Research article

Melatonin supplementation revives diabetic induced biochemical, histological and hematological impairments in rats

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ABSTRACT

Diabetes is very common all over the world, but still not curable and controlled; it causes alteration in all over body. It needs serious concern for the scientific community to find out some control measures. The current work was planned to explore the possible defensive effect of melatonin against the diabetes induced changes in whole blood profile. For this study albino rats were treated with streptozotocin [(STZ) (15 mg/kg for 6 days)] to induce diabetes. Induction was confirmed by blood glucose and serum sugar assessment. Total 36 rats were randomly selected for the experimental purpose and were divided into two major groups. Major group-1 consisting eighteen (18) and were further sub-divided into three (3) different groups viz. group-I served as normal control, group-II served as melatonin treated, group-III served as glibenclamide treated. Major group-2 consisting eighteen (18) rats were given streptozotocin (STZ) injection (15 mg/kg) for 6 days. After confirmation of diabetes by measuring blood glucose level, animals having blood glucose level above 250 mg/dl) confirmed as diabetic. Eighteen (18) Diabetic rats were three subdivided into following sub-groups and were given different therapeutic treatments, Viz group-IV served as Diabetic control, group-V treated with melatonin, group-VI treated with glibenclamide, respectively. Diabetic rats demonstrated inflection in all hematological variables. Diabetic animals revealed considerable reduction in RBCs count, HB content and its associated indices (HCT, RDW, MCV, MCH and MCHC). Decrease in WBCs and its related indices (polymorphs and lymphocytes). Platelet count showed significant increase, but platelet distribution width (PDW %) was found decreased. However administration of melatonin restored all the alterations in hematological parameters. Therefore, it can be concluded that melatonin will be better therapeutic molecule to revive hematological alterations during diabetes.

1. Introduction

Diabetes is a multifaceted metabolic disorder characterized by abnormal increase in blood glucose level or reduced secretion of insulin, or both [1]. Prolonged increase in the blood glucose level results in the activation of protein kinase C, increases glycation of proteins and lipids, and increase the rate of sorbitol formation [2]. Glycation of proteins changes the action of enzymes; however, sorbitol induces harmful hyperosmalrity [2]. Chronic hyperglycemia has several deadly negative impacts upon the structure and functions of different organs, by inducing neuropathy, retinopathy, nephropathy, hepatic diseases, and cardiovascular impediments [2]. Generation of superoxide radicals (O^{2*}), hydroxyl peroxide and hydroxyl radicals (OH*) through the auto-oxidation during hyperglycemic condition. Experimental studies on animal models by the induction of diabetes via streptozotocin by the

damage of pancreatic β-cells [3]. It is hypothesized that generation of Reactive Oxygen Species (ROS) aids the toxic action of streptozotocin on cells [3].

Melatonin an indoleamine (N-acetyl-5-methoxytryptamine) is an native neuro-hormone chiefly synthesized and released from the pinealocytes of pineal gland in mammals in a rhythmic manner [4, 5]. Melatonin is synthesis during the dark period in amplified manner and its synthesis and secretion decreases during the light period. Melatonin is mainly synthesized by the pineal gland; however 25% production is reported from extra-pineal sites [6]. It has been reported that melatonin plays its role in several physiological processes including the regulation of reproduction [7], circadian rhythms [8]. Recent studies revealed that melatonin acts as most potent scavenger of different ROS [9], such as hydroxyl and peroxyl radicals [8] including immune modulation and well recognized as antiageing. Melatonin is different from the other oxidants

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that it has the ability to cross all morphological barriers, i.e., the blood-brain, the placenta, and is well distributed throughout all cells [10].

Hepatorenal system plays crucial role in the maintenance, performance and regulating homeostasis [11]. These are the principal site for intense metabolism, detoxification of various drugs, purification and excretion. Both play an important role in every biochemical pathways of growth, protection against diseases, supplies nutrients and source of energy. Further, liver is closely associated with metabolic machinery and together these two hepatorenal constitutes hepatorenal metabolic factory. Participates in filtration, purification and excretion of excess metabolic products and formation of urine, maintain osmolarity of blood. The major population of kidney comprises cortical nephrons, thus any injury in renal cortex may lead some deleterious effects in the whole body.

Therefore, the current work was planned to elucidate the impact of external melatonin supplementation against diabetic induced alteration on free radical load, antioxidative defense system GSH cycle (GPX, GR and G6PDH), GST, glycogen quantification and total protein content in the hepatorenal system. Further, assessment of hematological variables, electrolyte quantification, hormonal profiles (Insulin) and serum protein (albumin and globulin) to check the functional status of hepatic and renal system.

2. Materials and methods

2.1. Chemicals, reagents and instruments

Chemicals and reagents (analytical grade) streptozotocin (STZ), melatonin (MEL), citrate monohydrate, sodium citrate, glutathione oxidized (GSSG), NADPH (0.12mM), NaH_2PO_4 (sodium dihydrogen phosphate), Na_2HPO_4 (disodium hydrogen phosphate), EDTA, reduced glutathione (GSH), sodium azide, nicotine adenine dinucleotide phosphate (NADPH), tris HCl, magnesium chloride, NADP+, dichlorophenol indophenols, phenazine methosulphate (PMS), glucose 6-phosphate, 2-choloro-2,4-dinitrobenezene (CDNB), potassium hydroxide (KOH), ethanol, anthrone, sulphuric acid (H_2SO_4), trichloro acetic acid (TCA), sodium hydroxide (NaOH), sodium bicarbonate (Na_2CO_3), copper sulphate ($CuSO_4$), Folin's Ciocalteau reagent, and distilled water. Required for the experimentation are procured from Himedia limited, India, and Sisco Research limited, India. Insulin ELISA kit procured from Biogenuix Med system Pvt. Ltd. Streptozotocin (STZ) and melatonin (MEL) was acquired from Sigma Aldrich, insulin (INS) was obtained from Actrapid, Novo Nordisk, A/S-Denmark. Egyptian Drug Trading Company, Glibenclamide (GB), from Ahmadabad, Gujrat, India. Centrifuge (Remi C 24BL)

and Perkin Elmer UV Visible Spectrophotometer (LAMBA, Serial No. 501812090010) and ELISA reader, TECAN.

Albino rats were procured from Defence Research and Development Establishment (DRDE) Gwalior, M.P. India and weight of rats ranged from 175-190 ± 10 g. Rats were adapted under standard husbandry conditions [(25 ± 2) ^{0}C temp, 60%–70% relative humidity, 14 h light and 10 h dark]. After period of acclimatization rats were indiscriminately divided into six groups each group contained six rats. All the procedures and protocols were approved by the national and international guidelines and rules. These protocols and procedures were also approved by the institutional ethical committee [(institutional practices of Institutional Animal Ethics Committee, SLT institute of Pharmaceutical, Sciences]) (IAEC), Guru Ghasidas Vishwavidyalaya (Registration Number:994/Go/ERe/S/06/CPCSEA) (Reference No. 157/IAEC/Pharmacy/2016).

2.2. Induction and confirmation of diabetes

Diabetic condition in rat was induced in the rats by the intraperitoneal administration of streptozotocin 15 mg/kg for six days in 0.1M citrate buffer (pH-7.4). Glucose in blood was examined using glucometer (ACCUCHECK) after 72 h of streptozotocin treatment. Total thirty six (36) male albino rats were randomly selected for the study and were
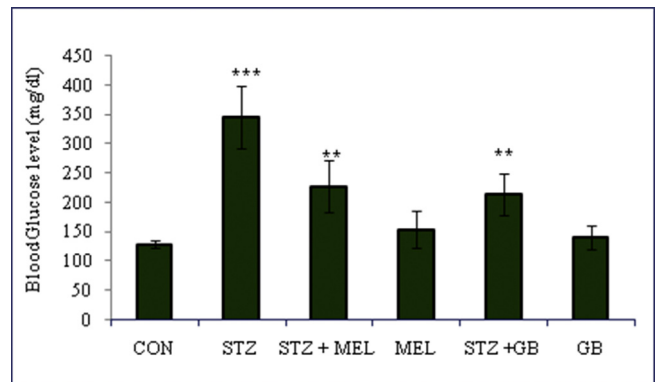

Figure 1. Mean difference in blood glucose level in different experimental groups. STZ: Streptozotocin, MEL: Melatonin, GB: Glibenclamide. Data represents Mean ± SE., N = 6. F-value = 6.512, Significant at 5% for ANOVA. STZ vs. CON at ***p < 0.001. STZ vs. STZ + MEL at **p < 0.01. STZ vs. STZ + GB at **p < 0.01.

Table 1. Effect of exogenous melatonin in diabetic rat model with respect to the weekly blood glucose level and serum random sugar level in different experimental groups.

Groups	Weekly changes in blood glucose level (mg/dl)				Week-IV	Random sugar level in serum (mg/dl)
	Before induction	Week-I	Week-II	Week-III		
CONT	130 ± 7	126 ± 6	128 ± 8	132 ± 3	1130 ± 6	64.18 ± 3.29
STZ	127 ± 10	380 ± 7**	386 ± 14***	360 ± 9***	367 ± 10**	97.675.80**
STZ + MEL	124 ± 8	408 ± 9*	398 ± 12**	290 ± 10*	221 ± 12*	75.68 ± 5.97*
MEL	128 ± 12	134 ± 4	130 ± 16	128 ± 8	125 ± 11	67.17 ± 4.37
STZ + GB	129 ± 4	412 ± 8*	370 ± 7*	280 ± 15*	232 ± 9*	78.0 ± 5.0*
GB	134 ± 5	130 ± 9	140 ± 9	125 ± 5	125 ± 6	69.34 ± 69.34

@F Value = 6.512.
Data are mean ± S.E.; N = 6.
Abbreviations: CONT = Control; STZ = Streptozotocin; MEL = Melatonin; GB = Glibenclamide.
@ Significant at 5% for ANOVA.
STZ vs CONT.
STZ vs STZ + MEL.
STZ vs STZ + GB.
Superscripts denotes; * = 0.05; ** = 0.01 and *** = 0.001.

divided into two major groups viz major group 1 and majr group 2. Major group-1 consisting of eighteen normal rats were subdivided into three (3) different groups viz. group-I served as normal control, group-II served as melatonin treated, group-III and group-III served as glibenclamide treated. Eighteen (18) rats were streptozotocin (STZ) injection (15 mg/kg) given for 6 days. After confirmation of diabetes by measuring blood glucose level, animals having blood glucose level above 250 mg/dl) considered as diabetic (Table1; Figure 1). Evaluation of sugar level in serum was also examined by using commercial kit (Table 1). Major group 2 consisting eighteen (18) STZ induced diabetic rats were subdivided into following sub-groups and were given different therapeutic treatments, Viz group-IV served as Diabetic control, group-V treated with melatonin, group-VI glibenclamide treated respectively. Diabetes was developed and stabilized over a period of 7 days. All the experimental groups were named and numbered as represented in the experimental design, also whole experimental design has been explained in the flow chart diagram.
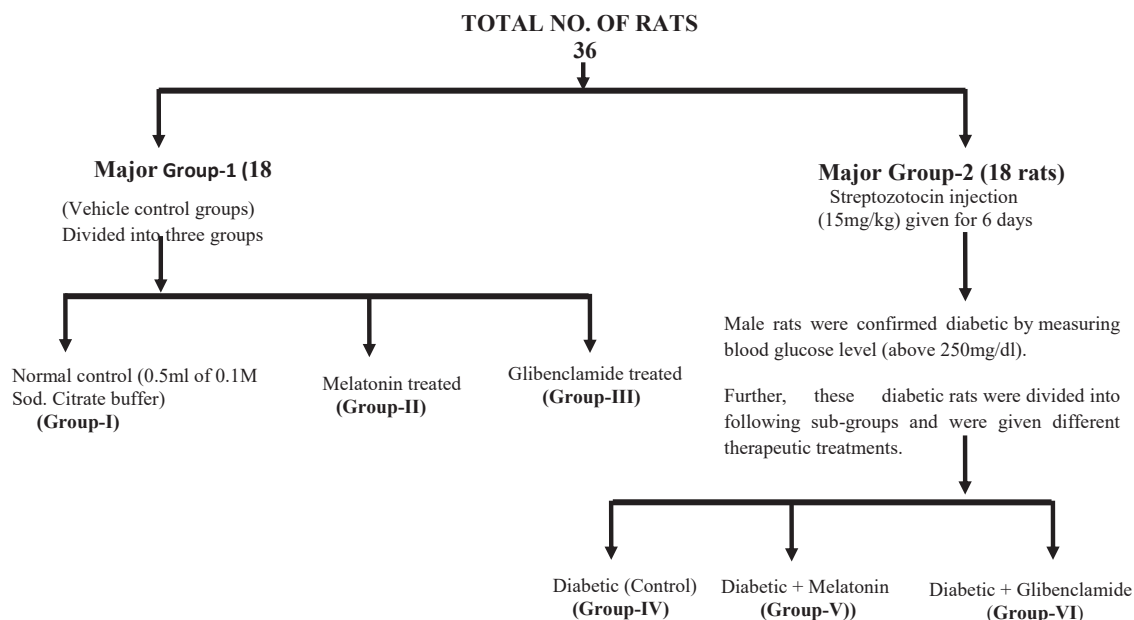
2.3. Experimental design

Group I: Normal control
Group II: MEL (1 mg/kg b. w. 4 weeks)
Group III: GB (0.5 mg/kg b. w. 4 weeks)
Group IV: Diabetic control [(STZ 15 mg/kg b. w. 6 days, intraperitoneal (i.p)]
Group V: STZ + MEL [STZ 15 mg/kg (6days) + 1 mg/kg b. w. 4 weeks]
Group VI: STZ + GB [(STZ 15 mg/kg + 0.5 mg/kg b. w. 4weeks, Per Os (p.o.)]

3. Evaluation of parameters

3.1. Tissue biochemistry

3.1.1. GSH cycle

This cycle includes three enzymes Glutathione reductase (GR), Glutathione peroxidase (GPX) and Glucose-6-phosphate dehydrogenase (G6PDH).

Glutathione reductase (GR) (E.C.1.6.4.2) assay was conducted by using the protocol of Goldberg and Spooner [12]. Homogenate (10%) was prepared in 1.15% KCl. The sample was processed for centrifugation for 10 min to obtain the supernatant and was proceed for GR. Whole reaction mixture was incubates for 10 min at room temperature and then 0.1ml NADPH was added to begin the reaction and note the change in absorbance/minute was recorded for 5 min at 340 nm.

Glutathione peroxidase (GPX E.C.1.11.1.9) activity was conducted following the modified method [13]. Homogenate (10%) was prepared in 1.15% KCl and it was centrifuged at 10,000 rpm in cold at room temperature and then supernatant was collected in tubes and used for further analysis. The sample was vortexed to mix up properly and then incubated for 10 min at room temperature. Reaction was started by adding 0.05 ml H_2O_2 reaction and change in absorbance/minute was noted for 5 min at 340 nm μmol NADPH oxidized/min/m protein was expressed as specific activity taken extinction coefficient of NADPH as 6300/m/cm.

Glucose-6-phosphate dehydrogenase (G6PDH EC 1.1.1.49), reaction mixture was incubated at optimum temperature for 10 min to allow the oxidation. The reaction was initiated by adding of 0.5 ml of glucose-6-

TOTAL NO. OF RATS
36

Flow chart representation of experimental design and allocation of rats into different experimental groups.

On the completion of experimental period (4weeks), all the group rats were euthanized and then sacrificed. Liver and renal cortex was processed for GSH cycle (GPX, GR and G6PDH) and GST estimation, glycogen quantification and total cellular protein content. Blood was centrifuged (at 3000g) in order to separate out serum and then stocked at -20 °C for serum biochemistry assessment. Different serum parameters were analyzed for total serum protein content (albumin and globulin), bilirubin, electrolytes (Na^+ & K^+) and insulin (INS). Further, blood was processed for the assessment of alterations in hematological and immunological variables.

phosphate. Absorbance was measured at 640nm by taking distilled water as blank; at one minute interval for 3.5 min in UV spectrophotometer [14].

3.1.2. Glutathione-S-transferase (GST; E.C. 2.5.1.18)

100 mg tissue was homogenized in 1ml of 1.15% KCl and then centrifugation was conducted at 10,000 rpm at room temperature for 10 min. The supernatant was taken out from the tube and processed for assay. Reaction mixture was prepared and then incubation of the sample was done at room temperature for 10 min. Reaction was started by adding 25μl GSH and absorbance was taken 3 readings per minute at 340 nm for 3 min [15].

3.1.3. Glycogen quantification in tissue (A direct method)

Glycogen was quantified in hepatic and renal cortex by the method of Seitfer et al. 1951 [16] with some modifications. Freshly dissected out liver and kidney cortex were weighed and then poured into the test tube in which 3 mL of 30% KOH solution were poured. Liver and renal cortex tissues were digested by heating the tubes for 20 min in boiling water in water bath, digestion was followed by cooling, and then 1.25 mL of 95% ethanol was added. The sample was again boiled and then allowed to cool, after cooling the whole sample was centrifuged at 3000rpm for 20 min at room temperature. Following the centrifugation the supernatant was discarded and to the pellet 1ml distilled water and 1.25 ml 95% ethanol was added and then bring to boil again. Then the samples were cooled followed by centrifugation at 3000 rpm for 20 min at 30°C. 1+4 ml distilled water was added to the pellet obtained after centrifugation. Take 1 ml of the sample and then add 4ml of distilled water. Finally 10 ml of Anthrone solution (200 mg/100 ml of Anthrone in 95% H_2SO_4) added to the sample on chilled water. Samples were cooled and then boil again in boiling water and again cooled and absorbance is taken at 620 nm.

3.1.4. Total protein quantification

Total protein content of hepatic and renal cortex tissues was estimated by modified method of Lowry et al., 1951 [17]. Total protein content in liver and kidney cortex samples were estimated by preparing the reaction mixture containing (0.2ml for homogenate was added with 0.1ml D.H_2O) were taken and proteins were precipitated by 0.2ml 10% TCA. Color was developed by addition of 0.5ml Folin's reagent and then samples were kept in dark for 30 min. Absorbance was recorded at 625 nm.

3.1.5. Hematological variables

5ml of blood were collected from veins aseptically through the retro-orbital sinus puncture [18] poured in EDTA tubes. Red blood cells (RBCs), hemoglobin (HB), hematocrit (HCT), leukocytes, platelets, lymphocytes, polymorphs, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution (RDW), platelet distribution width (PDW) were examined through the hematological analyzer as per the standard calibration of the instrument (Analytical, India).

3.2. Serum biochemistry

Serum was analyzed for the assessment of total protein (albumin and globulins) content in circulatory serum, bilirubin, electrolytes (Na$^+$ and K$^+$) and insulin through the commercial analytic kits purchased from ERBA diagnostics Mannheim GmbH Mallaustr, Germany), following the standard method available within the kit.

3.2.1. Histopathological observations

Small pieces of liver and renal cortex were washed in normal saline and fixed in Bouin's fixative. Liver and kidney cortex pieces of each experimental group were dehydrated by via various graded sequence of ethanol. Liver and renal cortex pieces were cleared through xylene and fixed in paraffin wax to prepare blocks. Liver and kidney cortex embedded within the blocks were cut into ribbons/sections (4–5 μm thick) using rotary microtome (Leica RM 2125-RT 5). The sections were further processed for staining through Hematoxylin and Eosin (H and E), mounted with DPX and examined under light microscope (Magnus, India, using 10x and 40x).

3.3. Statistical analysis

Results were represented as the mean $\pm$ SE, each group containing six animals. Results were analyzed through SPSS (IBM 20.0 version software), groups were compared with each other by using One-way analysis of variance (ANOVA) followed by student's t-test computed at $p \leq 0.05$ [19].

4. Results

4.1. Estimation of weekly changes in blood glucose level

Blood glucose screening is basic diagnostic parameter for the detection of diabetes. Diabetic rats revealed well marked increase in blood glucose level. Moreover, melatonin and positive hypoglycemic drug glibenclamide significantly normalized the blood glucose from week first upto week fourth as shown in Table 1.

4.2. Assessment of random serum sugar level

Melatonin and glibenclamide administered to diabetic rats revealed considerable decrease of sugar level in serum compared to diabetic control rats. Estimation of serum sugar level further support our results, as documented in Table 1.

4.3. Assessment of glycogen, total cellular protein content and antioxidant status in hepatorenal system

Total protein and glycogen content were reduced in diabetic rats in liver and renal cortex. Rats received the treatment the melatonin revealed considerable rise in the total protein and glycogen content in liver and kidney cortex. However, melatonin treatment alone does not revealed any adverse change in glycogen and total cellular protein content (Tables 2 and 3). Diabetes causes significant supression of glutathione peroxidase (GPX), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), and glutathione-S-transferase (GST) in hepatocytes and renal cortex. Supplementation of melatonin revealed considerable recovery in glutathione dependent antioxidative enzymatic activities near to the control range. Findings of the results showed that only supplementation of melatonin to the rats does not showed any change in these enzyme activities (Tables 2 and 3).

4.4. Serological studies

Diabetes induced glucotoxicity resulted significant increase in bilirubin, total protein content (albumin and globulin) and electrolytes (Na$^+$ and K$^+$) in comparable to control group of rats (Tables 3, 4, and 5). Treatment of melatonin showed recovery as well as restoration in all the serum variables. The efficiency of melatonin was comparable to the glibenclamide treated group of rats. Rats received only treatment of only melatonin does not revealed any adverse effect in all the serum parameters (Table 4). Diabetic rats revealed considerable reduction in insulin level in plasma compared with control rats. Melatonin treated animals revealed significant increase in plasma insulin level comparable to control group of rats (Table 4).

4.5. Hematological analysis

Red Blood Cells (RBCs), Hemoglobin (HB), Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular Hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) revealed significant reduction in diabetic control rats. However, treatment of melatonin showed considerable restoration in all the decreased indices. Red cell distribution width (RDW), Platelet distribution width (PDW) also revealed significant decrease while as lymphocyte count, platelet count, leukocytes and polymorphs demonstrated noteworthy raise in diabetic rats. Whereas, exogenous administration of melatonin barred further augmentation in lymphocyte, platelet, leukocytes and polymorphs about the control level (Tables 5 and 6).

Table 2. Percent protective effect of exogenous melatonin in an STZ induced diabetic rat model with respect to the different hepatic biochemical parameters.

Groups	Glycogen (µg/mg of tissue)	GSH Cycle (Unit/min/mg protein)			GST Unit/min/mg protein	Protein content (µg/mg)
		GR	GPX	G6PDH		
CONT	2773.29 ± 214.3	47.07 ± 2.8	598.14 ± 32.4	37.52 ± 4.7	598.14 ± 32.4	35.23 ± 2.2
STZ	1441.19 ± 128.9***	32.09 ± 2.8**	357.19 ± 32.2	19.57 ± 2.5**	357.19 ± 32.2**	14.82 ± 1.6***
STZ + MEL	2256.47 ± 82.8*	44.57 ± 4.4*	501.67 ± 15.8	33.89 ± 3.4*	501.67 ± 15.2*	31.5 ± 1.9**
%Protection by MEL	61.10%	67.62%	65.65%	79.75%	62.08%	81.64%
MEL	2674.69 ± 261.4	49.09 ± 3.5	611.92 ± 22.6	38.77 ± 4.3	611.92 ± 22.6	35.05 ± 2.4
STZ + GB	2091.69 ± 174.7*	35.84 ± 2.8*	497.46 ± 25.5	32.17 ± 2.7*	497.46 ± 25.5*	27.75 ± 2.6**
%Protection by GB	48.75%	25.02%	36.83%	70.60%	60.08%	61.78%
GB	2598.88 ± 199.7	48.7 ± 4.3	578.22 ± 52.5	36.84 ± 4.7	578.22 ± 52.5	34.15 ± 2.3
@F Value	8.556	5.369	7.327	4.179	10.144	16.081

Data are mean ± S.E.; N = 6.
Abbreviations: CONT = Control; STZ = Streptozotocin; MEL = Melatonin; GB = Glibenclamide; GR = Glutathione Reductase; GPx = Glutathione Peroxidase; G6PDH = Glucose-6-Phosphate Dehydrogenase and GST = Glutathione-S-Transferase.
@ Significant at 5% for ANOVA.
STZ vs CONT.
STZ vs STZ + MEL.
STZ vs STZ + GB.
Superscripts denotes; * = 0.05; ** = 0.01, *** = 0.001.

Table 3. Percent protective effect of exogenous melatonin in diabetic rat model with respect to the on different renal cortex biochemical parameters.

Groups	Glycogen (µg/mg of tissue)	GSH Cycle (unit/min/mg protein)			GST unit/min/mg protein	Total protein content (µg/mg)
		GR	GPX	G6PDH		
CONT	2243.72 ± 145.66	45.9 ± 4.61	7.21 ± 0.54	39.09 ± 3.12	579.62 ± 56.84	32.04 ± 1.91
STZ	1075.92 ± 100.83***	26.17 ± 2.07**	3.2 ± 0.30***	18.4 ± 2.01**	314 ± 56.98***	15.25 ± 1.87***
STZ + MEL	1935.65 ± 128.96*	43 ± 4.57*	6.19 ± 0.38*	36.09 ± 2.47*	557.91 ± 23.69**	28.02 ± 2.71**
%Protection by MEL	68.86%	85.30%	74.35%	85.75%	91.76%	76.07%
MEL	2225.46 ± 236.49	45.4 ± 5.03	7.19 ± 0.75	45.87 ± 4.06	534.79 ± 40.84	33.94 ± 2.08
STZ + GB	1901.77 ± 66.44*	39.69 ± 3.10*	5.60 ± 0.50*	33.04 ± 3.47*	528.79 ± 39.25**	28.61 ± 1.82*
%Protection by GB	70.72%	26.17%	59.84%	69.42%	80.08%	81.61%
GB	2230.59 ± 168.79	45.71 ± 3.40	7.75 ± 0.55	34.75 ± 4.74	560.7 ± 18.66	32.37 ± 3.03
@F Value	10.588	4.516	8.514	7.148	6.666	8.326

Data are mean ± S.E.; N = 6.
Abbreviations: CONT = Control; STZ = Streptozotocin; MEL = Melatonin; GB = Glibenclamide; GR = Glutathione Reductase.
GPX = Glutathione Peroxidase; G6PDH = Glucose-6-Phosphate Dehydrogenase and GST = Glutathione-S-Transferase.
@ Significant at 5% for ANOVA.
STZ vs CONT.
STZ vs STZ + MEL.
STZ vs STZ + GB.
Superscripts denotes; * = 0.05; ** = 0.01, *** = 0.001.

5. Histological observations

Control group of animals showed regular histocellular architecture of hepatocytes having well structured nucleus, regular arrangement of hepatic cords and preserved sinusoidal spaces. However diabetic group of rats demonstrated a range of deformities in hepatocytes. Diabetic rats showed wear and tear in hepatocytes, irregular and injured hepatocytes, having hepatic lesions, necrosis, vacuolation, and weakened sinusoids. Treatment of melatonin given to diabetic rats exhibited significant renewal in central vein impediment, normal shape and alignment of lobules having polygonal hepatocytes with prominent nucleus, and broader sinusoidal spaces (10X and 40X) (Figures 2 and 3). Diabetic rats showed deterioration and degeneration in glomeruli formation of vacuoles, thickened basement membrane and conjunction of capillaries in diabetic rats. Supplementation of exogenous melatonin to the diabetic rats during evening time for one month revealed remarkable regeneration in glomeruli and reduced size of vacuole and growth of capillaries.

6. Discussion

The eradication of the diseases is enormously complex due to poverty, diet issues, change in food intake, and advancement in technological approached, urbanization, sedentary habits cause various health issues in the population globally. Earlier studies demonstrated that diabetes causes excessive production of ROS that down regulates the local antioxidant system. Reactive free radicals such as hydroxyl radicals (-OH), superoxide anion (O_2-), lipid peroxyl radicals (LOO-), peroxyl radicals (ROO-) and hydrogen peroxide (H_2O_2) are mostly formed through advanced stages of diabetes.

In the present study remarkable decrement was observed in GR, GST, GPX and G6PDH activities cortical in hepatic and renal following the STZ intoxication. Present study was supported by the earlier findings [20, 21]. In melatonin treated diabetic rats, revealed elevated activity of GPX, GR, G6PDH and GST. Thus from this study, it could understood that melatonin might have neutralized the free radicals and improved cellular

Table 4. Percent protective effect of exogenous melatonin in diabetic rat model with respect to the serum biochemistry of diabetic.

Groups	Electrolytes (mmol/L)		Serum proteins (g/dl)			Bilirubin (mg/dl)	Insulin (ng/ml)
	Sodium (Na$^+$)	Potassium (K$^+$)	Total protein	Albumins	Globulins		
CONT	116.34 ± 2.78	2.79 ± 0.38	7.89 ± 0.53	4.37 ± 0.18	3.5 ± 0.32	0.25 ± 0.02	1.58 ± 0.11
STZ	137.17 ± 3.17***	4.5 ± 0.35*	3.95 ± 0.67**	2.55 ± 0.48**	1.40 ± 0.41***	0.717 ± 0.05***	0.59 ± 0.08***
STZ + MEL	122.67 ± 2.31**	2.79 ± 0.29*	6.745 ± 0.52*	3.92 ± 0.29*	2.84 ± 0.20**	0.445 ± 0.09**	0.99 ± 0.10**
%Protection by MEL	**69.61%**	**99.70%**	**71.05%**	**81.40%**	**66.74%**	**58.20%**	**41.71%**
MEL	118.84 ± 2.14	2.85 ± 0.17	7.52 ± 0.57	2.30 ± 0.33	3.45 ± 0.26	0.325 ± 0.05	1.50±±0.03
STZ + GB	123 ± 4.35**	3.5 ± 0.28*	6.48 ± 0.53*	3.80 ± 0.25*	2.66 ± 0.16*	0.5134 ± 0.05**	0.83 ± 0.04*
%Protection by GB	**87.99%**	**58.13%**	**64.07%**	**77.27**	**58.31%**	**43.56%**	**24.08%**
GB	119.34 ± 2.59	2.9 ± 0.25	7.45 ± 0.72	3.97 ± 0.30	3.49 ± 0.37	0.354 ± 0.03	1.46 ± 0.12
@ F Value	**2.45**	**6.41**	**6.966**	**8.888**	**8.775**	**6.484**	**23.407**

Data are mean ± S.E.; N = 6.
Abbreviations: CONT = Control; STZ = Streptozotocin; MEL = Melatonin; GB = Glibenclamide.
@ Significant at 5% for ANOVA.
STZ vs CONT.
STZ vs STZ + MEL.
STZ vs STZ + GB.
Superscripts denotes; * = 0.05; ** = 0.01, *** = 0.001.

Table 5. Percent protective effect of exogenous melatonin in diabetic rat model with respect to the different red blood cell indices and lymphocytes.

Groups	(RBC x 10^6/μl)	Hemoglobin (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (%)	Lymphocytes
CONT	8.98 ± 0.75	14.85 ± 0.46	40.97 ± 2.12	59.22 ± 30	20.44 ± 1.63	40.84 ± 2.18	23.03 ± 1.87
STZ	5.34 ± 0.43**	0.75 ± 0.84**	28.56 ± 2.01**	46.95 ± 4.68*	13.39 ± 0.82**	36.1 ± 1.37**	10.95 ± 1.39**
STZ + MEL	7.2 ± 1.04*	12.12 ± 0.81*	34.09 ± 1.82*	57.54 ± 1.48*	18.64 ± 1.32*	37.7 ± 1.06*	20.01 ± 1.65**
% Protection by MEL	**51.00%**	**33.40%**	**45.25%**	**86.30%**	**74.46%**	**33.75%**	**65.86%**
MEL	8.24 ± 0.47	13.57 ± 0.58	38.4 ± 2.29	63.64 ± 2.92	19.14 ± 1.21	39.9 ± 0.94	22.39 ± 1.04
STZ + GB	6.92 ± 0.96*	11.87 ± 85**	32.62 ± 1.81^{NS}	55.74 ± 2.77*	17.87 ± 1.75*	38.88 ± 1.87*	17.77 ± 1.57**
% Protection by GB	**43.10 %**	**27.31%**	**33.22%**	**71.63%**	**63.54%**	**58.64%**	**42.91%**
GB	7.83 ± 0.63	12.37 ± 0.56	38.34 ± 2.72	59.14 ± 3.27	19.8 ± 0.99	40.36 ± 2.21	23.45.07 ± 0.89
@F Value	**3.382**	**5.016**	**5.443**	**3.751**	**4.655**	**1.209**	**11.871**

Data are mean ± S.E.; N = 6.
Abbreviations: CONT = Control; STZ = Streptozotocin; MEL = Melatonin; GB = Glibenclamide; RBCs = Red Blood Cells; HCT = Hematocrit; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean Corpuscular Hemoglobin Concentration.
@ Significant at 5% for ANOVA.
STZ vs CONT at ≤0.01.
STZ vs STZ + MEL at $P \leq 0.05$.
STZ vs STZ + GB at $P \leq 0.05$.
Superscripts denotes; * = 0.05; ** = 0.01, *** = 0.001.

antioxidant enzymatic functions. The activities of GR, GPX, GST and G6PDH were restored when test drug (melatonin) was administered for four weeks which showed that H_2O_2 was detoxified in accelerated fashion. Therefore, exogenous treatment of melatonin for four weeks significantly restored the GPX enzymatic activity. It is involved in the maintenance of reduced state of GSH through quick deduction in oxidized glutathione (GSSG) to GSH via steeling electrons from NADPH. During physiological oxidative stress it acts as reducing component [22]. Melatonin donates electron to assist the GR to neutralize the reactive free radicals. The C_3OH M derivative of melatonin functions to detoxify free radicals [23]. The successive metabolites of melatonin including N-acetyl–N-formyl-5-methoxykynuramine (AFMK) and N-acetyl-5-methoxykynurmine (AMK) [24, 25] generates defensive cascade. Therefore, provides defense against the free radical induced damages [26]. Thus, confirms that first, second and third generations metabolites are proved very outstanding scavenger of free radicals [27].

Glutathione-S-transferase belongs to the phase II detoxification enzyme system that catalyzes the coupling of the different types of endogenous and exogenous electrophilic compounds. Hence, it has central importance in liver for the eradicating toxic compounds by coupling them with glutathione [28]. GST might be bind on melatonin, to function like an enzyme in conjunction reactions of GSH, because of its lipophilic nature. In response to ROS generation, melatonin has potential to modify the level of GSH in hepatocytes. Thus, it could be inferred that melatonin protect the cells from free radical damages.

Examination of blood parameters is a common as well as principal pinpointing medical tool for the assessment of health [29]. It provides detailed functions of different blood components [30]. Investigations of hematological indices are not only implemented for the evaluation of deadly effects of diabetes on the blood profile of animals. In diabetic rats hemoglobin level was noted significantly decreased in comparison to control. However, supplementation of melatonin considerable restored hemoglobin level. This explains for decrease in Hb level is because of the glycosylation of Hb and it is converted into HbA1c in diabetic control rats. The excess amount of glucose reacts with causes abnormal glycosylation of proteins (i.e., carbohydrate coating) [31]. Prolonged diabetic condition increases the level of glycosylated hemoglobin. However; exogenous melatonin might be decreasing the level of glucose by regeneration and enhancement in insulin secretion from beta cells. In diabetic normal Hb level decrease, which might be inhibiting function of red blood cells (RBCs) and also altering the life span of these cells resulting in decrease in RBCs formation. Decrease in RBCs level might be

Table 6. Percent protective effect of exogenous melatonin in diabetic rat model with respect to the on different white blood cell indices.

Groups	RDW (%)	PDW (%)	Platelets (x 10^3/μl)	Leukocytes (/Cmm)	Polymorphs (%)
CONT	23.04 ± 1.58	26.04 ± 1.54	88.34 ± 3.34	20222 ± 1046.12	71.4 ± 1.42
STZ	13.03 ± 1.57***	22.99 ± 1.37**	71 ± 3.42*	11858.33 ± 292.12***	43.36 ± 1.72***
STZ + MEL	19.44 ± 0.72**	23.32 ± 1.78*	87.333 ± 3.03*	13983 ± 858.25**	67.98 ± 1.51**
% Protection by MEL	64.03%	13.44%	106.1%	74.5%	87.80%
MEL	21.59 ± 1.46	26.34 ± 1.66	89.33 ± 4.22	14345 ± 1309.75	70.61 ± 3.00
STZ + GB	17.767 ± 1.63[NS]	23.4 ± 1.22*	87.83 ± 3.43*	11053 ± 882.86**	67.61 ± 1.69**
% Protection by GB	47.32%	10.81%	118.34%	70.26%	61.51%
GB	21.3 ± 0.86	24.3 ± 1.26	90.34 ± 4.00	12175 ± 1102.01	68.3 ± 1.45
@ F Value	8.476	1.152	0.233	14.184	38.480

Data are mean ± S.E.; N = 6.
Abbreviations: CONT = Control; STZ = Streptozotocin; MEL = Melatonin; GB = Glibenclamide; RDW = Red Blood Cell Distribution Width.
PDW = Platelet Distribution Width.
@ Significant at 5% for ANOVA.
STZ vs CONT.
STZ vs STZ + MEL.
STZ vs STZ + GB.
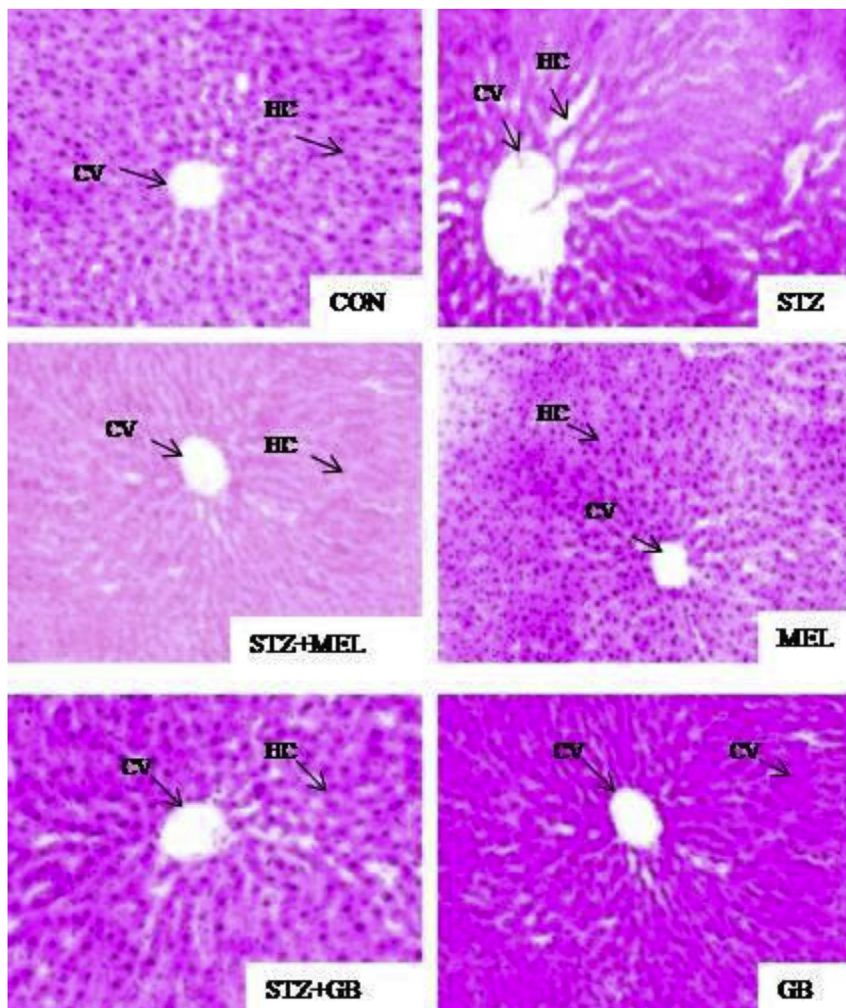Superscripts denotes; * = 0.05, ** = 0.01, *** = 0.001.

Figure 2. Histomicrograph of liver showing effect of melatonin in all experimental groups. CON- Control showing normal hepatocytes and well developed central vein (CV), STZ: Diabetic group of rats showing damaged central vein and amassing of cell debris in central vein of hepatocytes, vacuole formation, necrosis, dilation of sinusoid and distorted hepatic cords. STZ + MEL: Streptozotocin (STZ)+ Melatonin (MEL) injection has restored the cellular damages, MEL-Melatonin treatment restored the normal cellular structure of hepatocytes and central vein (CV), STZ + GB Glibenclamide + Streptozotocin restored and retained the cell damages and central vein wear and tear and GB-Glibenclamide treatment as a antidiabetic drug sustained the normalcy in liver cells.

creating some respiratory problems such as oxygen and carbon dioxide transportation in the cells. In diabetic rats significant decrease in RBC count was observed in comparison to control group. This indicates that the balance between the formation and destruction of RBCs alters during diabetes. However, exogenous melatonin injected to the diabetic group significantly increased the RBC count towards the control. This might be explained that melatonin normalizes the blood glucose level which in turn prevents the immature destruction of red blood corpuscles. The Packed cell volume (PCV) was observed to be decreased in diabetic rats, whereas, melatonin administration restored the PCV count towards the
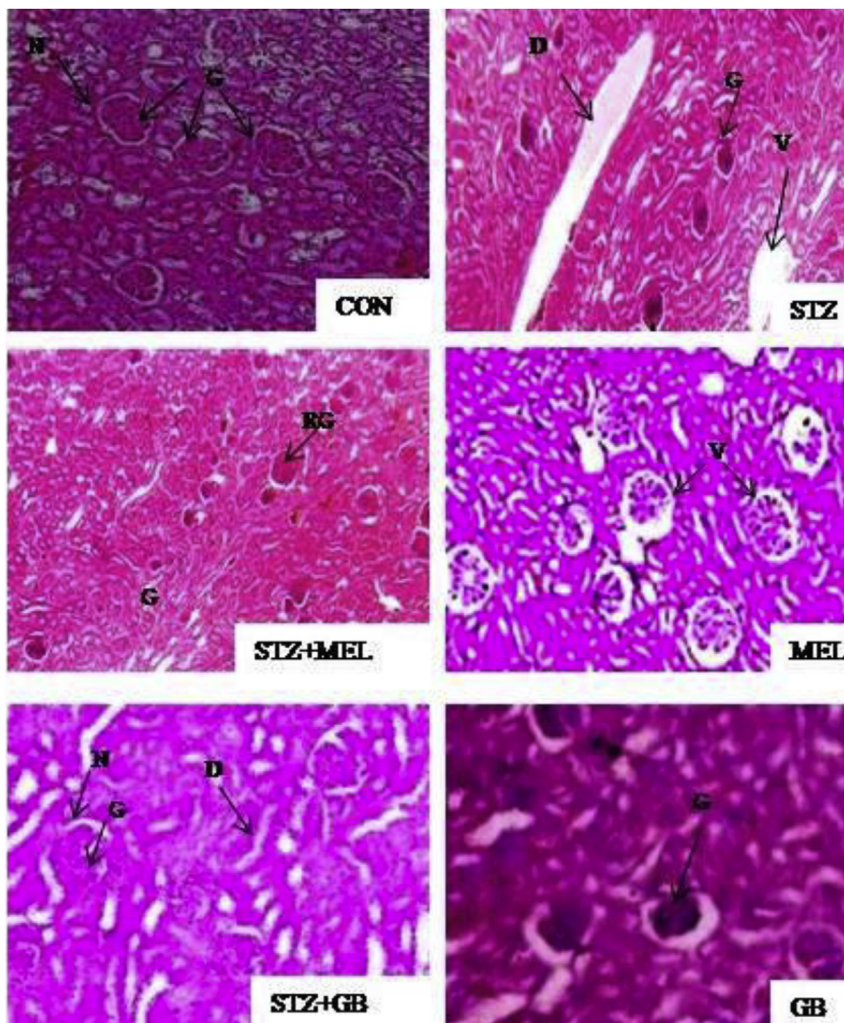
Figure 3. Histomicrograph of kidney of diabetic rats showing effects of melatonin and glibenclamide. Control (CON) exhibiting normal glomeruli and basement membrane. Streptozotocin (STZ) treatment caused thickening of basement membrane and damaged glomeruli. Streptozotocin (STZ)+Melatonin (MEL) treatment has decreased the thickening of basement membrane and restored the damage of glomeruli. Melatonin (MEL) administration maintained the normal cellular architecture of glomeruli. Glibenclamide (GLIBEN)+Streptozotocin (STZ) a standard antidiabetic molecule reversed the STZ induced damage in glomeruli. Glibenclamide (GLIBEN) maintained normal structure of renal tissues.

normal. The decrease in PCV is directly correlated with glycosylation of hemoglobin [32]. The evident role of melatonin on glucose homeostasis might be helped in the prevention or controlling the rate of hemoglobin glycosylation. Results of the present study were confirmed by comparing with hypoglycemic molecule (Glibenclamide) which was given to the group-III of animals. However, alone supplementation of melatonin and glibenclamide does not showed alteration. It was indicated from previous reports that during diabetes, level of MCV, MCHC and MCH augmentation. These variables are related with individual red blood cells [33]. It has been studied that alteration in MCH and MCHC to normocytic or hypochromic anaemia. This anemic state might be caused due to the diabetes induced metabolic dysfunctions. The level of the MCV, MCHC and MCH were found to be restored by the administration of exogenous melatonin.

Findings of the current study revealed that diabetic animals showed considerable increase in platelet count. Finding of our study coincides with the earlier results of Hillson (2015) who reported, during diabetes different alterations occurs in the body including hyperactivity of platelets, more adhesiveness, activation and clumping [34], all these variation leads to the insulin resistance. Alteration in platelets are associated with increased rate of blood clotting, delay in clot degradation and decreased function of endothelial cells. During diabetes these alterations together augments the chances of artherothrombotic attacks [35]. However, supplementation of melatonin notably restored platelet count near to control range, which in turn inhibits the platelet hyperactivity.

Diabetic rats showed reduction in overall WBC count and its related variables. Present finding coincides with earlier studies which reported that white blood cells (WBCs) and lymphocyte count decreased during diabetes [36]. Reduction in WBC and lymphocyte count might be associated with suppression of leukocytosis in bone marrow. This suppressed process of leukocytosis could be because of reduced protection against infection [37]. Exogenous administration of melatonin received by diabetic rats continuously for four weeks showed remarkable normalization in WBC and lymphocyte count upto the normal range. Chronic poor glycemic control occurs due to insufficient/absence of insulin [38].

Decreased RBCs, Hematocrit, HB, and PCV indicate anemic condition and reduced availability of iron content. The melatonin treatment might be having anti-anemic property which may be due to its electron rich indole ring and its conjugation with metal ions. In diabetic rats WBC count showed remarkable increased in comparison to control group of rats. This indicates some alteration in their normal physiological processes. Results of current study revealed that diabetes causes toxic manifestation and immune compromization. Leukocytosis is directly related with seriousness of disease and might associated with amplified leukocyte mobilization. In diabetic rats lymphocyte count also showed considerable increase in contrast compared to the control group of rats.

The glycogen level in diabetic rats was significantly decreased in the liver and renal cortex. Whereas melatonin supplemented to the diabetic rats significantly recovered glycogen content towards the control level. This decrease in glycogen content in the hepatocytes depends on the availability/concentration of glucose in extracellular matrix. Further, it

also depends on the insulin level, because insulin directly up-regulates the synthesis of glycogen [39].

Proteins responsible for the transportation of different small molecules. Albumin is the predominant protein present in circulating serum/plasma; it constitutes around 60 % of the total protein in serum. Albumin has ascertained with different cellular functions (physiological as well as pharmacological) such as transportation of fatty acids, metals, cholesterol, and bile pigments as well as drugs. A recent report suggested that albumin acts as antioxidant in plasma; trapping of free radical [40, 41]. This statement proposes that hypoalbumenemic patients suffer from decreased potential of ROS scavenging ability [42]. One promising function of albumin is endocytosis in transport albumin-bound nutrients into cells [43]. Thus, any adverse factor reducing the albumin level may indicate pathogenesis in different oxidative stress associated ailments, including diabetes, neuro-degeneration, and cardiovascular dysfunction and in some cases as cancer. Moreover, common complication of diabetes is reduction in serum albumin level, as albumin contributes in the modulation of osmotic pressure of plasma. Findings of current study revealed that considerable decrease in albumin level in blood serum in diabetic rats. Results of our study corresponds with earlier results of Tietz, 1986, which reported that diabetes causes considerable decrease in albumin level in blood serum [44]. Decreased level of serum albumin occurs because of elimination of albumin along with urine, which is an indicator of diabetic nephropathy. However, exogenous melatonin significantly restored the albumin level by preventing the formation of reactive oxygen species and abnormal glycation of proteins. Decrease in albumin and globulin concentration in the serum in untreated diabetic rats revealed diminished synthetic function of liver. Administration of melatonin protects from the free radical induced hepatic biochemical alterations caused by diabetes induced intoxication by autoxidation of glucose and non-enzymatic protein glycation.

Diabetic showed significant increase in serum bilirubin, whereas, exogenous supplementation of melatonin given to diabetic revealed significant restoration in bilirubin content. Bilirubin is formed during the catabolism and destruction of red blood cells and is excreted out by liver [45]. Therefore, interference of bilirubin with normal physiological function of liver, influences both conjunction and excretion rate. Hence, more bilirubin is general biomarker to examine the functional status of liver and also excretion of bile as well.

Histological observations exhibited that diabetic rats showed deterioration and collapsing of hepatocytes, injuries of hepatocytes, excessive rate of necrosis, formation of vacuoles, indistinct/broken hepatic cords, and obliterated sinusoids. Melatonin treatment remarkable recovery with prominent central vein, normal lobular pattern with well-developed polygonal hepatocytes having conspicuous nucleus, and wider sinusoidal spaces. The antidiabetic drug also showed recovery in these structural deformities and almost regained their normal pattern. Histophotomicrographs of kidney diabetic group of rats exhibited unclear renal structural design with deteriorated tubular structure, swelled glomeruli along with loss of glomerular space, vacuole formation at some regions which could have resulted because of thickening in basement membrane of endothelial lining and compression of capillaries in diabetic rats. Exogenous melatonin to diabetic rats showed significant reversal and restoration and hence restoration in histological changes in renal cortex, having normal glomeruli, even glomerular space, retained endothelial lining, healthy developed Bowman's capsule as well as normal tubules having normal lumen was noted comparable to gliben-clamide as an diabetic therapeutic molecule confirming the protective nature of melatonin.

7. Conclusion

Results of this study concluded that diabetes causes impairments in antioxidative system, cellular total protein, glycogen content and histo-architecture of liver and kidney cortex. In addition this diabetes also causes alteration in serum electrolytes (N^+ and K^+) and in the whole blood profile. These changes in tissue biochemical variables and in hematological variables hamper physiological processes (anemia, oxygen carrying capacity, weakness in immune compromisization and delayed healing of wounds. Treatment of melatonin restored the changes in the whole blood profile near the control level.

Declarations

References

[1] M. Versini, P.Y. Jeandel, E. Rosenthal, Y. Shoenfeld, Obesity in autoimmune diseases: not a passive bystander. Mosaic of Autoimmunity, Academic Press, 2019, pp. 981–1000.

[2] R.P. Robertson, J. Harmon, P.O. Tran, V. Poitout, β-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes, Diabetes 53 (1) (2004) 119–124, 2004.

[3] T. Szkudelski, The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas, Physiol. Res. 50 (6) (2001) 537–546.

[4] D.P. Cardinali, J.M. Rosner, Ocular distribution of hydroxyindole-O-methyl transferase (HIOMT) in the duck (Anas platyrhinchos), Gen. Comp. Endocrinol. 18 (2) (1972) 407–409, 1972.

[5] W.B. Quay, Humoral interrelations of the pineal gland with lateral eyes and orbital glands. The Pineal Gland and its Endocrine Role, Springer, Boston, MA, 1983, pp. 395–416, 1983.

[6] Y. Djeridane, B. Vivien-Roels, V. Simonneaux, J.M. Miguel, P. Pevet, Evidence for melatonin in rodent Harderian gland: a dynamic in vitro study, J. Pineal Res. 25 (1) (1998) 54–64.

[7] S. Rai, M. Basheer, H. Ghosh, D. Acharya, Y.A. Hajam, Melatonin attenuates free radical load and reverses histologic architect and hormone profile Alteration in female rat: an in vivo study of pathogenesis of letrozole induced poly cystic ovary, J. Clin. Cell. Immunol. 6 (2015) 6, 2015.

[8] M. Allegra, R.J. Reiter, D.X. Tan, C. Gentile, L. Tesoriere, M.A. Livrea, The chemistry of melatonin's interaction with reactive species, J. Pineal Res. 34 (1) (2003) 1–10.

[9] R.J. Reiter, J.C. Mayo, D.X. Tan, R.M. Sainz, M. Alatorre-Jimenez, L. Qin, Melatonin as an antioxidant: under promises but over delivers, J. Pineal Res. 61 (3) (2016) 253–278, 2016.

[10] C. Rodriguez, J.C. Mayo, R.M. Sainz, I. Antolin, F. Herrera, V. Martin, R.J. Reiter, Regulation of antioxidant enzymes: a significant role for melatonin, J. Pineal Res. 36 (2004) 1–9.

[11] S. Bandiera, S. Pfeffer, T.F. Baumert, M.B. Zeisel, miR-122–a key factor and therapeutic target in liver disease, J. Hepatol. 62 (2) (2015) 448–457.

[12] D.M. Goldberg, R.J. Spooner, Glutathione reductase, in: H.U. Bergmeyer, Edition (Eds.), Methods Enzymol. 3 (1983) 258–265.

[13] D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70 (1) (1967) 158–169.

[14] H.A. Ellis, H.N.A. Kirkman, Colorimetric method for assay of erythrocyte glucose-6-phosphate dehydrogenase, Exp. Biol. Med. 106 (1961) 607–609.

[15] W.H. Habig, M.J. Pabst, W.B. Jakoby, Glutathione S-transferases the first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (22) (1974) 7130–7139.

[16] S. Seifter, S. Dayton, B. Novic, E. Muntwyler, The estimation of glycogen with the anthrone reagent, Arch. Biochem. 25 (1950) 191–200.

[17] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[18] V. Riley, Adaptation of orbital bleeding technic to rapid serial blood studies, Proc. Soc. Exp. Biol. Med. 104 (1960) 751–754, 1960.

[19] G.W. Snedecor, W.G. Cochran, Statistical Method, eighth ed., Affiliated East-West Press, Ames, Iowa, 1989, pp. 217–236.

[20] B. Ramesh, K.V. Pugalendi, Antioxidant role of Umbelliferone in STZ-diabetic rats, Life Sci. 79 (3) (2006) 306–310, 2006.

[21] S. Bhatia, R. Shukla, S.V. Madhu, K.J. Ganbhir, K.M. Prabhu, Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy, Clin. Biochem. 36 (2003) 557–562.

[22] S. Li, H.Y. Tan, N. Wang, Z.J. Zhang, L. Lao, C.W. Wong, Y. Feng, The role of oxidative stress and antioxidants in liver diseases, Int. J. Mol. Sci. 16 (11) (2015) 26087–26124.

[23] D.X. Tan, R. Hardeland, L.C. Manchester, A. Galano, R.J. Reiter, Cyclic 3-hydroxymelatonin (C3OHM), a potent antioxidant, scavenges free radicals and suppresses oxidative reaction, Curr. Med. Chem. 21 (13) (2014) 1557–1565.

[24] A.R. Ressmeyer, J.C. Mayo, V. Zelosko, R.M. Sáinz, D.X. Tan, B. Poeggeler, I. Antolín, B.K. Zsizsik, R.J. Reiter, R. Hardeland, Antioxidant properties of the melatonin metabolite N1-acetyl- 5- methoxykynuramine (AMK) scavenging of free radicals and prevention of protein destruction, Redox Rep. 8 (4) (2003) 205–213.

[25] M. Schaefer, R. Hardeland, The melatonin metabolite N-acetyl-5-methoxykynuramine as a potent singlet oxygen scavenger, J. Pineal Res. 46 (1) (2009) 49–52.

[26] D.X. Tan, L.C. Manchester, R.J. Reiter, B.F. Plummer, J. Limson, S.T. Weintraub, W. Qi, Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway for melatonin biotransformation, Free Radic. Biol. Med. 29 (11) (2002) 1177–1185.

[27] R.J. Reiter, D.X. Tan, A. Galano, Melatonin reduces lipid peroxidation and membrane viscosity, Front. Physiol. 5 (2014) 377.

[28] J. Grellier, J. Bennett, E. Patelarou, R.B. Smith, M.B. Toledano, L. Rushton, D.J. Briggs, M.J. Nieuwenhuijsen, Exposure to disinfection by-products, fetal growth, and prematurity: a systematic review and meta-analysis, Epidemiology 120 (2010) 300–313.

[29] A.V. Hoff brand, J.E. Pettit, Haematological Parameters in Essentials of Haematology, fourth ed., Blackwell Science, 2000, pp. 21–25.

[30] M.T. Yakubu, M.A. Akanji, A.T. Oladiji, Hematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogia agrestis stem, Phcog. Mag. 3 (2007) 34, 2007.

[31] S. Dhandapani, V.R. Subramanian, S. Rajagopal, N. Namasivayam, Hypolipidemic effect of Cuminum cyminum L. on alloxan-induced diabetic rats, Pharmacol. Res. 46 (3) (2002) 251–255.

[32] R.K. Mathews, J.J. Sistino, In-vitro evaluation of the hemolytic effects of augmented venous drainage, J. Extracor. Tech. 33 (1) (2001) 15–18.

[33] A.H. Adebayo, A.O. Abolaji, T.K. Opata, I.K. Adegbenro, Effects of ethanolic leaf extract of Chrysophyllum albidum G. on biochemical and haematological parameters of albino Wistar rats, Afr. J. Biotechnol. 9 (14) (2010) 2145–2150.

[34] R. Hillson, Diabetes and the blood–white cells and platelets, Practical Diabetes 32 (5) (2015) 159–160.

[35] J.L. Ferreiro, J.A. Gómez-Hospital, D.J. Angiolillo, Platelet abnormalities in diabetes mellitus, Diabetes Vasc. Dis. Res. 7 (4) (2010) 251–259, 2010.

[36] S.O. Oyedemi, M.T. Yakubu, A.J. Afolayan, Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in streptozotocin induced diabetic rats, J. Med. Plants Res. 5 (1) (2011) 119–125.

[37] E. Jarald, S.B. Joshi, D.C. Jain, Diabetes vs herbal medicines, Iran. J. Pharmacol. Ther. 7 (2008) 97–100, 2008.

[38] C.G. Sheela, K.T. Augusti, Antidiabetic effects of allyl cysteine sulphoxide isolated from garlic Allium Sativum Linn, Indian J. Exp. Biol. 30 (6) (1992) 523–526.

[39] M. Roche, P. Rondeau, N.R. Singh, E. Tarnus, E. Bourdon, The antioxidant properties of serum albumin, Fed. Eur. Biochem. Soc. Lett. 582 (2008) 1783–1787.

[40] E. Bourdon, D. Blache, The importance of proteins in defense against oxidation, Antioxidants Redox Signal. 3 (2) (2001) 293–311, 2001.

[41] J.P. Nicholson, M.R. Wolmarans, G.R. Park, The role of albumin in critical illness, Br. J. Anaesth. 85 (4) (2000) 599–610.

[42] Sidman, C. L., Lymphocyte surface receptors and albumin. J. Immunol. 127(4): 1454–1458.

[43] K.T. Park, C.H. Yun, C.S. Bae, T. Ahn, Decreased level of albumin in peripheral blood mononuclear cells of streptozotocin-induced diabetic rats, J. Vet. Med. Sci. (2014) 13–631.

[44] N. Tietz, Textbook of Clinical Chemistry, WB Saunders, Philadelphia, 1986, p. 1279.

[45] K. Usha, G.M. Kasturi, P. Hemalatha, Hepatoprotective effect ofHygrophila spinosa andCassia occidentalis on carbon tetrachloride induced liver damage in experimental rats, Indian J. Clin. Biochem. 22 (2) (2007) 132–135.