ORIGINAL ARTICLE

Protective Role of Melatonin and Insulin in Streptozotocin induced Nephrotoxicity in Albino Rats

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ABSTRACT

Aims: To evaluate the protective role of melatonin and insulin on the morphology of proximal convoluted tubules(PCT) of albino rats made nephrotoxic by a chemotherapeutic drug like streptozotocin (STZ).
Study design: Prospective experimental study.
Place and duration of study: Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Post Graduate Medical Center (JMPC), Karachi, for 6 weeks from March to April, 2012.
Material and methods: 60 male albino rats were divided into 4 groups, containing 15 animals each. Group(gp) A was treated as control, gp B , C and D received 37 mg/kg STZ Intraperitoneally (I/P) once at the start of experiment, whereas gp C additionally received 10mg/100 ml of melatonin (MEL) 3-days prior to STZ administration, and gp D received MEL at the same dose along with subcutaneous INS at a dose of 1 unit per 100 grams body weight. Serum glucose was measured weekly. Body weights were recorded at the start and end of experiment, and the relative kidney weights were recorded after sacrificing the animals. The kidneys were processed for histological examination and periodic Acid Schiff Haematoxylin (PAS-H) stained sections were viewed under the light microscope for detailed morphological examination of the proximal convoluted tubules in all groups of rats.
Results: The microscopic examination revealed marked epithelial, cytoplasmic and nuclear changes in the P.C.T. of STZ treated gp B, a significant reduction in the severity of these changes in MEL treated gp C and complete restoration of morphology in MEL and INS treated group D. Serum glucose was significantly increased in both gp B and C and significantly restored in group D. STZ significantly decreased body weight of animals and increased relative weights of kidneys in gp B. MEL treatment in gp C significantly resorted the relative kidney weights but the body weights could not be restored in gp C, whereas in gp D, the animals gained a normal amount of body weight during 6 weeks as they did in control gp A and the relative kidney weights were also similar to those of control gp .
Conclusions: The results of the investigation indicated that concomitant administration of MEL and INS suppressed the progression of renal injury induced by nephrotoxic drugs like STZ.MEL alone significantly suppressed the histopathological damage to the P.C.T. but It could not decrease STZ induced hyperglycemia.Therefore,to counteract the nephrotoxicity and hyperglycemia induced by STZ,both MEL and INS should be administered concomitantly.
Key words: Streptozotocin, Melatonin, Nephrotoxicity, Oxidative stress, Oxygen Free Radicals, Reactive Oxygen species, Proximal convoluted tubules.

INTRODUCTION

Humans are exposed to a variety of potential nephrotoxic substances on a rather frequent basis[1]. Several therapeutic agents have known nephrotoxic potential; classic examples include anti-microbial agents, chemotherapeutic agents, analgesics, and immunosuppressive agent[2]. Medications commonly used in patients with cancer are notoriously nephrotoxic. The major groups of agents causing acute tubular toxicity are antibiotics, NSAIDS and chemotherapeutic agents. The proximal renal tubular cells vulnerability to the direct toxic action of chemicals is largely due to the role played by this portion of the nephron in absorption and secretion[3] Nephrotoxic drugs have become a common cause of acute renal failure and have replaced heavy metals as the prevailing cause of nephrotoxic renal injury. Changes in tubular morphology following exposure to a nephrotoxin can be of a variety of types, ranging from subtle changes in cell organelles detectable only by electron microscopy to extensive necrosis. Nephrotoxicity is intrinsic to the pharmacological effect of certain anticancer drugs. Because antineoplastic agents have a narrow therapeutic index, the amount of drug required to significantly reduce tumour burden usually induces significant

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nephrotoxicity. Philosophically, greater toxicity is acceptable for curative therapy as opposed to palliative therapy[4].STZ is amongst one of the most nephrotoxic chemotherapeutic compounds in frequent use for the treatment of pancreatic islet cell carcinoma and carcinoid tumors[5]. The effects of STZ on different organs have been extensively studied. It is diabetogenic, hepatotoxic, nephrotoxic and also causes gastric ulceration[6].

Renal toxicity is the major dose limiting side effect of STZ[7]. The site of injury involves both the glomerulus and tubules, based on histologic changes[8]. By producing hyperglycemia and hypoinsulinemia, STZ alters various metabolic and enzymatic functions of kidney, resulting in various pathologic lesions[5]. Formation of reactive oxygen species (ROS) is thought to be a mediator of the cytotoxic actions of STZ[8]. Organisms have developed several defence mechanisms to protect their cells against ROS. Such mechanisms include use of antioxidant enzymes and antioxidant molecules such as vitamin C, E and flavonoids.

Oxidative stress has a critical role in the pathophysiology of several kidney diseases. Enhanced generation of reactive oxygen species can overwhelm cells intrinsic antioxidant defences, resulting in oxidative stress. Cells under oxidative stress display various dysfunctions due to lesions caused by reactive oxygen species to lipids, proteins and DNA. In its role as the primary eliminator of exogenous drugs and toxins, the kidney is vulnerable to develop various forms of injury. Antioxidants are compounds that either reduce the formation of free radicals or react with and neutralize them. However, when a condition of oxidative stress establishes, the balance between free radicals production and the level of antioxidant molecules tilts towards excess of free radicals, and the defence capacities against ROS become insufficient. Melatonin (N-accetyl-5 methoxytryptamine), the chief secretory product of the pineal gland, is a multi-facetted free radical scavenger and a strong antioxidant[9]. It breaks down many free radicals, such as highly toxic hydroxyl and peroxyl radicals and oxygen free radicals (OFR). Melatonin can penetrate all the morphophysiological barriers in the human body due to its lipophilic and hydrophilic characteristics[10]. Thus MEL can effectively protect cell walls, organelles and nuclei from damage by free radicals. MEL functions as a modulator of sleep, sexual behaviour, immune functions and circadian rhythm. Moreover, MEL has a potent ROS scavenger activity chiefly because of its capacity to act as an electron donor[11]. It decreases inflammation and impedes the progress of tissue edema[12]. It inhibits the accumulation of neutrophils in the damaged renal tissue[12].

Insulin is essential for energy management in the body. Insulin receptors are believed to be widely expressed in the kidney. However, its role in the kidney is less defined [13]. Absorption of glucose by the tubular cells is independent of insulin action and hence makes the renal tubule vulnerable to glucotoxicity in periods of hyperglycemia[14].However, maintenance of the blood glucose concentration is critical for survival. If little or no insulin is present, glucose cannot be utilized properly by the cells and accumulates in the blood. In many studies involving STZ induced hyperglycemia in albino rats; insulin has been used to restore glucose values towards normal. Studies have been conducted in which various insulin treatment protocols have been compared in STZ induced diabetic rat models.

In the light of the proceeding statements, this study was designed to study the protective role of MEL and INS on the morphology of P.C.T. under the light microscope in albino rats made nephrotoxic by STZ. The effect of STZ, MEL and INS on body weights, and relative weights of the kidneys were also observed and the effects of these drugs on serum glucose levels were also monitored.

MATERIAL AND METHODS

This study was conducted in the department of Anatomy, BMSI, JPMC, Karachi for a period of 6 weeks. In this study, 60 healthy male albino rats, 90-120 days old, weighting around 250-290 gms were obtained from the animal house of BMSI and divided into 4 gps, each gp containing 15 animals.All the animals were kept under observation for 1 week prior to the commencement of study, for the assessment of their health status. All the animals were marked by ear punching and weighed. They were kept in propylene cages, equipped with drinking water bottles and wood chip floor bedding under natural environment. Food and water were supplied ad libitum. Serum glucose of all the animals was determined by a glucometer from the tail vein.

Gp A was taken as control. The animals of gp B, C and D were fasted overnight and administered STZ I/P in a dose of 37mg/kg[15] dissolved in 1 ml of citrate buffer at 4 PH, only on the first day of the experiment. Gp C additionally received 10 mg/100ml[12] of MEL. The water bottles were covered with aluminum foil to prevent degradation of MEL by sunlight. Clean water bottles and freshly prepared MEL solutions were provided each day. Serum glucose of Gp B, C and D animals was closely monitored throughout the experimental period. The animals were weighed and sacrificed at the end of their respective treatment. They were anaesthetized in a glass container, fixed on a dissecting board and the abdomen was opened

by giving a midline Incision. Both the kidneys were exposed and dissected. After washing with normal saline, they were fixed in buffered neutral formalin for 24 hours and then kept in 70% alcohol overnight. Dehydration of the tissues was done with a ascending strengths of alcohol, cleared in xylene and infiltrated with paraffin at 59 degrees. Paraffin blocks of tissue were made and 5microns thick longitudinal sections were cut by a rotatory microtome, mounted on labelled glass slides and stained with PAS-H for a detailed morphological examination of the P.C.T. of the cortex under the light microscope. A minimum of 10 fields of each kidney slide were examined and scored semi quantitatively for severity of changes. The scoring was done as none (-) mild (+), moderate (++) and severe (+++).

RESULTS

The lining epithelial cells of the P.C.T. were regularly arranged on an intact and well defined basement membranes and distinct brush borders (Fig 1). The animals of control group A gained a normal amount of body weight throughout the 6 weeks study (Table 1). The mean relative weights of the kidneys also corresponded to the body weights (Table 2). The mean values of serum glucose were 88.06±5mg/dl (Table 2) and no lesions were observed upon Pathological grading (Table 3).

Most of the P.C.T. showed dilatation with severe sloughing and degeneration, while others showed shrinkage in size with necrotic changes (Fig-2) Most of the cells showed vacuolated appearance obscuring cytoplasmic details. The brush borders and basement membranes were highly discontinuous and distorted (Fig-2). There was a significant increase in relative wts of kidneys(Table-2) and a marked decrease in body wts as compared to control (Table-1). The mean values of serum glucose were 379.12±15mg/dl, which were highly significant as compared to control (Table 1). Diffuse lesions were observed upon grading of the tubular damage (Table 3).

There was an overall improvement and preservation of the morphology of P.C.T. as compared to gp B (Fig. 3). Most of the cells had well defined and intact brush borders and basement membranes, however, marked cytoplasmic vacuolation and hypertrophy of the tubular cells was observered (Fig. 3). A significant restoration of the relative kidney weights was observed(Table 2), but the body weights could not be restored (Table-1) The mean values of serum glucose (360.18±7mg/dl) were highly significant as compared to control gp, high lighting the insignificant effect of MEL on serum glucose. The extension of tubular injury was significantly reduced by MEL (Table 3).

Normal morphology of the P.C.T. was observed (Fig-4), same as control gp (Fig-1). Insulin treatment restored body weights of animals and produced a statistically equal relative kidney weights as compared to the control group A(table 1).The mean values of serum glucose were markedly similar to the control gp (table-2) and no lesions were observed upon grading of the tubular damage (table-3).

Table 1: Changes in body weights of control Gp A,STZ treated Gp B,STZ and MEL treated GpC, and STZ, MEL and INS treated Gp D during 6 weeks of experiment.

Groups	Parameters	
	Initial body weight	**Final body weight**
A(Control)	261.30±1.63	283.70±1.76
B(STZ)	279.63±1.03	254.81±2.83**
C(STZ+MEL)	265.31±2.00	269.61±2.49*
D(STZ+MEL+INS)	276.69±1.88	279.81±1.34

Values are expressed as Mean ±SEM of 15 rats in each group.
*Significant P < 0.01, ** Highly Significant P < 0.05as compared to control.

Table-2: Mean relative kidney weights and blood glucose of albino rats in controlGp A, STZ treated Gp B, STZ & MEL treated Gp C and STZ, MEL and INS treated Gp D after 6 weeks of experiment.

Groups	Parameters	
	Relative kidney wt (gms)	**Serum Glucose (mg/dl)**
A (Control)	0.514±0.005	88.06±5.12
B (STZ)	0.670±0.012**	379.12±15.29**
C (STZ+MEL)	0.592±0.002*	360.18±7.36**
D(STZ+MEL+INS)	0.513±0.001	84.02±5.02

Values are expressed as Mean±SEM of 15 rats in each group.
*Significant P<0.01,**Highly Significant P<0.05 as compared to control.

Table 3: Severity of histological changes of the P.C.T. using scores on a scale as none (-), mild (+), moderate (++) and severe (++++).

Lesions of P.C.T.	Groups			
	A	B	C	D
Degeneration of Tubular Epithelium	**-**	**+++**	**+**	**-**
Tubular Dilatation	**-**	**++**	**+**	**-**
Cytoplasmic Vacuoles	-	**+++**	**+**	**-**
Distortion of brush border membrane	-	**+++**	**+**	**-**
Distortion of basement membrane	-	**+++**	**+**	**-**
Interstitial inflammation	-	**++**	**+**	**-**

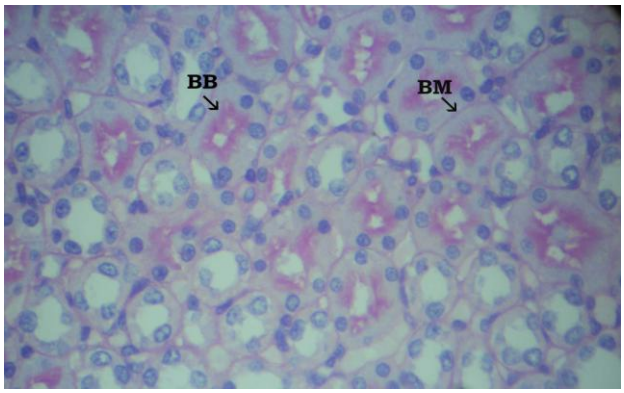

Fig. 1: Photomicrograph of 5 microns thick PAS-H stained section from cortex of kidney in Group A (control) rat showing normal architecture of proximal tubules with intact Brush Borders(BB)and Basement Membranes(BM)X400.

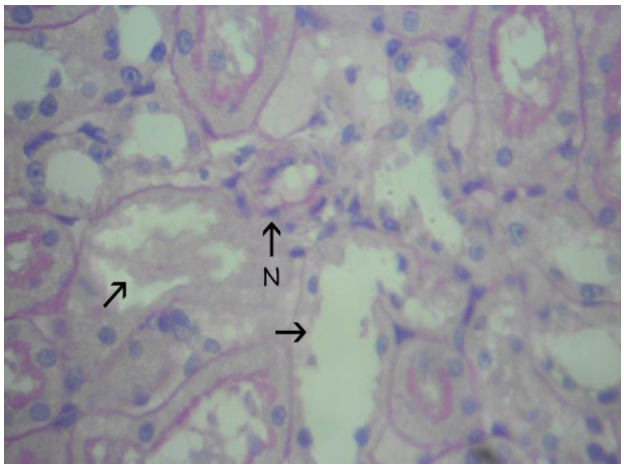

Figure-2: Photomicrograph of 5 microns thick PAS-H stained section from cortex of kidney in STZ treated group B showing disturbed architecture with indistinct BB and BM, nuclear and epithelial debris in the lumina and tubules showing necrotic changes.x400.

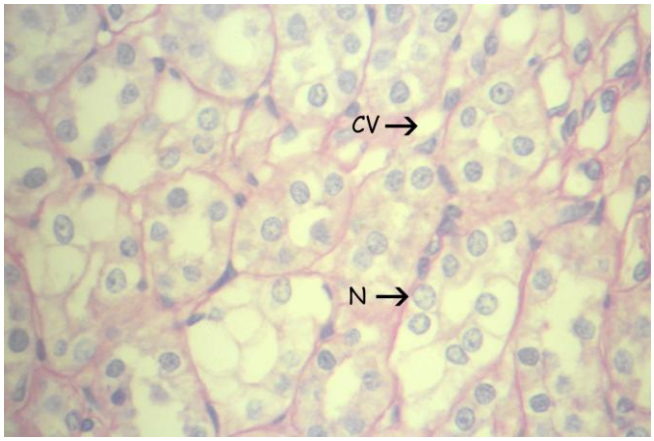

Figure 3: PAS-H stained 5 micron thick longitudinal section of kidney from STZ and MEL treated group C showing indistinct brush borders, enlarged nuclei (N) and cytoplasmic vacuoles (cv).x400.

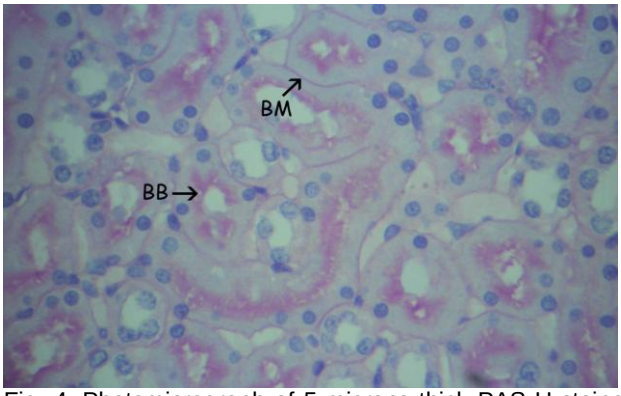

Fig. 4: Photomicrograph of 5 microns thick PAS-H stained section from cortex of kidney in Group D (MEL and INS treated)rat showing no change in proximal tubules with intact BB and BMx.400.

DISCUSSION

The present study demonstrated the significance of MEL in reducing the severity of renal damage in animals exposed to a nephrotoxic drug like STZ. However, significant restoration could only be achieved by concomitant administration of insulin. Since the kidney is highly susceptible to the toxic injury by a multitude of different drugs, it is not surprising that several antineoplastic agents may exert potent nephrotoxicity. STZ generates ROS which contributes to DNA fragmentation and evoke other deleterious changes in the cells[16].Petzold and Swenberg in 1978[17] demonstrated that a single I.V. dose of STZ induces strand breaks in kidneys and liver of rats. Alejandro D and Martha S in 2002[8] stated that STZ induces cell death by apoptosis and necrosis, which are in agreement to our results. Rodriguez et al in 2004[18] stated that oxidative stress and its constant companion inflammation play a major role in the pathogenesis of the progression of renal injury. MEL has potent antioxidant and anti-inflammatory properties and its production is impaired in chronic renal failure.

Glucose entry into proximal tubular cells is insulin independent which makes the proximal tubular cells particularly sensitive to hyperglycemia[19] .However, in a study conducted by Jan Melin et al in 2002[20] demonstrated that insulin treatment reduced renal alternations such as tubular vacuolization, glomerular basement membrane thickening and disturbed renal functions seen in STZ induced diabetes in rats. Maintenance of the blood glucose concentration is critical for survival. If little or no insulin is present, glucose cannot be utilized properly by the cells and accumulates in the blood, further aggravating the accumulation of free radicals .In a study conducted by Kim et al in 1999 [21],STZ caused

a marked reduction in body weights in albino rats, whereas insulin treatment caused a significant restoration of body weights.

The favourable results seen with melatonin administration in our experiment are likely related to the antioxidant and anti-inflammatory properties of this compound resulting from its strong ROS scavenger properties. MEL has been shown to ameliorate inflammation by blocking transcriptional factors and tumour necrosis factor alpha[22] .In those situations where free radical production is enhanced, MEL has demonstrated to be more effective than other antioxidants with the advantage that lower doses are needed[12].It has been proved earlier that MEL could effectively neutralize the impaired anti oxidative status in rats with STZ induced diabetes. MEL has been shown to be effective in protecting against severe free radical mediated toxicity in a variety of conditions including chemotherapy[23], ischemia reperfusion injury[24], acute renal failure caused by mercuric chloride and gentamycin[25]. In a study conducted by Naqvi A in 1992[15], STZ at the dose of 37mg/kg for 6 weeks produced marked hyperglycemia in albino rats which is in agreement with our results. In the present study, STZ resulted in significant hyperglycemia and MEL supplementation did not affect this parameter. In a similar study conducted by Sudnikovich et al in 2007[26],STZ administration to albino rats for 25 days resulted in significant hyperglycemia, increased levels of glycated hemoglobin and retarded growth of animals, whereas melatonin administration did not effect these parameters.

Our experimental study reveals that the renal injury caused by STZ is not only due to hyperglycemia, but due to its direct toxic effects on the morphology of the kidneys. M.Akmali in 2010[7] stated that STZ forms ROS which is responsible for its cytotoxicity. Our study further reveals that MEL preserves the morphology of the kidneys without producing a significant effect on blood glucose levels. Sudnikovich et al in 2007[26] stated that STZ caused a marked decrease in body weight and significant increase in kidney weights in albino rats which is consistent with our results. In the present study, administration of MEL could not restore body weights in STZ treated group of animals, which is similar to the result of a previous study conducted by Prunet et al in 2003[27]. This result is consistent with melatonin's ability to increase leptin expression by adipocytes[28]. However, melatonin did restore the relative weights of the kidneys in STZ treated group C. Similarly, Adewole et al[29] in 2010 demonstrated that melatonin significantly restored relative kidney weights in carbon tetrachloride induced renal injury in wistar rats.

CONCLUSION

In conclusion, this study demonstrates that MEL administration suppresses the progression of renal injury induced by nephrotoxic drug like STZ.MEL could not restore the weight of animals or decrease hyperglycemia in STZ treated group C, but it did preserve the renal morphology of the proximal convoluted tubules damaged by free radicals generated by STZ whereas in group D, serum glucose levels were significantly restored along with complete restoration of renal morphology .We may conclude that dietary supplementation of MEL could be an easy and inexpensive method of protecting cancer patients from renal damage caused by oxidative stress. However, it could not restore serum glucose levels in STZ induced hyperglycemia or prevent the formation of free radicals due to increased serum glucose levels in albino rats, without insulin administration.

REFERENCES

1. Perazella MA Renal Vulnerability to Drug Toxicity Clin J Am Soc Nephrol 2009; 4: 1275–1283.
2. Schetz M, Dasta J, Goldstein S, Golper T: Drug-inducedacute kidney injury. *Curr Opin Crit Care2005;*11: 555–565.
3. Pfaller W, Gstraunthaler G, Willinger CG Morphology of renal tubular damage from nephrotoxins. Toxicol lett.1990;53:39-43.
4. M.E. De Bore, G.A. Porter, W.M. Bennett and G.A. Verpooten. Clinical Nephrotoxins,2nd Ed., 353-372.2003 Kluwer Academic Publishers,Netherlands.
5. Zafar M, Naqvi NH, Ahmed M, KaimKhani Z.A. Altered kidney morphology and enzymes in STZ induced diabetic rats.Int.J. of Morphol 2009; 7(3) 783—790.
6. Piyachaturawat P, Poprasit J,Glinsukon T Gastric mucosal secretions and lesions by different doses of STZ in rats. Toxicol. Let,., 1990;55:21-29.
7. F. Ries and J. Klasttersky Nephrotoxicity induced by cancer chemotherapy with special emphasis on cisplatintoxicity. Amer. J. of kid.dis. 1986; Vol 8(S):368-379.
8. Alejandro D. Bolzan, Martha S. Bianchi. Genotoxicity of Streptozotocin. Mut. Res. 2002; 512:121:-134.
9. M. Akmali, R. Ahmadi, M. Vessal Pre- and post treatment of STZ administered rats with melatonin. Arch Iran Med. 2010,Vol 13(2).105-110
10. Reiter RJ, Tan DX, cabreroj D, Sainz RM, Mayo JC and Ramos S.The oxidant/antioxidant network :Role of melatonin Biol signals recept 1999;8(2):56-63.
11. C. Col, K. Dinler, O. Hasdemir, O. Buyukasik and G. Bugdayci Oxidative stress and lipid peroxidation products: effect of pinealectomy or exogenous melatonin injections on biomarkers of tissue damage during acute pancreatitis. HepatobiliaryPancreat Dis. Int; 2010; Vol 9(1):76-82.
12. Y.Quiroz, A.Ferrebuz, F.Romero, N.D. Vaziri and B. Rodriguez Iturbi. Melatonin ameliorates Oxidative

stress, inflammation, proteinuria and progression of renal damage in rats with renal mass reduction Amer. J. Of Phys. Renal Physiol. 2008;Vol 294 (2); F336-344.

13. Swasti Tiwari, Veerendra K.M. Halagappa,Shahla Riazi, Xinqun Hu. Reduced Expression of Insulin Receptors in the Kidneys of Insulin-Resistant Rats.J.Amer.Soc.Neph2007;18:2661-2671.

14. Jones SC,Saunders HJ,Pollock CA.High Glucose Increases Growth And Collagen Synthesis In Cultured Human And Tubulointerstitial Cells. Diabet Med 1999;16:932-938.
15. Naqvi A. The effect of streptozotocin on duodenal mucosa in albino rats. M. Phil thesis, Basic medical sciences institute, Jinnah post graduate medical centre, Karachi ,1992.
16. Takasu N, Komiya I, Asawat, NagdsawaY,Yamada T STZ and alloxan induced H2 O2 generation and DNA fragmentation in pancreatic Islets. Diabetes 1991; 40: 1141-1145.
17. G.L. Petzold, K.A. Swenberg Detection of DNA damage induced in vivo following exposure of rats to carcinogens. Cancer Res.1978; 38: 1589-1594.
18. Carmen Rodriguez, Juan C.Mayo, Rosa M.Sainz, I saacAntolin, Federico Herrera, Vanesa Martin, RusselJ.Reiter. Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal Res.2004;36 (1):1–9.
19. Volker Vallon The proximal tubule in the pathophysiology of the diabetic kidney AJP - Regu Physiol 2011; 300:R1009-R1022.
20. Jan Melin,Olof Hellberg , Erik Larsson, Lilian Zezina and Bengt C, Fellström. Protective effect of insulin on ischemic renal injury in diabetes mellitus *Kidney International*.2002; **61**: 1383–1392.
21. Eun-Mee Kim, Martha K.Grace,Catherine C.Welch,Allen `S.Levine. STZ induced diabetes decreases and insulin normalizes POMC mRNA in Arcuate Nucleus and Pituitary in rats.AJP-Regu Physiol 1999;276:R1320-R1326.
22. Ronald R.W. Melatonin in the promotion of Health, 2nd edition, CRC Press, USA, 2011.
23. Gultekin F, Hicycilmuz H. Renal deterioration caused by carcinogens as a consequence of free radical mediated tissue damage: review of the protective action of melatonin, Arch Toxicol 2007; 81:675-681.
24. Kurcer Z, Ogul E, Ozbilge H Baba F, Alsov N, Melatonin protects from Ischemia reperfusion-induced renal injury in rats. J pineal Res.2007;43:172-178.
25. Sener G, Sehirli AO, Altunbas HZ, Ersov Y, Paskaldglu K, Arbak S, Ayanoghi-Dugler G. Melatonin Protects against gentamicin-induced nephrotoxicity in rats.J pineal Res 2002; 32 : 231-236.
26. Elena JU Sudnikovich, Yuri Z. Maksimchik, Svetlana V.Z. Abroadskaya, Valeri L. Kubyshin, Elena A. Lapshina , Maria Bryszewska , Russell J. Reiter Melatonin attenuates metabolic disorders due to STZ-induced diabetes in rats . Eur .J. of Pharm. 2007;569:180-187
27. Prunet-Marcassus B, Desbazeille M. Bros A, Louche K, Delagrange P, Renard P, casteilla L, Penicaud L Melatonin reduces body weight in Sprague Dawley Rats with diet-induced obesity. Endocrinology 2003;1449(12): 5347-52.
28. Anclreotti S, Peres S.B., Anhe G.F. dcis Naves Borges –Silva C, neto JC, Lina F.B. Mei enhances leptin expression by rat adipocytes in the presenceof insulin. Am J. Physiol. Endocrinal Met,2005; 288 : E-805-812.
29. S.O.Adewole, A.A.Salako,O.W.Doherty and T.Naicker Effect of melatonin on carbon tetrachloride induced kidney injury in wistarrats.Afr.J.Biomed.Res.2010;10:153-164.