

## Biochemical and Histopathological Inflections in Hepato-renal Tissues of Streptozotocin (STZ) Induced Diabetic Male Rats: Impact of Exogenous Melatonin Administration

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### Abstract

**Objective:** To evaluate the therapeutic efficacy of exogenous melatonin (MEL) on hepato-renal tissue in a diabetic rat model.

**Methodology:** Streptozotocin (STZ) was used to establish diabetic rat model. Diabetes was confirmed by monitoring the blood glucose level, animals having glucose level above 250 mg/dl were considered as diabetic and were divided into six different groups. Model control group, diabetic group, melatonin treatment to diabetic rats, melatonin per se group, glibenclamide (a standard hypoglycemic drug) treatment to diabetic rats and glibenclamide (standard control) alone respectively. The model control was given 0.5 ml (0.1 M) citrate buffer, experiment was conducted for one month. After the completion of experiment, rats were sacrificed. Blood was collected and centrifuged at 3000 rpm for 10 minutes to obtain the serum. Serum was kept at -80°C for further analysis of liver and renal function tests and lipid profile. Liver and kidney tissues were weighed, fixed in Bouin's fixative for histopathological studies. Further tissues were processed for Lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT).

**Major findings:** Administration of MEL to STZ induced diabetic rat showed a significant decrease of lipid peroxidation (TBARS) in kidney and liver tissue comparable to the control and GLIBEN group of rats. In addition MEL prevented the decrease in antioxidative enzyme parameters viz. superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) of hepato-renal tissues. Parameters of liver functions (alanine amino transaminase (ALT), aspartate amino transaminase (AST) and alkaline phosphatase (ALP) and renal function (urea, uric acid and creatinine) were noted restored following MEL treatment. MEL administration further maintained the normal levels of lipid profiles i.e., triglyceride, cholesterol, low and high density lipoprotein (LDL, HDL) to that of the control group of rats. Histological architecture of liver and kidney tissues were noted repaired and rescued as judged by cellularity of hepatocytes and renal cells.

**Conclusion:** The present finding strongly indicates the protective effect of exogenous melatonin for hepato-renal tissues from the damages and impairment observed and noted in the experimentally induced STZ male rat model.

**Keywords:** Melatonin; Streptozotocin; Oxidative stress; Anti-oxidative enzymes; Kidney; Liver

### Introduction

Diabetes mellitus is a state of physiological stress, during which homeostasis of carbohydrate and lipid metabolism is improperly regulated by the insulin, leading to the elevation in plasma glucose level. Among several endocrine disorders, diabetes mellitus is the supremely diagnosed endocrinopathies in humans and other household pets which can easily be induced in experimental animals models [1,2]. Diabetes is characterized by hyperglycemia, because of  $\beta$ -cell dysfunction/resistance against insulin by the other peripheral cells of the body.

Liver is one of the major metabolic regulatory organs involved in the biotransformation and detoxification of drugs, toxic chemicals. During such biotransformation in liver these insoluble molecules are made hydrophilic [3]. In diabetes mellitus oxidative stress causes the

breakdown of liver cells, which may lead leakage of proteins and other major constituents of cells into the blood stream. Altered glucose homeostatic balance can lead to adverse effects in the whole body of an organism.

The second vital organ kidney acts potentially to release glucose into the blood because gluconeogenesis occurs in this organ. It has the potential to release glucose into the blood. Kidney as the excretory and filtration unit of the body is highly exposed to the heavy free radical load and therefore may lead to various anomalies in the normal functioning of its own physiology [4]. Nonetheless, various literatures support heavy free radical production and physiological stress as a major causative agent for diabetes [5]. Such physiological stresses during diabetic condition may impair the renal functions due to hyper-production of urea, uric acid and creatinine in blood serum which constitutes the basic parameters for renal function analysis [6]. Hyperglycemic condition leads oxidative stress, due to the production of superoxide anion, hydroxyl radicals, and non-enzymatic glycation of proteins and autooxidation of glucose.

Hyperglycemic status induces several changes in the antioxidative defense system which may accelerate the misappropriate generation of free radicals [4]. These reactive species damage and alter the membrane fluidity, non-enzymatic glycation of protein of any cells in terms of their permeability. The cell membrane which is normally selectively permeable, now becomes permeable to any molecule, because of the free radicals (ROS and other reactive species) leading to numerous oxidative cellular damage of the biological membrane. Such injury to cell membrane changes the functions of the cells and ultimately affects the internal cellular environment and therefore finally leading to cellular death [7-9]. The chain of free radicals generated from cells weakens/downregulates the antioxidative enzyme system thus making unabling to scavenge them. Glutathione reduced (GSH) which is primary antioxidant system plays a crucial role in the defense of cells from reactive free radicals and other oxidants species [10]. Further, superoxide dismutase (SOD), catalase (CAT), GSH cycle (GPx, GR and G6PDH) are powerful antioxidant molecules which always remains active during their reduced state in order to be compatible to scavenge free radical. Melatonin a chronobiotic molecule is reported to have pleiotrophic effects such as immune regulator in seasonally breeding rodents [11], immunosuppressive effect on synthetic glucocorticoids, dexamethasone [12], immunoprotective [13] and antiapoptotic [14], thermoregulation [15] and antiageing [16] mediating through endocrine axis [17,18]. It also helps to reduce the inflammatory response [19]. Melatonin declines the oxidative load and initiates the proliferation of lymphoid cellular organs in rodents [16]. The reduction of NO formation is considerable as it limits the rise in the intensity of the pro-oxidant metabolite, peroxy nitrite, and of free radicals derived from this compound (i.e. NO<sub>2</sub>, CO<sub>3</sub> and OH radicals) [19]. Clinically melatonin is considered as neuroprotective [20,21], to treat sleep disorders [22,23], and its use in several diseases of neonates, pediatric and children [20,24,25]. The action of melatonin is mediated by two ways: membrane receptors (MT<sub>1</sub>, MT<sub>2</sub>) and non-receptors mediated. Several findings suggest many therapeutic molecules to regulate the free radical of liver and kidney to treat diabetes.

Melatonin is nowadays reported a more effective antioxidant even than vitamin E and C [26]. However, very less attention has given towards such medication. Therefore, this study has been designed to evaluate histological and biochemical alterations of liver and kidney tissues in a streptozotocin (a chemotherapeutic alkylating molecule) induced diabetic rat model. The role of melatonin was compared with anti-diabetic molecule (Glibenclamide) to check and confirm the efficacy of melatonin.

## Materials and Methods

### Chemicals

Melatonin (Mel), Streptozotocin (STZ), Citrate monohydrate, Sodium citrate Thiobarbituric acid (TBA), Tris-hydrochloric acid (Tris-HCl), Phosphoric acid and Butylated Hydroxy Toluene (BHT), Glutathione reduced (GSH), Glibenclamide (GLIBEN), Phenazine methosulphate (PMS), Glacial acetic acid, H<sub>2</sub>O<sub>2</sub>, Dithio-bis 2-nitrobenzoic acid (DTNB), Nitroblue tetrazolium salt (NBT), Nicotine Adenine Dinucleotide Phosphate (NADPH) of analytical grade procured from sigma and Himedia. Analysis of liver and renal function tests (LFTs and RFTs) and lipid profile under blood serum biochemistry were done using commercial kits from rapid scientific, USA.

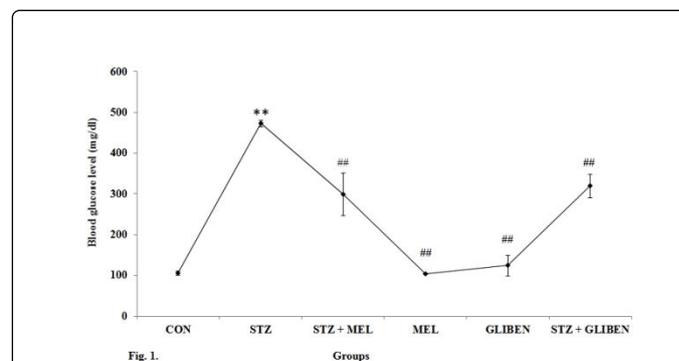
### Animal maintenance

Male rats (Wistar strain) weighing approximately 150 ± 10 g of same age groups were procured from Defence Research and Development Establishment (DRDE) Gwalior, MP, India and were kept under standard laboratory conditions (25 ± 2°C temperature, 60-70% humidity and a controlled 12 h light/dark cycle). Rats were fed with pellet diets and water ad libitum (Table 1). All experimental procedures was approved in accordance with guide lines for care and use of laboratory animals of institutional animal ethics committee (IAEC), Guru Ghasidas Vishwavidyalaya, Bilaspur (CG) India (Registration number: 994/Go/ERE/S/06/CPCSEA). Rats were acclimatized for two weeks and were grouped as follows.

Groups	Dosage	No. of animals	Duration (in days)
Control (CON)	0.5 ml (0.1 M ) citrate buffer	6	30
Streptozotocin (STZ)	15 mg/kg	6	30
STZ+MEL	15 mg/kg+10 mg/kg	6	30
Melatonin (MEL) (per se)	10 mg/kg	6	30
Glibenclamide (GLIBEN)	0.5 mg/kg	6	30
STZ+GLIBEN	15 mg/kg+0.5 mg/kg	6	30

**Table 1:** Grouping and acclimatization of Rats for two weeks.

### Induction and assessment of diabetes



**Figure 1:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on blood glucose level of streptozotocin induced diabetic male rat showing significantly increased blood glucose level in diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

Streptozotocin (STZ) was dissolved in 0.1m citrate buffer (pH 7.4) (15 mg/kg) and administered intraperitoneally for six consecutive days. Blood glucose level of the animals was monitored using Glucometer (ACCU CHECK) after 72 hours of streptozotocin treatment. Rats with blood glucose level exceeding higher than 250 mg/dl upto 6th day are confirmed as diabetic model (Figure 1). Diabetes was further confirmed by estimation of the sugar level in urine samples. Urine

sugar of diabetic rats exceeded continuously from traces upto 4+. Animals were divided into different groups and were kept for experimentation for 30 days as under the following experimental design (Table 2). After the completion of experiment, rats were sacrificed. Blood was collected and centrifuged at 3000 rpm for 10 minutes to obtain the serum. Serum was kept at -80°C for further analysis of liver and renal function tests and lipid profile. Liver and tissues were weighed, fixed in Bouin's fixative for histopathological studies and were also processed for Lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT).

### Parameters studied

**Gravimetric analysis:** The body weight of all the rats were taken before the experimental setup and assessed weekly to note the average change in body weight (Table 2).

Groups	0-Day	7-Day	14-Day	21-Day	28-Da	Final
Control	180 ± 5	175 ± 5	175 ± 5	179 ± 5	182 ± 5	185 ± 5
Streptozotocin (STZ)	175 ± 5	160 ± 5	155 ± 5	160 ± 5	150 ± 5	149 ± 5
STZ+MEL	175 ± 5	160 ± 5	165 ± 5	168 ± 5	170 ± 5	172 ± 5
Melatonin (MEL)	175 ± 5	168 ± 5	170 ± 5	173 ± 5	176 ± 5	175 ± 5
Glibenclamide (GLIBEN)	175 ± 5	160 ± 5	163 ± 5	165 ± 5	169 ± 5	173 ± 5
STZ + GLIBEN	178 ± 5	166 ± 5	164 ± 5	168 ± 5	170 ± 5	174 ± 5

**Table 2:** Showing variation in body weight of rats of each group during experimentation.

### Thiobarbituric acid-reactive substances (TBARS) assay

Thiobarbituric acid reactive substances (TBARS) are produced during oxidative damage to cell membrane. Malondialdehyde (MDA), one of the major lipid breakdown product and commonly used parameter to assess lipid peroxidation. Liver and kidney were excised and weighed for the preparation of 10% tissue homogenates in 20 mM Tris Hydrochloride (HCl) buffer (pH-7.4). The homogenates were centrifuged at 3000 g for 15 min at 4°C and supernatant was subjected to Thiobarbituric acid (TBA) assay by mixing it with 8.1% SDS, 20% acetic acid, 0.8% TBA and boiling for 1 h at 95°C. The reaction mixture was immediately cooled in running water and vigorously shaken with n-butanol and pyridine reagent (15:1) and centrifuged for 10 min at 1500 g. The absorbance (A) of the upper phase was measured at 534 nm. LPO was expressed as TBARS in nmol/g tissue weight by taking 1,1,3,3 tetraethoxypropane (TEP) as standard. The standard curve was calibrated using 10 nM concentration of TEP [27].

### Glutathione reduced (GSH)

The thiol content of liver and kidney was measured by using the modified method of [28]. 0.1 ml of tissue homogenate was added to 1.5 ml of 0.2M Tris buffer (pH 8.2) and 0.1 ml of 0.01 M dithio-bis (2-nitrobenzoic acid, DTNB), methanol was added to adjust total volume upto 10 ml. The sample mixture was incubated at 37°C for 30 minutes (min). Following incubation period of 30 minutes the mixture was centrifuged at 3000 rpm for 15 minutes. The absorbance of the yellow colored supernatant was measured at 412 nm using Perkin Elmer

Spectrophotometer (Lambda 25, serial No. 501812090210). The molar extinction coefficient of 13,100 was used to calculate GSH content.

### Super oxide dismutase (SOD) activity

Superoxide dismutase (SOD) level in liver and kidney tissues was assessed by using the modified method of [29]. 0.2 ml of tissue homogenate and to this whole reaction mixture containing (1.2 ml of sodium pyrophosphate, pH 7.0, 0.052 Mm, 0.1 ml of phenazine methosulphate (PMS), 185 µl and 0.3 ml of NBT (300 µM) ). NADH (0.5 µM) was added to each tube at 30°C. Reaction was stopped by the addition of 2 ml of glacial acetic acid. The reaction mixture was stirred and 4 ml of n-butanol and allowed to stand for 10 minutes. The mixture was centrifuged to separate butanol layer containing chromogen and absorbance was taken at 560 nm. The pure butanol was used as blank.

### Catalase (CAT) activity

The catalase (CAT) activity in liver and kidney was analyzed by modified method of [30]. 2.5 ml of phosphate buffer and 0.1 ml of liver and kidney tissue homogenate was taken in test tubes, incubated at 25°C for 30 minutes. The reaction was initiated by adding 650 µl of H<sub>2</sub>O<sub>2</sub> and absorbance was measured at 240 nm for 3 minutes.

### Blood serum analysis for LFTs, RFTs and lipid profile

The assessment of serum marker enzymes of liver including alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP). Renal marker enzymes like Creatinine, urea, and uric acid and Lipid profile assessment the level of Triglyceride, Cholesterol, low and high density lipoproteins (LDL and HDL) was done (Rapid serum autoanalyser following instructions provided with the manual of commercial kits).

### Histological preparation

Liver and kidney of all the six groups were harvested, cleared and fixed in Bouin's fixative for twenty four hours. The liver and kidney tissues were processed and blocks were prepared in paraffin wax. Liver and kidney histological sections of 5 µm thick using the rotary microtome (Leica RM 2125RT5) and then stained with hematoxylin and eosin. Histophotomicrographs of all six groups were captured in Trinocular research microscope under 400x magnification.

### Statistical analysis

The data was analyzed by one way ANOVA followed by post hoc students t-tests by IBM SPSS 19.0 software. The data was significant at p<0.05 and p<0.01.

### Results

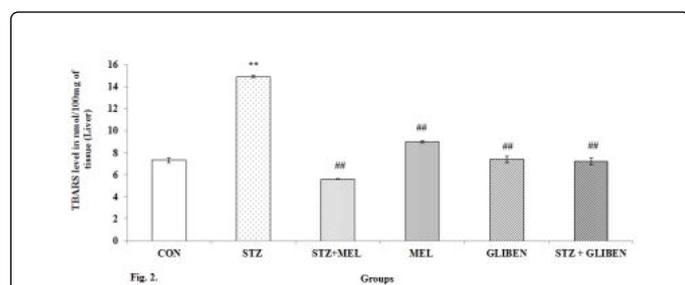
#### Effect of exogenous melatonin on body weight

Diabetic rats showed significant decrease in body weight, whereas melatonin co-administration to the diabetic rats increases the body weight in similar trend like the glibenclamide. The values of change in body weight as calculated and was significant at p<0.05 level (Table 2).

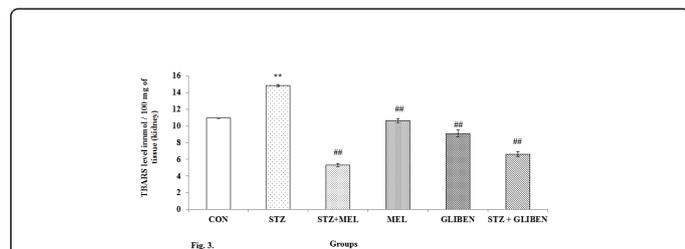
### Effect of exogenous melatonin on blood glucose level

The glucose level of the experimental groups was assessed firstly at 72 hours and then weekly to note the change in the blood glucose level using Glucometer (ACCU Check). Diabetic control showed significant increase in the blood glucose level, whereas melatonin (10 mg/kg) treatment for 30 days to the diabetic rats showed gradual decrease level in blood glucose in the similar way as the glibenclamide treated rats. Melatonin and glibenclamide administration alone does not showed any abnormal change in blood glucose level, but regulated the glucose level toward the normal range. The values of blood glucose level were significant ( $p < 0.01$ ) (Figure 1).

### Effect on lipid peroxidation (LPO) level on liver and kidney tissues



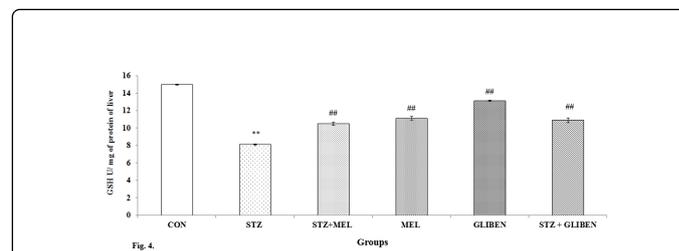
**Figure 2:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on lipid peroxidation (LPO) in hepatocytes of Streptozotocin induced diabetic male rat showing significant increase of LPO analyzed in terms of TBARS in diabetic rats. Histogram represent Mean + SE; N=6; \*\* $p < 0.01$ : CON vs. STZ; ## $p < 0.01$ : STZ vs. STZ + MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.



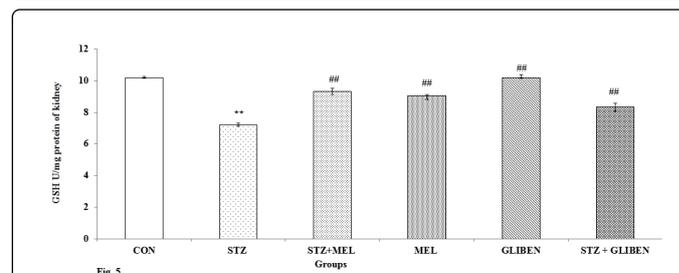
**Figure 3:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on lipid peroxidation (LPO) in renal cells of Streptozotocin induced diabetic male rat showing significant increase of LPO analyzed in terms of TBARS in diabetic rats. Histogram represent Mean+SE; N=6; \*\* $p < 0.01$ : CON vs STZ; ## $p < 0.01$ : STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

The free radical production was noted significantly higher in liver and kidney tissues of streptozotocin (STZ) induced diabetic rats. While as streptozotocin (STZ) induced diabetic rats treated with melatonin and glibenclamide showed significant decrease in TBARS level of liver and kidney tissues. Melatonin and glibenclamide alone showed significant ( $p < 0.01$ ) regulation of free radical generation (Figures 2 and 3).

### Effect on superoxide dismutase (SOD) on liver and kidney tissues



**Figure 4:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on glutathione reduced (GSH) in hepatocytes of Streptozotocin induced diabetic male rat showing significant decrease of GSH analyzed in terms of thiol content in diabetic rats. Histogram represent Mean+SE; N=6; \*\* $p < 0.01$ : CON vs. STZ; ## $p < 0.01$ : STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.



**Figure 5:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on glutathione reduced (GSH) in renal cells of Streptozotocin induced diabetic male rat showing significant decrease of GSH analyzed in terms of thiol content in diabetic rats. Histogram represent Mean +SE; N=6; \*\* $p < 0.01$ : CON vs. STZ; ## $p < 0.01$ : STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

A significant decrease in SOD activity was observed in streptozotocin (STZ) induced diabetic rats in both liver and kidney tissues, whereas melatonin administration to the diabetic rats showed improvement in antioxidant enzyme levels. SOD activity showed comparably increase like the glibenclamide and control rats. The values were significant at ( $p < 0.01$ ) (Figures 4 and 5).

### Effect on catalase (CAT) activity on liver and kidney tissues

Streptozotocin (STZ) induced diabetic rat model showed significant decrease in catalase (CAT) activity (enzyme which cleaves the  $H_2O_2$  into water and molecular oxygen) of liver and kidney tissues (Figures 5 and 6). However, liver tissue was noted more effective and sensitive. Melatonin treatment to streptozotocin (STZ) induced diabetic model showed significant increase ( $p < 0.01$ ) in catalase activity and compared to the control and glibenclamide (Figures 6 and 7).

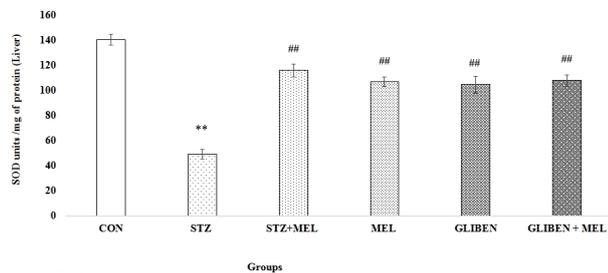


Fig. 6.

**Figure 6:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on superoxide dismutase activity (SOD) in hepatocytes of Streptozotocin induced diabetic male rat showing significant decrease of SOD analyzed in terms of SOD activity in diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

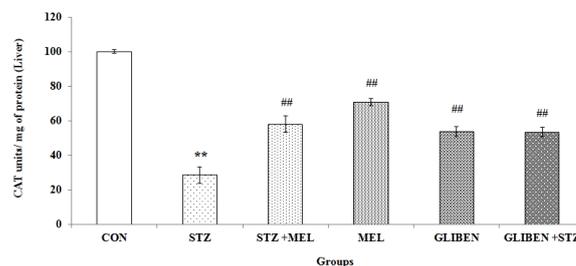


Fig. 8.

**Figure 8:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on catalase activity (CAT) in hepatocytes of Streptozotocin induced diabetic male rat showing significant decrease of CAT analyzed in terms of CAT in diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

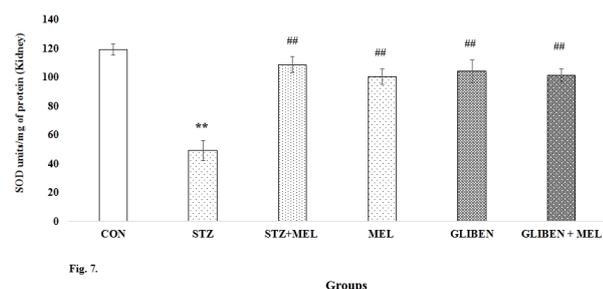


Fig. 7.

**Figure 7:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on superoxide dismutase activity (SOD) in renal tissues of Streptozotocin induced diabetic male rat showing significant decrease of SOD analyzed in terms of SOD in diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

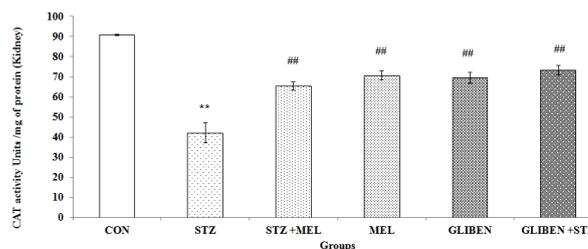


Fig. 9.

**Figure 9:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on catalase activity (CAT) in renal tissues of Streptozotocin induced diabetic male rat showing significant decrease of CAT analyzed in terms of CAT in diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

### Effect on glutathione reduced (GSH) of liver and kidney tissues

The GSH level was noted decreased significantly in streptozotocin (STZ) diabetic rats and (p<0.01) in liver and kidney both the tissues. Melatonin supplemented to streptozotocin diabetic rats showed gradual increase in thiol content in the hepatocytes and renal cells. The results were compared with standard antidiabetic molecule (Glibenclamide) treated group (p<0.01) (Figures 8 and 9).

### Effect of exogenous melatonin on liver function tests (LFTs) of circulating blood serum

The serum markers of liver injury (ALT/SGOT, AST/SGPT and ALP) showed significant increase in diabetic rats. The melatonin treatment reduces the level of marker enzymes in the serum by reversal of liver damage. The results were compared with glibenclamide treated group (p<0.01) (Figures 10-12).

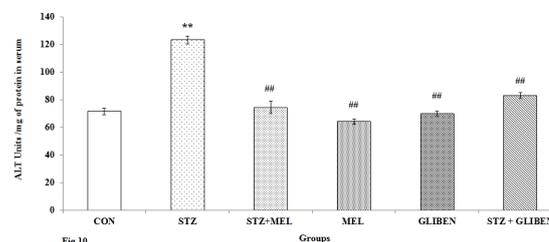


Fig. 10.

**Figure 10:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on alanine amino transaminase (ALT) in serum of Streptozotocin induced diabetic male rat showing significant increase of ALT analyzed in terms of units of ALT in the serum of diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

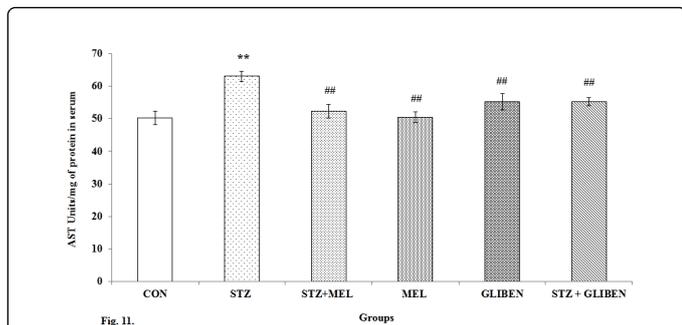


Fig. 11.

**Figure 11:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on aspartate aminotransaminase (AST) in serum of Streptozotocin induced diabetic male rat showing significant increase of AST analyzed in terms of units of AST in the serum of diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

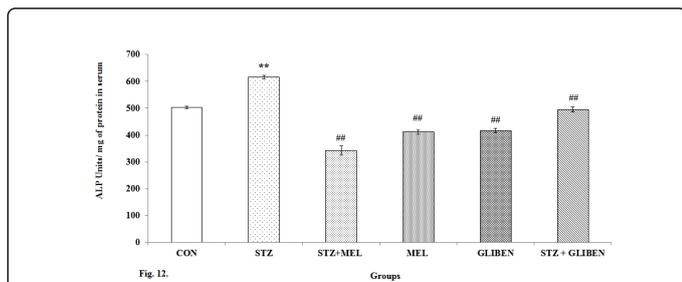


Fig. 12.

**Figure 12:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on alkaline phosphatase (ALP) in the serum of Streptozotocin induced diabetic male rat showing significant increase of ALP analyzed in terms of units of AST in the serum of diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

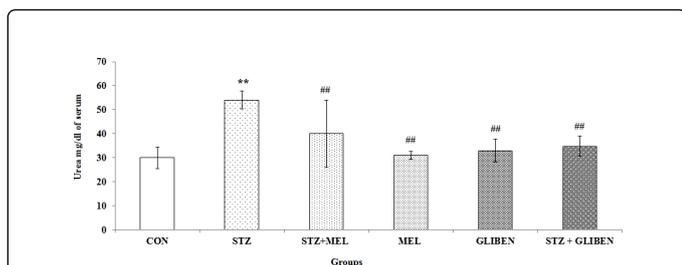


Fig. 13:

**Figure 13:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on urea in serum of Streptozotocin induced diabetic male rat showing significant increase of urea analyzed in terms of mg/dl of serum in diabetic rats. Histogram represent Mean + SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

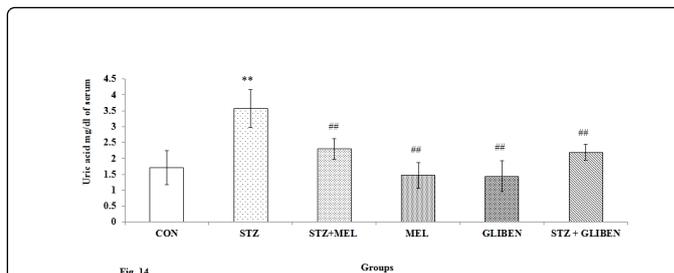


Fig. 14.

**Figure 14:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on uric acid in serum of Streptozotocin induced diabetic male rat showing significant increase of uric acid analyzed in terms of mg/dl of serum in diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

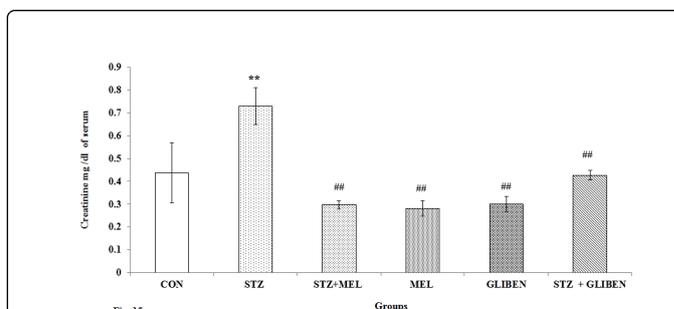


Fig. 15.

**Figure 15:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on Creatinine in serum of Streptozotocin induced diabetic male rat showing significant increase of creatinine analyzed in terms of mg/dl of serum in diabetic rats. Histogram represent Mean+SE; N=6; \*\*p<0.01: CON vs. STZ; ##p<0.01: STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

### Effect of exogenous melatonin on renal function tests (RFTs) of circulating blood serum

Serum analyzed for Creatinine, urea and uric acid showed a very significant (p<0.01 and p<0.01) increase in diabetic rats in kidney marker enzymes (Urea, Uric acid and Creatinine). Melatonin treatment significantly declines level of Urea, Uric acid and Creatinine in the serum, when compared with control and Glibenclamide treated rats (p<0.01) (Figures 13-15).

### Effect of exogenous melatonin on Lipid Profile of circulating blood serum

Triglyceride (TG), cholesterol, low and high density lipoprotein (LDL and HDL) levels was measure in diabetic rats, the level of cholesterol LDL and TG showed significant increase in diabetic rats. The melatonin co-administration has significantly reduced the level toward the normal when compared with control and Glibenclamide rats. The HDL level was significantly decreased in diabetic rats, melatonin supplementation to the diabetic rats significantly increased the level of HDL in similar trend of control and antidiabetic molecule

(Glibenclamide). Results were significant at ( $p < 0.01$ ), (Figures 16-18) (Table 3).

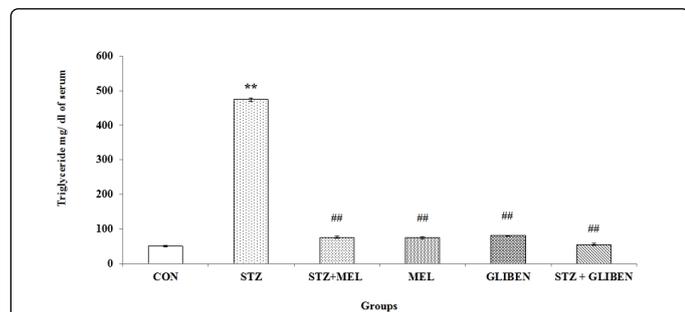


Fig. 16.

**Figure 16:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on triglyceride (TG) in serum of Streptozotocin induced diabetic male rat showing significant increase of triglyceride analyzed in terms of mg/dl of serum in diabetic rats. Histogram represent Mean +SE; N=6; \*\* $p < 0.01$ : CON vs. STZ; ## $p < 0.01$ : STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

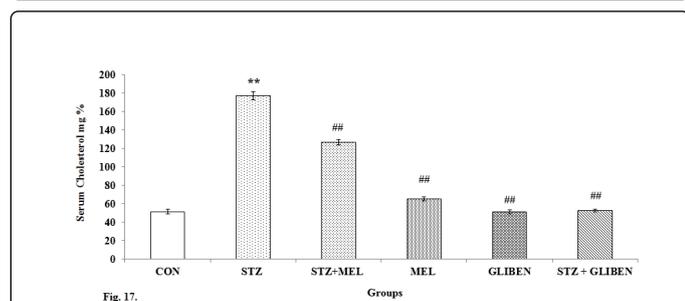


Fig. 17.

**Figure 17:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on cholesterol in serum of Streptozotocin induced diabetic male rat showing significant increase of cholesterol analyzed in terms of % of cholesterol in the serum of diabetic rats. Histogram represent Mean +SE; N=6; \*\* $p < 0.01$ : CON vs. STZ; ## $p < 0.01$ : STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

Groups	HDL % mg/dl
Control	13
STZ	24**
STZ+MEL	16.5**
MEL	19**
STZ+GLIBEN	21.25**
GLIBEN	19.75**

Values are mean±SEM (N=6), analyzed by one way ANOVA followed by student t-test; \*\* $p < 0.01$ : CON vs STZ; ## $p < 0.01$ : STZ vs STZ+MEL; STZ vs MEL; STZ vs STZ+GLIBEN; STZ vs GLIBEN.

**Table 3:** Table showing the effect of melatonin on the level of HDL % mg/dl in serum of streptozotocin (STZ) induced diabetic male rat.

## Histopathology of hepato-renal tissues

Streptozotocin exposed (STZ) rats showed damage in the hepatic cord, plasma membrane, in the central vein, loss of nuclei and accumulation of cell debris and necrotic hepatocytes. Exogenous melatonin administration to STZ induced diabetic rats showed developed central vein, nucleated hepatocytes, comparable to the antidiabetic molecule (Figure 19). Kidney tissues of STZ group of rats has showed fragmented glomeruli. Treatment of melatonin to the diabetic rats restored the degeneration of glomeruli comparable to the glibenclamide (Figure 20).

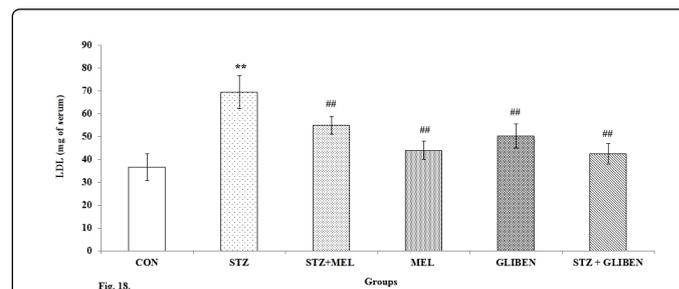


Fig. 18.

**Figure 18:** Effect of melatonin (MEL) and glibenclamide (GLIBEN) on low density lipoprotein (LDL) in serum of Streptozotocin induced diabetic male rat showing significant increase of LDL analyzed in terms of mg of LDL in the serum of diabetic rats. Histogram represent Mean+SE; N=6; \*\* $p < 0.01$ : CON vs. STZ; ## $p < 0.01$ : STZ vs. STZ+MEL; STZ vs. MEL; STZ vs. STZ+GLIBEN; STZ vs. GLIBEN.

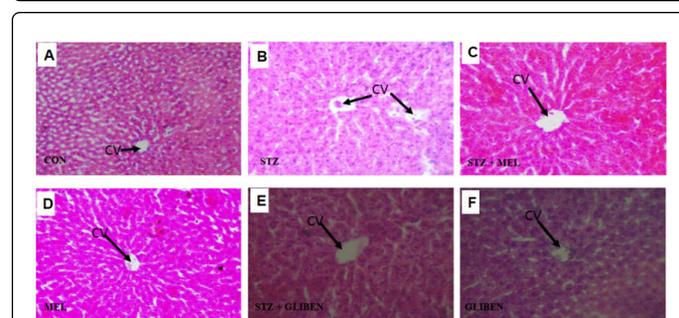


Fig. 19.

**Figure 19:** Histomicrograph of liver of Streptozotocin (STZ) induced diabetic rat model showing effect of melatonin and glibenclamide. A-Control (Con) showing normal hepatocytes and well developed central vein (CV), B- Streptozotocin (STZ) treated group showing damaged central vein and accumulation of cell debris in central vein of hepatocyte, C-Streptozotocin (STZ) + Melatonin (MEL) injection has restored the cellular damages, D- Melatonin (MEL) treatment restored the cellularity of hepatocytes and central vein (CV), E- Glibenclamide (GLIBEN) + Streptozotocin (STZ) glibenclamide recovered the cell damage and central vein deterioration and F- Glibenclamide (GLIBEN) treatment as an antidiabetic drug maintained the normalcy in liver cells. Magnification - 400X.

## Discussion

Streptozotocin induced diabetic rats showed a significant increase in blood glucose confirming the status of diabetes. Melatonin administration (10 mg/kg) checked the further increase in blood glucose level to the normal. Standard hypoglycemic drug glibenclamide decreased the glucose level in blood in rats previously given streptozotocin, when compared to untreated diabetic control rats. Blood glucose level was measured in all the experimental groups. The interesting finding of the present study of the present study is that melatonin significantly lowered the level of glucose upto the normal then the standard antidiabetic molecule (Glibenclamide). Melatonin per se treatment alone noted more effective in maintaining the glucose level than the standard antidiabetic molecule (Glibenclamide). Therefore might be participating in glucose homeostasis with other metabolic hormones.

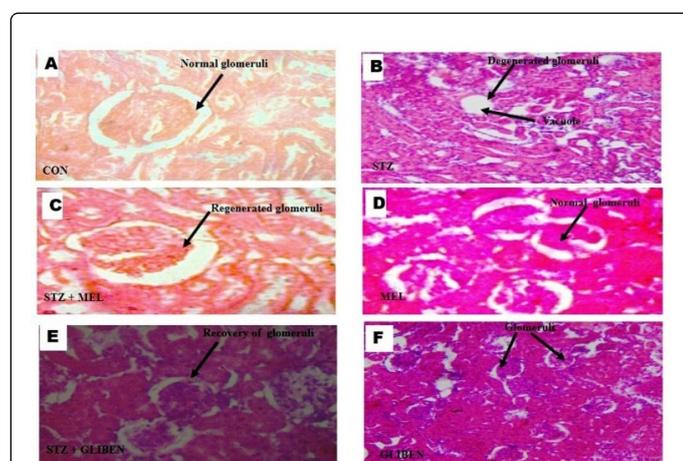


Fig. 20.

**Figure 20:** Histomicrograph of kidney of Streptozotocin (STZ) induced diabetic rat model showing effect of melatonin and glibenclamide. A-Control (Con) showing normal glomeruli and basement membrane, B-Streptozotocin (STZ) administration caused thickening of basement membrane and damaged glomeruli C-Streptozotocin (STZ) + Melatonin (MEL) treatment has reduced the thickening of basement membrane and restored the damage in glomeruli, D Melatonin (MEL) treatment maintained the normal cellularity of glomeruli, E Glibenclamide (GLIBEN) + Streptozotocin (STZ) a standard antidiabetic restored the STZ induced cellular damage in glomeruli and F- Glibenclamide (GLIBEN) maintained normalcy in renal tissues. Magnification - 400X.

The present study was carried out on a streptozotocin (STZ) induced diabetic male rats which exhibit relationship with the human physiological status. Streptozotocin (STZ) was used as a chemotherapeutic alkylating molecule for the treatment of metastatic pancreatic islet cell tumors and other malignancies [31] in the patients whose pancreas cannot be removed through surgery. The streptozotocin was first used as an anticancer drug for the treatment of pancreatic cancer [32]. Lipid peroxidation (LPO) considered a very common biomarker of oxidative stress measured in terms of TBARS level. Present finding suggests that excess glucose level might have resulted because of the metabolic stress which along with overproduced free radical generation influence the natural

physiological antioxidative defense system as justified in terms of TBARS, GSH, SOD and CAT. These results are in accordance with previous study [33]. Report suggests that chemotherapeutic treatment of different malignancies using STZ results in the formation of antibodies [34], which may in turn starts the destruction of insulin producing  $\beta$ -cells and finally leads deficiency of insulin [35]. Therefore leading to the excess glucose level in the body. The surplus glucose gets autoxidised becomes the origin of free radical production.

Diabetes induced free radical generation noted in present finding could have led to peroxidative damage to the liver and kidney tissues, which further might have caused the excessive level of MDA formation, the interesting finding coincides with earlier results [36]. Administration of melatonin reversed the abnormalities evoked by oxidative stress which is assumed as the strong reason in the patients of diabetes mellitus and may lead to tissue dysfunction and hyperglycemia [37]. Such dysfunctions drive non-enzymatic glycation and oxidation of proteins and lipids responsible for diabetic complications [38]. Moreover, previous studies reported that melatonin reduces the level of lipid peroxidation and protein glycosylation, glucose [39,40]. The free radicals generated during the different glucose metabolizing pathways following STZ induction of diabetes might have scavenged by melatonin, because of its high potential of antioxidative property, as free radical scavenger which in turn led to enhanced and up regulated the antioxidative enzymes (GSH, SOD and CAT). Thus simultaneously down regulating free radical production. Therefore, melatonin may be a potent agent in reducing the oxidative damage caused by Streptozotocin in diabetic patients. Melatonin restored antioxidative enzyme level as that of antidiabetic molecule glibenclamide treated group of rats.

The level of liver marker enzymes increases in case of acute hepatic damage or during the mild damage or injury in hepatocytes [41]. AST and ALT were noted to be elevated revealing hepatic damage or liver injury in streptozotocin induced diabetes, which is in parallel with the finding of Inoguchi et al. [42] and Sharmila et al. [43]. Increase in AST and ALT in serum can be explained on the basis destructive changes in hepatocytes as a result of intoxicemia observed under cellular architecture of histology [44]. High level of ALP in blood serum was noted in diabetic rats, might be due to its own increased synthesis by cells lining over bile canaliculi in response to cholestasis and excessive biliary pressure. The administration of melatonin to the streptozotocin induced diabetic rats showed significant decrease in level of AST, ALT and ALP in serum. Indicating hepato-protection by melatonin as judged by reduced hepato-cellular damage, such hepato-protection may further prevent the leakage of marker enzymes from cell cytoplasm to blood stream. Therefore, further adds support of the antioxidative free radical scavenging capability of exogenous melatonin against hepatic injury due to diabetes [45]. Renal functions are highly affected, as polyuria and glycosuria is very common in diabetic patients. In the present study it has been observed that streptozotocin induced diabetic male rats showed significant elevation of serum urea, uric acid and creatinine level in serum in comparison with control rats. Elevation in urea, uric acid and creatinine concentration inferred clearly diminished physiological ability of kidneys regarding filtration of waste products from the blood then excrete to them in the urine. Treatment of exogenous melatonin restored normal level of urea, uric acid and creatinine level in blood serum. Reports till date suggest that melatonin has potential to boost the normal functioning of renal system.

Diabetes alters lipid and lipoprotein level as noted in the present finding. Recent reports revealed that vascular NADPH oxidase driven ROS production can play an important role in pathophysiology of many vascular diseases, like hypercholesterolemia, atherosclerosis and hypertension [46,47]. In the present study significantly increased level of cholesterol, LDL, TG was noted, but interesting finding that it simultaneously decreases level of HDL-C in diabetic rats also noted compared with control group. Deficiency of insulin is associated with the elevation in cholesterol levels due to the accelerated mobilization of lipids from the adipose tissue to the plasma [48]. Melatonin injection to the diabetic rats lowered the cholesterol, triglyceride, low density lipoproteins, but increased the concentration of high density lipoproteins (HDL-c) in diabetic rats. This particular effect of melatonin can be explained on the basis as melatonin injection might have stimulated the specific receptor to enhance the uptake of circulatory LDL-C in liver cell [49]. Further increased LDL-C concentration in plasma of diabetic rats might have resulted because abnormality in low density lipoprotein- C receptor [41]. Accelerated increase of LDL-C, VLDL however may leads to decrease in HDL-C, because there is reciprocal relation between concentration of LDL-C and HDL-C. HDL-C is a protectant as it reverses cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effects of oxidized low density lipoproteins [41].

Histopathological observations of liver and kidney tissues showed damage in the central in liver and also deterioration in the hepatic cells in the streptozotocin (STZ) induced group of rats. Histopathological study diabetic rats receiving melatonin injection daily evening time showed marked recovery and restoration of the liver central vein and also showed restoration in hepatic cell morphology, healthy systematized hepatic cords were separated by narrow blood sinusoids. In kidney glomeruli showed degeneration of glomeruli and vacuole formation, thickening of basement membrane and compression of capillaries in streptozotocin (STZ) induced rats. Diabetic rats treated with exogenous melatonin injection during evening daily for one month showed significantly regeneration in glomeruli and vacuole size decreased and expansion of capillaries.

STZ a toxic glucose analogue, with replacement at C2 with an N-methyl-N-nitrosourea group [50,51] transported via GLUT2 transporter and gets preferentially accumulated in the pancreatic  $\beta$ -cells. This C2 group is cytotoxic and damages  $\beta$ -cells. The hypothesis for toxic mechanism of streptozotocin initiates when STZ gets decomposed and leads the production of free radicals. The other well-known properties of streptozotocin is that it causes the DNA alkylation, due to which double stranded DNA becomes fragmented and finally causes DNA damage. Streptozotocin inhibits the synthesis of DNA in both bacteria and mammals [52], because of its alkylating properties. Since, melatonin possesses an aromatic indole ring, this ring functions as electron donor thereby reduces and repairs electrophilic radicals [53]. Melatonin has a distinguishing property it does not undergo redox cycling and is considered a suicidal or terminal antioxidant [54]. This statement clearly reveals that melatonin becomes irreversibly oxidized and is recycled. Melatonin has a well-known dose dependent antioxidative effect, it provides protective role against damage from free radical a potent radical scavenger. Literature suggests that melatonin significantly promotes hepatic GSH content due to stimulation of  $\gamma$ - glutamyl-cysteine synthase and potentiation of GSH recycling by increasing GSH-reductase [55]. Since the hexose monophosphate shunt is impaired in diabetes NADPH availability is reduced the ability to recycle GSSG to GSH is decreased [56]. Decreased GSH content in liver during diabetes is probably due to its

increased utilization by the hepatic cells as to attempt to counteract the increased formation of lipid peroxide. Nevertheless, melatonin has very well-known cytoprotective functions via paracrine or autocrine effect. Present findings suggest that both the tissue liver as well as kidneys were very sensitive to wards exogenous melatonin in the streptozotocin induced diabetic rat model. The cytoprotective action of melatonin on liver and kidney tissues can be judged based on the recovery of histological cellularity and various biochemical parameters like liver and kidney function test.

Present finding regarding hepato-renal protective action of melatonin can be explained by two possible proposed mechanisms, first via melatonin's free radicals scavenging action and antioxidative property which have acted up on the various free radicals generated during the metabolic change in diabetic model and in addition enormously highly toxic peroxynitrite [57] and peroxy radicals. Second way of action is activation of endogenous enzymes and enhancing antioxidative defense system that screen out free radicals and improved the hepato-renal physiological and biochemical state of functions. Histological repair and restoration of liver and kidney tissue following melatonin strongly suggests that these two tissues were sensitive to melatonin supplementation and hence is recovery to normal healthy rats groups. Finding advocate regarding proliferative impact of melatonin injection on the endogenous pancreatic beta-cells which intern might have resulted in the reappearance circulatory insulin level and hence happen to cause decrement in the blood level during the diabetic condition of rat [58]. It is further suggested and supported that exogenous melatonin administration influences the glucose metabolism by increasing activity of glucose-6-phosphate dehydrogenase (GSPDH) in the blood which might have resulted due to the increase in number of substrate for G6PD (stimulating the flow of glucose into cells and its phosphorylation) and its direct action [59].

Melatonin being lipophilic molecule and less toxic with its any kind of dose therefore may be suggested as a therapeutic molecule for the treatment of diabetes and hepato-renal functional anomalies during diabetic condition.

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## Author Contribution

The present scientific study was conceived and designed with experimental protocol by: SR YAH. The execution of experiment and analysis of the data was done by SR YAH MB HG. SR and YAH have major contribution towards the writing of the paper.

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