP2E1 Activities, Gene Expression and Other Studied Parameters in Diabetic Groups (n = 30)

	hepatic CYP2E1 activities (nmol/min/mg protein)		CYP2E1 gene expression	
variables	r	p	r	p
β-hydroxybutyrate (mmol/L)	0.49	<0.001	0.50	<0.001
plasma AOPP (μmol/L)	0.37	<0.05	0.40	<0.05
serum PON 1 activity (nmol/min/mL)	−0.40	<0.05	0.43	<0.05
liver MDA (nmol/g tissue)	0.39	<0.05	0.45	<0.01
liver GPx activity (nmol of NADPH oxidized/min/mg)	−0.42	<0.05	0.48	<0.001
hepatic CYP2E1 activities (nmol/min/mg protein)			0.50	<0.001

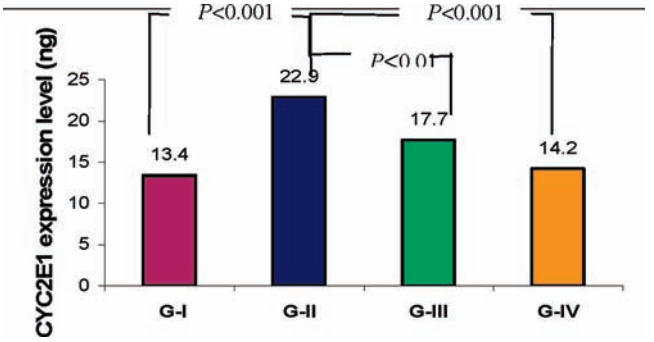

Figure 2. Comparison of CYC2E1 RT-PCR expression between the studied groups: G-I = control group; G-II = untreated STZ induced diabetic group; G-III = melatonin treated diabetic group; G-IV = taurine treated diabetic group.

Oxidative Stress. Table 2 shows that plasma AOPP and liver MDA (oxidative stress markers) were significantly elevated in G-II compared with G-I. Melatonin or taurine decreased significantly their levels as compared with G-II, with no significant differences as compared with G-I, while serum PON1 and hepatic GPx were significantly decreased in G-II as compared with the control, and melatonin or taurine caused significant increase in serum PON1 activities (with marked increase in G-IV), but only taurine treatment can elevate liver GPx to near normal, with no significant effect for melatonin..

Catalytic Activity and Expression of CYP2E1. In Table 2 liver microsome CYP2E1 activities in diabetic rats were significantly higher than those in nondiabetic rats. Taurine or melatonin supplementation in diabetic rats caused significant decrease in CYP2E1 activity in liver microsomes as compared with G-II with no significant differences for taurine supplementation, but still significant differences for melatonin supplementation when compared with G-I.

A significant correlation exists not only between CYP2E1 activities and gene expression but also between both CYP2E1 activities and gene expression and the studied parameters (Table 3).

The expression of CYP2E1 in different studied groups is represented in Figures 1and 2. Similar to the results of enzyme activity, there were significant differences between all the studied groups except between G-I and G-IV (with $F = 23.1$, $P < 0.0001$), in which hepatic CYP2E1 mRNA expression in untreated diabetic rats (22.9 ± 4.8 ng) was significantly increased ($P <$ 0.001) as compared to nondiabetic control (13.4 ± 0.9 ng). Melatonin and taurine supplementation in diabetic rats caused significant decreases in hepatic CYP2E1 mRNA (17.7 ± 2.3 ng and 14.2 ± 1.8 ng, respectively) compared to levels in untreated diabetic rats but with more significant decrease ($P < 0.001$) in taurine than in melatonin (Figure 2).

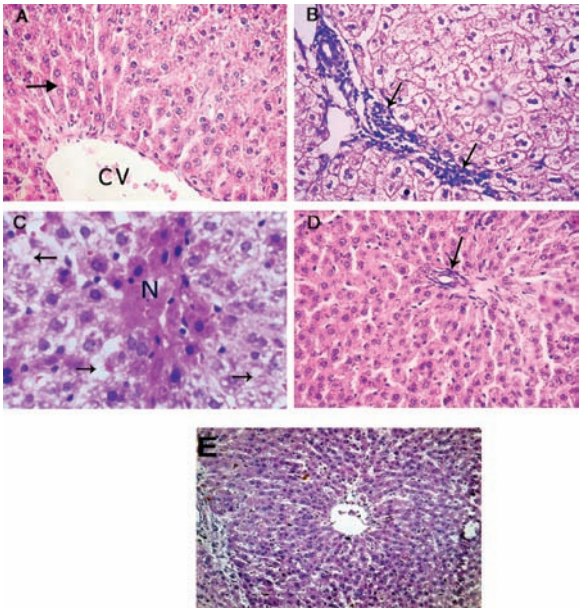

Figure 3. Light microscopical photomicrograph of liver tissue of control group (A) showing the normal hepatocytes with rounded nuclei forming hepatic cell cords (→) radiating from the central vein (CV), liver tissue of diabetic rat (B) showing the hypertrophied and vacuolated hepatic cells and the heavy cellular infiltration (→) and (C) shows small areas of focal necrosis and vacuolated hepatocytes. Liver tissue of diabetic rat (D) treated with taurine, treated with melatonin (E) showing the apparently normal hepatic cells with few inflammatory cells around a blood vessel (→) (H&E, magnification ×400).

Histopathological Pictures of the Liver. These are presented in Figure 3A−E.

DISCUSSION

Both human and experimental animal models of diabetes exhibit high oxidative stress due to persistent and chronic hyperglycemia. It has been shown that dietary supplementation with natural antioxidants attenuated the oxidative stress and diabetic state induced by STZ.[5]

In this study, in STZ induced diabetic rats, decreased body weight and increased plasma glucose level were observed; neither melatonin nor taurine was able to prevent weight loss, but taurine has the capability to return plasma glucose to near normal level (Table 1), which may be due to a diminished rate of renal

gluconeogenesis,[20] inhibition of oxidative stress[21] or the potentiation of the effects of insulin.[22] On the other hand, melatonin did not significantly prevent the development of hyperglycemia in the STZ-induced diabetic rats similar to the results of other researchers.[23,24] Also, treatment of the diabetic rats with either melatonin or taurine resulted in a marked decrease in serum triglycerides, total cholesterol and liver enzymes levels with more decrease in the taurine treated group, which may be due to upregulation of hepatic LDL receptor and/or through improving the binding of LDL to them.[25]

Furthermore, elevated liver MDA levels (Table 2) in STZ induced diabetic rat demonstrated herein were reduced after melatonin or taurine treatment; it could be related to their direct antioxidant effects through their direct scavenging action.[20−26] In the current study the significant higher plasma AOPP levels reported in untreated diabetic rats came in agreement with those of Kalousova et al.,[27] but upon melatonin or taurine treatment, AOPP formation could be decreased similar to the studies of Eskiocak et al.[28] and Kilic and Yildirim.[29] The high level of lipid peroxidation marker in the diabetic rats is a reflection of insufficiency of antioxidant defenses in combating ROS-mediated damage.[30] In this study, the hepatic GPx activity (Table 2) was decreased in the diabetic tissues, which may be due to hyperglycemia, leading to glycation of antioxidant enzymes causing its inactivation.[30] However, upon taurine treatment hepatic GPx activity was increased, which came in accordance with Franconi et al.;[31] since cysteine is a precursor of taurine and GSH, taurine supplementation may cause enhancement in GSH levels by directing cysteine into the GSH synthesis pathway, but melatonin has no significant effect, and this came in accordance with the result of Oktem et al.[32] However, regarding PON1, it was significantly lowered in diabetic rats, which might be related to increased lipid peroxide levels and/or glycation of HDL which may increase its turnover and reduce its efficiency. It was reported that there are some inhibitors against PON1 activity in circulating blood of diabetic patients such as glycosylated proteins.[33] Low concentration and enzymatic activity of PON1 were thought to be independent predictors of cardiovascular events specially in diabetic patients.[34] However, it was observed that taurine or melatonin supplementation increased serum PON1 activities in STZ induced diabetes in rats, which came in agreement with Dirican et al.,[35] Tas et al.,[36] and El Mesallamy et al.[37] Because lipid peroxidation products have been reported to inhibit PON1 activity, the reduced tissue MDA levels, which reflected an improvement in the oxidative−antioxidative balance, may be a contributing factor to the increased serum PON1 activities.[38] This effect may be one of the antiatherosclerotic activities of taurine, so, taurine supplementation may be beneficial for diabetic patients with decreased PON1 activity to make them less susceptible to coronary heart diseases. Furthermore, Topsakal et al.[39] reported that melatonin may play a role in increasing the reduced PON1 concentrations by improving the glucose metabolism and/or oxidative stress.

CYP2E1 is inducible in several pathophysiological states including diabetes, and its expression parallels increased ROS production and oxidative stress in various tissues of STZ-induced diabetes rats.[3] In the current study, both the hepatic CYP2E1 enzyme activity and gene expression (Table 2, Figures 1 and 2), were increased in STZ-induced diabetic rats as compared with control and both were significantly correlated with the studied oxidative stress parameters and with β-hydroxybutyrate (Table 3). These results supported previous observations on the CYP2E1-dependent liver injury in STZ-induced diabetic rats.[40] This link stems from the unusually high capacity of CYP2E1 to generate free radicals which are thought to result in lipid peroxidation and thus contribute to liver disease.[41] Diabetes is commonly associated with the development of nonalcoholic steatohepatitis. Therefore, it is possible that the increased expression of CYP2E1 can lead to liver disease in diabetics.[42] Also, it has been suggested that the increased ketones and other small organic molecules in diabetes act as inducers of CYP2E1. Although the mechanism is not fully understood, it may be related to an enhanced rate of gene transcription.[43] Therefore, this study further demonstrated that the production of CYP2E1 gene expression and enzyme activity can be prevented not only by taurine but also by melatonin supplementation, which is less than that of taurine, so they can protect the liver from the hepatic injury induced by type 1 diabetes. In correlation with these, this study showed that elevated levels of ketone bodies (β-HB) by STZ were reduced by taurine more than melatonin supplementation, so that the suppression of ketone production can be one of the mechanisms for reduction in CYP2E1 gene expression in diabetic rats. These results came in agreement with Yao et al.,[9] who showed that taurine supplementation caused a significant decrease in plasma β-HB levels in diabetic rats compared with untreated diabetic animals. Furthermore, the exact mechanism of inhibition of diabetes-induced CYP2E1 elevation by taurine warrants further studies.

Finally in the present study, the hepatic protective role of melatonin and taurine in STZ-induced diabetic liver damage was established by normalization of hepatic histological picture upon treatment of STZ induced diabetes by either taurine or melatonin (Figure 3). So we can conclude that taurine has the capabilities more than melatonin in protecting the liver from hepatic injury induced by type 1 diabetes, by either reducing oxidative stress or restoring CYP2E1 activity and gene expression. Therefore antioxidants might prove beneficial as an adjuvant treatment to insulin in type 1 diabetes associated with manifestations of liver injury.

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ABBREVIATIONS USED

AOPP, advanced oxidation protein product; ALT, alanine aminotransferase; AST, aspartate aminotransferase; β-HB, β-hydroxybutyrate; CYP2E1, cytochrome P450 2E1; GPx, glutathione peroxidase; MDA, malondialdhyde; PON1, paraoxonase-1; ROS, reactive oxygen species; STZ, streptozotocin

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