

The possible protective role of melatonin on the changes in the cerebral cortex and meninges of streptozotocin-induced diabetes in adult male albino rats (histological and immunohistochemical study)

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Introduction: Diabetes mellitus is a serious common metabolic disease. It causes a variety of functional and structural disorders in the central nervous systems. It induces alterations in the brain glucose metabolism and increase oxidative stress. Melatonin is a potent free radical scavenger and stimulates the major antioxidant enzymes.

Aim: This study was aimed to evaluate the possible protective role of melatonin on the histological changes in the cerebral cortex and meninges after induction of diabetes in a rat model.

Materials and Methods: In this study, forty adult male albino rats were divided into four groups (ten rats for each): Group I control rats, group II rats received intraperitoneal injection of Streptozotocin (STZ) (60 mg/kg, single dose), group III rats received intraperitoneal injection of melatonin (10 mg/kg/d) for six weeks, group IV received same previous doses of STZ and melatonin for six weeks. At the end of experiment, the cerebral cortex was dissected and processed for light microscopic examinations and also for glial fibrillary acidic protein (GFAP) to demonstrate the astrocytes. Morphometrical and statistical analyses were carried out.

Results: Examination of cerebral cortex of group II showed separation of the pia mater, congestion in the blood vessels and hemorrhage in intermediate lamella. There were multifocal histological changes and depletion of the cellular elements. The neuropil showed vacuolation. There were multiple areas of microinfarction and pericellular halos. Cresyl Violet stained sections showed karyolysis and immunohistochemical study showed significant increase in GFAP positive astrocytes. In contrary, Examination of cerebral cortex of group IV showed apparent improvement in almost all layers. Cresyl Violet stained sections showed darkly stained Nissel's granules. Immunohistochemical study showed significant decrease in GFAP positive astrocytes.

Conclusion: Melatonin can ameliorate the effect of diabetes on the cerebral cortex and meninges through its antioxidant effect.

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Key Words: Cerebral Cortex, karyolysis, melatonin, streptozotocin.

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INTRODUCTION

The Cerebral cortex is the source of neural transactions that enhance memory, cognition, speech and intellectual activity^[1]. The cytoarchitectural structure of the cortex is characterized by the presence of six – layered laminated pattern of cells arranged from outside to inside; molecular, outer granular, outer pyramidal, inner granular, inner pyramidal and the multiform layer^[2, 3].

The arrangement of the meninges covering the cerebral cortex in their intervacular segments are from in to out; subpial space; inner pial layer; pial space; outer pial layer; inner arachnoid layer; arachnoid space; outer arachnoid layer; neurothel; inner dural layer; dura mater. The inner pial layer attached to the brain surface follows the perivascular glia into deeper portions of the brain^[2]. The double-layered inter- mediate lamella (IL), composed of

the outer pial and inner arachnoid layers, dissociates when joining the blood vessel, resulting in a widening of the intercellular clefts^[4].

On the other hand, Diabetes mellitus (DM) is a common progressive serious metabolic disease. It is considered one of the most common chronic diseases worldwide^[5].

DM is a disease characterized by chronic hyperglycemia and requires long-term management. Chronic changes in the level of glycemic induce alterations in the brain glucose metabolism; increase oxidative stress and can lead to various complications, affecting the CNS. This complication is referred to as diabetic encephalopathy and is characterized by impairments in cognitive functions and electrophysiological changes. These functional changes are accompanied by structural and neurochemical abnormalities as well as degenerative changes in the brain^[6, 7]. Recent

clinical evidence suggests that diabetes leads to increased incidences of vascular dementia, ventricular hypertrophy, lacunar infarcts, and hemorrhage and may be a predisposing factor for Alzheimer's disease^[8].

The Streptozotocin (STZ)-induced diabetes in rats serves as an excellent model to study the molecular, cellular and morphological changes in the brain induced by stress in DM. STZ is often used to induce DM in experimental animals because of its toxic effects on pancreatic-cells. It provides a relevant example of endogenous chronic oxidative stress as a result of hyperglycemia. During hyperglycemia, enhanced formation of oxygen free radicals occurs in the tissues. These oxidant radicals contribute to increased neuronal death by oxidizing proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes^[6, 9].

Melatonin is a neurohormone synthesized primarily in the pineal gland during the dark phase of the light. From physiological stand point, melatonin induces sleepiness, decreased alertness and slow reaction time, sensitizing the brain to sleep-inducing factors. It is a multitask molecule that influence sleep patterns and circadian rhythms, with an effective antioxidant properties^[8].

Hence, melatonin easily crosses the membranes and the blood-brain barrier, having a potent free radical scavenger action and stimulates the major antioxidant enzymes. Melatonin has previously been shown to exhibit neuroprotection under a variety of circumstances^[10].

Numerous publications have shown that melatonin can protect brain and other tissues from toxicity of many environmental and chemical insults both in vivo and in vitro^[11].

Therefore, this study was designed to investigate the effect of STZ induced DM on the structure of the cerebrum and meninges and the protective effect of melatonin on them.

MATERIALS AND METHODS

Forty five adult male albino rats of Wistar strain weighing 180-200 g were used in the present study. Animals were obtained from the animal house of Research Center and Bilharzial Research Unit of Faculty of Medicine, Ain Shams University.

Rats were allowed free access to water and food and were housed in rooms with 12 hours day and night cycle, good hygienic conditions, good ventilation and a temperature of 21±3°C. Animals were left one week for acclimatization before the start of the experiment. All animal procedures were approved by the animal care and use committee of faculty of medicine Ain Shams University.

Used drugs

- Streptozotocin: powder (STZ; Sigma Chemical Co., St. Louis, MO, USA).
- Melatonin: Tablet form from GNC/USA.

Experimental design and drug administration

- At the onset of the study, a blood sample was collected from the tail vein of each rat for the measurement of blood glucose levels to exclude DM.
- Rats were divided into four groups as follow:

Group I: (control and Sham control): Fifteen rats subdivided into

Ia: Five rats were not subjected to any procedure and served as a control.

Ib: Five rats were administered a single intraperitoneal injection of 0.1 ml saline as a vehicle for STZ.

Ic: Five rats were administered intraperitoneal injection of 0.1 ml ethanol throughout the experiment as a vehicle for melatonin.

Group II (rats received STZ):

Included ten rats and used to induce experimental diabetes. STZ was dissolved in saline. Each rat was administered a single intraperitoneal injection of STZ at a dose of 60 mg/kg body weight freshly dissolved in 0.1 ml saline under anesthesia^[9].

Three days after STZ injection, fasting blood samples^[5] taken from the dorsal vein of rats' tails to confirm diabetes induction. The blood glucose level of all rats was estimated by the glucose-oxidase method using (Accu-check Active; Roche Diagnostics, Mannheim, Germany). The diabetic level of blood glucose is ensured once a week.

All rats that presented with a fasting blood glucose level higher than 250 mg/dl were considered diabetic^[9].

Group III (rats received melatonin):

Ten rats received melatonin (dissolved in 0.1% ethanol). It was administered intraperitoneal injection at a dose of 10 mg/kg/d throughout the experiment.

The administration schedule for melatonin, i.e., daily just prior to lights off, was the commonly used schedule^[6].

Group IV (diabetic rats received melatonin):

Included ten rats and used after induction of diabetes and confirmation as group II. Diabetic rats were administered intraperitoneal injection at a dose of 10 mg/kg/d of melatonin throughout the experiment^[6]. Melatonin was prepared as in the group III.

After 6 weeks, all the rats were anaesthetized with intraperitoneal injection of thiopental sodium 25mg/kg body weight. The brain was extracted and the right cerebrum was dissected after the brain was split in the mid-sagittal plane and processed for:

I. Histological study:

The specimens were fixed in 10% formol saline, processed to prepare 5 µm-thick paraffin sections. Histological sections were stained with haematoxylin and eosin (H&E) and

0.1% cresyl violet acetate (Sigma certified stain, C- 5042), prepared in acetate buffer (pH3.5) [5].

II. Immunohistochemical study:

Sections were incubated with a monoclonal antibody glial fibrillary acidic protein (GFAP; Sigma, St Louis, Missouri, USA) used for detection of astrocytes. Detection of the antibody was carried out using a biotin–streptavidin detection system with 0.05% diaminobenzidine as a chromogen (Amersham, Little Chalfont, UK) for GFAP[9].

All stained sections were examined with the light microscope and photographed with Olympus E330 camera.

III. Quantitative morphometric study:

Images were analyzed using computer-based image analysis software (Leica Qwin 500; Imaging Systems, Cambridge, UK) computer assisted image analysis was performed in Anatomy Department, Faculty of Medicine, Ain shams University. Astrocytes cells was counted in an area of 20 000 μm^2 and selected randomly in the GFAP stained sections using light microscopy at $\times 400$ magnification.

IV. Statistical study

GFAP-positive astrocytes was presented as mean \pm SD. Statistical analysis was carried out using unpaired student's t-test, where the level of significance (P) value was set at 0.05 (one-way analysis of variance).

RESULTS

I. Histological and immunohistochemical results:

Group I and III (control group and rats received melatonin):

Light microscopic examination of hematoxylin and eosin (HxandE) stained sections of both groups I and III showed similar finding with no observable difference and revealed that the cerebral cortex was formed of four distinguished layers from outside inward with no sharp boundaries. These layers were; molecular layer formed of fibers traveling parallel to the surface with relatively few cells, outer granular layer, outer pyramidal layer and inner granular layer (Fig. 1). The cerebrum was covered with leptomeninges (pia and arachnoid mater; they were obvious with intermediate lamella (IL) in-between containing blood vessels (Fig. 2).

The pyramidal and granule cells in addition to neuroglial cells could be observed. The pink stained background; the neuropil, was detected as a mat of neuronal and glial cell processes (Fig. 3). Pyramidal cells had open face nuclei with prominent nucleoli and basophilic Nissel granules (Fig. 4).

Cresyl Violet stained sections showed darkly stained Nissel's granules in the cytoplasm of pyramidal cells surrounding the large vesicular nucleus with prominent nucleolus (Fig. 5).

Immunohistochemical study showed GFAP positive Astrocytes; they appeared few, dispersed and organized

around blood vessels in the granular and molecular layers (Fig. 6).

Group II (diabetic group):

Examination of HandE stained sections obtained from diabetic group revealed multifocal histological changes an apparent depletion of the cellular elements of most of the cortical layers was noticed as compared to the control group (Fig. 7).

An obvious finding was the discontinuity and separation of the pia mater as well as congestion in the blood vessels. Vacuolation of the vessel wall and hemorrhage in IL were detected (Figs. 8, 9, 10).

Many vacuoles of variable sizes either single or multiple appeared intercellular and intracellular in all layers (Fig. 10). There was degeneration in the molecular layer (Fig. 9). Hyalinization between the outer granular and the outer pyramidal layer was also seen (Fig. 10).

Between the molecular layer and the outer granular layer there was an area of microglia adjacent to an inflammatory mononuclear perivascular cuff. Most of nerve cells appeared shrunkened. They were surrounded by pericellular halos (Fig. 11).

There were multiple areas of disorganized deeply acidophilic tissue patches; most probably areas of microinfarction (Fig. 12).

The pyramidal cells were mostly affected; they became disfigured and lost their processes (Fig. 13). They had bizarre shaped deeply stained nuclei (Fig. 14). The neuropil among the nerve cells and neuroglia were separated by empty spaces (Fig. 14).

Cresyl Violet stained sections showed light stained Nissel's granules in the cytoplasm of pyramidal cells that showed karyolysis (Fig. 15).

Immunohistochemical study showed abundant GFAP positive astrocytes with multiple processes, in the granular and molecular layers (Fig. 16).

Group IV (diabetic rat received melatonin):

Examination of HandE stained sections obtained from diabetic group received melatonin revealed an apparent improvement in almost all layers.

The covering meninges showed regular continuous pia (Fig. 17), in spite of being still congested the vessel wall in IL was devoid of vacuolation in comparison to that in the diabetic group. The molecular layer was apparently normal as compared to that of the control group (Fig. 18).

Most of the pyramidal cells were more or less as that of control group. They had open face nuclei, prominent nucleoli and basophilic Nissel's granule however pericellular halos were still present (Fig. 19). The neuropil was compact (Figs. 19 and 20).

Cresyl Violet stained sections showed darkly stained

Nissel granules in the cytoplasm of pyramidal cells surrounding the large vesicular nucleus with prominent nucleolus (Fig. 21).

Immunohistochemical study showed GFAP positive astrocytes; they appeared small, multiple, scattered fragmented and nearby blood vessels (Fig. 22).

II. Morphometric results:

Significant increase in number of astrocytes ($P < 0.001$) was observed in rats of group II as compared with the control groups I.

In group IV (diabetic rat receiving melatonin), there was significant decrease in number of astrocytes ($P < 0.001$) compared with the diabetic groups II (Table 1 and Histogram 1).

Non-significant changes were detected in the mean number of astrocytes in rats of both groups III, IV as compared to group I.

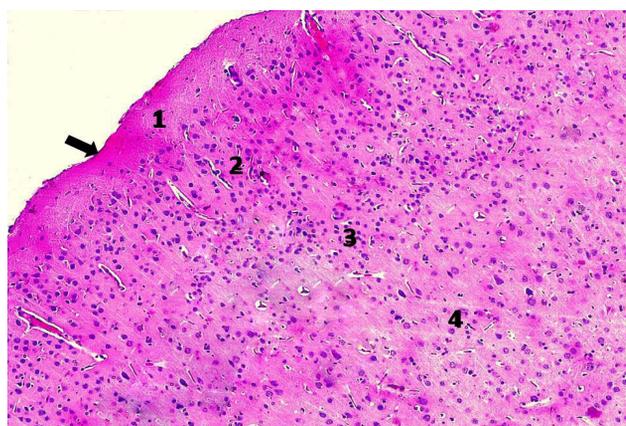


Fig. 1: A photomicrograph of a section in the cerebral cortex of control male albino rat showing the pia matter (arrow) and part of the cerebral cortical layers; the molecular layer (1), the outer granular layer (2), the outer pyramidal layer (3) and the inner granular layer (4). Hx and E x400

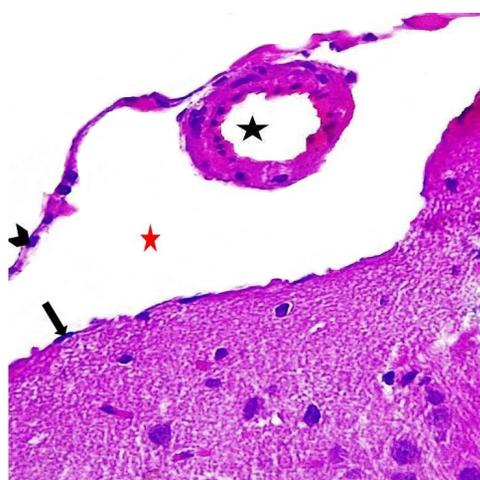


Fig. 2: A photomicrograph of a section in the cerebral cortex of control male albino rat showing layers of meninges the arachnoid layer (arrow head), pia matter (arrow) and the blood vessel (black asterisk) in intermediate lamella (red asterisk). Hx and E x400

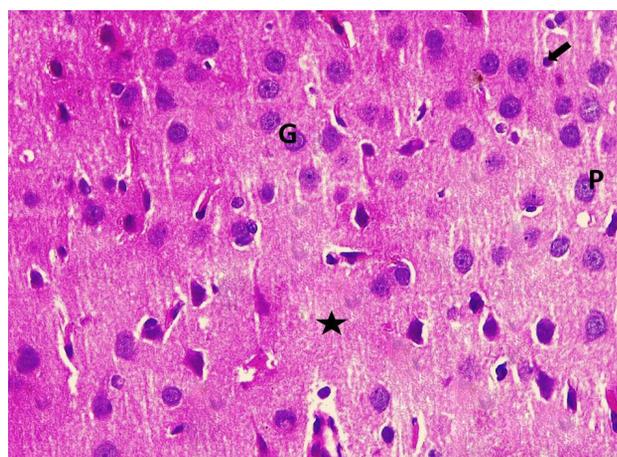


Fig. 3: A photomicrograph of a section in the cerebral cortex of control male albino rat showing pyramidal cells (P), granular cells (G). Notice the neuroglial cells (arrow) and the surrounding neuropil (asterisk). Hx and E x400

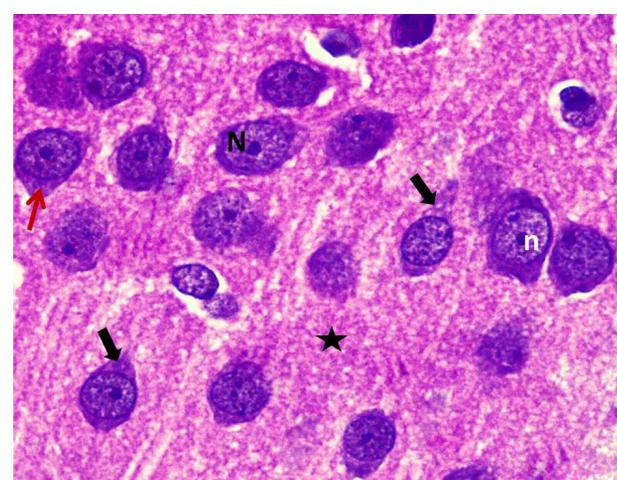


Fig. 4: A photomicrograph of a section in the cerebral cortex of control male albino rat showing pyramidal cells (arrow) with open face nuclei (N) with prominent nucleoli (n). Note the basophilic Nissel's granule (red arrow) and the neuropil (asterisk) in-between the cells. Hx and E x1000

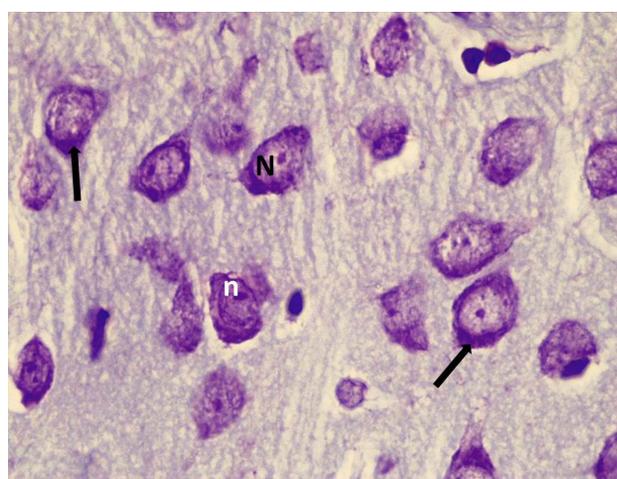


Fig. 5: A photomicrograph of a section in the cerebral cortex of control male albino rat showing darkly stained Nissel's granules in the cytoplasm of pyramidal cells (arrow) surrounding the large vesicular nucleus (N) with prominent nucleolus (n). Cresyl Violet x1000

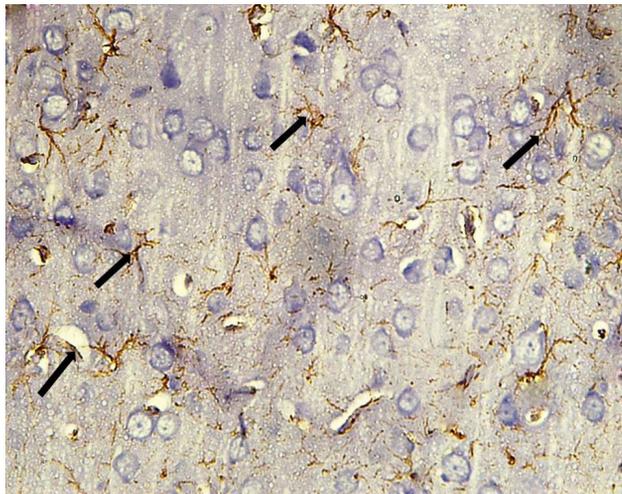


Fig. 6: A photomicrograph of an immunohistochemically stained section in the cerebral cortex of control male albino rat showing GFAP positive Astrocytes; they appear few, dispersed and organized around blood vessels in the granular and molecular layers (arrow). GFAP $\times 400$

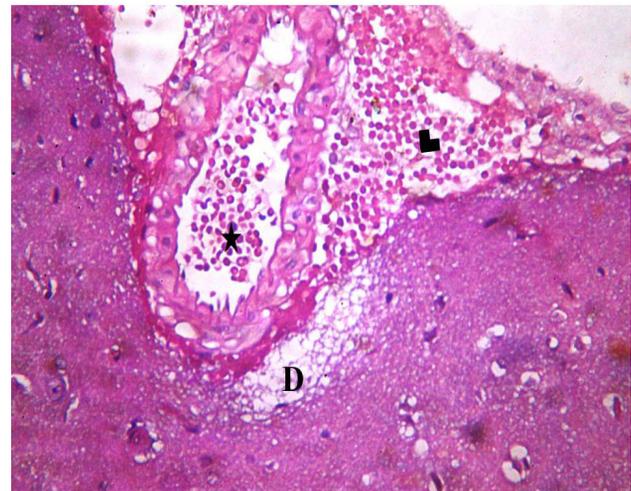


Fig. 9: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing a congested blood vessel (asterisk) and hemorrhage (arrow head) in intermediate lamella. Note the degeneration (D) in the molecular layer. Hxand E $\times 400$

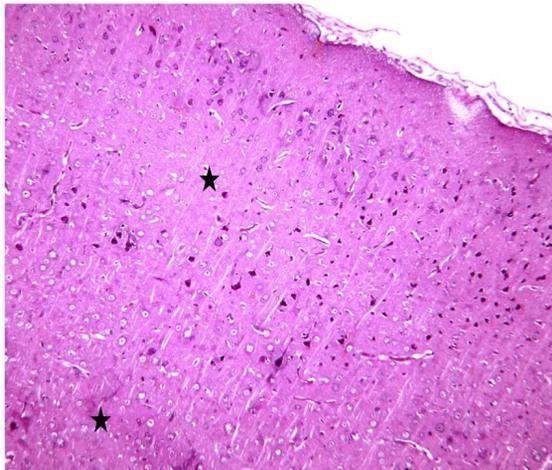


Fig. 7: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing an apparent depletion of the cellular element of most of cortical layers (asterisk). Hxand E $\times 100$

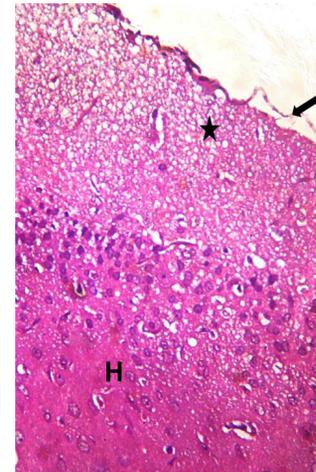


Fig. 10: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing separation of pia matter (arrow), vacuolation in the molecular layer (asterisk). Hyalinization (H) between second and third layers of the cerebral cortex is obvious. Hxand E $\times 400$

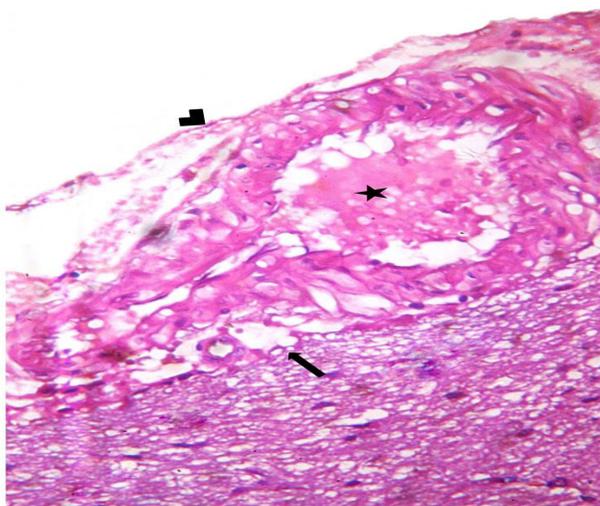


Fig. 8: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing congested blood vessel in intermediate lamella (asterisk) with discontinuity in the pia mater (arrow). Note the arachnoid mater (arrow head). Hxand E $\times 400$

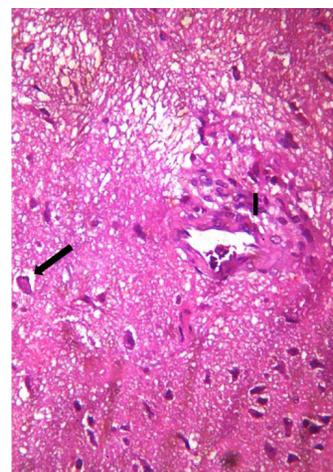


Fig. 11: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing microglia adjacent to an inflammatory mononuclear perivascular cuff (I). Note the pericellular halos (arrow). Hxand E $\times 400$

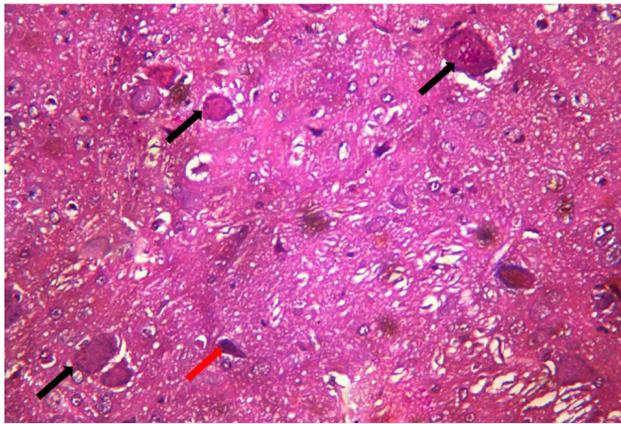


Fig. 12: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing deeply acidophilic tissue patches (black arrow). Some pyramidal cells degenerated with acidophilic cytoplasm and pale karyolitic nuclei (red arrow). Hxand E ×400

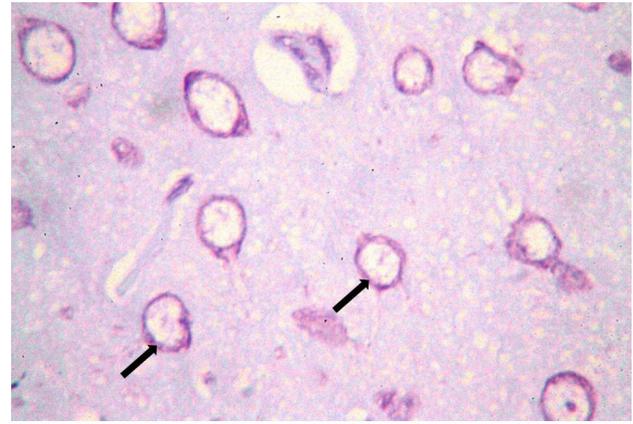


Fig. 15: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing light stained Nissel's granules in the cytoplasm of pyramidal cells (arrow). Cresyl Violet ×1000

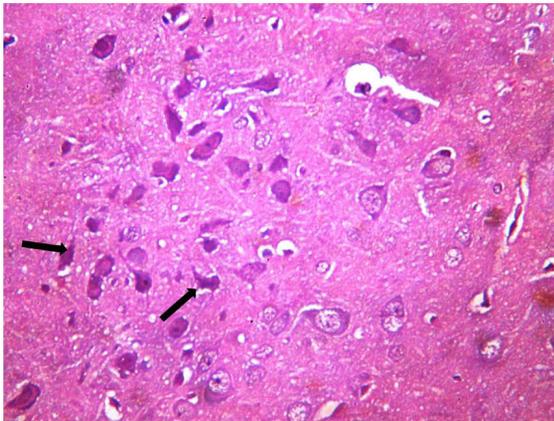


Fig. 13: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing irregular shrunken pyramidal cells (arrow). Hxand E ×400

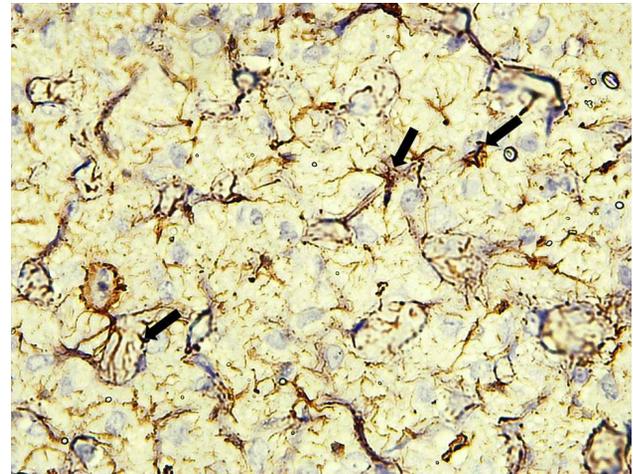


Fig. 16: A photomicrograph of an immunohistochemically stained section in the cerebral cortex of diabetic male albino rat showing abundant GFAP positive Astrocytes multiple processes, in the granular and molecular layers (arrow). GFAP ×400

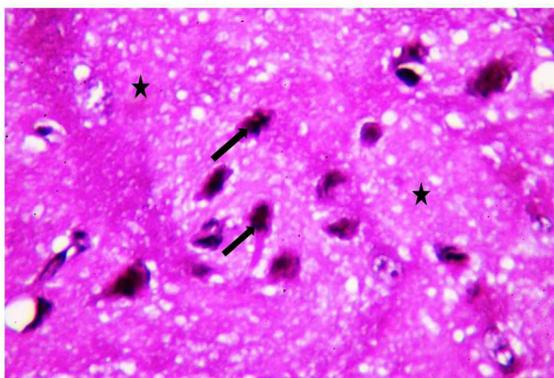


Fig. 14: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat showing bizarre shaped darkly stained nuclei of pyramidal cells (arrow). Note the surrounding neuropil are separated by multiple spaces (asterisk). Hxand E ×1000



Fig. 17: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat received melatonin showing regular pia matter (arrow). Hxand E ×100

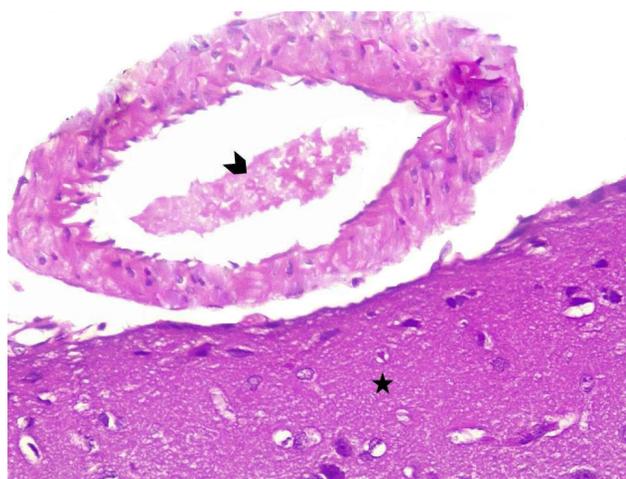


Fig. 18: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat received melatonin showing congested blood vessels in intermediate lamella (arrow head) and apparently normal molecular layer (asterisk). Hxand E ×400

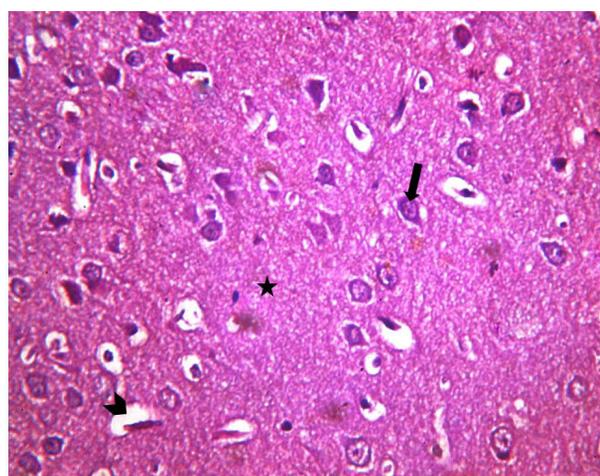


Fig. 19: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat received melatonin. Most of pyramidal cells (arrow) are normal and compact neuropil (asterisk). Note the pericellular halos (arrow head). Hxand E ×400

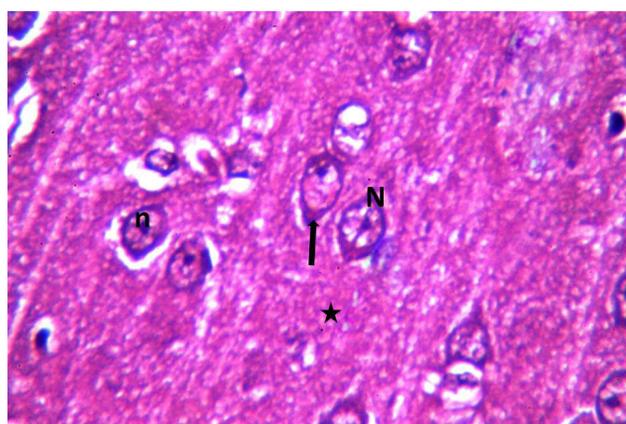


Fig. 20: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat received melatonin showing apparently normal pyramidal cells with open face nuclei (N), prominent nucleoli (n) and basophilic cytoplasm (arrow). Note the compact neuropil (asterisk). Hxand E ×1000

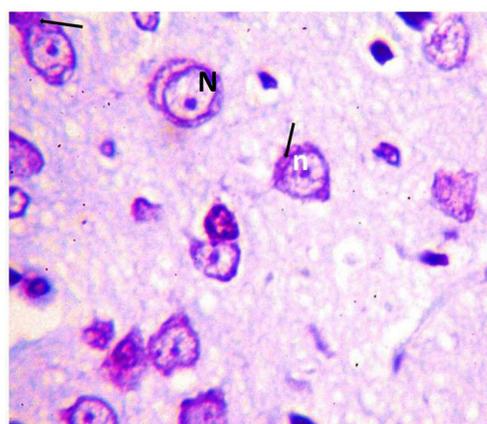


Fig. 21: A photomicrograph of a section in the cerebral cortex of male diabetic albino rat treated with melatonin showing darkly stained Nissel's granules in the cytoplasm of pyramidal cells (arrow) surrounding the large vesicular nucleus (N) with prominent nucleolus (n). Cresyl Violet ×1000

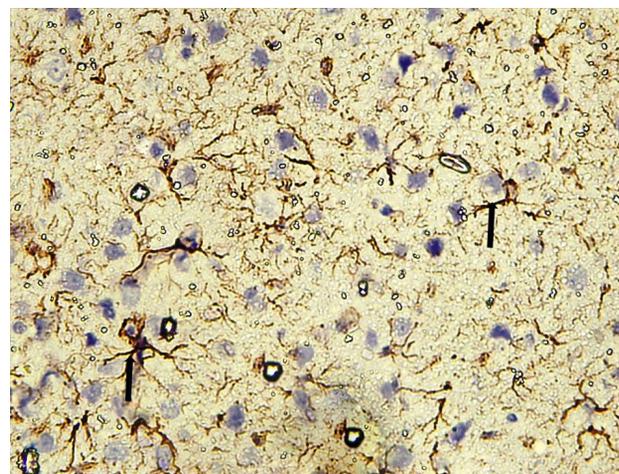
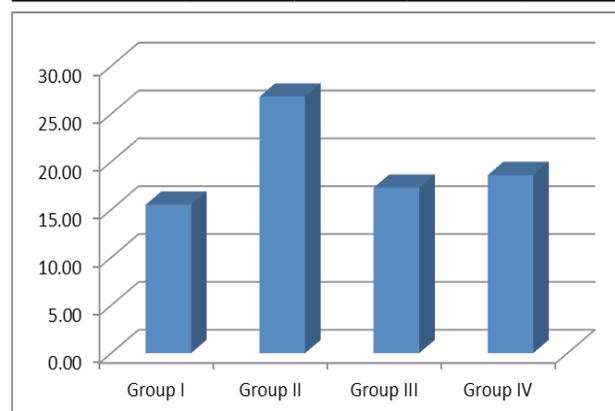


Fig. 22: A photomicrograph of an Immunohistochemically stained section in the cerebral cortex of diabetic male albino rat received melatonin showing GFAP positive astrocytes. They appear small, multiple, scattered fragmented and nearby blood vessels. GFAP ×400

Table 1: Mean and SD of astrocyte number/20 000 μm² in the groups studied

Parameter	Group I	Group II	Group III	Group IV
Astrocyte number	15.5 ± 3.2	26.8 ± 4.3	17.2 ± 4.1	18.6 ± 2.4



Histogram 1. Astrocyte number in the different groups studied.

DISCUSSION

Diabetes mellitus is a common progressive serious metabolic disorder. It causes a variety of functional and structural disorders in the central as well as the peripheral nervous systems^[12, 13]. During diabetes, the glucose utilization in the brain gets decreased because it's glucose-dependent organ, which can be damaged by hyperglycemia as well as hypoglycemia. So the brain is more vulnerable to the critical pathological events^[14].

STZ-induced diabetes animal model is widely used to elucidate the diabetes associated complications. This model provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia^[6, 15].

In the present work, there was a depletion of the cellular elements of most of the cortical layers. Baydas *et al.* (2003) stated that hyperglycemia causes autoxidation of glucose, glycation of proteins, and activation of polyol metabolism. These changes enhanced formation of reactive oxygen (ROS) and nitrogen species with depletion of antioxidant defense system, including reduced glutathione (GSH) and glutathione peroxidase (GSH-Px), a key antioxidative enzyme.

That is causing an imbalance between pro-oxidants and antioxidants. ROS can attack the polyunsaturated fatty acid in the biomembrane and induce free radical chain reactions. This contributes to increased neuronal death, damaged DNA, and augmented levels of lipid peroxidation products in cellular membranes. In addition, hyperglycemia effectively makes more substrate available for aerobic glycolysis in the brain, leading to acidosis^[3, 9].

The brain tissues are highly susceptible to oxidative damage due to its high utilization of oxygen and it's poorly developed antioxidant defense mechanism^[3].

In the current study, STZ administration induced multifocal histological changes

These were in agreement with numerous previous studies that described similar structural changes occurring in cerebral cortex of diabetic rats^[6, 13-16].

In the present work, congested blood vessels in intermediate lamella space and blood vessels in cortex with perivascular swelling and hemorrhage were observed. This result was in agreement with other investigators who reported that the disturbances of neuronal glucose transport and metabolism in both hyperglycemia and hypoglycemia can induce vascular damages^[17, 18].

Additionally, there was discontinuity and separation of the pia mater. Reviewing the literature, these findings couldn't be detected. It was suggested that the separation of pia mater observed in these rats might affect the vascularity and the nutrition of the neurons of the cortex with subsequent disturbance of their function.

In the current study, intercellular and intracellular vacuolation were seen of variable sizes as pale spots dispersed almost uniformly throughout the thickness of

cortical layers of STZ treated group.

Mohamed *et al.* (2014) previously explained that the spaces around the neurons might be attributed to the shrinkage of cells and withdrawal of their processes secondary to cytoskeletal affection leaving peri-cellular spaces. They are indicative of neuronal death and are consistent with neuronal necrosis as seen in early stages of ischemic, hypoxic/ischemic, hypoglycemic and excitotoxic states^[3, 20].

Regarding the cytoplasmic vacuolation in the nerve cells might be a result of lipid peroxidation theory, in addition to damage of the cell membrane as well as membranes of other organelles. Such damages specifically followed by an increase in the sodium permeability which exceeds the capacity of pump to extrude the sodium. Accumulation of sodium in the cell leads to an increase in water content in the cell leading to its swelling^[3, 21, 22].

However^[23], Scott *et al.* (2008) believed that the neuropil vacuoles represented the swollen neuronal processes and presynaptic nerve endings, while the cytoplasmic vacuoles corresponded with swollen mitochondria.

Moreover, deposition of acidophilic homogenous substance inbetween layers of the cortex which is known as hyalinization was seen in the current study. Similar finding were reported before by Schreiber *et al.* (2015)^[24] in the peripheral nerves and explained that an important physiological evidence of microvasculature alteration. As a result, nerve ischemia occurs, caused by raise in wall thickness and hyalinization of the basal lamina of vessels that nurse peripheral nerves, together with luminal reduction. These alterations are caused by plasma protein seape of capillary membrane to endoneurium, promoting swelling and augmented interstitial pressure in the nerves, accompanied by higher capillary pressure, deposition of fibrin and thrombus development.

In the present study, there was an area of microglia adjacent to an inflammatory mononuclear perivascular cuff in HxandE stained cortical sections of diabetic group. This is in agreement with previous work of Abcouwer (2012)^[25]. The author suggested that microglial activation might be linked to hyperglycemia by the formation of advanced glycation end products (AGE) or other protein glycation products^[26]. It is also possible that microglial activation is a response to systemic low-grade inflammation caused by diabetes. Chronic low-grade inflammation is a central theme in many diabetic complications^[27].

Additionally, areas of microinfarcts were observed. Neuropathological studies have repeatedly identified increased cerebrovascular diseases, specifically cerebral infarct, in association with diabetes^[28, 29]. Moreover, the brain has been recognized as a target organ for microvascular complications due to diabetes^[30].

Microvascular dysfunction was triggering neuronal, glial and vascular injury pathways, while pathological neurovascular remodeling and angiogenesis increases

the risk of edema and hemorrhage after ischemic stroke and reperfusion. Glial and neuronal cell damage may also play a part in blood brain barrier disruption and cognitive impairment^[31, 32].

On the other hand, the histopathological changes in diabetic cortex of the STZ-induced animals many degenerative changes in the pyramidal cells. The cells appeared small, contracted, disfigured and some neurons surrounded by halos. The nucleus is basophilic, hyperchromatic, small and pyknotic and moves to more peripheral position and the nucleolus disappear. These findings were in agreement with Mohamed *et al.* (2014)^[19] who stated that cerebral ischemia or anoxia led to eosinophilic degeneration, mostly of pyramidal cells of cerebral cortex as the whole cell shrinks, contracts, the cytoplasm loses its Nissl granules and becomes eosinophilic. This was in accordance with Malone *et al.* (2008)^[33-36], Martinez-Tellez *et al.* (2005), Hernandez-Fonseca *et al.* (2009), Huang *et al.* (2012) and Faheem and El Askary (2017).

Furthermore, cresyl violet stain showed light stained Nissl granules in the cytoplasm of pyramidal cells that showed karyolysis^[37]. Pamidi *et al.* (2014) supported this finding as they found that the untreated diabetes mellitus coupled with stress can induce highly significant damage in the neurons of rat cerebral cortex which was shown by a decrease in the number of surviving neurons of cresyl violet stained sections.

It had generally been suggested that hyperglycemia enhances neuronal damage; in addition astrocytes may also be the target^[38]. The current work showed that the number of GFAP-positive astrocytes significantly increased in STZ-treated rats. This was in accordance with Golalipour *et al.* (2011) and Selim and Selim (2013).

Neurons have been the primary focus of studies related to the effects of oxidative stress and antioxidants in the central nervous system. It was obvious that neuronal survival depends on neuronal-glial interaction^[6]. Glial cells play a vital role in the homeostatic regulation of the central nervous system; these cells are involved in neurotransmitter uptake, neuronal metabolic support, pH regulation, and protection against toxic episodes such as excitotoxicity and oxidative stress. Astrocytes preserve neuronal survival through inactivation of ROS^[7].

The alterations in astrocyte that observed in the present work are possibly because of oxidative stress and free radical formation^[6]. Also, these findings were in agreement with those of a study that deduced that mechanical and chemical insults to the brain stimulate the proliferation and hypertrophy of astrocytes with increased synthesis of glial fibrillary acidic protein (GFAP), is an intracellular intermediate filament protein^[7]. This phenomenon is called reactive gliosis, which is a universal reaction of astrocytes with specific structural and functional changes^[40].

During reactive gliosis, astrocytes secrete neurotoxic substances such as inflammatory cytokines and free

radicals, which actively attack protein molecules within neurons, resulting in neuronal damage, and contribute toward the pathogenesis of neurodegenerative diseases. These evidences indicate that altered astrocyte activity contributes toward the central nervous system pathophysiology in diabetes mellitus^[9, 39].

Melatonin (N-acetyl-5-methoxytryptamine) is one of the strongest antioxidants. It is secreted with a daily rhythm by the pineal gland^[41]. The peak concentration is around 10 pg/ mL in blood and 3 pg/ mL in the saliva^[42]. It has a variety of physiologic, immunological and biochemical functions. It is an endogenous free-radical scavenger and exerts chemoprotective, immunostimulatory and myelostimulatory effect^[43, 44].

It is thought that melatonin may be useful in the management of several diseases, such as depression, insomnia, obesity, diabetes, cancer, and immune and cardiac disorders^[45, 46]. Melatonin is a biological modulator of mood, sleep, sexual behavior and circadian rhythm at physiological concentration in human^[47].

In the present study, examination of stained cortical sections of diabetic rats received melatonin showed apparently reversed most of the histopathological changes caused by diabetes in cerebral cortex.

The covering meninges showed regular continuous pia, in spite of being still congested the vessel wall in IL was devoided of vacuolation in comparison to that in the diabetic group. The molecular layer was apparently normal as observed in the control group.

Most of pyramidal cells were more or less as that of control group. They had large vesicular nuclei, prominent nucleoli and basophilic Nissl's granule as evidenced by Cresyl Violet stained sections in a comparable way to the control group. However pericellular halos were still present. The neuropil appeared compact. Additionally the astrocytes appeared small, multiple, scattered fragmented and nearby blood vessels. There was significant decrease in Astrocytes number that was demonstrated through GFAP immunostaining.

Reiter *et al.* (2000)^[48] previously explained that Melatonin was discovered to be a direct free radical scavenger. Besides its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, it stimulates several antioxidative enzymes which increase its efficiency as an antioxidant. Baydas *et al.* (2003) reported that administration of melatonin to STZ-treated rats significantly reduced the levels of lipid peroxidation products and increased the GSH concentrations.

Borlongan *et al.* (2000)^[49] investigated the effects of melatonin on the glial cell response during a hypoxic insult. They reported that melatonin significantly enhanced survival of glial cells and markedly reduced infarct volume following middle cerebral artery occlusion^[6].

The current results proved that melatonin provide

a neuroprotective effect through suppression of glial reactivity and promotion of antioxidant defense system of glial cells. Also, it had a property as a potent scavenger of ROS. That was in agreement with Baydas *et al.* (2002)^[50], Allegra *et al.* (2003) and Baydas *et al.* (2003)^[51].

CONCLUSION

The findings of the present study introduced a new insight into the pathogenesis and treatment of neurodegenerative diseases; diabetic cerebral complications and it was cleared that melatonin treatment attenuated STZ– induced diabetic changes in cerebral cortex and meninges.

CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربي

الدور الوقائي المحتمل للميلاتونين على التغيرات في القشرة الدماغية والأغشية السحائية لمرض السكري المستحدث بالاستربتوزوتوسين في ذكور الجرذان البيضاء البالغة (دراسة هستولوجية و هستوكيميائية)

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قسم التشريخ وعلم الأجنة ، كلية الطب ، جامعة عين شمس ، القاهرة ، مصر

مقدمة: داء السكري هو اضطراب خطير و شائع في التمثيل الغذائي.و يسبب مجموعة متنوعة من الاضطرابات الوظيفية والهيكلية في الجهاز العصبي المركزي. فانه يحفز تغييرات في تمثيل الجلوكوز في المخ وزيادة الضغط التأكسدي. الميلاتونين هو عامل قوي مضاد للأكسدة ويحفز الإنزيمات الرئيسية المضادة لها.

الهدف من البحث: تقييم الدور الوقائي المحتمل للميلاتونين على التغيرات النسيجية في القشرة الدماغية والأغشية السحائية التي يسببها مرض السكري المستحدث في الجرذان البالغة.

المواد وطرق البحث: استخدم في هذه الدراسة عدد اربعون من الجرذان الذكور البيضاء البالغة وقسمت الي اربع مجموعات (عشر جرذان لكل منهما):

المجموعة الاولى: كمجموعة ضابطة, المجموعة الثانية: تلقت حقنة واحدة في الغشاء البريتوني ٦٠ ملج/كج من الاستربتوزوتوسين , المجموعة الثالثة: تلقت عقار الميلاتونين عن طريق الحقن في الغشاء البريتوني بجرعة 10 مجم لكل كجم من وزن الجسم كل يوم لمدة ستة اسابيع. المجموعة الرابعة : تلقت عقار الاستربتوزوتوسين والميلاتونين بفس الطريقة والمدة والجرعات السابقة . بعد انتهاء اخر جرعة في كل مجموعة تم التضحية بالحيوانات و اخذت العينات من قشرة المخ وتمت معاملتها للفحص بالمجهر الضوئي و أيضا لبروتين GFAP للتدليل على الخلايا النجمية وأجريت تحليلات مورفومترية وإحصائية.

النتائج: فحص القشرة الدماغية للجرذان المصابة بداء السكر أظهرت فصل للأم الحنون وكذلك احتقان في الأوعية الدموية ، ونزيف في الصفيحة المتوسطة. كانت هناك تغيرات نسيجية متعددة في المخ ونفاد للعناصر الخلوية خاصة في الخلايا الهرمية. كانت هناك مناطق متعددة من جلطات صغيرة ، وهالات شبه خارجية تحيط بمعظم الخلايا العصبية. وأظهر فحص الشرائح وجود تحلل بنواة الخلايا والدراسة هستوكيميائية المناعية أظهرت زيادة كبيرة في الخلايا النجمية. علي العكس، بفحص القشرة الدماغية للجرذان المصابة بداء السكر ومعالجة بالميلاتونين أظهرت تحسن شبه كامل في كل الطبقات.

الخلاصة: يمكن للميلاتونين تخفيف تأثير مرض السكر على القشرة الدماغية والأغشية السحائية من خلال تأثيره كمضاد للأكسدة.