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Modulation of antioxidant status, carbohydrate and lipid metabolism by melatonin on streptozotocin induced diabetic rats

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Abstract

Melatonin, "synchronizer of the biological clock" is major hormones secreted from the pineal gland have various therapeutic effects. The present study was designed to explore the modulatory effect of melatonin on antioxidant status, glucose and lipid metabolism in streptozotocin (STZ) induced diabetic rats. Male Wistar rats weighing 180-200 g were made diabetic by administration of streptozotocin (STZ) (40 mg/kg body weight) intraperitoneally. Melatonin was administered intraperitoneally at a dose of 2 mg/kg body weight to STZ-induced diabetic rats for 30 days. Body weight, blood glucose, carbohydrate metabolic enzyme, lipid profile, antioxidant and lipid peroxidation status were assessed. The level of the blood glucose, carbohydrate metabolic enzymes (glucose-6-phosphatase and fructose-1,6-bisphosphatase) and lipid peroxidative marker (TBARS) were increased in STZ induced diabetic rats while the melatonin treatment revert back to the near normal condition. In contrast, administered melatonin resulted in an increased in body weight and insulin secretion in diabetic rats. The enzymatic antioxidants (SOD, CAT and GPX) and non-enzymatic antioxidants (GSH, vitamin C and vitamin E) were also increased by melatonin treatment. The cholesterol and phospholipids which were elevated in diabetic rats were normalized by the melatonin administration. Hence these findings indicate that melatonin protects against STZ induced oxidative stress and thus explain its use in treatment of diabetes by modulating lipid and glucose metabolism.

Keywords*:* Melatonin, diabetes, blood glucose, antioxidant enzymes, lipid peroxidation.

Introduction

Diabetes mellitus is a condition of disordered metabolism, frequently owing to hereditary and environmental causes, which leads to abnormal levels of blood glucose (American Diabetes Association 2005). Hyperglycemia causes oxidative stress which leads to generation of free radicals (Imlay et al. 2003). Lipid peroxidation is a process in which ROS may act with a variety of biomolecules such as lipids, carbohydrates, proteins, nucleic acid and macromolecules of connective tissue, causing oxidative damage of polyunsaturated fatty acid (PUFA) forming cytotoxic aldehyde (Rattan et al. 2006). Streptozotocin is extensively used to induce diabetes in experimental animals by destroying pancreatic β-cells (Szkudelski et al. 2001). In diabetic state, oxidation of low density lipoprotein (LDL) is closely associated with concurrent oxidation and glycation process. Furthermore, in diabetic coronary heart disease patient's the high lipid peroxidation level was found in LDL (Lve et al. 1991). In many studies, antioxidants have been shown to inhibit the development of coronary heart disease (CHD) and it has both antioxidative and hypolipidemic properties will be applicable in the treatment of CHD related with diabetes (Surekha et al. 2007).

Diabetes is the most common endocrine disease and it is a burden not only on the affected individuals but also on the society in all countries of the world. According to World Health Organization estimates, by 2025 total 300 million of the worldwide population will be affected by diabetes (Wild et al. 2004). There is no definitive treatment regimen for the diabetic patients in developing countries and hence it is of great interest to develop synthetic agents for the prevention and treatment of ailments. In recent time, the safety and efficacy of synthetic drug have been validated for conducting clinical and laboratory experiments. But these drugs are only aimed to reduce the level of blood glucose and moreover in most cases, it causes some side effects such as hypoglycemia (Rosenbloom et al. 2003).

Melatonin (*N*-acetyl-5-methoxy tryptamine), showed in (Fig.1) is a pineal hormone has the ability to neutralize the free radical and its related toxicants can readily enter into subcellular compartment and nucleus via acting as a lipophilic agent. Recent reports suggest that melatonin has both free radical scavenging and antioxidant properties (Reiter et al. 2000), which prevents the increase in plasma glucose levels in STZ induced diabetic rats (Andersson et al. 2001). There is evidence that alternative antioxidant is a valuable approach for the treatment of oxidative stress related diseases (Sewerynek et al. 2002). To our knowledge, there is no scientific studies were reported on the antidiabetic effect of melatonin against

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STZ-induced diabetes. Therefore, the main objective of our study was designed to provide scientific validity for the antidiabetic potential of melatonin on streptozotocin-induced diabetic rats.

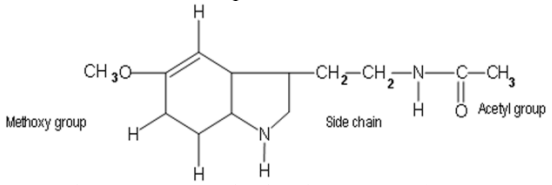

Figure 1: Structure of melatonin (Lerner *et al*., 1958).

Materials and Methods

Animals

4-7 week old male albino Wistar rats were purchased from the Tamilnadu Veterinary Animal Science (TANUVAS), Chennai, India. Rats weighing (180-200 g) were maintained under standard conditions of humidity temperature (28 ± 2 ºC) and light (12 h light/dark). The animals were housed in polypropylene cages (45 × 24 × 15 cm), food pellets and tap water ad libitum were available to the animals throughout the experimental period of replenished daily. Animals were handled according to the university and institutional legislation, regulated by the ethical committee on Animal care of Annamalai University. All the procedures performed on the animals were approved and conducted in accordance with the National Institute of Health Guide (Reg. No.487/160/1999/CPCSEA).

Experimental induction of diabetes

The male albino Wistar rats weighing 180-200 g were made diabetic by intraperitoneal injections of STZ. The animals were allowed to fast for 24 h and were given STZ injection (40 mg/kg bw) with freshly prepared aqueous solution of citrate buffer as vehicle, pH 4.5. The control animals received buffer alone. STZ treated animals were allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia. After 48 hours of STZ administration, the blood glucose range of (200-300 mg/dL) were considered as diabetic rats and used for the experiment.

Experimental design

Animals were randomized and divided into four groups of six animals each

Group I	:	Control rats given with citrate buffer (pH 4.5) orally.
Group II	:	Rats were made diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg bw) with citrate buffer (pH 4.5).
Group III	:	Diabetic rats treated with (2 mg/kg bw) of melatonin daily by intraperitoneal administration for 30 days.
Group IV	:	Control rats treated with (2 mg/kg bw) of melatonin daily by intraperitoneal administration for 30 days.

After the termination of the experiment all the animals were anaesthetized between 8:00 am and 9:00 am using ketamine chloride (24 mg/kg bw) and sacrificed by cervical dislocation after an overnight fast. Blood was collected and kept at -20 ºC until use. Plasma and serum were separated after centrifugation and used for various biochemical estimations. Tissues (liver and kidney) were collected and stored at 4 °C for the measurement of various enzyme activities.

Preparation of erythrocyte lysate and tissue homogenate

Erythrocyte lysate was prepared after the separation of plasma, the buffy coat was removed and the packed erythrocytes were washed thrice with cold physiological saline. A known volume of the erythrocyte was lysed with cold hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 2000 rpm for 10 min and the supernatant was used for the estimation of enzymic antioxidants. For the tissue homogenate preparation the known volume of liver and kidney tissues were homogenized in Tris-HCL/Phosphate buffer pH 7.0 using Potter-Elvehjam homogenizer with Teflon pestle. The homogenates were centrifuged at 1000 rpm for 10 minutes. The supernatant was separated and used for various biochemical estimations.

Biochemical analysis

Blood glucose was determined by the method of O-toluidine using the modified reagent of (Sasaki et al. 1972). Plasma insulin was assayed by the solid phase system amplified sensitivity immunoassay using reagent kits obtained from Medgenix-INS-ELISA. The assay was based on the oligoclonal system in which several monoclonal antibodies (Mabs) directed against distinct epitopes of insulin were used (Burgi et al. 1988). Glucose-6-phosphatase and fructose-1,6-bisphosphatase was assayed by (Koide et al. 1992) and (Gancedo et al. 1971). The estimation of thiobarbituric acid reactive substances (TBARS) by the method of (Niehaus et al. 1968). Enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) by the methods of (Kakkar et al., 1978), (Sinha, 1972) and (Rotruck et al., 1973), respectively. The activities of non enzymatic antioxidants, vitamins C and E and reduced glutathione were estimated by the methods of (Omaye et al. 1979), (Baker et al., 1980) and (Ellman et al. 1959). Plasma and tissue lipids were extracted by the method of (Folch et al. 1957). The total cholesterol and phospholipids were determined by the methods of Zlatkis (1953) and Zilversmit (1950).

Statistical analysis

All quantitative measurements for control and experimental animals were expressed as means ± SD for six rats in each group.The data were analysed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using statistical package of social science (SPSS) Version 10.0 for Windows.

Results

Table 1 shows the changes in the body weight, blood glucose and plasma insulin level of experimental rats by melatonin administration. The mean body weight of diabetic rats decreased significantly ($P<0.05$) compared to all other groups. After treatment with melatonin, there was an increase in body weight of group III and group IV rats when compared with initial body weight. No significant changes were observed between group I and group IV rats. Diabetic rats resulted in significant ($P<0.05$) hyperglycemic induced by STZ group II. After melatonin administration in group III and group IV there was a significant decrease in blood glucose and an increase in insulin secretion when compared to diabetic rats. There were no significant changes between group I and group IV rats.

Table 1. Effect of melatonin on body weight, blood glucose and plasma insulin level on experimental rats

Groups	Body weight (g)		Blood glucose (mg/dL)	Plasma insulin (µg/mL)
	Initial (0 day)	Final (30 days)		
Control	191.12 ± 17.50[a]	218.21 ± 18.01[a]	87.45 ± 7.71[a]	16.24 ± 1.64[a]
Diabetic control	194.23 ± 13.31[a]	159.15 ± 12.4[b]	290.91± 13.33[b]	5.24 ± 0.15[b]
Diabetic + Melatonin (2 mg/kg bw)	190.07 ± 14.5[a]	183.22 ± 15.26[c]	98.73 ± 8.21[c]	11.49 ± 1.03[c]
Control+ Melatonin (2 mg/kg bw)	189.06 ± 17.43[a]	216.22 ± 19.05[a]	85.55 ± 7.77[a]	17.19 ± 1.25[a]

Table 2. Effect of melatonin on glucose-6-phosphatase in plasma and tissues of experimental rats.

Groups	Glucose-6-Phosphatase		
	Plasma (mg/dL)	Liver (µg /mg protein)	Kidney (µg /mg protein)
Control	0.29 ± 0.03[a]	0.18 ± 0.02[a]	0.13 ± 0.03[a]
Diabetic control	0.55 ± 0.04[b]	0.40 ± 0.04[b]	0.34 ± 0.02[b]
Diabetic + Melatonin (2 mg/kg bw)	0.11 ± 0.01[c]	0.27 ± 0.02[c]	0.25 ± 0.02[c]
Control + Melatonin (2 mg/kg bw)	0.28 ± 0.02[a]	0.16 ± 0.01[a]	0.11 ± 0.01[a]

Table 3. Effect of melatonin on fructose-1,6-bisphosphatase in plasma and tissues of experimental rats

Groups	Fructose 1,6-bisphosphatase		
	Plasma (mg/dL)	Liver (µg /mg protein)	Kidney (µg /mg protein)
Control	0.41± 0.03[a]	0.36 ± 0.03[a]	0.32 ± 0.02[a]
Diabetic control	0.62 ± 0.03[b]	0.62 ± 0.06[b]	0.58 ± 0.06[b]
Diabetic + Melatonin (2 mg/kg bw)	0.49 ± 0.04[c]	0.45 ± 0.02[c]	0.42 ± 0.03[c]
Control + Melatonin (2 mg/kg bw)	0.39 ± 0.02[a]	0.35 ± 0.03[a]	0.35 ± 0.03[a]

Table 4. Effect of on melatonin on lipid peroxidation (TBARS) status in plasma and tissues of experimental rats.

Groups	TBARS		
	Plasma (mg/dL)	Liver (mg/100g tissue)	Kidney (mg/100g tissue)
Control	0.18 ± 0.03[a]	0.75 ± 0.06[a]	1.34 ± 0.13[a]
Diabetic control	0.41± 0.04[b]	3.28 ± 0.29[b]	2.79 ± 0.21[b]
Diabetic + Melatonin (2 mg/kg bw)	0.22 ± 0.02[c]	1.41 ± 0.12[c]	1.65 ± 0.15[c]
Control + Melatonin (2 mg/kg bw)	0.17 ± 0.10[a]	0.73 ± 0.07[a]	1.27 ± 0.12[a]

Table 5. Effect of melatonin on the activities of enzymatic antioxidants (SOD, CAT and GPx) in erythrocyte lysate of experimental rats.

Groups	Erythrocytes lysate		
	SOD (U^A/mg Hb)	CAT ($U^{A/}$ mg Hb)	GPx (U^A /mg Hb)
Control	7.1 ± 0.56[a]	148.14 ±12.71[a]	13.16 ± 1.93[a]
Diabetic control	4.65 ± 0.27[b]	74.27 ± 5.61[b]	7.49 ± 0.61[b]
Diabetic + Melatonin (2 mg/kg bw)	6.18 ± 0.64[c]	127.22 ± 11.52[c]	10.31 ± 0.95[c]
Control + Melatonin (2 mg/kg bw)	7.19 ± 0.53[a]	147.39 ± 12.43[a]	12.93 ± 1.2[a]

Table 6. Effect of melatonin on the activities of enzymatic antioxidants such as SOD, CAT and GPx in the liver and kidney of experimental rats.

Tissue samples	Groups	Enzymatic antioxidants		
		SOD (U^Amg /protein)	CAT(U^Bmg /protein)	GPx (U^Cmg /protein)
Liver	Control	10.25 ± 0.81[a]	73.09 ±3.90[a]	8.72 ± 0.71[a]
	Diabetic control	4.52 ± 0.36[b]	42.15 ± 2.56[b]	4.33± 0.24[b]
	Diabetic + Melatonin (2 mg/kg bw)	7.15 ± 0.60[c]	62.57 ± 3.86[c]	7.26 ± 0.59[c]
	Control + Melatonin (2 mg/kg bw)	10.09 ± 0.96[a]	73.43 ± 4.72[a]	8.51 ± 0.86[a]
Kidney	Control	13.51±1.06[a]	31.98 ± 3.80[a]	8.49 ± 0.86[a]
	Diabetic control	8.50 ± 0.65[b]	16.10 ± 2.20[b]	3.89 ± 0.29[b]
	Diabetic + Melatonin (2 mg/kg bw)	12.65 ± 1.09[c]	25.31± 2.14[c]	6.69 ± 0.56[c]

Table 7. Effect of melatonin on the activities of non-enzymatic antioxidants such as GSH, vitamin C and vitamin E in plasma of experimental rats.

Groups	Plasma		
	GSH (mg/dL)	Vitamin C (mg/dL)	Vitamin E (mg/dL)
Control	28.97 ± 2.81[a]	1.82 ± 0.18[a]	1.55 ± 0.33[a]
Diabetic control	17.24 ± 1.71[b]	0.80 ± 0.07[b]	0.86 ± 0.07[b]
Diabetic + Melatonin (2 mg/kg bw)	23.48 ± 2.02[c]	1.27 ± 0.12[c]	1.21 ± 0.08[c]
Control + Melatonin (2 mg/kg bw)	27.99 ± 2.58[a]	1.81 ± 0.17[a]	1.68 ± 0.16[a]

Table 8. Effect of melatonin on the activities of non-enzymatic antioxidants such as GSH, vitamin C and vitamin E in liver and kidney of experimental rats.

Tissue samples	Groups	Non enzymatic antioxidants		
		GSH (µg/mg protein)	Vitamin C (µg/mg protein)	Vitamin E (µg/mg protein)
Liver	Control	40.55 ± 2.56[a]	1.44 ± 0.13[a]	0.90 ± 0.83[a]
	Diabetic control	24.30 ± 1.33[b]	0.75 ± 0.07[b]	0.59 ± 0.03[b]
	Diabetic + Melatonin (2 mg/kg bw)	31.51 ± 2.31[c]	1.21 ± 0.11[c]	0.77 ± 0.06[c]
	Control + Melatonin (2 mg/kg bw)	39.51 ± 3.04[a]	1.38 ± 0.14[a]	0.89 ± 0.07[a]
Kidney	Control	36.16 ± 1.49[a]	1.10 ± 0.11[a]	0.70 ± 0.07[a]
	Diabetic control	24.49± 1.81[b]	0.38 ± 0.05[b]	0.36 ± 0.05[b]
	Diabetic + Melatonin (2 mg/kg bw)	30.73 ± 1.24[c]	0.82 ± 0.06[c]	0.58 ± 0.05[c]
	Control + Melatonin (2 mg/kg bw)	35.17 ± 1.89[a]	1.04 ± 0.07[a]	0.68 ± 0.06[a]

Table 9. Effect of melatonin on lipid profiles in plasma and tissues of experimental rats.

Lipid Profile	Groups	Samples		
		Plasma (mg /dL)	Liver (mg /100g tissue)	Kidney (mg /100g tissue)
Total Cholesterol	Control	95.25 ± 4.54[a]	158.12 ± 12.74[a]	183.02 ± 12.3[a]
	Diabetic control	138.1 ± 12.45[b]	317.27 ± 21.85[b]	341.22 ± 21.65[b]
	Diabetic + Melatonin (2 mg/kg bw)	120.17 ± 10.2 [c]	220.19 ± 16.46[c]	335.4 ± 23.58[c]
	Control + Melatonin (2 mg/kg bw)	94.21 ± 6.3[a]	150.43 ± 13.38[a]	181.06 ± 12.34[a]
Phospholipids	Control	3.74 ± 0.31[a]	1.61 ± 0.11[a]	1.38 ± 0.12[a]
	Diabetic control	4.12 ± 0.28[b]	2.64 ± 0.13[b]	2.03 ± 0.21[b]
	Diabetic + Melatonin (2 mg/kg bw)	2.43 ± 0.13 [c]	2.13 ± 0.11[c]	1.65 ± 0.05[c]
	Control + Melatonin (2 mg/kg bw)	3.52 ± 0.08[a]	1.98 ± 0.13[a]	1.42 ± 0.04[a]

Values are means ± SD (standard deviation) for six samples from 6 rats in each group. Values not sharing a common superscript differ significantly at $p \leq 0.05$ Duncan's Multiple Range Test (DMRT).

Table 2 and 3 depicts the effect of melatonin on carbohydrate metabolic enzymes in plasma and tissues of experimental rats. Glucose-6-phosphatase and fructose-1,6-bisphosphatase was found to be enhanced in group II rats when compared to other groups. Melatonin administration in group III and group IV rats showed significantly (P<0.05) reduced level of both these enzymes when compared with group II rats. No significant changes were observed between group I and group IV rats.

Table 4 shows the effect of on melatonin on lipid peroxidation (TBARS) status in plasma and tissues of control and experimental animals. The level of TBARS in STZ treated rats (group II) alone were significantly (P<0.05) increased when compared to control. In group III and group IV rat, the level of TBARS was significantly decreased when compared with group II.

Table 5 and 6 shows the effect of melatonin on the activities of enzymatic antioxidants (SOD, CAT and GPx) in erythrocyte lysate and tissues (liver and kidney) of control and experimental rats. The activities of enzymatic antioxidants were markedly decreased in diabetic rats when compared to group II diabetic rats. No significant changes were observed between group I and group IV rats.

Table 7 and 8 shows presents the effect of melatonin on the activities of non-enzymatic antioxidants (GSH, Vitamin C and Vitamin E) in plasma and tissues of control and experimental rats. Group II diabetic rats showed a significant reduction in non-enzymatic antioxidants levels when compared to group I control rats. In group III and group IV animals melatonin administration significantly (P<0.05) modulated the non-enzymatic antioxidants when compared to diabetic rats. No significant changes were observed between group I and group IV rats.

Table 9 indicates the effect of melatonin on lipid profiles in plasma and tissues of experimental animals. The levels of cholesterol and phospholipids found to be elevated significantly (P<0.05) in diabetic rats when compared to group I rats. Upon melatonin administration the levels were significantly (P<0.05) lowered in group III and group IV rats compared to group II. No significant changes were observed between group I and group IV rats.

Histopathological changes in the pancreas, liver and kidney

Fig. 2 shows the histopathological examination of pancreatic tissues of control and experimental animals (H and E 10X). Diabetic group rats showed shrinkage of islet cells and growth of adipose tissue in the pancreas. Treatment with melatonin in diabetic rats showed reduced shrinkage of islet cells in the pancreas, which supports the biochemical analysis.

Fig. 3 shows the histopathological examination of liver tissues of control and experimental animals (H and E 10X). Rats showed marked reduction in fatty changes and inflammatory cell infiltration in the liver and melatonin control rats showed no changes in liver compared to control. Diabetic liver showed fatty changes and

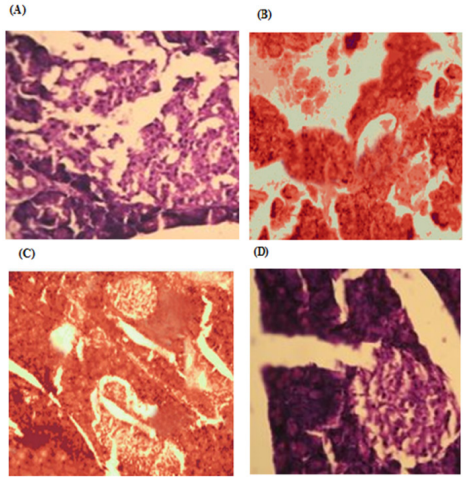

Figure 2: Histopathological examination of pancreatic tissues of control and experimental animals (H and E 10X). (A) CON:Showing normal islet cells, (B) DIABETIC: Showing shrinkage of islet cells and growth of adipose tissue, (C) DIABETIC+MEL (2 mg/kg body wt): Showing reduced shrinkage of islet cells, (D) CONTROL+ MEL (2 mg/kg body wt.):Showing small normal islet cells.

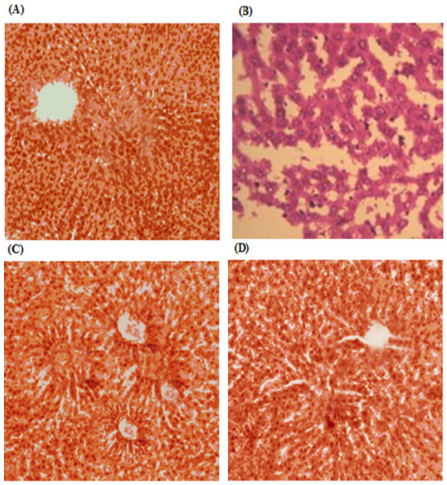

Figure 3: Histopathological examination of liver tissues of control and experimental animals (H and E 10X). (A) Control: Showing normal liver, globular architecture, (B) Diabetic: Showing fatty changes and inflammatory cell infiltration, (C) Diabetic+Melatonin: (2 mg/kg b.w): Showing marked reduction in fatty changes and inflammatory cell infiltration, (D) Control + Melatonin (2mg/kg b.w): No change in normal liver.

inflammatory cell infiltration in the liver. Treatment with melatonin in diabetic

Fig. 4 shows the histopathology of kidney tissues of control and experimental animals (H and E 10X). Diabetic kidney showed larger area of hemorrhage, lymphocyte infiltration and fatty infiltration, which upon treatment with melatonin in diabetic rats showed marked reduction of hemorrhage and fatty infiltration and melatonin control rats showed normal tubules and glomeruli.

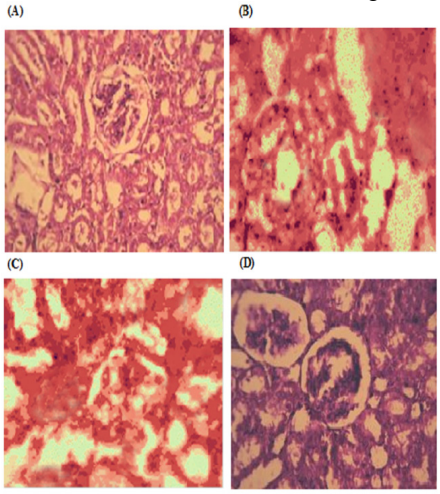

Figure 4: Histopathological examination of kidney tissues of control and experimental animals (H and E 10X). (A) Control: Showing normal liver, Showing normal tubular cells and glomeruli, (B) Diabetic: Showing hemorrhage, lymphocyte and fatty infiltration, (C) Diabetic + Melatonin: (2 mg/kg b.w): Showing marked reduction of hemorrhage and fatty infiltration, (D) Control + Melatonin (2mg/kg b.w): Showing normal tubular cells.

Discussion

In our experiment, STZ induced group showed a significant reduction in the body weight of diabetic rats, which may be due to dehydration, catabolism of fats and proteins. A better body weight gain was observed in melatonin treated animals when compared to diabetic group. Several studies have reported that an increase in food consumption in response to exogenous melatonin may be the reason for the increase in body weight and another likely explanation for short-term increase in food intake was found to be that melatonin induces a reinforcement of eating habits that usually accompany melatonin peak secretion (Angers et al. 2003).

Our results observed that there was an increase in blood glucose level and decrease in insulin secretion due to destruction of beta cells by STZ. Administration of melatonin resulted in a significant decrease in blood glucose level and slight increase in insulin secretion in diabetic rats. MT1 and MT2 receptors are known to be present in rat islets and human pancreatic tissue (Peschke et al. 2006). A putative signalling pathway of the pancreatic β-cell is influenced via MT1 and MT2 melatonin membrane receptor has been demonstrated in recent reports. The influence of melatonin on pancreatic β-cells and on insulin secretion is associated with intracellular cAMP, cGMP and IP_3 signal transduction pathways and this plays a role in maintaining glucose homeostasis (Eunyoung et al. 2006). In addition, melatonin increases the expression of forehead box A_2, which was recently discovered to regulate fatty acid oxidation and inhibited by insulin. Moreover, the most profound effect of melatonin administration is the prevention of increase in nitric oxide level in blood plasma during STZ treatment and this effect plays an important role in prevention of β-cell integrity (Rao et al. 2002).

In our experiment the decreased level of insulin was seen in diabetic rats when compared to other groups. Liver is an insulin dependent tissue and it is severely affected during diabetes. The balance between glucose production and its utilization in the liver is regulated primarily by insulin. Melatonin supplementation showed slight increase in insulin secretion and decrease in blood glucose level and this may be due to release of insulin which is stored in granules from existing pancreatic β-cells.

In our result, it was seen that there was an increase in gluconeogenic enzymes in diabetic rats. Activation of gluconeogenic enzymes increases the blood glucose level due to the state of insulin deficiency and impaired expression of gluconeogenic enzymes. As said in earlier reports the administration of melatonin increase the level of insulin secretion in diabetic rats and this may be the reason for the significant reduction in the level of gluconeogenic enzymes (Mehmet et al. 2006).

Further melatonin regulates cAMP which leads to inhibition of gluconeogenesis and increased glycolysis and thus decreasing the level of blood glucose (Mackenzie et al. 2002). It was reported that melatonin administration to diabetic rats also reversed the pentose phosphate enzymes, glucose-6-phospatase, transketolase and catalase activity in diabetic liver and thus helps in preventing an increase in blood glucose level (Baynes et al. 1999). Hence the melatonin reveals its antidiabetic action by decreasing blood glucose, increasing insulin secretion and by suppressing the activity of gluconeogenic enzymes.

Lipid peroxidation is found to be an important pathophysiological event in variety of disease particularly diabetes (Tan et al. 2002). It is evident from our result that an elevated levels of TBARS seen in plasma of diabetic rats when compared to other groups. In contrary melatonin administration significantly reduced the level of TBARS in plasma of diabetic rats which indicates that the drug possess a potent antilipid peroxidative effect and this action is mediated by acetyl group and methoxy group at position 5 of the indole nucleus reacts with free radicals by contributing an electron (Zhang et al. 2004).

The SOD and CAT and Gpx are the enzymatic antioxidants that scavenge free radicals against oxidative stress during diabetes. The present study reveals that the activity of SOD in diabetic rats was decreased due to their utilization by tissues and plasma to scavenge superoxide anion into H_2O_2 and O_2 radical. The activity of SOD level was increased in melatonin treated rats and this is due to its effect in neutralizing O_2 superoxide free radicals (Reiter et al. 2005; Mirunalini et al. 2004). Catalases are the enzymes that catalyze the conversion of H_2O_2 to H_2O by oxidizing reduced glutathione and decreased CAT activity was observed in diabetic rats when compared to control and a similar result was observed for GPx. This is due to radical induced inactivation and glycation of enzymes and their utilization by plasma and tissues to scavenge free radicals (Heales et al. 2008; Ran et al. 2007) whereas melatonin promotes the enhanced activity of these enzymes in diabetic rats which reveals that it is a free radical scavenger and powerful antioxidant as supported with earlier reports (Rodriguez et al. 2004).

In our result the reduced level of vitamin C and vitamin E were observed in plasma of diabetic rats. This is due to the presence of lipid peroxidation in plasma and so both the vitamins are being utilized to combat free radicals (Gultekin et al. 2001).

Administration of melatonin in diabetic rats increased the level of vitamin C and vitamin E.

In our findings it was observed that there was a reduced level of GSH in plasma of diabetic rats when compared to control rats and it is due to the increased utilization of GSH during oxidative stress (Reiter et al. 1995). GSH is a major endogenous antioxidant which counterbalance free radical medicated damage. It is well known that GSH involve in the protection of normal cell structure and function in maintaining the redox hemostasis, quenching of free radicals and by participating in destroying reactions (Lang et al. 1992). When treated with melatonin the diabetic rats showed elevated level of GSH which increases GSH dependent enzymes and both the vitamin levels also increased which prevents oxidation of glutathione ad spares reduced GSH. Melatonin acts synergistically with vitamin C, Vitamin E and GSH to destroy free radicals; Vitamin E inactivates fatty free radicals by adding electron. The resulting oxidized vitamin E is recycled back to reduced vitamin E by vitamin C (Gitto et al. 2001). A positive association between Diabetic mellitus and lipid abnormalities has demonstrated in several studies (Aravind et al. 2002). In our results the level of cholesterol and phospholipids in plasma and tissues of diabetic rats was increased. Our findings also agree that diabetes mellitus is accompanied in the risk of atherosclerosis and it is inhibited through drug therapy thus lowering the level of plasma lipids. The abnormally high concentration of serum lipids especially cholesterol in diabetes is mainly as a result in increase in mobilization of fatty acid from peripheral deposit because insulin inhibits the hormone sensitive lipase. Generally during diabetes lipogenesis is decreased with increase in lipolysis in tissues (Kelley et al. 1997).

The increase and decrease in lipoprotein levels is the reflection of total cholesterol level that is low density lipoproteins (LDL-C), very low density lipoprotein (VLDL-C) and increase or decrease in high density lipoproteins (HDL-C) level with total cholesterol that determines the lipoprotein metabolism (Lve et al. 1991). Earlier reports shown that melatonin was able to reduce plasma and tissue lipid levels and cholesterol concentrations containing in apolipoprotein B-containing lipoproteins such as VLDL, LDL in hypercholestremic rats (Papaharalambus et al. 2007). Melatonin may exert the effect by augumenting endogenous cholesterol clearance and in one vitro study revealed that because of its lipophilic and nonionized nature; melatonin can enter the lipid phase of LDL particles and prevent lipid peroxidation and thus acts as an amphiphilic chain breaking antioxidant in a lipid environment by scavenging free radicals. It also protected oxidized LDL-induced inhibition of nitric oxide production in human umbilical artery and also reported that the melatonin reduced oxidation susceptibility of HDL and increases HDL levels, this may potentiate anthiatherogenic effects (Wakatsuki et al. 2001). Our result also collaborated with this hypothesis by reducing the level of lipid profile in diabetic rats with melatonin administration which shows that it possesses strong antihyperlipidemic action.

As evidenced with earlier reports the mechanism whereby melatonin stimulates the activity of antioxidant defence system that detoxify O_2 based reactants remain is likely to be mediated via membrane/nuclear receptors during oxidative stress in diabetic condition (Reiter et al. 2005). Either or both involved in the mechanism by which melatonin promotes the activity of the antioxidant enzymes by inducing the mRNA gene expression. Thus when melatonin present at pharmacological or physiological range it seems to be mediated by via receptor and enhances antioxidant enzymes during diabetes (Kotler et al. 1998).

Conclusion

In conclusion, these findings indicate that melatonin, a ubiquitous molecule protects against hyperglycemia induced oxidative damage which shows that the melatonin possess antidiabetic and antihyperlipidemic actions. However, the detailed mechanism(s) of action will be elucidated by further studies.

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