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Evaluation of *in vivo* antidiabetic activity of melatonin in streptozotocin induced diabetic rats

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Abstract

The first line of treatment for diabetes mellitus includes oral hypoglycaemic agents; however, these drugs are often associated with adverse effects and may not completely prevent progression of diabetic complications. Melatonin, an endogenous indoleamine hormone, has been reported to possess antioxidant, cytoprotective and antidiabetic properties. The present study was undertaken to evaluate the *in vivo* antidiabetic efficacy of melatonin in streptozotocin-induced diabetic rats. Seven groups of rats were maintained as normal control, diabetic control, metformin-treated (100 mg/kg b.wt.), melatonin-treated at 5, 10 and 30 mg/kg body weight and a combination-treated group receiving melatonin (10 mg/kg body weight) with metformin. Diabetes was induced in male Wistar rats by streptozotocin administration. Body weight, serum glucose, histopathological changes and immunohistochemical expression of insulin in pancreatic islets were evaluated on the 15th, 30th and 45th day of the experiment. Normal control rats showed normal body weight and physiological status throughout the study period. Diabetic control rats exhibited significant hyperglycaemia, progressive reduction in body weight, degenerative changes in pancreatic islets and marked depletion of insulin-positive cells. In contrast, melatonin-treated groups showed significant reduction in serum glucose levels with gradual restoration of body weight. Histopathological examination revealed improvement in islet morphology, while immunohistochemistry demonstrated increased insulin-positive β -cells in treated groups. Among the tested doses, melatonin at 10 mg/kg b.wt. Showed optimum efficacy, whereas 30 mg/kg body weight did not confer substantial additional benefit. The combination-treated group showed the most pronounced improvement and was comparable or superior to metformin-treated rats. The findings of the present study indicate that melatonin possesses significant antihyperglycaemic activity and exerts protective effects on pancreatic tissue. Improvement in glycaemic control, body weight, histoarchitecture of islets and insulin immunoreactivity may be attributed to the therapeutic bioactivity of melatonin.

Keywords: Melatonin, Streptozotocin, Diabetes mellitus, Serum glucose, Histopathology, Immunohistochemistry

Introduction

Diabetes mellitus (DM) is a debilitating chronic metabolic disorder that has emerged as a major global public health challenge, affecting both developed and developing nations. The worldwide prevalence of diabetes continues to rise rapidly, particularly in Asia, Latin America and Africa, due to urbanization, sedentary lifestyle, obesity and dietary transitions. The disorder is characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action or both, leading to disturbances in carbohydrate, lipid and protein metabolism (American Diabetes Association, 2024; International Diabetes Federation, 2021) [11, 3]. Chronic diabetes is associated with several microvascular and macrovascular complications including atherosclerosis, coronary artery disease, nephropathy, retinopathy, neuropathy and progressive pancreatic dysfunction. Sustained hyperglycaemia also promotes oxidative stress, inflammation and mitochondrial dysfunction, which contribute significantly to the pathogenesis of diabetic complications (Brownlee, 2001; Forbes and Cooper, 2013) [10, 7]. The first line of treatment in DM includes the use of insulin and oral hypoglycaemic agents such as metformin and sulfonylureas. Although these therapies are

effective in controlling blood glucose, they may be associated with adverse effects such as gastrointestinal intolerance, hypoglycaemic episodes, weight changes and reduced long-term efficacy. Moreover, these agents may not completely arrest oxidative stress-mediated tissue injury or β -cell deterioration (Viollet *et al.*, 2012; Kalra *et al.*, 2015) [28, 13]. Therefore, there is growing interest in adjunct therapeutic agents that are safe, economical and capable of targeting multiple pathogenic pathways.

Melatonin (*N*-acetyl-5-methoxytryptamine) is an endogenous indoleamine hormone synthesized predominantly by the pineal gland and is widely recognized for regulation of circadian rhythm and sleep physiology. In recent years, melatonin has attracted substantial attention because of its potent antioxidant, anti-inflammatory, antiapoptotic and cytoprotective properties. Melatonin directly scavenges reactive oxygen and nitrogen species and also stimulates endogenous antioxidant defense enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Reiter *et al.*, 2000; Rodriguez *et al.*, 2004) [21, 24]. Several experimental studies have demonstrated that melatonin exerts beneficial effects in diabetes mellitus. It has been reported to lower blood glucose, improve insulin sensitivity, modulate pancreatic insulin secretion and preserve β -cell integrity. Melatonin receptors are expressed in pancreatic islets, suggesting a physiological role in glucose homeostasis and endocrine pancreatic function (Peschke *et al.*, 2013) [18]. In addition, melatonin has been shown to protect pancreatic tissue against oxidative damage and apoptosis induced by streptozotocin (Agil *et al.*, 2013; Reiter *et al.*, 2014) [1, 22]. Streptozotocin (STZ)-induced diabetes in rats is a widely accepted experimental model for evaluating antidiabetic agents, as STZ selectively damages pancreatic β -cells through DNA alkylation, nitric oxide generation and oxidative stress, resulting in insulin deficiency and hyperglycaemia (Lenzen, 2008; Szkudelski, 2001) [16, 26]. With this background, the present study was undertaken to evaluate the *in vivo* antidiabetic efficacy of melatonin administered alone or in combination with metformin in streptozotocin-induced diabetic rats and to compare its therapeutic response with a standard oral hypoglycaemic drug.

Materials and Methods

Animals

Genetically normal 126 adult male Wistar albino rats weighing 170 ± 20 g were procured and maintained under standard laboratory conditions with controlled temperature, humidity and normal light-dark cycle. The animals were housed in clean polypropylene cages and provided *ad libitum* access to standard commercial rat feed (Amruth Feeds, Bangalore) and clean reverse osmosis drinking water throughout the experimental period. The study was conducted after obtaining approval from the Institutional Animal Ethics Committee, Veterinary College, Hebbal, Bengaluru, in accordance with CPCSEA guidelines.

Animals were divided into seven groups:

Chemicals

Streptozotocin (STZ) was procured from Sigma Chemicals, USA. Melatonin (*N*-acetyl-5-methoxytryptamine) of analytical grade purity was obtained from a standard commercial supplier. Metformin hydrochloride tablets were procured from a local pharmacy and freshly prepared before

administration. All other chemicals and reagents used in the study were of analytical grade and obtained from Merck, HIMEDIA and other standard commercial sources.

Experimental Induction of Diabetes Mellitus

The rats were fasted overnight prior to induction of diabetes. Experimental diabetes mellitus was induced by a single intraperitoneal injection of freshly prepared streptozotocin at a dose rate of 45 mg/kg body weight dissolved in 0.09 M cold citrate buffer (pH 4.5) (Lenzen, 2008) [16]. Rats in the normal control group received citrate buffer alone.

After 72 hours of STZ administration, diabetic status was confirmed by estimating random blood glucose levels using a glucometer (Accu-Chek®, Roche, India). Blood samples were collected from the tail vein using a sterile insulin syringe fitted with a 31G needle. Animals showing blood glucose levels above 200 mg/dL were considered diabetic and selected for the study.

Animals were divided into seven groups

Treatments were administered orally once daily for 45 days. Body weight and serum glucose were recorded at periodic intervals. At the end of the experiment, pancreatic tissues were collected and fixed in 10% neutral buffered formalin. Sections were processed for routine hematoxylin and eosin staining for histopathological examination and separate sections were subjected to immunohistochemical staining for insulin. To study the progressive effects of the treatments administered to different groups, animals from each group were sacrificed humanely under Isoflurane anaesthesia on the 15th and 30th day, while the remaining animals were sacrificed on the 45th day of the experimental period. Blood samples were collected in both EDTA vacutainers and serum vacutainers containing clot activator for further analysis. Various parameters including body weight and serum glucose were evaluated during the study period.

During necropsy, pancreatic tissues were collected carefully and preserved in 10 per cent neutral buffered formalin (pH 7.4). The preserved tissues were processed routinely and subjected to histopathological examination and immunohistochemical demonstration of insulin.

Biochemical Estimation

Serum glucose concentration was estimated as per the manufacturer's protocol using standard commercial diagnostic kits (Trivitron, LabSystems).

Body Weight

The rats were weighed individually on the day of commencement of the experiment and subsequently on the 3rd, 15th, 30th and 45th day of the study prior to necropsy.

Histology: Representative pancreatic tissue samples measuring approximately 3-5 mm thickness were collected in 10 per cent neutral buffered formalin for histopathological examination. The tissues were processed by routine paraffin embedding technique. Sections of 4 μ m thickness were cut using a rotary microtome and stained with hematoxylin and eosin (H&E) as per standard procedures (Luna, 1968).

Immunohistochemistry: Sections of pancreas were subjected to immunohistochemistry for demonstration of

insulin in the β -cells of islets of Langerhans using mouse monoclonal *anti*-insulin primary antibody. The bound primary antibody was detected using horseradish peroxidase (HRP)-conjugated secondary antibody system. Tissue sections were subjected to heat-induced epitope retrieval using citrate buffer (pH 6.0) prior to staining. After completion of staining, insulin-positive β -cells were counted under high magnification. The number of positively stained cells among 1000 β -cells was recorded and expressed as percentage.

Results

The present study was conducted to evaluate the antidiabetic efficacy of melatonin in streptozotocin induced diabetes mellitus in rats and to compare its hypoglycaemic effect with that of metformin. The effects of melatonin were assessed based on body weight changes, serum glucose levels, histopathological alterations and immunohistochemical expression of insulin in pancreatic tissue.

Body Weight

- The mean ($\pm$ SE) body weight values of all experimental groups recorded at different intervals are presented in Table 1 and Fig. 1.
- The normal control rats (Group I) showed a gradual and consistent increase in body weight throughout the experimental period. The body weight increased from 168.5 $\pm$ 4.072 g on Day 3 to 330.85 $\pm$ 6.46 g on Day 45, indicating normal growth and metabolic activity.
- In contrast, diabetic control rats (Group II) exhibited progressive reduction in body weight throughout the study period. The body weight decreased from 175.67 $\pm$ 4.58 g on Day 3 to 121 $\pm$ 5.32 g on Day 45. The reduction became more evident from Day 15 onwards.
- Metformin-treated rats (Group III) showed gradual improvement in body weight during the experimental period. Body weight increased from 155.17 $\pm$ 2.19 g on Day 30 to 217.67 $\pm$ 9.54 g on Day 45, although the values remained lower than normal controls.
- Rats treated with melatonin at 5 mg/kg body weight (Group IV) showed moderate improvement in body weight with values increasing to 227.5 $\pm$ 9.35 g on Day 45.
- Group V rats treated with melatonin at 10 mg/kg body weight demonstrated marked improvement in body weight throughout the study period. The body weight increased to 245.5 $\pm$ 3.57 g on Day 45 and the values approached near normal levels.
- Similarly, Group VI rats treated with melatonin at 30 mg/kg body weight also showed progressive increase in body weight during the experimental period with values reaching 235.33 $\pm$ 2.65 g on Day 45.
- The combination-treated group (Group VII) receiving melatonin along with metformin exhibited maximum improvement in body weight among all treated groups. The body weight progressively increased from 171.33 $\pm$ 3.33 g on Day 30 to 297 $\pm$ 8.36 g on Day 45 and the values were comparatively closer to normal control rats.
- Overall, melatonin-treated groups showed significant improvement in body weight compared to diabetic control rats, with the 10 mg/kg dose and combination therapy showing better restoration of body weight.

Serum Glucose

- The mean ($\pm$ SE) serum glucose values of treatment and control groups at different intervals of the experimental period are presented in Table 2 and Fig. 2.
- The normal control rats (Group I) maintained relatively constant serum glucose levels throughout the study period without significant variation, indicating normal glucose homeostasis and pancreatic endocrine function. The serum glucose values ranged from 112.62 $\pm$ 3.47 mg/dL on Day 3 to 108.54 $\pm$ 5.86 mg/dL on Day 45.
- In contrast, diabetic control rats (Group II) showed marked and persistent hyperglycaemia throughout the experimental period. The serum glucose levels remained significantly elevated from 394.83 $\pm$ 18.11 mg/dL on Day 3 to 378.16 $\pm$ 16.67 mg/dL on Day 45.
- Metformin-treated rats (Group III) exhibited gradual and significant reduction in serum glucose levels during the treatment period. The glucose values decreased from 389.67 $\pm$ 29.52 mg/dL on Day 3 to 151.17 $\pm$ 16.74 mg/dL on Day 45, although the values remained comparatively higher than normal control rats.
- Group IV rats treated with melatonin at 5 mg/kg body weight showed moderate decline in serum glucose levels throughout the study period. The serum glucose values decreased from 402.31 $\pm$ 26.34 mg/dL on Day 3 to 202.63 $\pm$ 17.98 mg/dL on Day 45.
- Group V rats treated with melatonin at 10 mg/kg body weight demonstrated marked reduction in serum glucose levels throughout the experimental period. The serum glucose values progressively decreased from 398.33 $\pm$ 34.68 mg/dL on Day 3 to 163.38 $\pm$ 14.79 mg/dL on Day 45 and approached near normal levels by the end of the experiment.
- Similarly, Group VI rats treated with melatonin at 30 mg/kg body weight also showed progressive reduction in serum glucose levels during the study period. The glucose values declined from 388.12 $\pm$ 37.09 mg/dL on Day 3 to 209.10 $\pm$ 13.21 mg/dL on Day 45. However, the degree of reduction was comparable to Group V.
- The combination-treated group (Group VII) receiving melatonin along with metformin exhibited maximum reduction in serum glucose levels among all treated groups. The serum glucose values progressively declined from 383.86 $\pm$ 26.40 mg/dL on Day 3 to 148.18 $\pm$ 14.66 mg/dL on Day 45 and were comparatively closer to normal control values.
- Overall, melatonin-treated groups showed significant antihyperglycaemic activity when compared to diabetic control rats, with melatonin at 10 mg/kg body weight and combination therapy showing better glycaemic control.

Histopathology: Microscopic examination of pancreatic sections from Group I (normal control) rats revealed normal histoarchitecture characterized by well-arranged exocrine acini and distinct, round to oval islets of Langerhans composed of closely packed endocrine cells with normal cytoplasmic and nuclear morphology. No degenerative or inflammatory changes were observed throughout the experimental period.

In contrast, pancreatic sections from Group II (diabetic control) rats showed severe pathological alterations consistent with streptozotocin-induced β -cell injury. The lesions included marked reduction in the size and number of

islets of Langerhans, irregular and shrunken islets, depletion of endocrine cellularity, cytoplasmic vacuolation, pyknosis of nuclei, focal necrosis and disorganization of normal pancreatic architecture. In some sections, mild interstitial edema and separation of acinar tissue were also evident. These lesions progressively intensified from the 15th to the 45th day of the experiment.

Group III rats treated with metformin showed moderate histological improvement when compared with diabetic control rats. The islets appeared relatively larger with partial restoration of cellular population. Reduced vacuolar degeneration and lesser necrotic changes were observed, although complete normalization of islet structure was not achieved.

Pancreatic sections from rats treated with melatonin at a dose of 5 mg/kg (Group IV) revealed mild to moderate regenerative changes in the islets of Langerhans. An increase in endocrine cellularity was observed along with partial restoration of islet architecture. Degenerative alterations such as cellular vacuolation and shrinkage were comparatively reduced when compared to the diabetic control group. However, mild residual degenerative changes were still evident in some sections.

Pancreatic sections of Group V rats treated with melatonin at 10 mg/kg demonstrated marked histological improvement. The islets of Langerhans appeared well-defined with increased cellular density and restoration of normal endocrine architecture. Minimal cytoplasmic vacuolation and degenerative changes were observed. The pancreatic parenchyma closely resembled that of the normal control group, indicating substantial protective and restorative effects of melatonin at this dose.

Pancreatic sections from Group VI rats administered melatonin at 30 mg/kg also exhibited significant recovery of pancreatic architecture. The islets showed improved cellular organization and reduced degenerative lesions with restoration of endocrine components. However, the degree of improvement was not appreciably greater than that observed in Group V, suggesting that increasing the dose beyond 10 mg/kg did not produce additional marked histological benefit.

Pancreatic sections from melatonin-treated rats (Groups IV, V and VI) revealed dose-dependent protective and restorative changes. Group IV (5 mg/kg) showed mild to moderate regeneration of islets with increased endocrine cellularity and reduction in degenerative lesions. Group V (10 mg/kg) demonstrated marked improvement characterized by well-defined islets, increased cellular density, minimal vacuolation and near-normal pancreatic architecture. Group VI (30 mg/kg) also showed significant recovery, but the improvement was not appreciably greater than that observed in Group V.

Group VII rats receiving melatonin in combination with metformin exhibited the best histopathological response. The pancreas showed near-normal acinar arrangement and prominent islets of Langerhans with preserved cellularity, minimal degenerative changes and restoration of normal architecture. These findings indicate a synergistic protective effect of combination therapy.

The histological improvement observed in melatonin-treated groups may be attributed to its potent antioxidant, anti-inflammatory and antiapoptotic properties, which protect β -cells against streptozotocin-induced oxidative damage and facilitate tissue recovery.

Immunohistochemistry: Immunohistochemical staining for insulin was performed to assess the functional integrity of pancreatic β -cells. Group I (normal control) rats showed intense brown cytoplasmic immunoreactivity in the majority of β -cells within the islets of Langerhans, indicating normal insulin content and secretory activity.

Group II (diabetic control) rats exhibited marked reduction in insulin immunostaining intensity with severe depletion of positively stained β -cells. Many islets showed only scattered weakly reactive cells or complete absence of staining, indicating extensive β -cell destruction following streptozotocin administration.

In Group III (metformin-treated) rats, moderate restoration of insulin immunoreactivity was observed. The number of positively stained β -cells increased when compared with diabetic controls, suggesting partial recovery of β -cell function.

Pancreatic sections from Group IV rats treated with melatonin at 5 mg/kg showed mild to moderate increase in insulin immunoreactivity within the islets of Langerhans. An increase in the number of insulin-positive β -cells was observed compared to the diabetic control group. The staining intensity was moderately enhanced, indicating partial restoration of β -cell functional activity.

Group V rats treated with melatonin at 10 mg/kg demonstrated marked restoration of insulin immunoreactivity. Numerous strongly insulin-positive β -cells were distributed throughout the islets, with intense cytoplasmic staining and improved cellular organization. The findings indicated substantial recovery of β -cell population and endocrine function, closely resembling the normal control group.

Pancreatic sections from Group VI rats administered melatonin at 30 mg/kg also exhibited significant enhancement in insulin immunostaining. The islets contained a considerable number of insulin-positive β -cells with strong staining intensity and improved cellular distribution. However, the degree of immunoreactivity and percentage of positive cells were comparable to those observed in Group V, suggesting no substantial additional benefit at the higher dose.

Melatonin-treated Groups IV, V and VI showed progressive increase in insulin-positive cells in a dose-dependent manner. Group IV displayed mild to moderate enhancement in staining intensity and β -cell population. Group V demonstrated marked restoration with numerous strongly positive β -cells distributed throughout the islets. Group VI also showed significant improvement, but staining intensity and percentage of positive cells were comparable to Group V, indicating no substantial added advantage at the higher dose.

Group VII rats treated with melatonin and metformin showed the highest insulin immunoreactivity among treated groups. The islets contained abundant strongly stained β -cells with staining pattern closely resembling normal controls, suggesting maximal preservation or regeneration of endocrine pancreatic tissue.

The increase in insulin-positive cells following melatonin treatment indicates protection of surviving β -cells, possible stimulation of β -cell regeneration and improvement in insulin synthesis. Melatonin may exert these effects through free radical scavenging, stabilization of mitochondrial membranes and suppression of apoptosis in pancreatic tissue. Thus, immunohistochemical findings strongly support the antidiabetic efficacy of melatonin observed in the present study.

Table 1: The Mean ($\pm$ SE) body weight values of treatment and control groups at different intervals of time

Groups	Bodyweight -Days post treatment			
Day3	Day 15	Day 30	Day 45	
Group I (NC)	168.5 $\pm$ 4.072< aA>	191 $\pm$ 1.52< aA>	223 $\pm$ 9.55< bcA>	330.85 $\pm$ 6.46< dA>
Group II (DC)	175.67 $\pm$ 4.58< aA>	155.67 $\pm$ 2.34< abB>	120 $\pm$ 3.05< bC>	121 $\pm$ 5.32< bC>
Group III (M)	187.67 $\pm$ 4.46< abA>	158.17 $\pm$ 3.24< bB>	155.17 $\pm$ 2.19< bB>	217.67 $\pm$ 9.54< aB>
Group IV (MT 5)	185.83 $\pm$ 3.11< abA>	156.67 $\pm$ 3.4< bB>	160.33 $\pm$ 2.84< bB>	227.5 $\pm$ 9.35< aB>
Group V (MT 10)	173.83 $\pm$ 4.06< abA>	151.5 $\pm$ 3.13< bB>	155.83 $\pm$ 4.08< bB>	245.5 $\pm$ 3.57< aB>
Group VI (MT 30)	184.83 $\pm$ 5.06< abA>	149.83 $\pm$ 7.69< bB>	158.66 $\pm$ 2.34< bB>	235.33 $\pm$ 2.65< aB>
Group VII (M+MT)	175.83 $\pm$ 5.43< bA>	142 $\pm$ 2.40< abB>	171.33 $\pm$ 3.33< bAB>	297 $\pm$ 8.36< baD>

Note: Values bearing different superscripts between rows within a column vary significantly ($p \leq 0.05$), Values bearing different superscript in capital letters between the row vary significantly ($p \leq 0.05$).

Table 2: The Mean ($\pm$ SE) serum glucose (mg/dL) values of treatment and control groups at different intervals of time.

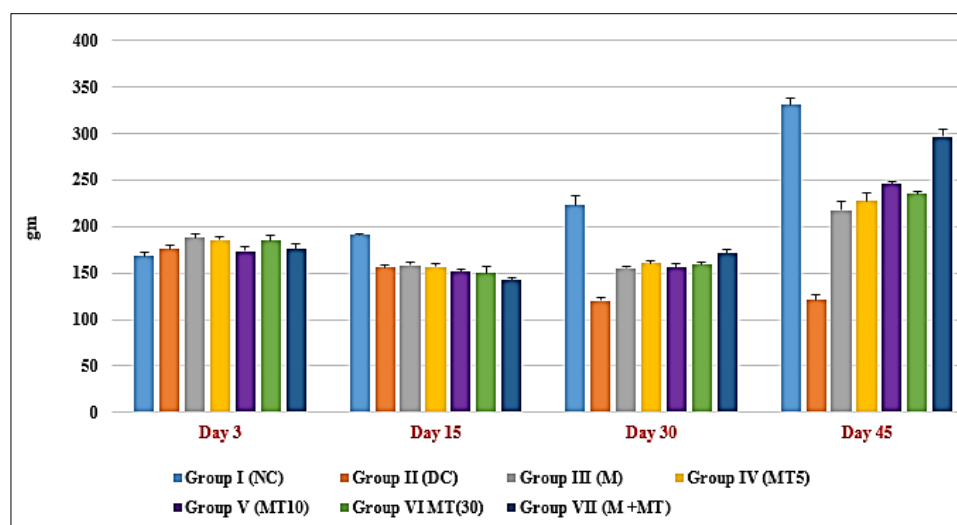
Groups	Serum glucose -Days post treatment			
Day3	Day 15	Day 30	Day 45	
Group I (NC)	112.62 $\pm$ 3.47< aB>	118.17 $\pm$ 0.94< aC>	110.61 $\pm$ 1.66< aC>	108.54 $\pm$ 5.86< aD>
Group II (DC)	394.83 $\pm$ 18.11< aA>	387.83 $\pm$ 14.15< aA>	364.76 $\pm$ 18.97< aA>	378.16 $\pm$ 16.67< aA>
Group III (M)	389.67 $\pm$ 29.52< aA>	283.56 $\pm$ 29.04< bB>	213.5 $\pm$ 19.52< bB>	151.17 $\pm$ 16.74< cC>
Group IV (MT 5)	402.31 $\pm$ 26.34< aA>	319.35 $\pm$ 26.04< bB>	244.17 $\pm$ 16.64< cB>	202.63 $\pm$ 17.98< dB>
Group V (MT 10)	398.33 $\pm$ 34.68< aA>	294.67 $\pm$ 23.09< bB>	226.83 $\pm$ 25.70< cB>	163.38 $\pm$ 14.79< dC>
Group VI (MT 30)	388.12 $\pm$ 37.09< aA>	320.11 $\pm$ 26.94< bB>	245.5 $\pm$ 21.75< cB>	209.10 $\pm$ 13.21< dB>
Group VII (M+MT)	383.86 $\pm$ 26.40< aA>	284.82 $\pm$ 24.34< bB>	226.13 $\pm$ 18.05< cB>	148.18 $\pm$ 14.66< dC>

Note: Values bearing different superscripts between rows within a column vary significantly ($p \leq 0.05$), Values bearing different superscript in capital letters between the row vary significantly ($p \leq 0.05$).

Table 3: The Mean ($\pm$ SE) percentage positivity of insulin secreting cells of treatment and control groups at different intervals of time

Groups	Insulin positive cells - Days Post Treatment			
15	30	45		
Group I (NC)	71 $\pm$ 5.09< bA>	74.33 $\pm$ 4.08< abA>	79.0 $\pm$ 7.66< aA>	
Group II (DC)	3.5 $\pm$ 0.62< bD>	5.66 $\pm$ 0.71< abD>	6.0 $\pm$ 0.68< aC>	
Group III (M)	8.5 $\pm$ 1.17< bc>	10.33 $\pm$ 2.22< bC>	47.91 $\pm$ 1.96< aB>	
Group IV (MT 5)	12.66 $\pm$ 1.39< cBC>	27.91 $\pm$ 2.3< bB>	39.91 $\pm$ 3.84< aB>	
Group V (MT 10)	17.08 $\pm$ 1.31< cBC>	33.25 $\pm$ 2.64< bB>	46.61 $\pm$ 2.64< aB>	
Group VI (MT 30)	13.0 $\pm$ 1.95< cBC>	31.66 $\pm$ 2.09< bB>	41.0 $\pm$ 2.46< aB>	
Group VII (M +MT)	22.8 $\pm$ 2.79< bB>	33.9 $\pm$ 2.1< abB>	52.8 $\pm$ 7.6< aB>	

Note: Values bearing different superscripts between rows within a column vary significantly ($p \leq 0.05$), Values bearing different superscript in capital letters between the row vary significantly ($p \leq 0.05$).

**Fig 1:** The Mean ($\pm$ SE) body weight values of treatment and control groups at different intervals of time

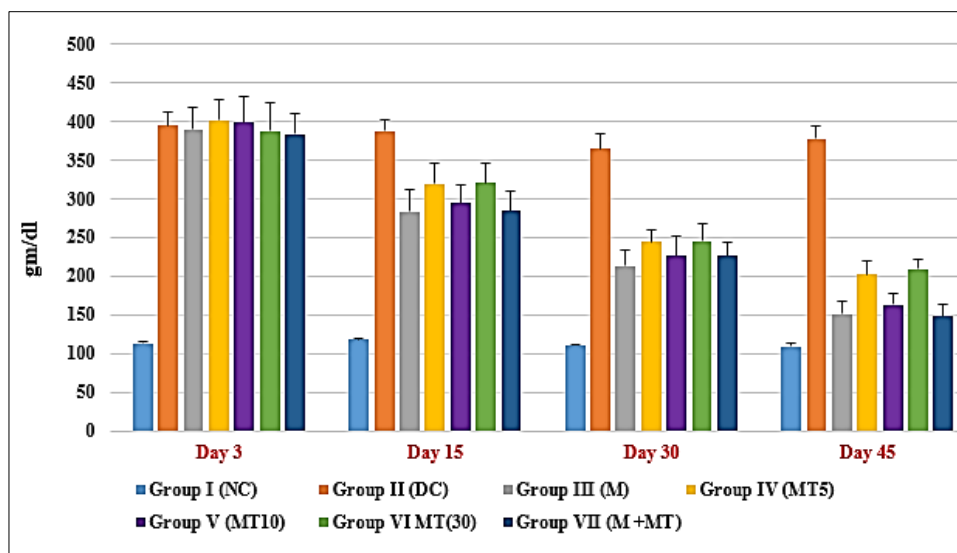


Fig 2: The Mean ($\pm$ SE) serum glucose (mg/dL) values of treatment and control groups at different intervals of time

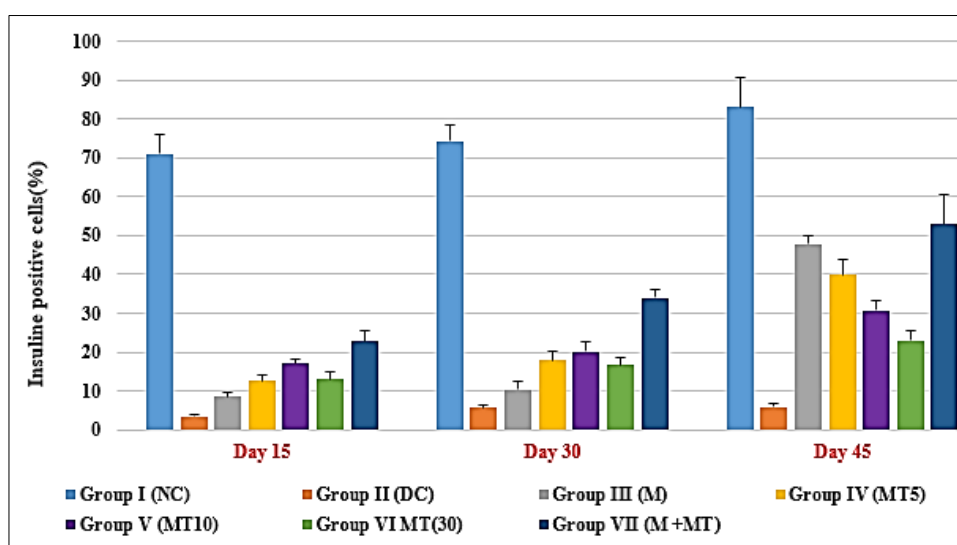


Fig 3: The Mean ($\pm$ SE) percentage positivity of insulin secreting cells of treatment and control groups at different intervals of time

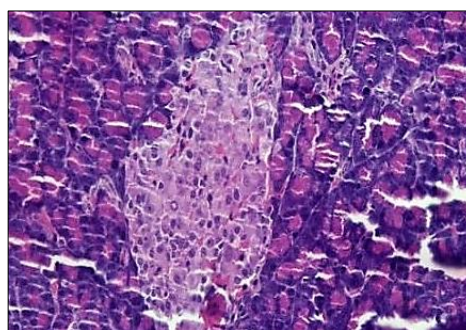
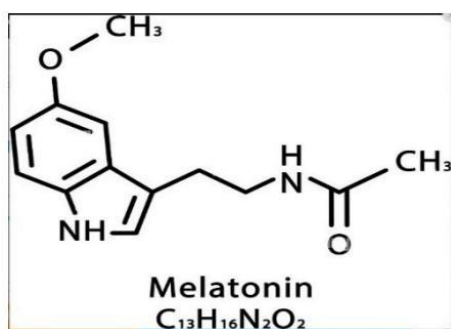


Plate 1: Structure of melatonin, **Plate 2:** Normal pancreas showing well- formed islets -200X

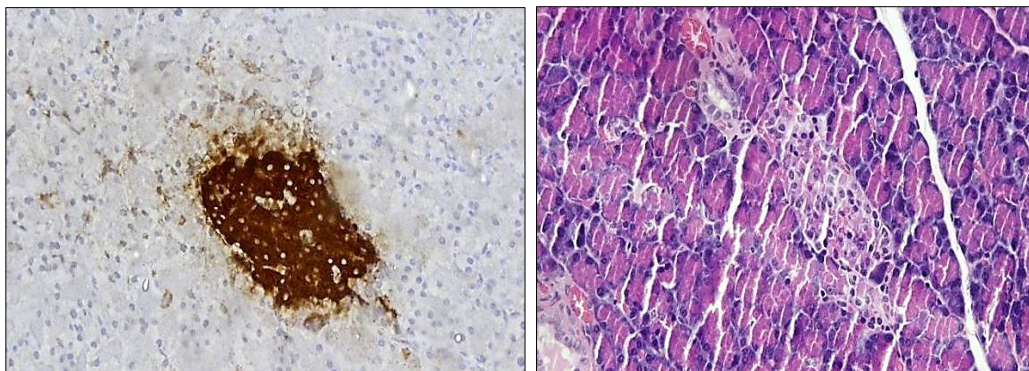


Plate 3: Normal pancreas showing the islets of Langerhans with numerous insulin-positive β -cells (400 $\times$)., **Plate 4:** Group II showing necrotic changes in the islets of Langerhans on the 45th day (400 $\times$).

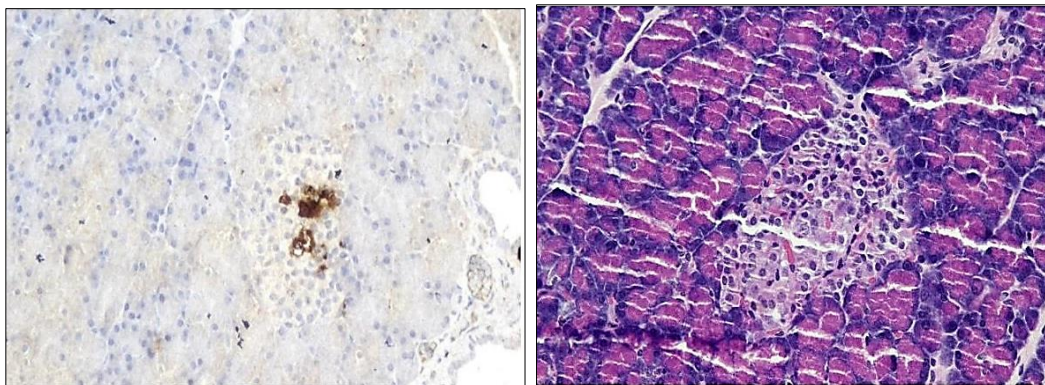


Plate 5: Group II showing lightly stained insulin-positive β -cells in the islets of Langerhans on the 45th day (400 $\times$). **Plate 6:** Group III showing improvement in the islets of Langerhans on the 45th day (400 $\times$).

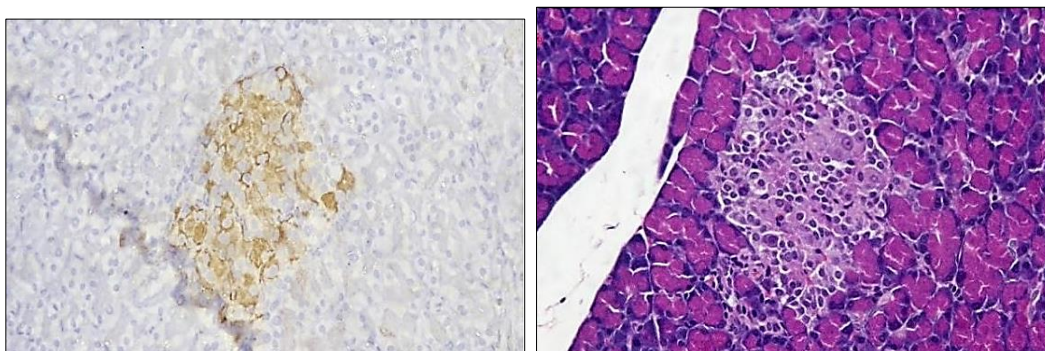


Plate 7: Group III showing an increased number of insulin-positive β -cells in the islets of Langerhans on the 45th day (400 $\times$). **Plate 8:** Group IV showing vacuolar degeneration of insulin-positive β -cells in the islets of Langerhans on the 45th day (400 $\times$).

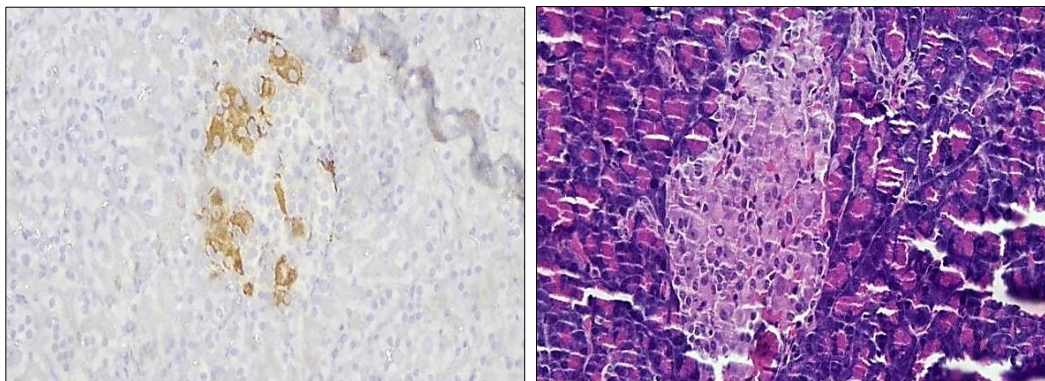


Plate 9: Group IV showing a moderate number of insulin-positive β -cells in the islets of Langerhans on the 45th day (400 $\times$). **Plate 10:** Group V showing improved cellularity of the islets of Langerhans without any degenerative changes on the 45th day (400 $\times$).

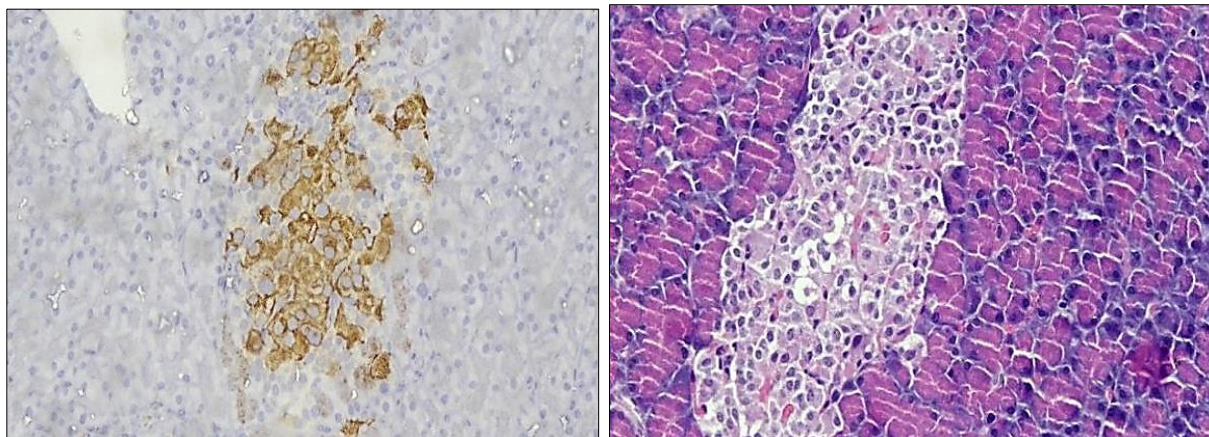


Plate 11: Group V showing a moderate number of insulin-positive β -cells in the islets of Langerhans on the 45th day (400 $\times$). **Plate 12:** Group VI showing a mild increase in the cellularity of the islets of Langerhans without any degenerative changes on the 45th day (400 $\times$).

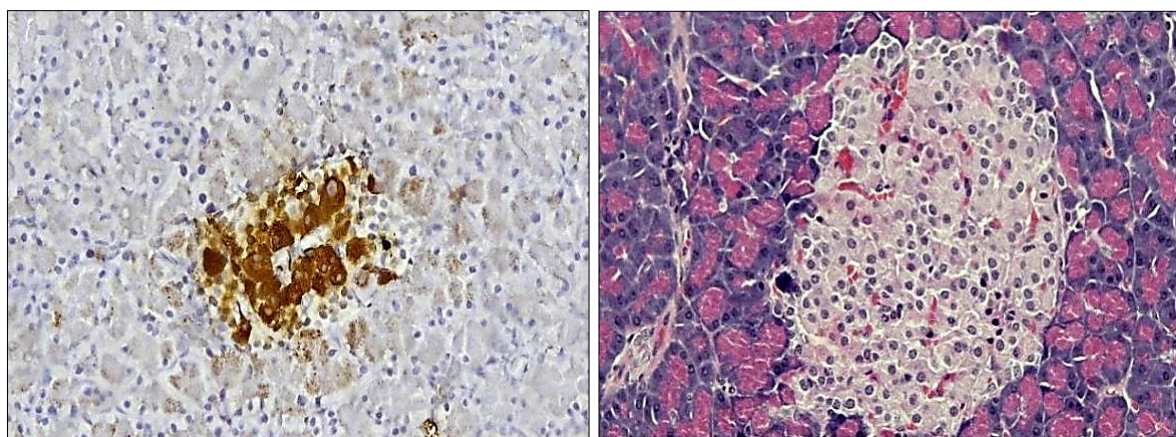


Plate 13: Group VI showing the majority of β -cells exhibiting a positive insulin immunoreaction in the islets of Langerhans on the 45th day (400 $\times$). **Plate 14:** Group VII showing well-preserved histoarchitecture of the islets of Langerhans on the 45th day (400 $\times$).

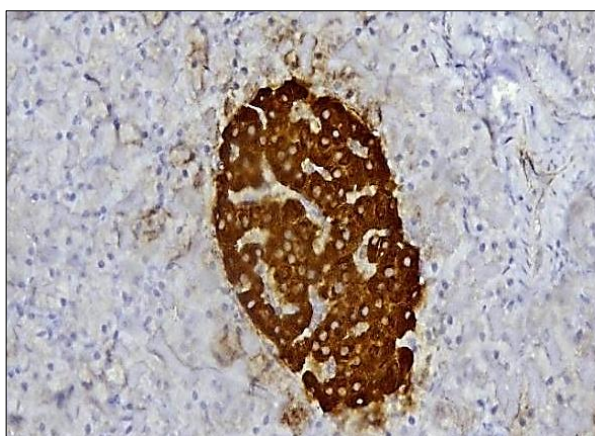


Plate 15: Group VII showing diffuse areas of insulin-positive β -cells exhibiting a positive immunoreaction on the 45th day (400 $\times$).

Body Weight

In the present study, streptozotocin-induced diabetic rats showed marked reduction in body weight throughout the experimental period, whereas melatonin-treated groups exhibited gradual restoration of body weight in a dose-dependent manner.

The normal control rats maintained progressive increase in body weight during the study period, indicating normal metabolic activity and efficient utilization of carbohydrates, proteins and lipids. In contrast, diabetic control rats exhibited significant reduction in body weight from Day 15 onwards. The progressive loss in body weight observed in diabetic rats may be attributed to severe insulin deficiency

caused by streptozotocin-induced destruction of pancreatic β -cells. Insulin deficiency impairs glucose uptake and utilization by peripheral tissues, resulting in enhanced catabolism of proteins and fats for energy production. Persistent hyperglycaemia further contributes to glycosuria, osmotic diuresis and dehydration leading to tissue depletion and muscle wasting (Szkudelski, 2001; Kavitha Rani, 2015) [26, 14]. Similar findings of body weight reduction in streptozotocin-induced diabetic rats were also reported by Prince and Menon (2001) [19], Ramesh and Pugalendi (2005) [20] and Agil *et al.* (2013) [1].

Metformin-treated rats showed gradual recovery in body weight during the experimental period. The improvement

may be attributed to enhanced insulin sensitivity, suppression of hepatic gluconeogenesis and improved peripheral utilization of glucose and nutrients. Similar observations were reported by Erejuwa *et al.* (2011) [9].

Melatonin-treated groups demonstrated progressive improvement in body weight when compared to diabetic control rats. Group IV rats treated with melatonin at 5 mg/kg body weight showed moderate restoration, whereas Group V rats treated with melatonin at 10 mg/kg body weight exhibited marked improvement with body weight values approaching near normal levels by the end of the experiment. Group VI rats treated with melatonin at 30 mg/kg body weight also showed significant increase in body weight; however, the improvement was comparable to Group V, indicating no substantial additional benefit at higher dose levels.

The beneficial effect of melatonin on restoration of body weight may be attributed to its potent antioxidant, anti-inflammatory and cytoprotective properties. Melatonin scavenges reactive oxygen species, reduces oxidative stress-induced cellular injury and improves mitochondrial function, thereby enhancing insulin secretion and glucose utilization (Reiter *et al.*, 2000; Rodriguez *et al.*, 2004) [21, 24]. Melatonin has also been reported to improve lipid and protein metabolism by reducing free radical-mediated tissue damage and enhancing endogenous antioxidant defense mechanisms (Tan *et al.*, 2015) [27]. Preservation of pancreatic β -cell integrity and improvement in endocrine pancreatic function may have further contributed to better metabolic utilization of nutrients and restoration of body weight.

The combination-treated group receiving melatonin along with metformin exhibited maximum recovery in body weight among all treated groups. The enhanced restoration observed in this group may be attributed to synergistic interaction between melatonin and metformin in improving glucose homeostasis, insulin sensitivity and metabolic efficiency. Similar synergistic protective effects of melatonin in diabetic models have been documented by Agil *et al.* (2013) [1] and El-Sokkary *et al.* (2019) [8].

Overall, the findings of the present study indicate that melatonin effectively ameliorates body weight loss associated with streptozotocin-induced diabetes mellitus and improves the general metabolic status of diabetic rats.

Serum Glucose

In the present study, streptozotocin-induced diabetic rats exhibited marked hyperglycaemia throughout the experimental period, whereas treatment with melatonin produced significant reduction in serum glucose levels in a dose-dependent manner.

The normal control rats maintained relatively constant serum glucose levels during the study period, indicating normal glucose homeostasis and proper endocrine pancreatic function. In contrast, diabetic control rats showed persistent elevation of serum glucose levels from Day 3 to Day 45. The sustained hyperglycaemia observed in diabetic rats may be attributed to selective destruction of pancreatic β -cells induced by streptozotocin, resulting in severe insulin deficiency and impaired peripheral utilization of glucose. Streptozotocin enters β -cells through *GLUT-2* transporters and induces DNA alkylation, oxidative stress and mitochondrial dysfunction leading to β -cell necrosis and decreased insulin synthesis (Szkudelski, 2001; Lenzen,

2008) [16, 26]. Reduced insulin secretion further enhances hepatic gluconeogenesis and glycogenolysis, thereby contributing to persistent hyperglycaemia. Similar observations were reported by Prince and Menon (2001) [19], Erejuwa *et al.* (2011) [9] and Agil *et al.* (2013) [1].

Metformin-treated rats showed progressive reduction in serum glucose levels throughout the experimental period. The antihyperglycaemic effect of metformin may be attributed to suppression of hepatic gluconeogenesis, improvement in insulin sensitivity and enhanced peripheral uptake of glucose (Viollet *et al.*, 2012; Rena *et al.*, 2017) [23, 28]. Similar findings were documented by Erejuwa *et al.* (2011) [9].

Melatonin-treated groups demonstrated gradual decline in serum glucose levels when compared to diabetic control rats. Group IV rats treated with melatonin at 5 mg/kg body weight showed moderate reduction in serum glucose levels, whereas Group V rats treated with melatonin at 10 mg/kg body weight exhibited marked antihyperglycaemic activity with glucose values approaching near normal levels by the end of the experiment. Group VI rats treated with melatonin at 30 mg/kg body weight also showed significant reduction in serum glucose levels; however, the degree of reduction was comparable to Group V, indicating no substantial additional advantage at higher dose levels.

The antihyperglycaemic activity of melatonin observed in the present study may be attributed to its potent antioxidant and cytoprotective properties. Melatonin directly scavenges reactive oxygen and nitrogen species, reduces oxidative stress-induced β -cell injury and improves mitochondrial function, thereby preserving insulin secretion and glucose metabolism (Reiter *et al.*, 2000; Rodriguez *et al.*, 2004) [21, 24]. Melatonin receptors present in pancreatic islets also regulate insulin secretion and glucose homeostasis (Peschke *et al.*, 2013) [18]. In addition, melatonin has been reported to improve insulin sensitivity, reduce inflammatory mediators and enhance endogenous antioxidant defense mechanisms, thereby protecting pancreatic tissue against streptozotocin-induced oxidative damage (Tan *et al.*, 2015) [27].

The combination-treated group receiving melatonin along with metformin exhibited maximum reduction in serum glucose levels among all treated groups. The enhanced antihyperglycaemic effect observed in this group may be attributed to synergistic interaction between melatonin and metformin in improving insulin sensitivity, preserving pancreatic β -cells and enhancing peripheral glucose utilization. Similar synergistic protective effects of melatonin in diabetic models have been reported by Agil *et al.* (2013) [1] and El-Sokkary *et al.* (2019) [8].

Overall, the findings of the present study clearly indicate that melatonin possesses significant antihyperglycaemic activity and effectively ameliorates streptozotocin-induced hyperglycaemia either alone or in combination with metformin.

Histopathology

In the present study, histopathological examination of pancreatic tissue was carried out to evaluate the protective effect of melatonin against streptozotocin-induced pancreatic damage in diabetic rats. The histopathological alterations observed in pancreatic sections of different experimental groups are illustrated in Plates 2, 4, 6, 8, 10, 12 and 14.

The pancreatic sections of normal control rats (Group I) revealed normal histoarchitecture characterized by well-organized exocrine acini and distinct, round to oval islets of Langerhans composed of closely packed endocrine cells with normal cytoplasmic and nuclear morphology (Plate 2). No degenerative or inflammatory changes were observed throughout the experimental period. Similar observations in normal pancreatic tissue have been reported by Junqueira and Carneiro (2013) [12].

In contrast, pancreatic sections from diabetic control rats (Group II) revealed severe pathological alterations characterized by marked reduction in the size and number of islets of Langerhans, irregular and shrunken islets, depletion of endocrine cellularity, cytoplasmic vacuolation, pyknosis and focal necrosis of β -cells (Plate 4). Mild edema and separation of acinar tissue were also evident in some sections. The lesions progressively intensified from Day 15 to Day 45 of the experiment indicating continuous oxidative and metabolic damage to pancreatic tissue.

The severe degenerative alterations observed in diabetic rats may be attributed to streptozotocin-induced oxidative stress and selective destruction of pancreatic β -cells. Streptozotocin enters β -cells through *GLUT-2* transporters and induces DNA alkylation, nitric oxide generation and mitochondrial dysfunction resulting in β -cell necrosis and insulin deficiency (Szkudelski, 2001; Lenzen, 2008) [16, 26]. Similar histopathological findings in streptozotocin-induced diabetic rats were reported by Prince and Menon, (2001) [19] Akbarzadeh *et al.* (2007) [2] and Arunachalam *et al.* (2014) [5].

In Group III rats treated with metformin, there was progressive improvement in pancreatic histoarchitecture during the experimental period. The islets appeared relatively larger with partial restoration of endocrine cellularity and reduction in degenerative changes when compared with diabetic control rats (Plate 6). However, complete normalization of pancreatic architecture was not observed. The improvement in histological appearance may be attributed to improved glycaemic control and reduction in oxidative stress following metformin treatment.

In Group IV rats treated with melatonin at 5 mg/kg body weight, the pancreatic sections revealed mild to moderate regenerative changes in islets of Langerhans. Increased endocrine cellularity and reduction in vacuolar degeneration were observed when compared to diabetic control rats, although mild residual degenerative changes were still evident in some sections (Plate 8).

Group V rats treated with melatonin at 10 mg/kg body weight demonstrated marked histological improvement throughout the experimental period. The islets of Langerhans appeared well-defined with increased cellular density and restoration of normal endocrine architecture. Minimal cytoplasmic vacuolation and degenerative changes were observed and the pancreatic parenchyma closely resembled that of normal control rats as illustrated in Plate 10. Similar pancreatic protective effects of melatonin have been reported by Anwar and Meki (2003) [4], Agil *et al.* (2013) [1] and Sudnikovich *et al.* (2007) [25].

Likewise, Group VI rats treated with melatonin at 30 mg/kg body weight also exhibited significant restoration of pancreatic architecture with improved islet organization and reduced degenerative lesions (Plate 12). However, the degree of improvement was comparable to that observed in

Group V indicating no substantial additional benefit at higher dose levels.

The beneficial effects of melatonin observed in the present study may be attributed to its potent antioxidant, anti-inflammatory and antiapoptotic properties. Melatonin directly scavenges reactive oxygen species, reduces lipid peroxidation and stimulates endogenous antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase (Reiter *et al.*, 2000; Rodriguez *et al.*, 2004) [21, 24]. Melatonin also stabilizes mitochondrial membranes and inhibits apoptotic cell death thereby protecting pancreatic β -cells against oxidative injury (Tan *et al.*, 2015) [27].

The rats in the combination-treated group (Group VII) receiving melatonin along with metformin exhibited the best histopathological response among all treated groups. The pancreas showed near-normal acinar arrangement and well-preserved islets of Langerhans with minimal degenerative changes indicating marked restoration of pancreatic architecture (Plate 14). The enhanced recovery observed in the combination-treated group may be attributed to synergistic action of melatonin and metformin in improving glucose homeostasis and reducing oxidative stress-induced pancreatic injury.

The overall histopathological findings of the present study clearly indicate that melatonin effectively ameliorates streptozotocin-induced pancreatic damage and promotes restoration of endocrine pancreatic tissue in diabetic rats.

Immunohistochemistry

In the present study, immunohistochemical demonstration of insulin was carried out to identify and enumerate pancreatic β -cells in various treatment groups using monoclonal anti-insulin antibody. The mean ($\pm$ SE) percentage positivity of insulin secreting cells in different experimental groups are presented in Table 3 and Fig. 3.

In the normal control group (Group I), most of the islets of Langerhans revealed intensely stained insulin-positive β -cells distributed uniformly throughout the islets. The β -cells exhibited densely stained brown cytoplasmic granules that were compactly arranged with distinct cellular boundaries, whereas the exocrine acini and non- β endocrine cells remained negative for insulin immunostaining (Plate 2). Similar observations in normal pancreatic tissue were reported by Kiernan (2015).

The pancreatic islets of diabetic control rats (Group II) revealed marked reduction in the number of insulin-positive β -cells throughout the experimental period. Most of the positively stained cells appeared swollen, irregular and weakly stained with scattered cytoplasmic granules indicating degeneration and depletion of insulin content (Plate 5). The percentage positivity of insulin secreting cells remained significantly lower ($P \leq 0.05$) when compared with normal control rats at all intervals of observation as presented in Table 3 and Fig. 3. The severe reduction in insulin immunoreactivity observed in diabetic rats may be attributed to streptozotocin-induced oxidative damage and destruction of pancreatic β -cells resulting in impaired insulin synthesis and secretion (Szkudelski, 2001; Lenzen, 2008) [16, 26].

In Group III rats treated with metformin, there was a progressive increase in the number of insulin-positive β -cells during the experimental period. The improvement in insulin immunoreactivity was significantly higher when

compared with diabetic control rats particularly on the 30th and 45th day of observation; however, the values remained comparatively lower than normal control rats (Table 3 and Fig. 3). The pancreatic islets showed moderate restoration of β -cell population with enhanced staining intensity as illustrated in Plate 7. Similar observations were reported by Erejuwa *et al.* (2011) [9].

In Group IV rats treated with melatonin at 5 mg/kg body weight, the pancreatic islets showed gradual improvement in insulin-positive β -cells at different intervals post-treatment. Moderate increase in staining intensity and β -cell population was observed when compared to diabetic control rats, although the values remained lower than normal control rats (Plate 9). The percentage positivity of insulin secreting cells progressively increased from Day 15 to Day 45 as presented in Table 3 and Fig. 3.

Group V rats treated with melatonin at 10 mg/kg body weight demonstrated marked restoration of insulin immunoreactivity throughout the experimental period. Numerous strongly stained insulin-positive β -cells with intense cytoplasmic granules were distributed uniformly throughout the islets (Plate 11). The improvement in percentage positivity of insulin secreting cells was significantly higher when compared with diabetic control rats and was comparable to metformin-treated rats on later intervals of observation. The marked increase in insulin immunoreactivity observed in this group suggests substantial preservation and functional recovery of pancreatic β -cells.

Similarly, Group VI rats treated with melatonin at 30 mg/kg body weight also showed progressive improvement in insulin-positive β -cells at regular intervals post-treatment (Plate 13). Strong insulin immunostaining with improved cellular distribution was observed; however, the degree of improvement was comparable to Group V indicating no substantial additional benefit at higher dose levels.

The rats in the combination-treated group (Group VII) receiving melatonin along with metformin also exhibited progressive increase in insulin-positive β -cells throughout the experimental period. The percentage positivity of insulin secreting cells was significantly higher than diabetic control rats and showed values approaching normal control rats by the end of the study period (Table 3 and Fig. 3). The pancreatic islets revealed abundant strongly stained β -cells with diffuse cytoplasmic positivity closely resembling normal pancreatic tissue as illustrated in Plate 15.

The progressive restoration of insulin immunoreactivity observed in melatonin-treated groups may be attributed to its potent antioxidant and antiapoptotic properties. Melatonin scavenges reactive oxygen species, stabilizes mitochondrial membranes and protects pancreatic β -cells against oxidative damage induced by streptozotocin (Reiter *et al.*, 2000; Peschke *et al.*, 2013) [18, 21]. The findings of the present study therefore indicate that melatonin effectively preserves pancreatic β -cell integrity and improves insulin synthesis in streptozotocin-induced diabetic rats.

8 Conclusion

The present study demonstrated that the test compound effectively alleviated streptozotocin-induced diabetes in rats, as evidenced by improved body weight, reduced serum glucose levels, restoration of pancreatic histoarchitecture and increased insulin-positive β -cells. These findings indicate significant antidiabetic activity, likely mediated

through pancreatic β -cell protection and regeneration, supporting its potential as a therapeutic agent for diabetes mellitus.

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