HISTOCHEMCAL AND BIOCHEMICAL EFFECTS OF MELATONIN ON PANCREATIC β-CELLS OF STREPTOZOTOCIN-TREATED DIABETIC RATS

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Running Title:

Protective effects of melatonin on pancreatic β-cells and enzymes of carbohydrate metabolism.

ABSTRACT

The present study was undertaken to investigate the effects of melatonin on (i) enzymes of carbohydrate metabolism [i. e., glucose-6-phosphate dehydrogenase (G-6-PDH), succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH)], (ii) pancreatic β-cell integrity, (iii) blood glucose concentrations, plasma insulin, triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, and (iv) oxidative stress experimentally-induced by streptozotocin (STZ) treatment in Wistar rats. Fifty male and female Wistar rats (200-250 g) were randomly divided into three experimental groups (i. e., control, STZ-treated, and STZ + melatonin-treated groups). Diabetes was induced in the diabetic animal groups by single intraperitoneal injections of STZ (75 mg/kg body weight), while 'control' animals received equal volumes of intraperitoneally-administered citrate buffer (pH 6.3) solution. In the third group of animals, melatonin (200 μg/kg/day, s.c.) was administered for 3 days prior to administration of STZ; and melatonin treatment was continued until the end of study period (30 days). Diabetes mellitus was confirmed by using Bayer Elite® Glucometer and compatible blood glucose test strips. The experimental animals were sacrificed at different time intervals (10^{th}, 20^{th} and up to 30^{th} day following treatment with STZ). The pancreases of the rats were excised and randomly processed for histochemical staining and pancreatic insulin content. In the diabetic state, pancreatic β-cell degeneration and weak histochemical staining for G-6-PDH, SDH, and LDH were observed. Treatment of the diabetic rats with melatonin markedly increased histochemical staining intensity of the enzymes. Biochemically, the specific activities of G-6-PDH, SDH and LDH were also significantly increased ($p<0.05$). Similarly, LDL, pancreatic insulin, and triglyceride contents were significantly increased ($p<0.05$). However, melatonin induced a significant decrease ($p<0.05$) in blood glucose concentrations and HDL of the animals. In summary, the findings of the present study indicate that melatonin has a protective effect on oxidative stress induced by STZ treatment in rats, and also preserves pancreatic β-cell integrity.

Key Words:

Diabetes mellitus, Streptozotocin, Melatonin, Glucose-6-phosphate dehydrogenase, Succinic dehydrogenase, Lactic dehydrogenase; Triglyceride and Cholesterol.

INTRODUCTION

Streptozotocin (STZ) is a specific pancreatic β-cell toxin, and it can be used to induce experimental diabetes mellitus in mice, rats and other laboratory, experimental animals. The precise mechanism of STZ-induced diabetes is unknown, but it has been suggested that oxygen radicals, especially hydroxyl radicals (OH^-), may be involved [1, 2]. Streptozotocin is believed to be taken up by pancreatic β-cells through the glucose transporter Glut-2 [3] where it decomposes intracellularly causing DNA damage; directly by alkylation [4], and indirectly via generation of nitric oxide (NO) [5], resulting in pancreatic β-cell death by necrosis [6]. There are two STZ-induced animal models of diabetes; (a) administration of a single, relatively high dose of STZ which induces diabetes within 48 h by directly destroying pancreatic β-cells, and (b) administration of multiple-low-doses of streptozotocin (MLDS) which causes gradual β-cell damage resulting in an immune cell response directed towards β-cells. MLDS is characterized by a progressive hyperglycemia and insulitis similar to that observed in a recent onset of type 1 diabetes [7, 8]. Islets infiltrating immune cells produce cytokines, nitrogen, and oxygen free radical species which cause pancreatic β-cell destruction [9]. Cytokine treatment of islets leads to β-cell expression of the inducible isoform of nitric oxide synthase (iNOS) [10], and subsequent production of nitric oxide (NO) and related free radical species. Cytokine-induced NO formation in β-cells inhibits insulin secretion [11], decreases cellular ATP levels [12], increases cyclic GMP levels [13], DNA damage [14] and cell death [15]. Chemically generated free radicals such as NO, superoxide and peroxynitrite, a potent reactive oxidant formed from the rapid reaction of NO and superoxide [15, 16], have been shown to functionally inhibit pancreatic β-cells. If cellular antioxidants are low, or the production of free radical species exceeds antioxidant defenses, oxidative stress develops [17]. Many diabetic complications that are provoked by oxidative stress usually develop into diabetes mellitus. Oxidative stress in diabetes mellitus is increased either by free radicals produced during increased glucose auto-oxidation, or by the corruption in regeneration of natural antioxidants.

Diabetes mellitus is associated with a high oxidative stress level, resulting from an imbalance between free radicals or reactive oxygen species (ROS) production, and the antioxidant systems [18]. Inhibition of these oxidative processes by co-adjuvant therapy could, therefore, prevent or at least delay, the onset and/or the development of long-term diabetic complications [19]. Chronic elevation of blood glucose impairs glucose-stimulated insulin secretion [20, 21, 22], and puts pancreatic islets at risk for oxidative damage [23]. Pancreatic β-cells are sensitive to oxidative stress because their intracellular antioxidative defense mechanisms are weak [24, 25]. Both *in vivo* rodent models of type 2 diabetes and *in vitro* type 1 diabetes, as well as high glucose concentrations increase the level of reactive oxygen species (ROS), leading to oxidative stress within the pancreatic islets [26, 27]. Thus, hyperglycemia induces chronic oxidative injury, which is initially responsible for impairment of insulin secretion and ultimately leads to pancreatic β-cell apoptosis. In the case of type 1 diabetes, increased glucose levels are associated with increased β-cell generation of cytokines [28], which are pro-oxidants. In a model of type 2 diabetes, high glucose concentrations increased intracellular peroxide levels in the islets [27]. This raises questions about the state of antioxidant host defenses within the islets, and whether augmentation of these defenses might be an appropriate therapeutic strategy to lessen

the impact of diabetes and hyperglycemia on pancreatic β-cells. Unfortunately, the islet is among the least well-endowed tissues in terms of intrinsic antioxidant enzyme expression, including superoxide dismutase (SOD-1, SOD-2), catalase, and glutathione peroxidase [24, 25]

Glucose-6-phosphate dehydrogenase (G-6-PDH) catalyzes the first step of the pentose phosphate pathway, producing reduced nicotinamide adenine dinucleotide (NADPH), the principal cellular reductant in all cell types. Although there are other metabolic pathways that produce NADPH, such as NADP-linked malic enzyme, which oxidatively decarboxylates malate to pyruvate, and mitochondria enzyme, NADP-linked isocitrate dehydrogenase. G-6-PDH is the predominant source of cellular defense against oxidative stress [29, 30]. Sekine *et al*. [31] have reported the low affinity of pancreatic islet cell membranes for the transport of lactate, which plays a potential role in nutrient sensing with subsequent insulin release.

Several years ago, it was recognized that the pineal hormone, melatonin, is the most efficient free radical scavenger and antioxidant [32, 33, 34]. Melatonin mediates physiological and endocrinological processes, particularly reproduction, and has several functions in terms of its antioxidant ability. Because of its high lipid solubility, it readily scavenges the most toxic free radicals, and detoxifies peroxy-nitrite anion, nitric oxide, singlet oxygen, and the peroxyl radical [32]. It may also stimulate several antioxidative enzymes, including superoxide dismutase and glutathione peroxidase. All these actions contribute to melatonin's ability to reduce oxidative damage [35, 36, 37, 38]. Melatonin's role in glucose metabolism and in diabetes mellitus is controversial. In rodents, the role of melatonin in glucose metabolism has been investigated using either exogenous melatonin administration, or a chronic suppression of endogenous melatonin synthesis by the pineal gland through surgical pinealectomy. Some investigators have reported that melatonin increases glycemia [39], whereas others have shown no effect [40, 41, 42], or even a decrease [43]. In rabbits, melatonin induces a decrease in plasma glucose concentration in basal condition, while it enhances hyperglycemia after a glucose load [44]. More recently, some investigators have shown that administration of melatonin prior to STZ treatment decreased serum glucose level in mice [45].

Because of the importance of the enzymes of carbohydrate metabolism in the generation of the principal cellular reductant (NADPH) against oxidative stress, not only to the pancreatic β-cells but also to all cell types, it was thought worthwhile to investigate the effects of melatonin as a scavenger on oxidative stress induced by STZ treatment on the cellular integrity and enzymatic activities of the pancreas.

MATERIALS AND METHODS

Animals

Fifty male and female Wistar rats (*Rattus norvegicus*), three months old and weighing 200-250 g, were randomly assigned to three (A, B and C) groups of 'test' and 'control' rats. They were kept and maintained under laboratory conditions of temperature, humidity and light; and were allowed free access to standard laboratory diet pellets (Ladoke Feeds, Ibadan, Nigeria) and water *ad libitum*. All the animals were fasted for 16 hours, but still allowed free access to water, before the

commencement of our experiments. The control group of animals (C) consisted of ten rats, while the treatment groups consisted of twenty rats each. Group A (20) rats received STZ (75 mg/kg i.p.) only, while Group B (20) rats received STZ (75 mg/kg i.p.) plus melatonin (200 μg/kg/day, s.c.).

Induction of experimental diabetes

Diabetes mellitus was induced (in groups A and B diabetic 'test' rats) by single intraperitoneal injections of STZ (75 mg/kg, freshly dissolved in 0.1mol/l citrate buffer, pH 6.3). Melatonin (200 μg/kg/day s. c.) was administered to group B diabetic rats, daily for 3 days before STZ treatment, and it was continued until the end of the study period (30 days). Control rats were injected with equal volume of citrate buffer solution intraperitoneally. The 'test' animals in both groups A and B became diabetic within 48 hours after STZ administration. Diabetic state was confirmed by measuring blood glucose concentrations 48 hours after STZ injection. At the end of the 16 hour fasting period – taken as zero time (i. e., 0 hour), blood glucose levels (initial glycaemia – G_o) of the fasted normal (normoglycemic) and STZ-treated, diabetic (hyperglycemic) rats were determined and recorded.

Determination of Blood Glucose Concentrations

Blood samples (20 μl each) were obtained from the caudal vein of the animals for determination of blood glucose levels. The samples were obtained 1 day before STZ-treatment, and on various days after induction of diabetes mellitus. Diabetes was allowed to develop and stabilize in the STZ-treated rats over a period of 1 day to 30 days. Blood glucose concentrations were determined by means of Bayer Elite® Glucometer and compatible blood glucose test strips [46]. The mean fasting blood glucose levels for non-diabetic rats were found to vary between 4.01±0.04 and 4.20±0.13 mmol/L. Fasted STZ–treated rats with blood glucose concentrations ≥18 mmol/L were considered to be diabetic, and used in this study. Differences in glucose concentrations between groups were analyzed statistically by one-way analysis of variance (ANOVA; 95% confidence interval), followed by Scheffe's multiple range comparison test. Differences were considered significant when the P value was less than 0.05. The rats received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by National Academy of Sciences, and published by the National Institutes of Health, Maryland, USA.

Pancreatic insulin content determination

The splenic regions of the pancreatic tissues from euthanized rats were weighed and homogenized, on various experimental days, in acid-ethanol solution (75% ethanol, 23.5% distilled water, 1.5% concentrated HCl). After overnight incubation at 4°C, the suspensions were centrifuged, and the supernatants were collected and assayed for insulin content, using a competitive ELISA kit [47]. Plates were coated with rabbit anti-guinea-pig Ig secondary Ab (Organon Teknoka, Durhan, NC), followed by incubation with a guinea-pig anti-human insulin Ab (Cortex Biochem, San Leandro, CA). Following two washing steps, various extract dilutions or insulin standards

(Linco Research, St. Louis, MO) were mixed with constant concentration of HRP-conjugated rat insulin (Organon Teknika) for 4 h at room temperature, or at 4^{o}C overnight, before competitive capturing was allowed for 3 h. After washing five times, Sigma FAST OPD tablets (Sigma, St. Louis, MO) were used as substrate. Results were analyzed using ceres 900 C ELISA-reader.

Plasma Insulin

Blood samples were collected sequentially before and on experimental days. The plasma was separated by centrifugation, using polyethylene glycol, and plasma insulin concentration was determined by Radioimmunoassay (RIA), with an anti-porcine insulin anti-body (INC; Biomedicals, Orsay, France) and pure rat insulin (Novo, Copenhagen, Denmark) as the reference standards [48, 49].

2.6. Histochemical Analysis of Islets of STZ-Treated Wistar rats

Pieces from snap-frozen pancreatic tissues were serially cut in a cryostat [50]. Sections of 10 μm-thick (using Lipshaw cryostat) were mounted onto the slide and incubated by the respective histochemical reaction. Several successive measurements were taken after starting the reaction by applying the assay mixture and covering the section with a coverslip. The following enzymes of carbohydrate metabolism were demonstrated: G-6-PDH, SDH and LDH [51, 52, 53], respectively.

2.7. Biochemical Estimation

The pancreatic tissues were put immediately in isotonic 1.1% potassium chloride solution and stored at –33^{o}C if not used immediately. The tissues were homogenized using ten volumes of cold 1.1% potassium chloride. All operations were carried out at -4^{o}C. The homogenates were centrifuged at 12,000 x g for 10 min, and the clear supernatants collected were used for G-6-PDH, SDH and LDH enzyme determination [54, 55, 56], respectively.

2.8. Determination of Plasma Cholesterol, Lipoproteins and Triglyceride

Whole blood was centrifuged at 4^{o}C for 20 min at 1600 × g to obtain plasma. Aliquots of plasma were taken for determination of total cholesterol, and plasma triglyceride were enzymatically determined [57, 58]. Plasma high density lipoprotein (HDL)-cholesterol was isolated by precipitation of lower densities with sodium phosphotungstate and magnesium chloride [59]. Estimation of low density lipoprotein (LDL)-cholesterol was done by using the equation of Friedeward *et al;* [60], as shown bellow.

LDL – Cholesterol = Total plasma cholesterol – HDL – cholesterol – (0.2 × Plasma triglyceride).

2.9. Statistical analysis

Data obtained from 'test' rats treated with STZ and STZ plus melatonin, as well as those obtained from citrate buffer-treated 'control' rats, were pooled and expressed as means (±SEM). The differences between them were analyzed statistically with one-way analysis of variance (ANOVA; 95% confidence interval), followed by Scheffe's multiple range comparison test. Values of $p<0.05$ were taken to imply statistical significance.

RESULTS

Characteristics of diabetic state

Forty-eight hours after STZ (75 mg/kg i.p.) administration, all animals that had been treated with STZ displayed glucosuria, ketonurea, hyperglycemia, hypoinsulinemia and a moderate loss of body weight. The pancreatic weight, plasma glucose, plasma insulin, and pancreatic insulin contents of both STZ-treated diabetic and non-diabetic rats were either significantly increased or decreased ($p<0.05$) in value respectively (Table 1).

3.2. Blood glucose and plasma insulin concentrations

In our control set of experiments, pretreatment of the rats with citrate buffer alone did not significantly modify ($p>0.05$) the plasma insulin and blood glucose concentrations of either the fasted normal or diabetic rats. As shown in Fig. 1, there was a gradual rise in blood glucose concentrations of the STZ-treated rats as from day 1 following injection of STZ, and the values were significantly higher ($p<0.05$) than those of control animals. Furthermore, high levels of blood glucose concentrations of the STZ-treated rats were persistently observed until the end of study period (20.2±0.1 mmol/l - Table 2). The blood glucose concentrations of group B rats treated with STZ plus melatonin significantly reduced in value ($p<0.05$) as from day 5 and throughout the period of melatonin treatment (4.5±0.2 mmol/l - Table 2). The plasma insulin concentration and pancreatic insulin contents were significantly decreased ($p<0.05$) in the STZ-treated diabetic rats. Significant reduction in blood glucose (55 to 60%-fold) became even more pronounced as from day 15, and remained high throughout the study period. Group B (STZ + melatonin-treated) rats maintained relatively normal insulin concentration level. Their insulin values were not significantly different ($p>0.05$) from those of control animals (Table 1).

Table 1.

Various parameters recorded in diabetic (40) and normal (10) rats just before and after removal of pancreas. Values given represent the means (± SEM) of at least 10 observations.

Treatment groups	Body weight (g)	Pancreas weight (g)	Plasma glucose (mmol/L)	Plasma insulin contents (μU/ml)	Pancreatic insulin contents (μU/mg)
Control rats	246.6±0.5	1.48±0.06	4.10±0.6	12.65±2.10	15.55±2.52
STZ diabetic rats	186.3±0.3*	0.74±0.08*	18.23±0.25*	5.25±1.12*	4.82±1.25*
STZ diabetic rats +melatonin	238.4±0.4	1.23±0.10	5.41±0.3	10.82±1.4	13.26±1.8

*p<0.05 vs control.

Table 2.

Changes in blood glucose concentrations, plasma and pancreatic insulin contents of group A diabetic rats treated with STZ, and group B rats treated with STZ + melatonin. Values given represent the means (±SEM) of 10 observations.

Experimental days	STZ diabetic rats			STZ diabetic rats + melatonin		
	Blood glucose (mmol/l)	Plasma insulin (μU/ml)	Pancreatic insulin (μU/mg)	Blood glucose (mmol/l)	Plasma insulin (μU/ml)	Pancreatic insulin (μU/mg)
0	4.1±0.4	12.1±0.4	15.3±0.6	4.2±0.5	12.1±0.4	15.3±0.6
5	16.4±0.4*	8.3±0.5	10.4±0.3	5.2±0.4	10.4±0.2	13.6±0.1
10	18.2±0.3*	6.4±0.3	8.6±0.2	4.8±0.3	10.6±0.3	13.9±0.5
15	18.8±0.6*	5.1±0.6*	6.9±0.4*	4.4±0.2	11.1±0.1	13.8±0.3
20	19.6±0.1*	4.8±0.2*	5.7±0.3*	4.6±0.5	11.6±0.4	14.6±0.2
25	20.2±0.4*	3.8±0.4*	5.4±0.5*	4.2±0.6	11.8±0.6	14.2±0.4
30	19.6±0.2*	3.5±0.2*	4.8±0.6*	4.5±0.2	11.2±0.5	14.8±0.3

*p<0.05 vs control

Table 3.

Biochemical estimation of G-6-PDH, SDH and LDH in the pancreatic β-cells of control, STZ- treated, and STZ + melatonin-treated rats. Values given represent the mean (±SEM) of at least 8 observations.

		Control rats	STZ-treated diabetic rat	STZ-treated diabetic rat + melatonin
Optical Density/min	G-6-PDH	0.010±0.01	0.001±0.02*	0.009±0.02
	SDH	0.007±0.02	0.001±0.03*	0.007±0.04
	LDH	0.032±0.03	0.008±0.04*	0.040±0.01
Enzyme Unit (mol/min/ml)	G-6-PDH	2.82±0.13	0.31±0.04*	2.41±0.22
	SDH	0.035±0.02	0.02±0.04	0.031±0.05
	LDH	16.21±0.13	3.52±0.16*	14.81±0.22
Specific Activity (mol/min/mg/protein)	G-6-PDH	2.10±0.02	0.01±0.04*	2.30±0.34
	SDH	1.55±0.02	0.02±0.03*	1.22±0.12
	LDH	7.26±0.62	1.51±0.21*	6.81±0.24
Protein Concentration (mg protein/ml)	G-6-PDH	2.82±0.16	1.62±0.14*	2.54±0.31
	SDH	2.64±0.13	1.12±0.04	2.51±0.22
	LDH	3.14±0.43	1.21±0.22	2.85±0.35

*$p<0.05$ vs control

Histochemical activities

Under normal physiological conditions, increases in blood glucose levels should stimulate and raise the activities of the enzymes of carbohydrate metabolism. However, the STZ diabetic rats expressed weak staining intensity for G-6-PDH, SDH and LDH when compared with the control and melatonin-treated diabetic rats (Fig.4). These findings are justified by the negative regression in biochemical estimation of the carbohydrate metabolizing enzymes. Biochemically, the entire profile of the enzymes, optical density, enzyme unit, specific activity and protein concentration of G-6-PDH, SDH and LDH were regressionally affected. Values for the control were 2.10±0.02, 1.55±0.02, 7.26±0.62 mol/min/mg protein respectively, while the STZ-treatment rats showed significant reductions ($p<0.05$) in the activities of G-6-PDH, SDH and LDH (0.01±0.04, 0.02±0.03, 1.51±0.21 mol/min/mg protein) respectively. The activities of these enzymes were protected by melatonin (Table 4 and Fig. 3). Total plasma HDL and LDL cholesterol and triglycerides in the control, STZ-treated and STZ + melatonin treated rats are shown in Table 4. Plasma LDL cholesterol and triglyceride concentrations significantly increased ($p<0.05$) in diabetic rats when compared with the control and melatonin-treated rats.

Table 4.

Plasma lipoprotein cholesterol fractions, total cholesterol and triglycerides in control, STZ-treated diabetic, and STZ + melatonin-treated groups. Values given represent the mean (±SEM) of 8 observations.

	STZ-treated diabetic rats				STZ + melatonin- treated diabetic rats			
Experimental days	HDL	LDL (mmol/l)	TC	TRIG (mmol/l)	HDL	LDL (mmol/l)	TC	TRIG (mmol/l)
0	0.98±0.4	1.2±0.3	2.4±0.1	1.5±0.2	0.88±0.2	1.12±0.2	2.7±0.2	1.9±0.3
5	0.72±0.3	1.6±0.2	2.9±0.3	1.9±0.3	0.92±0.4	1.40±0.4	2.7±0.1	2.0±0.2
10	0.64±0.4	1.9±0.5	3.2±0.2	2.4±0.5	1.22±0.3	1.12±0.3	2.6±0.3	1.7±0.4
15	0.56±0.3*	2.2±0.4*	3.6±0.4*	2.8±0.4*	1.24±0.4	0.98±0.1	2.5±0.2	1.4±0.3
20	0.48±0.1*	2.6±0.2*	3.5±0.6*	3.2±0.3*	1.42±0.1	0.94±0.4	2.5±0.2	1.2±0.1
25	0.46±0.2*	2.8±0.3*	3.6±0.4*	3.9±0.5*	1.51±0.5	0.91±0.2	2.3±0.2	1.2±0.4
30	0.41±0.3*	3.1±0.4*	3.8±0.3*	4.3±0.2*	1.64±0.2	0.87±0.3	2.2±0.1	1.1±0.2

*$p<0.05$ vs control

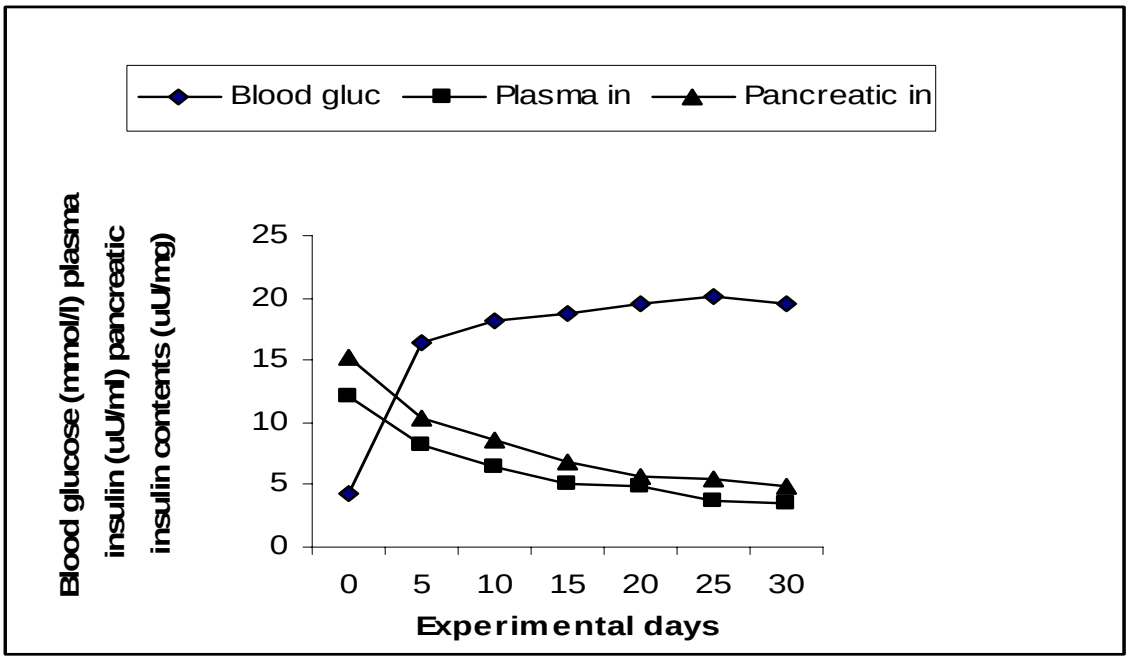

Figure 1.

Effects of streptozotocin (STZ) treatment on blood glucose concentrations, and plasma and pancreatic insulin contents. The rats became diabetic within 48 hours following STZ treatment, and remained hyperglycemic and hypoinsulemic throughout the study period.

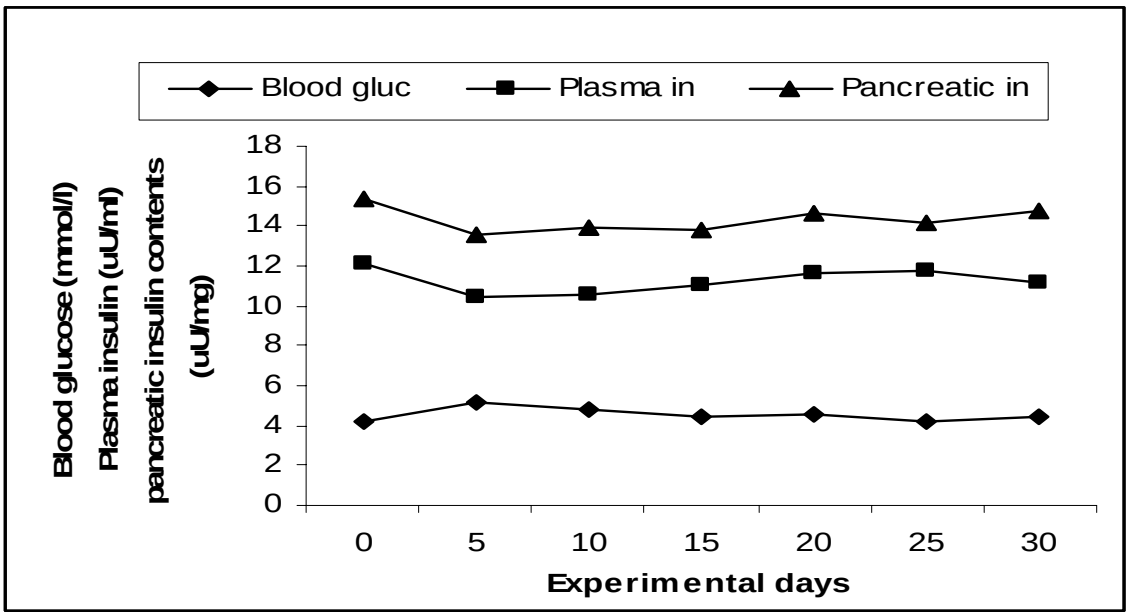

Figure 2.

Effects of streptozotocin (STZ) + melatonin treatments on blood glucose concentrations and plasma and pancreatic insulin contents. Melatonin showed protective effects on β-cells, and the rats were able to maintain their normoglycemic and normoinsulinemic status throughout the study period.

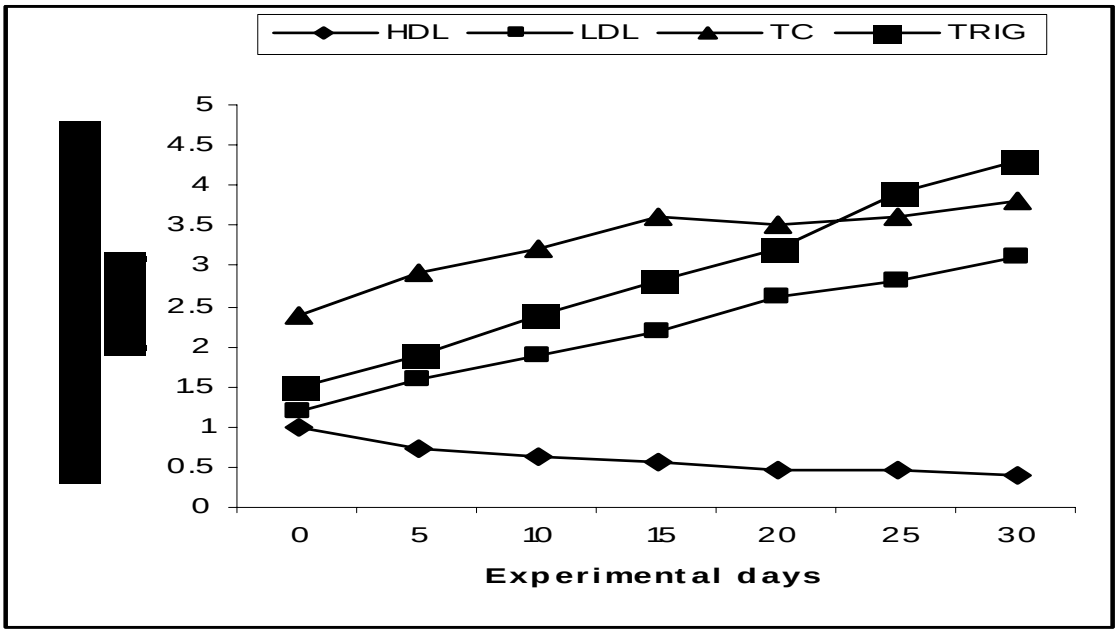

Figure 3.
Effects of streptozotocin (STZ) treatment on plasma triglyceride and cholesterol. There was a significant increase ($p<0.05$) in TC, LDL and TRIG. However, HDL decreased significantly ($p<0.05$) throughout the study period.

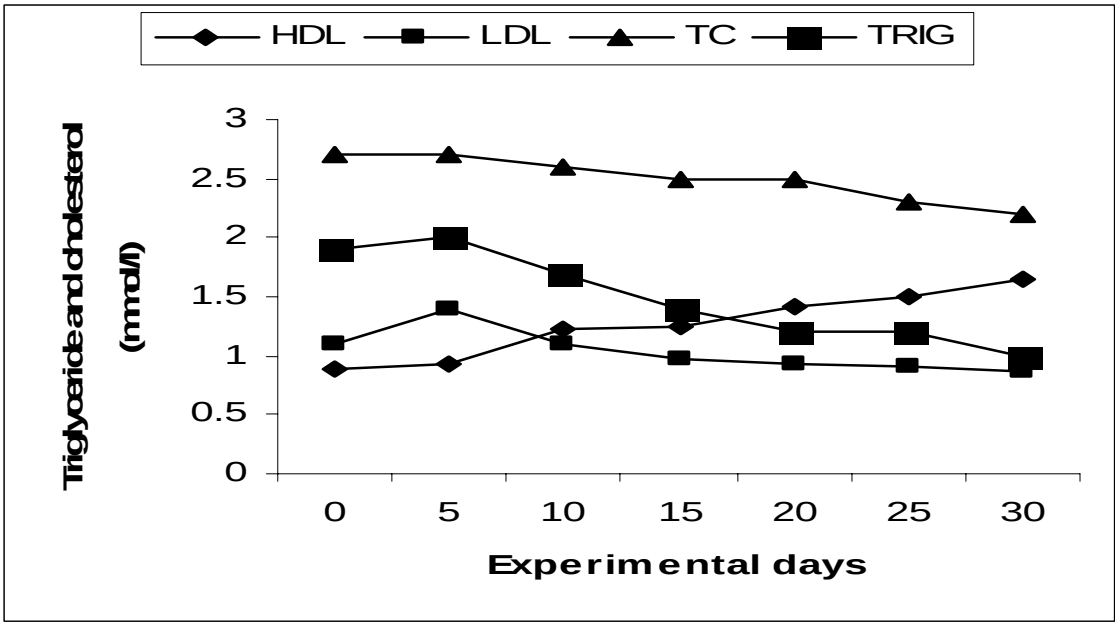

Figure 4.

Effects of streptozotocin (STZ) + melatonin treatment on triglyceride and cholesterol. Melatonin showed protective effects on the enzymes, and it stabilized TC, LDl, and TRIG. However, HDL level gradually rose during the study period.

DISCUSION

Chronic exposure of pancreatic β-cells to supraphysiologic concentrations of glucose causes defective and marked decreases in insulin content, and abnormal insulin secretion [61]. The present study examined the effects of melatonin on β-cell damage of STZ-induced diabetic rats. Free radical mechanisms and the possible sources of oxidative stress in the pathogenesis of diabetes and diabetic complications have been extensively studied in animal models and in human subjects [1]. Human diabetics and experimental animal models of diabetes exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby depleting the activity of oxidative defense system, and thus promoting *de novo* free radicals generation [62, 63]. Oxidative stress has recently been shown to be responsible, at least in part, for pancreatic β-cell dysfunction caused by glucose toxicity. Under hyperglycemia, production of various reducing sugars, such as glucose-6-phosphate and fructose, increases through glycolysis and the polyol pathway. During this process, reactive oxygen species (ROS) are produced, and they cause tissue damage [64, 65, 66].

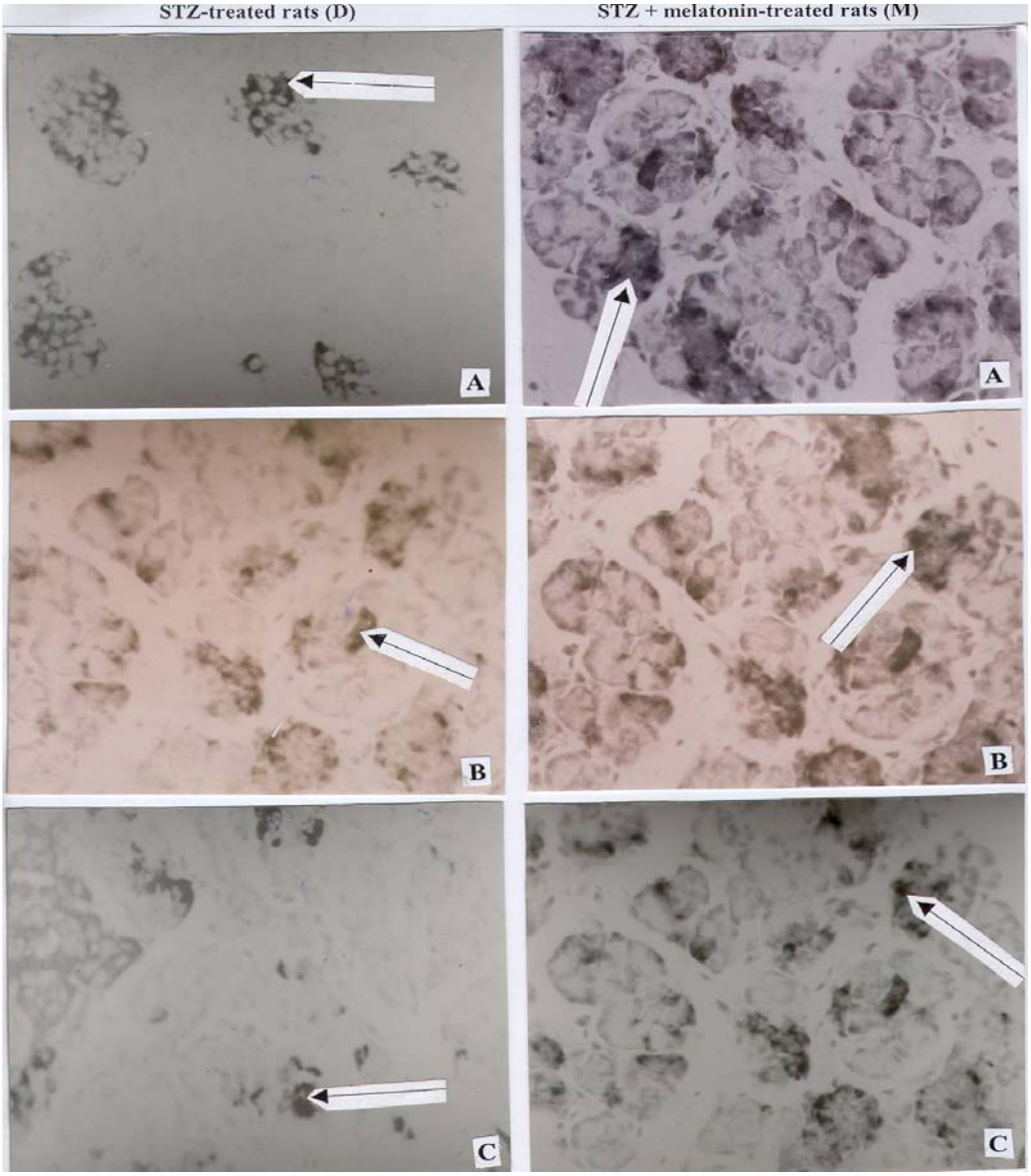

Figure 5.

Histochemical staining of the pancreatic tissues, represented by dark formazan. Columns on the left-hand-side represent STZ treated diabetic rats, while columns on the right-hand-side represent STZ + melatonin-treated rats. A, B and C show G-6-PDH, SDH and LDH staining respectively. The islets of melatonin-treated rats demonstrated distinct staining intensity for the 3 enzymes studied.

Melatonin has been shown to be an effective free radical scavenger and antioxidant, and might, therefore, be potentially capable of protecting pancreatic β-cells against oxidative stress and diabetes in Zucker diabetic fatty (ZDF) rats and db/db mice [67]. In the present STZ-induced experimental type 1 diabetes study, we have shown that melatonin is effective not only on insulin sensitivity, but also in β-cell protection. Melatonin also has therapeutic preventive and protective effects in diabetes by decreasing oxidative stress and preserving pancreatic β-cell integrity. Such damaged pancreatic β-cells often display extensive degranulation and oxidative

enzyme distortion (as observed in this study) when examined histologically or histochemically, and are clinically associated with development of diabetes in some experimental animal models of type 1 diabetes [26, 68]. Therefore, protection of pancreatic β-cells against chronic hyperglycemia-induced damage is an important target for the management, treatment and/or control of type 1 diabetes. To enable effective prevention and treatment of glucose toxicity to β-cells [69], it is essential to understand the biochemical aspects of the phenomenon. Streptozotocin, as an antibiotic and an anticancer agent, has been widely used for inducing diabetes in a variety of experimental animals by affecting degeneration and necrosis of pancreatic β-cells [70, 71]. Although the β-cell cytotoxic action of STZ is not fully understood, it is thought to be mediated via inhibition of free radical scavenger-enzymes, thereby enhancing the production of superoxide radicals. The later action has been implicated in lipid oxidation, DNA damage, and sulfhydryl oxidation. In the present study, almost all insulin-positive β-cells were degranulated, degenerated or necrosed in the STZ-treated rats, leading to a decrease in insulin secretion, and an increase in blood glucose, cholesterol and triglyceride concentrations. This gave rise to the consideration that deteriorating β-cell function in diabetic patients might be caused by chronic exposure to high concentrations of lipids; a concept termed: 'lipotoxicity hypothesis' [72]. Prolonged exposure of pancreatic β-cells to fatty acids has been reported to inhibit insulin gene expression, as well as enzymes of carbohydrate metabolism [73].

Another finding of this study is the severity of deficit in G-6-PDH, SDH and LDH activities; as well as increases in the plasma concentrations of cholesterol and triglyceride which are correlated to the severity of insulin deficiency. Activities of G-6-PDH, SDH and LDH normally protect β-cell function, and this is supported by the study of Monte-Alegre *et al*., [74]. G-6-PD in particular always helps to keep glutathione in its reduced form. This, in turn, acts in concert with glutathione peroxidase, as a scavenger for ROS [75]. Lipotoxicity results from high glucose concentrations, since elevated fatty acids are not readily oxidized in mitochondria, but are shunted towards esterification pathways. The adverse effect of this effort is the accumulation of triglycerides in β-cells, the common occurrence or antecedent in hyperglycemic and hypoinsulemic conditions [72]. Generally, diabetes is marked by characteristic alterations in lipoprotein levels, including an elevation of triglycerides and very low density lipoprotein (VLDL), and a decreased HDL concentration [76]. This observation is important because of its association with increased risk of developing cardiovascular diseases.

In conclusion, the findings of this study indicate that melatonin has therapeutic, preventive and protective effects in diabetes by reducing oxidative stress and blood glucose as well as preserving pancreatic β-cell integrity. Hence, it slows down islet infiltration and immune cell attack on the β-cells, thus preventing or delaying β-cell death. The current study also suggests that melatonin, possibly in combination with other therapies which inhibit β-cell apoptosis, may be a promising strategy to delay or prevent development of type 1 diabetes.

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