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Research Article
Repossession of Brain Complications in a Streptozotocin Induced Diabetic Rat by Exogenous Melatonin Administration

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Abstract

Background and Objective: Diabetes is one of the foremost culprit responsible in degrading the health of a person in this stressful life. The present study focused on protective effect of melatonin (MEL) on brain of streptozotocin (STZ) induced diabetic rat. **Materials and Methods:** Thirty six male rats were randomly divided into six groups, each group contain six rats, Control, STZ induced, STZ+Mel, Mel, STZ+GB (Glibenclamide) and GB. Streptozotocin was injected for six days continuously thereafter blood glucose level were censored after 72 h. The animals showing blood glucose level above 250 mg dL^{-1} were considered as diabetic and were administered with exogenous Mel for 4 weeks. Animals were euthanized after 4 weeks. Brain of all respective groups were dissected, weighed and fixed in Bouin's fixative for histological studies as well as processed for the assessment of biochemical variables viz lipid peroxidation (LPO), antioxidative defense system; reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity and total protein quantification. Data were analyzed by students t-test followed by one-way ANOVA to compare between different experimental groups. **Results:** Streptozotocin (STZ)-induced diabetic rats exhibited significant increase in rate of LPO ($p \leq 0.001$) but a significant decrease in organ weight, GSH, SOD, CAT and total protein content ($p \leq 0.05$, 0.01 and 0.001). However, melatonin treatment restored LPO, weight of brain, total protein, catalase (CAT) SOD, GSH ($p \leq 0.05$, 0.01 and 0.001). Decrement in hippocampal volume signifies the brain cell damaged might have resulted due to increased free radical load during diabetes. Further, histophotomicrographs of diabetic rats showed decrease in astrocyte number indicating compromised state of immune defense system. Melatonin administration however revived the brain architecture because of its antiapoptotic and antioxidant nature. **Conclusion:** Therefore, melatonin might be suggested as neuroprotective therapeutic molecule regulating morphological, anatomical and biochemical functions of brain during diabetes induced brain impairments.

Key words: Melatonin, streptozotocin, glutathione, astrocyte, glial cell

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes is a multi-faceted metabolic syndrome[1], it is one of the major health concerns in the public across the globe[2]. Approximately 220 million people are expected to be suffer from diabetes mostly Low and Middle Income Countries (LMIC)[3]. In 2008, diabetes has costed about 1.256 million deaths globally in LMIC[4]. The high level of blood sugar is one of the major risk factor for atherosclerosis, inflammatory disease of the arterial walls, due to the formation of fatty streaks and plaques. Atherosclerosis can lead to stroke or heart attack. Diabetes also affects the angiogenesis and wound healing[5]. It also hinders the supply of vitamin-C to both retina and brain which in turn results in the Blood-Brain Barrier (BBB) `permeability[1]. WHO reported that diabetes is the 8th leading cause of death and is mainly characterised by high rates of mortality and morbidity[6]. Diabetes occurs either because of lack of insulin or because of the presence of factors that oppose the action of insulin[7]. Symptoms of diabetes include frequent urination, increased thirst and increased hunger, as it provokes to numerous dysfunctions.

Brain is the central organ of the nervous system and it lies within the cranial cavity of the skull. It maintains the homeostasis by receiving sensory inputs, integrating the information and helps in making decisions[8]. Motor activities and behavioural postures are controlled by the brain[9,10]. Cells of the brain include neurons and astrocytes that maintain the BBB. Hyperglycaemic end products contribute to the pathologic substrate for Alzheimer's disease and other neurodegenerative diseases. It also leads to stress and depression[3]. Prolonged diabetes results in the decreased volume of white and gray matter[11] and also leads to the decreased number of astrocytes which might be down regulating the immune cells of the brain. Persistent diabetic episodes also lead to increase in hippocampal volumes[12]. Due to diabetes, number of astrocytes are altered disturbing the BBB which causes many neurodegenerative diseases by weakening the immune system of the brain.

Melatonin is the main secretory product of pineal gland and is known as pineal neurohormone[13]. It is a peptide hormone belonging to the indole compound[14]. Melatonin has pleiotropic functions as immune modulator[15], antioxidant and anti-apoptotic mediator[16]. It is also involved in bone metabolism[17]. Melatonin signal is also critical for glucose regulation in blood and in maintaining homeostasis[18]. Along with these, clinical investigation of melatonin therapy in elderly patients has demonstrated a relaxation effect[19] and the improvement of depressed mood and memory[20]. It has positive impact on angiogenesis and wound healing and is effective in improving sleep disturbances resulting from Alzheimer's diseases[21]. Melatonin restores the oxidative stress in different organs like liver, kidney and ovaries[22,23].

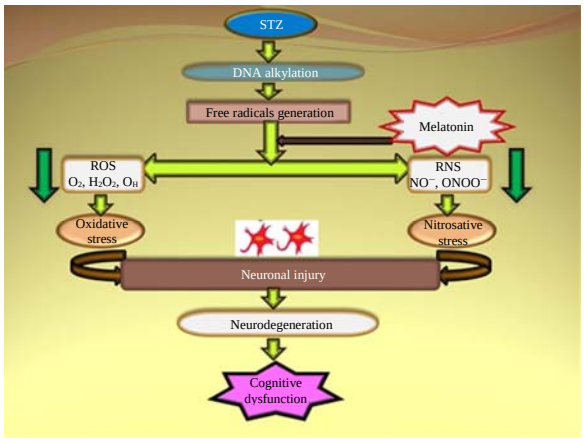

Fig. 1: Proposed neuroprotective role of exogenous melatonin

Streptozotocin is a permanent diabetogenic inducing drug and is synthesized by a soil microbe, *Streptomyces achromogenes* (a Gram-positive bacterium) having extensive spectrum of antibacterial properties. Due to selective toxicity to the beta cells of the pancreatic cells[24], it causes the DNA damage which induces the activation of poly ADP-ribosylation which leads to diabetes induction[25].

Therefore, present study was conducted to reveal biochemical followed by histologically alterations resulted during diabetic condition and further to evaluate protective role of melatonin to overcome diabetes induced brain complications.

Diagrammatic representation of STZ induced oxidative stress and free radical generated nervous damages and the hypothesized protective role of melatonin (Fig. 1).

MATERIALS AND METHODS

Animal model and maintenance: Male albino rats (*Rattus norvegicus*) weighing approximately 180±10 g of same age group were procured from Defence Research and Development Establishment (DRDE) Gwalior, M.P. and kept under standard laboratory conditions (25±2°C temperature, 60-70% humidity and 12 h photoperiod). The experimental study was carried out during the month of January-March, 2017 at Department of Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G), India. Rats were fed with pellet diets and water *ad libitum*. All experimental procedures for care and use of laboratory animals were approved by the Institutional Animal Ethics Committee (IAEC), Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G), India (Registration Number: 994/Go/ERe/S/06/CPCSEA). After two weeks of acclimatization, the rats were divided into following six different groups.

Experimental design:

Group I: Normal control rats (0.1M Sodium Citrate buffer)
Group II: Diabetic control (STZ 15 mg kg^{-1} b.wt., 6 days, i.p.)
Group III: STZ+MEL [STZ 15 mg kg^{-1} (6 days) + 1 mg kg^{-1} b. wt., 4 weeks i.p.]
Group IV: MEL (1mg kg^{-1} b.wt., 4 weeks)
Group V: STZ+GB (STZ 15 mg kg^{-1}+0.5 mg kg^{-1} b.wt., 4 weeks, p.o.)
Group VI: GB (0.5 mg kg^{-1} b.wt., 4 weeks)

The STZ dissolved in 0.1 M citrate buffer of pH 7.4 was administered intraperitoneally for consecutively 6 days. Blood glucose level of the animals was monitored using glucometer (ACCU CHECK) after 72 h of streptozotocin treatment. Rats with blood glucose level higher than 250 mg dL^{-1} up to 6th day were considered as diabetic model. After successful induction, MEL and GB were given for 4 weeks. At the end of 30th day, animals of each group were sacrificed following complete anaesthesia. Tissues were dissected out, cleaned and were fixed in Bouin's fixative and also stored at -20°C for the study of different parameters.

Chemicals required: BHT, Copper Sulphate, DNTB (0.01 M), Folin's Solution, Glacial Acetic Acid, Glibenclamide, H_2O_2, Melatonin, Methanol, NaOH (0.2 N), NADH, NBT, PBS Phosphate Buffer (50 mM), Phosphoric Acid, PMS, Sodium Carbonate, Sodium Potassium Tartrate, Sodium Pyrophosphate buffer (pH-7.0), Streptozotocin, Sucrose Buffer, TBA (0.6%), TCA (10%), Tris Buffer (0.2 M, pH-8.2), Tris-HCL Buffer (pH-7.4). All the chemicals and reagents used to carry out biochemical and histological analysis were of analytical grade.

Parameters studied

Morphological and gravimetric analysis: Brain of each group was carefully separated from cranium. They were weighed using the weighing balance and then photography was done with the help of camera (OLYMPUS CX2181ED) to compare with the different experimental groups.

Lipid Peroxidation Assay (LPO): Lipid peroxidation assay was conducted according to Ohkawa *et al.*[26]. The homogenate was prepared by Tris-HCl buffer (pH 7.4). Equal amounts of homogenate and BHT were taken and 3 mL of phosphoric acid and 1 mL of TBA was mixed to it. After the centrifugation at 3500 rpm pellet was decanted and only supernatant was taken. The absorbance was measured at 535 nm.

Glutathione Reduced Assay (GSH): GSH assay was performed according to Sedlak and Lindsay[27]. The 100 mg of fresh tissue was homogenised in 1 mL of sucrose buffer. To 100 µL of homogenate 1.5 mL of Tris Buffer (pH-8.2) was added and the volume was made up to 10 mL by methanol. After centrifugation at 3000 rpm for 15 min absorbance was measured at 412 nm.

Assay of Superoxide Dismutase (SOD): SOD was conducted following the method of Kakkar *et al.*[28]. The homogenate was prepared in Phosphate Buffer Saline. 0.2 mL of homogenate was centrifuged at 1500 rpm for 10 min and only the supernatant was taken. Then 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of PMS and 0.3 mL of NBT were added to the supernatant and finally absorbance was taken at 560 nm.

Catalase Assay (CAT): The assay was performed according to Beer and Sizer[29]. The homogenate was prepared in phosphate buffer. Phosphate buffer (ph 8.2) with volume of 2.5 mL was added to 0.1 mL of homogenate and centrifuged at 3000 rpm for 15 min. Forty microliter of the supernatant was taken and 3 mL of H_2O_2 phosphate buffer to it. The absorbance was taken at 240 nm.

Protein estimation: The total protein content was quantified following the method of Lowry *et al.*[30] with some modifications. Two hundred microliter (200 µL) of 10% TCA was added to the homogenate and centrifuged at 2000 rpm for 15 min. To the pellet 1 mL of NAOH (0.2 N) was added. From the above prepared sample 0.1 mL was pipette out, to this 0.4 mL of distilled water was added. Five milliliter of Solution C was added then added and then incubated for 10 min. Five hundred microliter (500 µL) of Folin's solution was added to develop color and absorbance was taken at 625 nm.

Histological studies: Tissues were fixed in Bouin's fixative for 24 h. They were processed through the various steps of dehydration and clearance and then embedded in paraffin wax. Tissue sections of 5 µm thick were cut using rotary microtome (Leica RM 2125RT5). Hematoxylin eosin stained slides were observed under light microscope for histopathological changes.

Statistical analysis: Results were expressed as Mean±SE. Data were analyzed by student's t-test followed by one-way ANOVA using IBM 20.0 version software[31]. Results were considered significant at different levels ($p \leq 0.05$, 0.01 and 0.001).

RESULTS

Morphological differences: Brain showed some well demarcated changes in hippocampal area. In diabetic rats the hippocampal region of the brain was shrinked and size decreases, while as melatonin treated diabetic rats showed significant restoration in the hippocampal area (Fig. 2).

Effect of melatonin on gravimetric analysis: Diabetic rats showed significant decrease in body weight ($p \leq 0.01$), whereas melatonin co-administration to the diabetic rats increases the body weight ($p \leq 0.05$) (Fig. 3).

Effect of melatonin on lipid peroxidation: The free radical production was noted significantly higher ($p \leq 0.001$) in brain of streptozotocin (STZ) induced diabetic rats. Administration of melatonin showed significant decrease ($p \leq 0.05$) in LPO level of brain in streptozotocin (STZ) induced diabetic rats (Fig. 4).

Effect of melatonin on reduced glutathione GSH): The GSH level was noted decreased significantly in brain of streptozotocin (STZ) diabetic rats ($p \leq 0.001$). Melatonin supplemented to streptozotocin diabetic rats showed significant increase ($p \leq 0.05$) in GSH level (Fig. 5).

Effect of melatonin on superoxide dismutase (SOD) activity: A significant decrease in SOD activity was observed in streptozotocin (STZ) induced diabetic rats in brain ($p \leq 0.001$), whereas melatonin administration to the diabetic rats showed improvement in antioxidant enzyme levels ($p \leq 0.05$) (Fig. 6).

Effect of melatonin on catalase activity: Streptozotocin (STZ) induced diabetic rats revealed significantly decreased catalase (CAT) activity ($p \leq 0.01$) (enzyme which cleaves the H_2O_2 into water and molecular oxygen) in brain. Melatonin treatment to streptozotocin (STZ) induced diabetic model showed significant increase in catalase activity ($p \leq 0.01$) (Fig. 7).

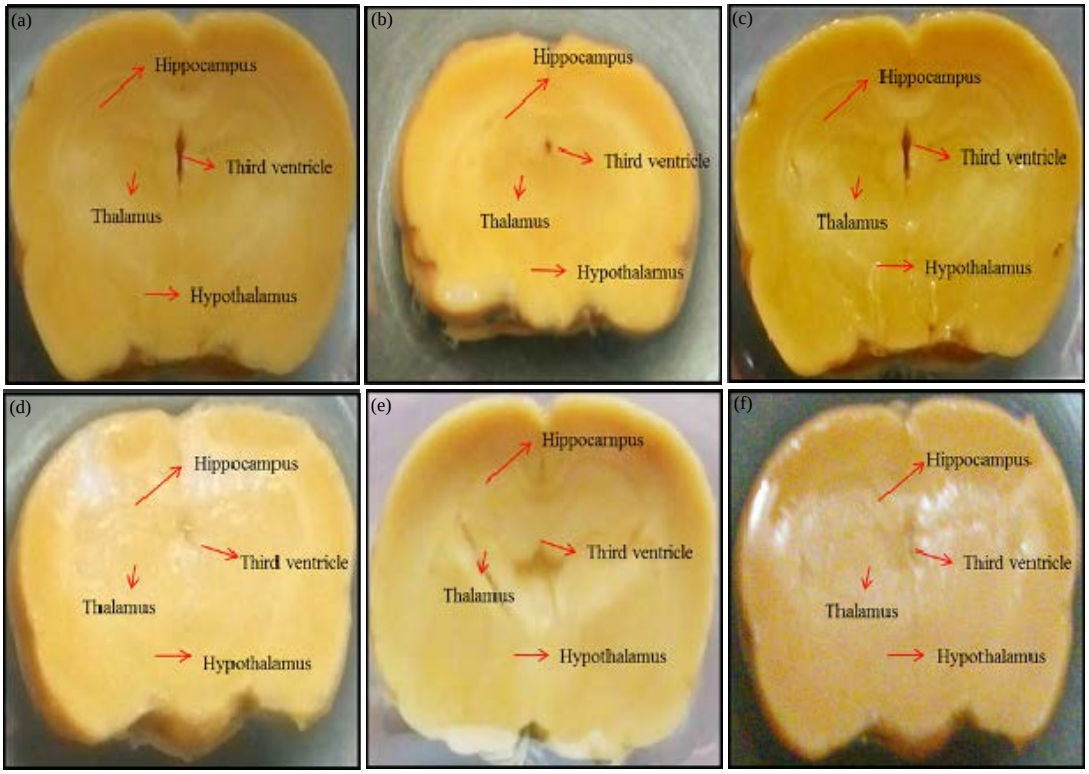

Fig. 2(a-f): Showing morphological changes in the hippocampal region of the brain in different experimental groups, (a) Con-(Control) group no change in size of hippocampus, (b) STZ (Streptozotocin) group the hippocampal area decreased in size (c) STZ +Mel (Streptozotocin+melatonin) group recovery in size of hippocampus, (d) STZ+GB (Streptozotocin+Glibenclamide) group showed recovery in the hippocampical region and (e-f) Mel and GB treated groups does not showed any change in the hippocampus

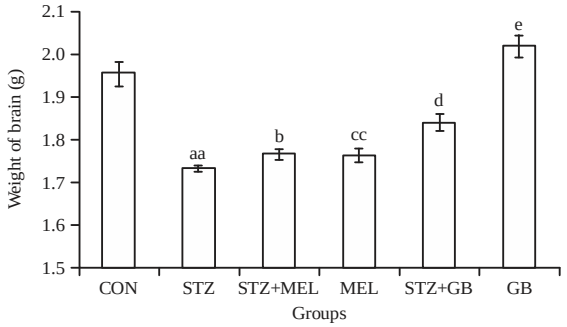

Fig. 3: Effect of melatonin on weight of brain

Histogram represents Mean±SE, N = 6, aa: p≤0.01; CON vs. STZ, b: p≤0.05; STZ vs. STZ+MEL, cc: p≤0.01; STZ vs. MEL, d: p≤0.05: STZ vs. STZ+GB, e: p≤0.05: STZ vs. GB

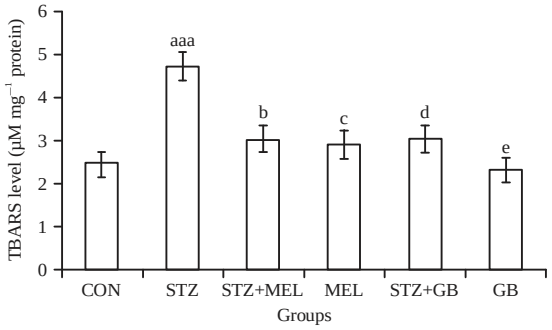

Fig. 4: Effect of melatonin on lipid peroxidation of brain

Histogram represents Mean±SE, N = 6, aaa: p≤0.01: CON vs. STZ, b: p≤0.05: STZ vs. STZ+MEL, c: p≤0.05: STZ vs. MEL, d: p≤0.05: STZ vs. STZ+GB, e = p≤0.05: STZ vs. GB

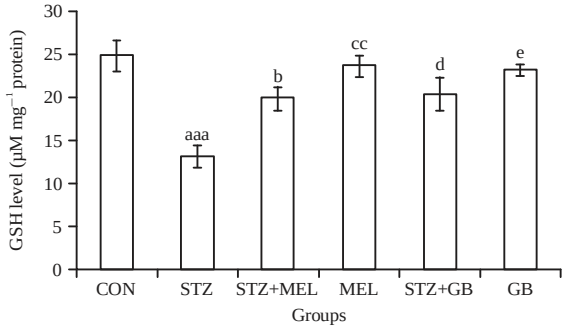

Fig. 5: Effect of melatonin on GSH level of brain

Histogram represents Mean±SE, N = 6, aaa: p≤0.001: CON vs. STZ, b: p≤0.05: STZ vs. STZ+MEL, cc: p≤0.01: STZ vs. MEL, d: p≤0.05: STZ vs. STZ+GB, e: p≤0.05: STZ vs. GB

Effect of melatonin on total protein estimation: The protein content was noted significantly decreased in brain of streptozotocin (STZ) induced diabetic rats (p≤0.05). Administration of melatonin significantly restored the total protein contents of brain in streptozotocin (STZ) induced diabetic rats (p≤0.05) (Fig. 8).

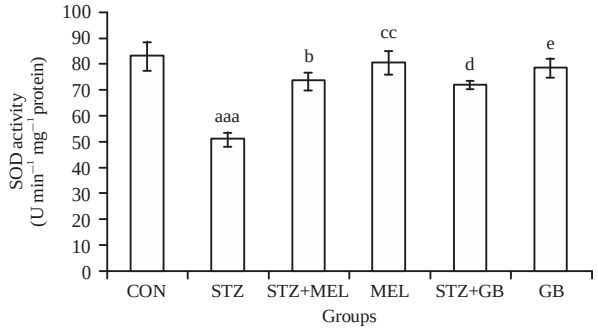

Fig. 6: Effect of melatonin on Superoxide dismutase (SOD) activity of brain

Histogram represents Mean±SE, N = 6, aaa: p≤0.001: CON vs. STZ, b: p≤0.05: STZ vs. STZ+MEL, cc: p≤0.01: STZ vs. MEL, d: p≤0.05: STZ vs. STZ+GB, e: p≤0.05: STZ vs. GB

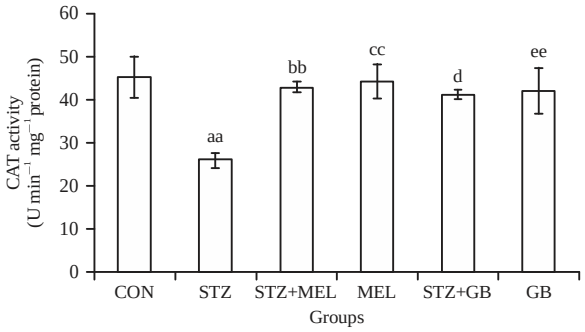

Fig. 7: Effect of melatonin on catalase (CAT) activity of brain

Histogram represents Mean±SE, N = 6, aa: p≤0.01: CON vs. STZ, bb: p≤0.01: STZ vs. STZ+MEL, cc: p≤0.01: STZ vs. MEL, d: p≤0.05: STZ vs. STZ+GB, ee: p≤0.01: STZ vs. GB

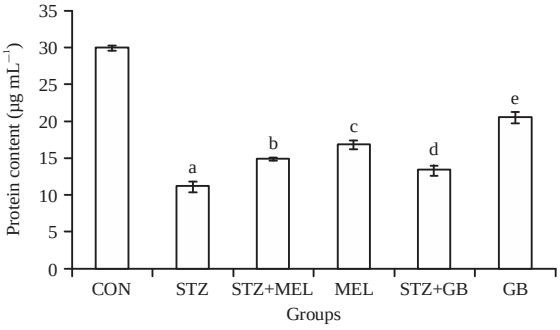

Fig. 8: Effect of melatonin on protein contents of brain

Histogram represents Mean±SE, N = 6, a: p≤0.05: CON vs. STZ, b: p≤0.05: STZ vs. STZ+MEL, c: p≤0.01: STZ vs. MEL, d: p≤0.05: STZ vs. STZ+GB, e: p≤0.05: STZ vs. GB

Effect of melatonin on histological architecture of brain: Brain of STZ induced diabetic rats showed decrease in astrocyte number indicating compromised state of immune defence system. Melatonin administration however, revived the brain architecture as compared to

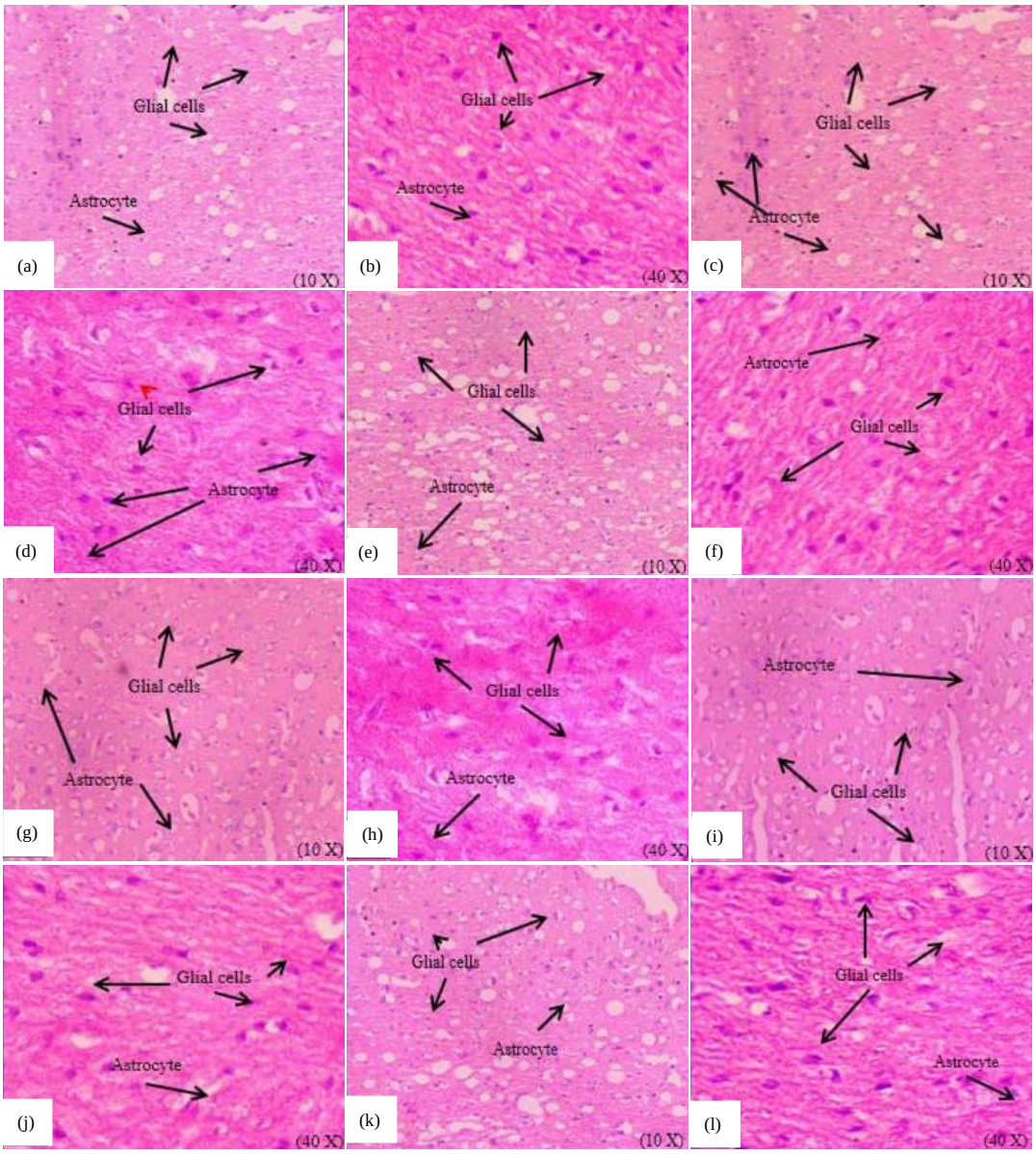

Fig. 9(a-l): Photomicrographs of rat brain sections in different experimental groups stained with Hematoxylin and Eosin (10X and 40X), (a-b) Histopathological sections of pancreas in control rat revealed normal astrocyte and glial cells, (c-d) Rat treated with MEL (*per se*) showing normal cellular structure with abundant astrocytes and glial cells, (e-f) GB (*per se*), hypoglycaemic drug, (g-h) STZ induced diabetic control rats revealed decreased astrocyte number and brain alterations, (i-j) Brain of rats treated with MEL (1 mg kg^{-1}) after induction of diabetes showed restoration in glial and astrocyte number and (k-l) STZ+GB, brain of rats treated with hypoglycaemic drug (glibenclamide) after successful induction of diabetes to compare with melatonin treated diabetic rats

control group and therefore, may be said as immune regulator of brain/CNS via immune modulation (Fig. 9).

DISCUSSION

Prolonged diabetes lead to an increased risk of acquiring vascular dementia, hemodynamic impairments (Stroke), Cognitive deficits, behavioral modulations, damages in the Blood Brain Barrier (BBB) and other neurodegenerative diseases. It is a condition in which the ultimate response of the insulin is decreased than normal[31]. This might be because of improper functioning of either the cells that produce insulin or by the cells that have to respond to the insulin[32]. In humans, the diabetogenic process appears to be caused by immune

destruction of the β- cells; part of this process is apparently mediated by white blood cell production of active oxygen species[33]. Diabetes can be characterized by a state of chronic hyperglycemia (peripheral insulin resistance), glucosuria, polyurea, polydipsia (excessive thirst), polyphagia (constant hunger), sudden weight loss, ketoacidosis and ketonuria (urinary ketones)[34,35] nephropathy, cardiovascular diseases etc.

Diabetes is the most common serious metabolic disorder[36], where a bunch of functional and morphological alterations can occur in the central nervous system[37]. These changes include abnormal expression of hypothalamic neuropeptides, astrogliosis in the hippocampus[38], decreased synaptic plasticity in the hippocampus, neurotoxicity and changes in glutamate neurotransmission[39]. Several evidences have shown that diabetes may be associated with learning and memory deficits in humans[40]. Studies examining the effects of STZ-induced diabetes on memory function in mice and rat models have also shown deficits in memory retention and retrieval, compared to non-diabetic animal models[41]. Astrocytes have effective roles in supporting the neurons, maintenance of the Blood Brain Barrier (BBB)[42], vascular reactivity, regulation of extracellular glutamate levels, energy metabolism and protection from reactive oxygen species in the central nervous system[43]. In the present study the astrocytes were found to be decreased in STZ-induced diabetic rats which might have disturbed the neurons and physiology of the brain. These astrocytes regulates immune functions of the neuron, decrement in the astrocytes weakens the immune system of the brain. Altered astrocyte activity contributes to the CNS pathophysiology in diabetes in different parts of the brain which affect coordination and working efficiency of the diabetic patients. Results of current study coincide with the previous studies which indicated that diabetes led to astroglial alterations by decreasing the number of astrocytes. Melatonin administration to the diabetic rats restored the number of astrocytes, as melatonin is known as immune modulator[44]. These results were confirmed by administering melatonin to normal rats which does not showed any alteration in astrocyte count. Both high and low dose of melatonin protects the astrocytes[45].

In the present study, weight of brain was found to be decreased in the STZ-induced diabetic rats. It has been reported that depression and stress caused by diabetic conditions are the two factors which decreases the weight of brain[46] type-1 and type-2 diabetes causes depression in patient, which might be the reason that diabetes leads to weight loss[47]. Melatonin might have reduced the stress and depression that may be a possible hypothetical protective role of melatonin to restore the weight of brain in the STZ-induced diabetic rats towards control group. The STZ-induced diabetic rats exhibited shorter hippocampus region in the brain morphological observations documented in the photomicrographs. The hippocampus is the region of brain where high receptors for insulin are found suggesting that it plays a major role in memory. The administration of MEL increases the hippocampus area towards normal because the antioxidants (i.e., melatonin) restores the cell damage and causes cell proliferation in the hippocampus region of brain[48] as shown in the Fig. 2.

Hyperglycemia is the main reason for significant damage to the body through the accumulation of metabolic toxins such as superoxides and Advanced Glycation End Products (AGEPs), causing ischaemia, hypoxia, oxidative stress and inflammation. It also can also cause apoptosis of neurons and gliosis, which might be lead to cognitive impairment and even central nervous system disorders such as Alzheimer's Disease (AD)[49]. Multiple genes or proteins play important roles in diabetic neuropathy. Oxidative stress is a process during which balance between free radical generation and antioxidant defence gets disturbed. These free radicals damage the cells of any organ system. Lipid peroxidation is widely used procedure to measure the oxidative stress in blood, serum or any other organ[50]. Oxidative stress is associated with several indices of adiposity and a low antioxidant defence[51]. In present study rate of lipid peroxidation level was found to be higher in the STZ-induced diabetic rats, our result was supported by previous findings of Vincent *et al.*[52]. It has well documented that melatonin is an antioxidant that can easily cross cell membranes and the BBB[53]. It directly as well as indirectly act as an antioxidant to scavenge Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) including H_2O_2, OH^-, O_2^- and NO^- [54]. The MEL normalised the LPO level by activating the antioxidant enzymes as the MEL functions as to enhance the potential antioxidant enzymatic systems. Because of these mutilated defensive antioxidant properties, melatonin might have reduced the lipid peroxidation in STZ-induced diabetic rat. The findings of this study were confirmed by administration of melatonin and standard hypoglycaemic drug glibenclamide to the other groups of rats which does not showed any abnormal change in free radical production.

Diabetes is associated with decreased cellular glutathione concentrations[55]. Oxidative stress and ROS formation are markedly increased by uncontrolled hyperglycaemia[56]. Streptozotocin causes reduction and weakening in the antioxidative defence like glutathione reduced (GSH) as was observed in STZ injected group of rats. While scavenging

property of MEL might be responsible in the treatment of increased glutathione formation in STZ-induced diabetic rat, this increment in the GSH level in the tissue might be due to the donation of electron by the indole ring of melatonin. The results were supported by the alone treatment of melatonin and glibenclamide to separate group of animals which showed regulatory roles to maintain the glutathione levels in the tissues.

The free radicals generated during the different glucose metabolizing pathways following STZ induction of diabetes might have been scavenged by melatonin. Because of its high potential of antioxidative property, as free radical scavenger which in turn led to enhanced and up regulation of the antioxidative enzymes (GSH, SOD and CAT)[22]. Thus, simultaneously down regulating the free radical production. Therefore, melatonin may be a potent agent in reducing the oxidative damage caused by Streptozotocin in diabetic patients. In the present study the SOD and catalase activity was found to be decreased in STZ-induced diabetic conditions which is similar to previous findings of Cojocaru[57]. Melatonin restored antioxidative enzyme level as that of antidiabetic molecule glibenclamide treated group of rats[22]. It is proposed that MEL suppresses the degradation of the nuclear factor-E2-related factor (Nrf2) and increases its nuclear translocation via its interacting with ubiquitin ligase in the nucleus and then Nrf2 stimulates the transcription of antioxidant enzymes (e.g., MnSOD and catalase) through binding to the antioxidant response element[58]. This property of MEL might have been the reason that catalase activity was increased in administration of MEL in STZ-induced diabetic rat.

During the diabetic condition the proteins mostly becomes glycosylated and non-functional. The oxidative stress causes the disintegration of plasma membrane, leading to leakage of intracellular proteins and other enzymes into blood plasma. The protein level was found to be decreased in STZ-induced diabetic conditions. The MEL might have been beneficial in the view related to protein contents that protein content was found to be increased in administration of MEL in streptozotocin-induced diabetic rats. These finding could be because of MEL, which influences the insulin secretion and homeostasis[59] and its oral administration exert anti- hyperglycemic effect in young rats as insulin sensitizer and by improvement in β-cell function[60]. Clinical results indicate that MEL improves glycemic control in blood[59].

CONCLUSION

It is concluded that melatonin is a powerful antioxidant molecule. It can be used as a neuroprotective either as supplement/adjuvant therapeutic molecule to improve various deficits associated with diabetic neuropathy. Thus, it might provides a useful therapeutic option in humans to reduce oxidative stress and the associated neuronal damages in diabetic patients.

SIGNIFICANCE STATEMENT

Finding of present study discovers the antagonising effect of exogenous administration of melatonin against the neurodegeneration and damages in the rat brain during STZ induced diabetic condition. Melatonin normalized the oxidative stress and reversed the brain damage by renormalizing of antioxidant system. Therefore, antioxidants might be the promising therapeutic approach in reducing diabetes induced neuronal damages and its related complications. Therapeutic properties of melatonin may help making independent melatonin based drug discovery process in future. However such novel therapy may be further explored to un turn various pharmacokinetic in order to implement in medical science.

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