ADJUVANT PROTECTIVE EFFECT OF MELATONIN AGAINST DIABETOGENIC STRUCTURAL CHANGES OF ALLOXAN ON ADULT ALBINO RATS CEREBELLAR CORTEX

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ABSTRACT:

Background: A prevalent metabolic disease with well-known, dangerous secondary consequences is diabetes mellitus. Damage to the central nervous system is also linked to it. The impairment of cerebellar functioning together with histochemical and structural abnormalities are characteristics of this injury.

Aim of the study: Was to determine how the adult albino rats' cerebellum histological structure was affected by diabetes mellitus brought on by alloxan as well as any potential preventive effects of melatonin taken orally against the rats' cerebellar structural damage.

Material and methods: Throughout the course of the investigation, four equal groups of twenty-four adult albino rats were formed at random. The control group was Group I, while the diabetic group was group II , which received one alloxan injection at a 150 mg/ kg/ b.w. dosage, Group III (the positive control group) consumed melatonin orally at a dose of 10 mg/kg/b.w. daily for four weeks, and Group IV (the diabetic group treated with melatonin) received oral melatonin at a dose of 10 mg/ kg/ b.w. daily for four weeks following the induction of diabetes by alloxan monohydrate intraperitoneal in a dose of 150 mg/ kg/b.w. Cerebella samples were obtained, prepared into paraffin blocks, and examined under a light microscope after a month.

Results: In group II, the cerebellar cortex showed signs of degeneration, particularly in the Purkinje cell layer. Strong evidence of gliosis was present, validated by an elevated level in the astrocyte count. In group IV there was an alleviation of the cerebellar morphology following melatonin treatment. Melatonin improved cerebellar thickness, reduced the quantity of astrocytes while regaining Purkinje cell number and morphology.

Conclusion: The melatonin plays a role in the protection of the cerebellum from diabetic-induced neuronal damage.

Keywords: Cerebellum, Alloxan, Melatonin, Diabetes mellitus, Purkinje cells.

INTRODUCTION:

One of the most prevalent and dangerous metabolic disorders is diabetes mellitus (DM), which is defined by hyperglycemia and alterations in the metabolism of lipids, carbohydrates, and proteins [(1)]. Twenty to thirty percent of elderly persons have diabetes as a result of age-related increases in the disease's frequency, while another ten to twenty-five percent have impaired glucose tolerance [(2)]. Long-term diabetes has been

linked to an increased risk of brain shrinkage, lacunar infarcts, and white matter abnormalities. Cognitive impairment and mobility abnormalities were among the functional and behavioral effects of diabetic brain complications [3&4]. The fundamental central nervous system hub for motor coordination has long been acknowledged to be the cerebellum [5&6]. Diabetes mellitus type I has been linked to structural cerebellar abnormalities [7]. In adult rats, hyperglycemia induced by alloxan monohydrate enhanced apoptosis in the cerebellar Purkinje cells and pyramidal neurons in the brain [8]. Additionally, un-controlled gestational hyperglycemia has neurotoxic consequences on the offspring's cerebellar Purkinje neurons [9]. In addition to its function in protecting the tissues from oxidative damage caused by a variety of free radical-generating substances and processes, melatonin, the primary secretory hormone of the pineal gland, has been shown to possess antioxidant and free radical-scavenging properties [10].

AIM OF THE STUDY:

To determine how the adult albino rats' cerebellum histological structure was affected by diabetes mellitus brought on by alloxan as well as any potential preventive effects of melatonin taken orally against the rats' cerebellar structural damage.

MATERIAL AND METHODS:

Experimental Rats:

The current investigation employed twenty-four adult albino rats. They were kept locally at Ain Shams University in the Medical Research Center's animal shelter. Three rats were housed in medium-sized cages that were 22–25ºC, with regular light and dark cycles, and were fed normal pellet chow with unlimited access to water. Four groups of six rats each were created from the animals:

Rats in Group I, also known as the control group, were given a single intraperitoneal injection of normal saline (0.9% NaCl).

Rats in Group II (diabetic group) were given a single dose of alloxan monohydrate dissolved in normal saline (150 mg/kg bodyweight) intraperitoneal injection. Blood samples from the tail were utilized to measure blood glucose using a glucometer (Roche Diagnostics, Mannheim, Germany) and commercial test strips after three days of induced hyperglycemia. Rats with a blood glucose level proven to be higher than 250 mg/dl once a week were classified as diabetic.

Group III (positive control group): rats were given melatonin only. After being dissolved in hundred percent ethanol, melatonin was diluted with drinking water until it had ethanol concentration below 0.1% to yield a dose of 10 mg/kg body weight /day for four weeks.

For four weeks following their induction of diabetes, rats in Group IV (treatment group) were given drinking water containing 10 mg/kg bodyweight of melatonin every day for 4 weeks. As in group II, rats in group four were injected with intraperitoneal injection of one dose of 150 mg/kg body weight of alloxan monohydrate dissolved in normal saline for induction of Diabetes. After three days of induction of hyperglycemia, blood glucose was assayed by blood samples from the tail using commercial test strip and a glucometer (Roche diagnostics, Mannheim, Germany). A blood sugar level of ≥ 250 mg/dl in rats used to demonstrate DM, and the diabetic level of blood glucose was ensured once a week. For obtaining melatonin dose, it was dissolved in hundred percent ethanol. Then, melatonin was diluted with drinking water until it had ethanol concentration below 0.1% to yield a dose of 10 mg/ kg body weight /day for four weeks. After 4 weeks all rats were anaesthetized with intraperitoneal injection of thiopental sodium 50 mg/kg body weight. The brain was

extracted and then the cerebellum was removed. Parts of the cerebella were fixed in 10% neutral formalin for 10 days, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and processed for paraffin blocks. Midsagittal serial sections of the cerebellum were cut at 7 µm thickness and stained with Hematoxylin and Eosin, Cresyl violet and Glial Fibrillar Acid Protein (GFAP). Using a light microscope (Olympus, M268, Germany, in Anatomy Department, Faculty of Medicine, Ain Shams University), every section was analyzed and photographed.

Processing of tissues for Glial Fibrillar Acid Protein **(**GFAP) immunohistochemical analysis: GFAP immunostaining is used to look at how astrocytes are distributed throughout a tissue and how they react to neurological degeneration or damage as seen by astrocyte distribution. The intermediate GFAP was used to show astrocytes where it colorizes their cell membrane and cytoplasm brown using the modified Avidin-Biotin immune peroxidase approach [(10,11)]. The tissue slices underwent deparaffinization and were dipped in phosphate buffered saline (PBS, pH 7.4) from Sigma Chemical Co. After that, they were placed in cold methanol (BDH Laboratory Supplies, UK) for four minutes and repeatedly rinsed in phosphate buffer saline (PBS) (BDH Laboratory Supplies, UK). Prior to administering the primary antibody, tissues were treated for 30 minutes with 3% H2O2 (dilution from 30%; Fluka Chemika, Switzerland). They were then rinsed with PBS. After being diluted 1:20 in PBS (Sigma Chemical Co.), the primary antibody, rabbit anti-GFAP, was added and incubated for an hour. In order to provide a control, the biotinylated secondary anti-rabbit antibody was added first, and it was diluted 1:20 in PBS containing 1% bovine serum albumin (BSA; Sigma Chemical Co.) and left for 30 minutes. The extra avidin-peroxidase (Sigma Chemical Co.) was then added to the slides and incubated for 30 minutes after being washed three times in PBS. Following three PBS washes, the slides were exposed for 30 seconds to diaminobenzidine tetrahydro-chloride (Bio-Rad Laboratories, Canada). Finally, the slides were washed in distilled water. The counter stain using Mayer's Hematoxylin (0.75% in 70% alcohol) was done for 5 seconds. After that, it was cleaned with running tap water, dried, and put in xylene for five minutes before being mounted with DPX (Fluka Chemika, Switzerland). It colorized the cell membrane and cytoplasm of astrocytes brown. Using a light microscope (Olympus, M268, Germany, in Anatomy Department, Faculty of Medicine, Ain Shams University), every section was analyzed and photographed.

Ethical consideration:

This research was done after receiving approval (NO. FMASU MD189/2015) from the Research Ethics Committee (REC), Faculty of Medicine, Ain Shams University. The Faculty of Medicine, Ain Shams University Research Ethics Committee (FMASU REC) is organized and operated according to guidelines of International Council on Harmonization (ICH) and the Islamic organization for Medical Sciences (IOMSs), the United States Office for Human Research Protections and the United States Code of Federal Regulations and operates under Federal Wide Assurance (NO. FWA 000017585).

RESULTS:

1. Hematoxylin and Eosin staining:

Sections in the cerebellar cortex of rats show the control cerebellar cortex (Group I) being made up of a molecular cell layer, a Purkinje cell layer, a granular cell layer and white matter (Figure 1A). Stellate and basket cell fibers make up the majority of the molecular cell layer. Big piriform cells with a big vesicular nucleus and an apparent nucleolus are organized in a single row to form the Purkinje cell layer. There are Bergman cells around Purkinje cells. The small granular cells with deeply stained

nuclei makeup the granular cell layer of cerebellar cortex. (Figure 1B). The diabetic cerebellar cortex (Group II) shows a molecular cell layer, distorted-shaped shrunken Purkinje cells, and a granular cell layer with the appearance of congested blood vessels (Figure 2B). Degenerated Purkinje cells with pyknotic eccentric nuclei and a significant number of Bergman cells are visible in the Purkinje cell layer. Degenerated granular cell layer were noted (Figure 2B). Group III, the positive control cerebellar cortex, exhibits the Purkinje, granular, and molecular cell layers near those of the control group. Appearance of vacculations in the granular cell layer. In comparison to the control group, the Purkinje cells seem to be somewhat fewer in number (Figure 1C). The Purkinje cells regain their piriform shape with an apical cytoplasmic cone with more or less normal shape. Note degenerated Purkinje cells appeared with a pyknotic dark nucleus. When compared to the control group, the Purkinje cells seem to be somewhat fewer in number (Figure 2C). The diabetic rats treated with melatonin in the cerebellar cortex (Group IV) show, the molecular cell layer, the granular cell layer, the Purkinje cell layer and white matter have an appearance similar to that of the control group. The Purkinje cells appear slightly reduced in number compared with the control group (Figure 1D). Purkinje cells regain their piriform shape with an apical cytoplasmic cone and a large vesicular nucleus with more or less normal shape. Other Purkinje cells are shrunken and degenerated. Note the high number of Bergman cells and halos around Purkinje cells (Figure 2D).

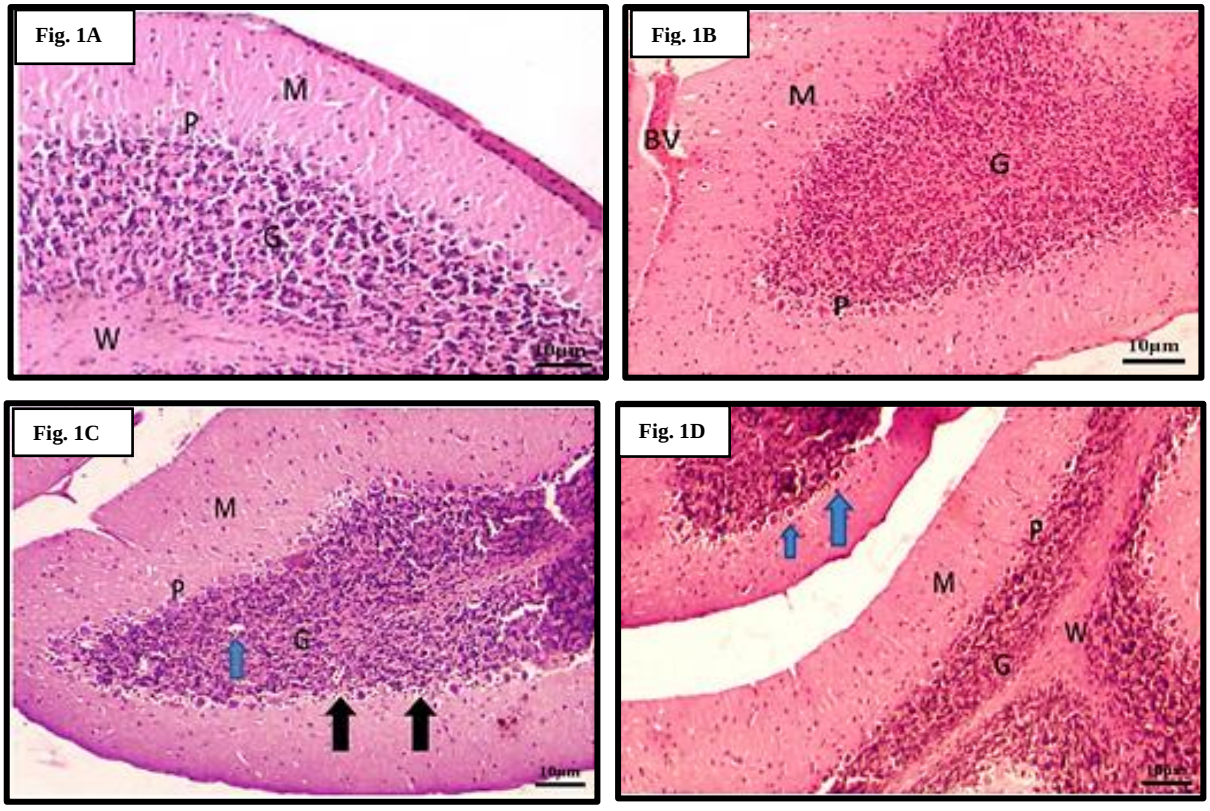

Figure 1: Photomicrographs of sections in the cerebellar cortex of rats (H&Ex100): **(Fig. 1A):** The control cerebellar cortex (group I) is composed of three cell layers: granular (G), Purkinje (P), and molecular (M). Note the core of white matter (W). (**Fig. 1B):** The diabetic cerebellar cortex (group II) shows a molecular cell layer (M), a Purkinje cell layer with distorted-shaped shrunken cells (P), and a granular cell layer (G). Note the congested blood vessel (BV). **(Fig. 1C):** The positive control cerebellar cortex (group III) shows a molecular cell layer (M), a granular cell layer (G), and a Purkinje cell layer (P) similar to the control group. Note the vacculations in the granular cell layer (blue arrow). In comparison to the control group, the Purkinje cells seem to be somewhat fewer in number. Note areas of loss of Purkinje cells (black arrow). (**Fig.** 1**D):** The diabetic rat treated with melatonin (group IV) the cerebellar cortex shows a molecular cell layer (M), a granular cell layer (G), a Purkinje cell layer (P) and white matter (W) as the group of control, The cells of Purkinje seem to be somewhat fewer in number in comparison of group of control (blue arrow).

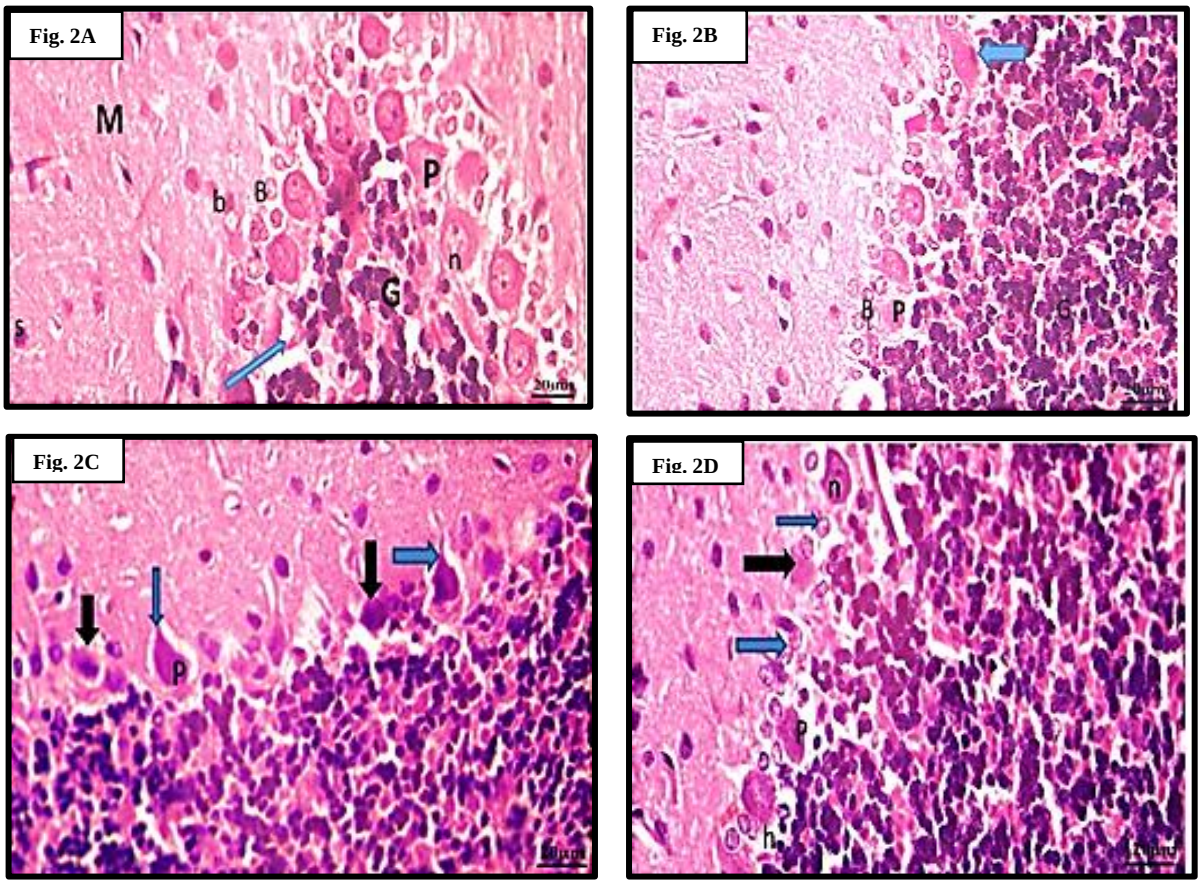

Fig. 2: Photomicrographs of sections in the cerebellar cortex of rats (H&Ex400) show:
(Fig. 2A): The control cerebellar cortex (group I) shows (M) a molecular layer, (P) a Purkinje layer, and (G) a granular cell layer. Fibers with few cells, basket cells (b) and stellate cells (s) makeup the molecular layer. The layer of Purkinje cell (P) consists of big piriform cells that ordering in one row. Note the large vesicular nucleus (n) with prominent nucleolus. There are Bergman cells (B) around the Purkinje cells. Small granule cells with darkly stained nuclei makeup the granular cell layer (arrow). **(Fig. 2B):** The diabetic cerebellar cortex (group II), the layer of Purkinje cells shows some degenerated cells (P) with eccentric pyknotic nuclei (n). Note the large number of Bergman cells (B). Note degenerated granular cell layer (G). **(Fig. 2C):** The positive control cerebellar cortex (group III) shows the Purkinje cells piriform shaped cells (P) with apical cytoplasmic cone (blue arrow) with more or less normal shape. Note degenerated Purkinje cells with pyknotic dark nucleus (black arrow). The cells of Purkinje seem to be somewhat fewer in number in comparison of group of control. **(Fig. 2D):** The diabetic rat treated with melatonin (group IV) cerebellar cortex shows the Purkinje cells regain their piriform shape (P) with apical cytoplasmic cone and large vesicular nucleus (n) with more or less normal shape. Other Purkinje cells are shrunken and degenerated (black arrow). Note the high number of Bergman cells (blue arrows). Note the spaces (halos) around Purkinje cells (h).

2. Cresyl violet staining:

Cerebellar cortex sections of control rat group (group I) demonstrate folia of cerebellum with deeper fissures, and each folium is formed of a well-developed cortex covering a core of white matter (Figure 3A). The cerebellar cortex of control displays molecular cell layer formed of a few small stellate and basket cells. The big piriform cells that make up the Purkinje cell layer. The small, spherical cells with densely pigmented nuclei that are closely packed together make up the granular cell layer. Note that the Nissl substance appears more deeply purple than the nucleus (Figure 4A). The diabetic cerebellar cortex (group II) shows cerebellar folia with a fissure in between. There are vacuolations between cells of all layers of the cerebellum (Figure 3B). There are some degenerative cells with pyknotic nuclei visible in the layer of Purkinje cells. Purkinje cell loss was present in several places. Note the increase in Bergman cells (Figure 4B). Similar to the group of control, the molecular

cell layer, Purkinje cell layer, and granular cell layer are visible in the positive control cerebellar cortex (group III). The cells of Purkinje (in group III) seem to be somewhat fewer in number in comparison of group of control. Note areas of loss of Purkinje cells (arrow) and vacuolations in the granular cell layer (Figure 3C). Purkinje cells appear in a piriform shape. They are surrounded by Bergman cells (Figure 4C). The diabetic rat treated with melatonin (group IV) in the cerebellar cortex shows that the Purkinje cell layer has an appearance similar to that of the control group. The Purkinje cells appear slightly reduced in number compared with the control group. Note areas of loss of Purkinje cells (Figure 3D). Purkinje cells acquire an apical cone and resume their piriform shape. Others are pyknotic, irregular, and shrunken (Figure 4D).

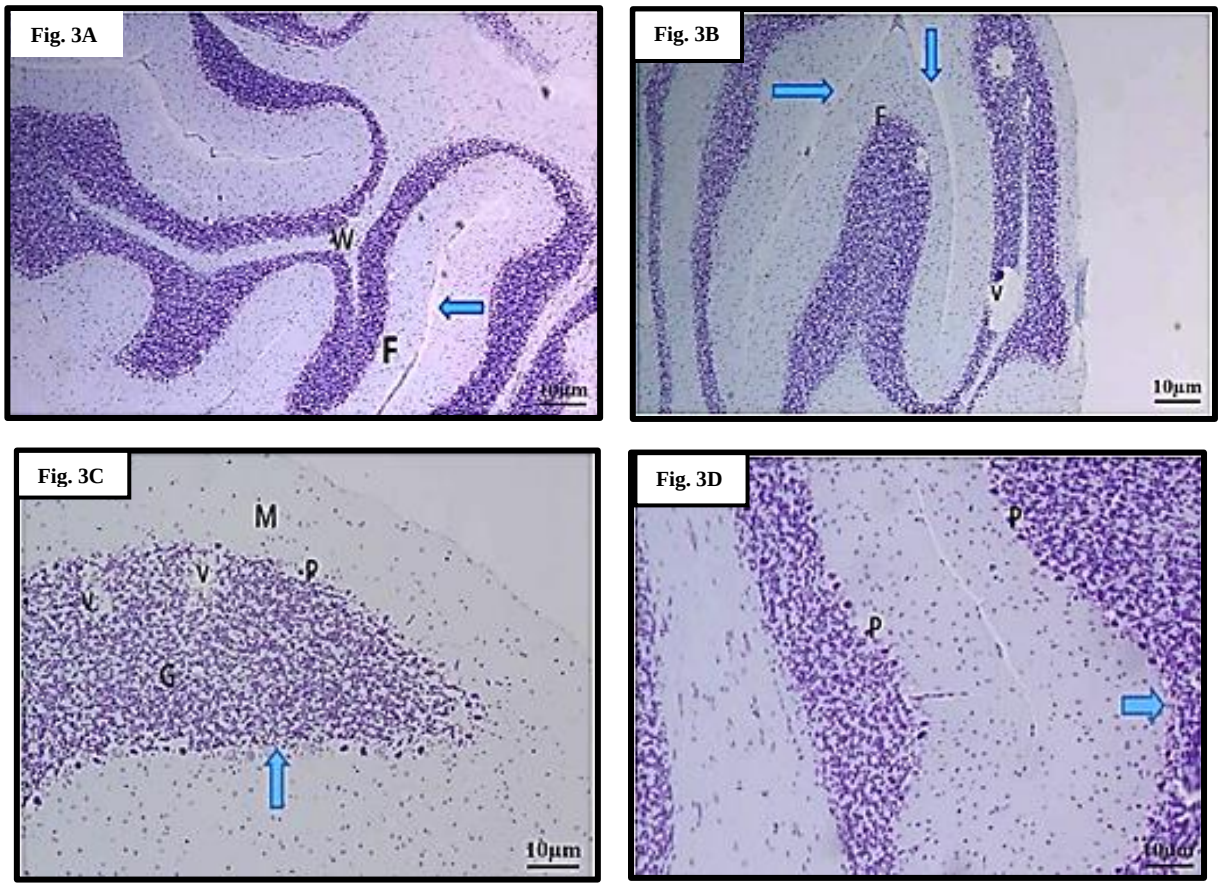

Fig. 3: Photomicrographs of sections in the cerebellar cortex of rats (Cresyl violet x100) show **(Fig. 3A):** The control cerebellar cortex (group I) with appearance of folia of cerebellum (F) with deeper fissures (arrow) and each folium is formed of a well-developed cortex enveloping a white matter core (W). **(Fig. 3B):** The cerebellar cortex of group of diabetes (group II) shows cerebellar folia (F) with fissure (arrows) in between. There are vacuolations between cells of all layers of cerebellum. **(Fig. 3C):** Group III, the positive control cerebellar cortex, displays the molecular cell layer (M), the granular cell layer (G), and the Purkinje layer cells (P), which resemble the control group's cells. The cells of Purkinje seem to be somewhat fewer in number in comparison of group of control. Note areas of loss of Purkinje cells (arrow) and vacuolations in the granular cell layer (v). **(Fig. 3D):** The diabetic rat treated with melatonin cerebellar cortex (group IV) shows the cells of Purkinje layer (P) looks like to group of control. The cells of Purkinje seem to be somewhat fewer in number in comparison of group of control. Note areas of loss of Purkinje cells (arrow).

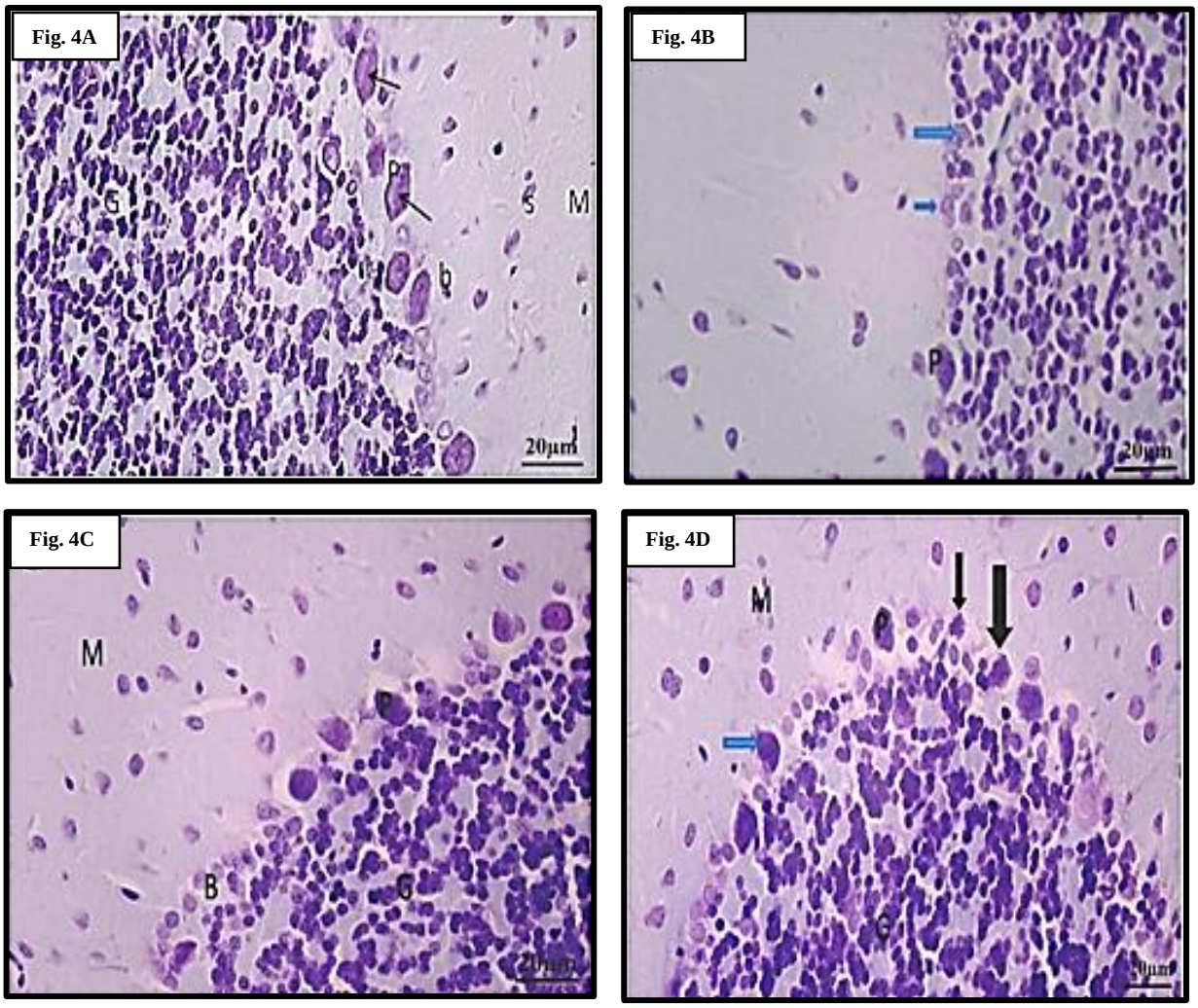

Fig. 4: Photomicrographs of sections in the cerebellar cortex of rats (Cresyl violet x400) **(Fig. 4A):** The control cerebellar cortex (group I) displays a molecular cell layer (M) formed of few small stellate (S) and basket cells (b). Large piriform cells make up the Purkinje cell layer (P) (arrow), while small, rounded cells with dark nuclei are closely packed in the granular cell layer (G). Note that Nissl substance appears more deep purple than the nucleus (arrows). **(Fig. 4B):** The diabetic cerebellar cortex (group II) shows the layer of Purkinje with some degenerative cells (**P**) with nuclei appear pyknotic. There were sites of Purkinje cell loss. Note the increased number of Bergman cells (arrows). Note wide areas of separations between Purkinje cells. **(Fig. 4C):** Group III, the positive control cerebellar cortex, displays the molecular cell layer (M), the granular cell layer (G), and the Purkinje layer cells (P), which resemble the control group's cells. The Purkinje cells appears piriform in shape (P). The Bergman neuroglia cells (B) encircle them. **(Fig. 4D):** The diabetic rat treated with melatonin (group IV) shows (M) a molecular layer, (P) a Purkinje layer, and (G) a granular layer. Some Purkinje cells restore their piriform shape with apical cone (blue arrow). Others are irregular, pyknotic, and shrunken (black arrows). Note the reduced number of Purkinje cells.

3. GFAP immunohistochemistry:

Sections in the control cerebellar cortex (group I) show GFAP mild positive reaction for astrocytes (Figure 5, 6A). The control cerebellar cortex shows brownish, star-shaped cells in the granular cell layer. Elongated cells and their processes appear in the molecular cell layer with a faint brownish immune reaction (Figure 6A). The diabetic cerebellar cortex (group II) shows a strong positive reaction to GFAP immunostaining (Figure 5, 6B). The astrocytes can be detected as dark brown star-shaped cells with their dendrites in the granular cell layer (Figure 6B). The positive control cerebellar cortex (group III) displays a mildly positive GFAP response for the dendrites and cell axons of

astrocytes in every layer (figure 5, 6C). They appear as deep brown star-shaped cells in the granular cell layer, with their dendrites in the molecular cell layer (Figure 6C). The diabetic cerebellar cortex treated with melatonin (group IV) shows GFAP mild positive reaction for astrocyte dendrites and cell axons in every layer. In (Figure 5D). The white matter and granular layer show vacuolations. There are fewer astrocytes with thin processes compared with diabetic group (Figure 6D).

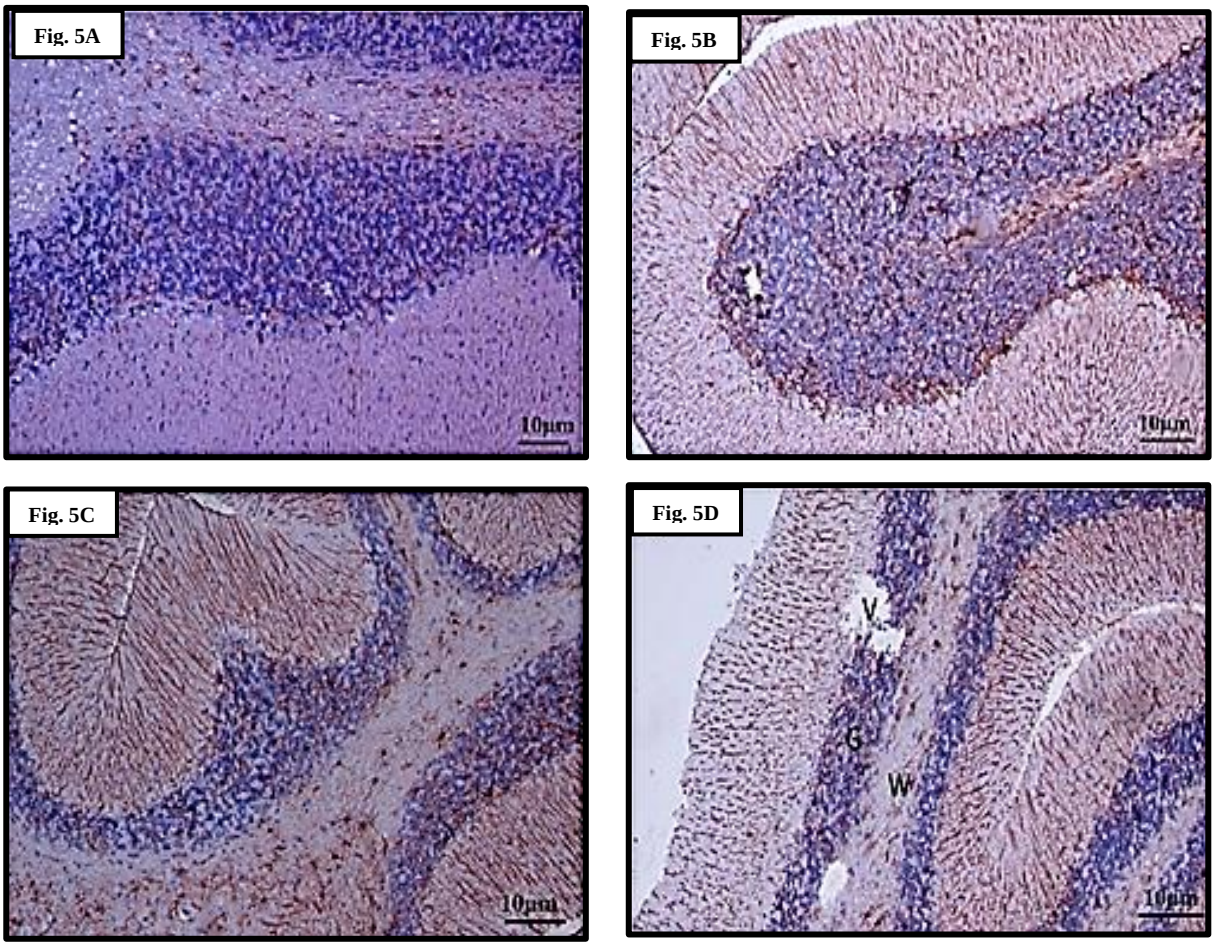

Fig. 5: Photomicrographs of cortical sections of cerebellum (GFAP x100) **(Fig. 5A):** The cerebellar cortex of group of control (I) shows GFAP with mild positive reaction for astrocytes. **(Fig. 5B):** The cerebellar cortex of diabetic group (group II) shows GFAP with strong positive reaction. **(Fig. 5C):** The positive control cerebellar cortex (group III) shows GFAP with mild positive reaction for astrocytes axons and dendrites in every layer. **(Fig. 5D):** The diabetic cerebellar cortex treated with melatonin (group IV) shows GFAP with mild positive reaction for astrocytes axons and dendrites in every layer. Note the white matter (W) and the granular layer (G) with vacuolations (V).

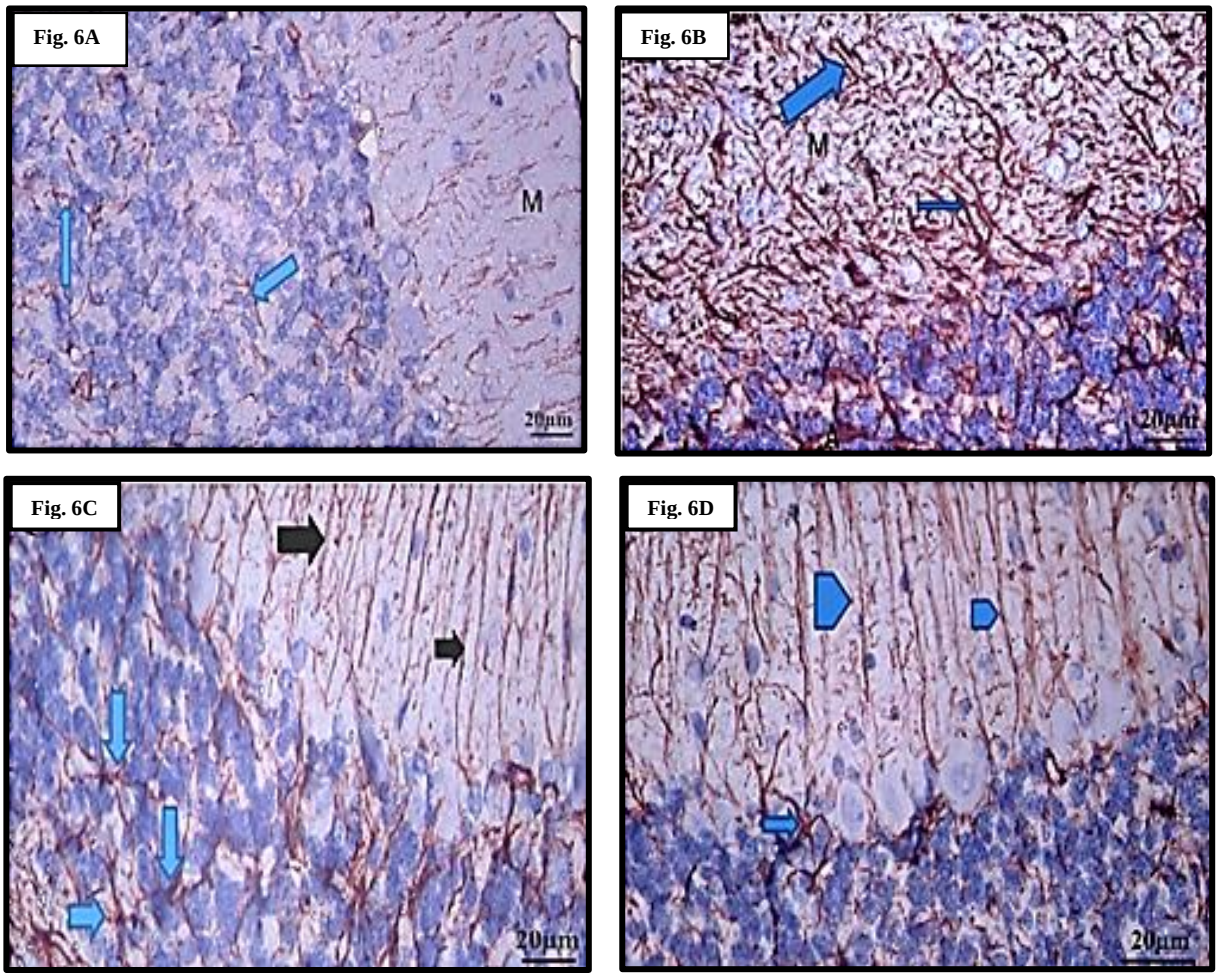

Fig. 6: Photomicrographs of cortical sections of cerebellum (GFAP x400) **A:** The cerebellar cortex of group of control (I) shows brownish star shaped cells (arrows) in the granular cell layer. Elongated cells and their processes appear in the molecular cell layer (M) with faint brownish immune reaction. **B:** The diabetic cerebellar cortex (group II) shows a strong positive reaction to GFAP immunostaining. Astrocytes can be detected as dark brown star-shaped cells with their dendrites (arrow) in the granular cell layer (**M**). **C:** The positive control cerebellar cortex (group III) shows mild positive reaction to GFAP immunostaining. In the granular cell layer, astrocytes (blue arrows) are identifiable as deep brown, star-like cells, while their dendrites (black arrows) are seen in the molecular cell layer. **D:** The diabetic cerebellar cortex treated with melatonin (group IV) shows smaller numbers of astrocytes (arrow) with thin processes (arrow heads) compared with diabetic group.

DISCUSSION:

The present work was done to evaluate the effects of DM induced by alloxan on the cerebellar tissue on adult albino rats and to assess the role of oral intake of melatonin in preventing these effects. That was achieved by using histological and Immunohisto-chemical stains to the different experimental groups.

In the present investigation, the cerebellum of diabetic rat's underwent significant degenerative alterations. The cells of Purkinje showed eccentric, pyknotic nuclei and seemed atypical. This was consistent with a prior study which demonstrated the functional and morphological changes in the cerebellum of uncontrolled diabetes in animals. The main hallmark was an increase in neuronal death [11&12]. They discovered that uncontrolled hyperglycemia was linked to

neuronal degeneration, neuroglial dysfunction-ion, and myelin sheath disarray in the cerebellar cortex. They proposed that oxidative stress associated with diabetes also occurs with a decrease in antioxidant status, which may intensify the deleterious effects of free radicals [11].

In the present study, there was a decreased number of Purkinje neurons, and a halo space appeared around the Purkinje cells with gliosis appeared between cells of all cerebellar layers with a decrease in cerebellar thickness. Rats with streptozotocin-induced diabetes showed less cognitive impairment than normoglycemic controls, according to a previous study[13]. While the exact cause of diabetes-related cognitive impairment is unknown, factors like oxidative stress, vascular problems, and metabolic impairment are thought to be potential contributors [14].

Tissues become more vulnerable to problems due to stress of oxidation stress brought on by hyperglycemia. Reactive oxygen species and lipid peroxidation are produced more frequently under oxidative stress. Glycation of proteins, autoxidation of glucose, and activation of polyol metabolism are all effects of hyperglycemia. These modifications quicken the production of ROS, increasing the oxidative modifications of proteins, DNA, and lipids [15]. Additionally, hyperglycemia effectively increases the amount of reduced substrate that is needed for neurological glycolysis, which elevates the blood acidity and increases the production of free radicals by lowering the amounts of antioxidant endogenous compounds[16]. Furthermore, a further analysis revealed that hyperglycemia is one of the main causes of central nervous system problems. The central nervous system is primarily dependent on glucose that can suffer harm from both hyper- and hypoglycemia [17&18].

According to the current study, GFAP-positive astrocytes were found in much higher numbers in Alloxan-induced diabetic rats than in control animals. These findings are consistent with earlier research by *Jahanshahi et al*.[19] and *Golalipour et al*.[20], which discovered higher astrocyte numbers in the dentate gyrus of diabetic rats. Other researchers discovered more GFAP in the brain tissue of diabetic rats in various locations, primarily in the cerebellum and limbic system[12,16]. The astrocyte number changes are possibly due to stress of oxidation and production of free radicals. These are consistent with another research demonstrated that Damage to the brain that is both mechanical and chemical causes astrocytes to proliferate and grow larger while producing more intermediate glial filaments. Reactive gliosis is the term for this process, which is a common response of astrocytes with certain functional and structural alterations [16]. Reactive gliosis causes astrocytes to release neurotoxic chemicals including free radicals and inflammatory cytokines, which actively target protein molecules inside neurons to cause neuronal damage and aid in the development of neurodegenerative disorders [21]. These data suggest a role for altered astrocyte activity in the CNS pathophysiology-logy of diabetes mellitus [20]. *El-Akabawy* and *ElKholy*[21], who attributed it to changes in blood glucose levels and oxidative stress, discovered the same phenomenon. Purkinje cell degeneration coexisted with reactive astrocytic growth in a mouse model of spinocerebellar ataxia [22].

GFAP expression was found to be lower in the cerebellar and cerebral corti of untreated diabetic rats in a different study [23]. There is little doubt that this disagreement calls for more clinical and experimental research. These earlier results suggested that GFAP, a hallmark of reactive astrocytosis, is produced and degraded differently in diabetics. GFAP expression be a valuable marker for demonstrating the neurological degenerative changes in DM. In this research, concurrent melatonin administration lessened

the harmful effects of diabetes mellitus on the cerebellum. The granular, Purkinje, and molecular cell layers appeared almost normal. The Purkinje cells did, however, show darker nuclei and cytoplasm and were somewhat smaller and fewer in number when compared to the control group. This is consistent with a prior study that looked at the melatonin's protective function on metabolic parameters and the oxidant effect of aluminum on rats. It was discovered that melatonin treatment lessened the detrimental effects of aluminum on the rat cerebellum. This was highlighted by the molecular, Purkinje, and granular cell layers appearing almost normal following concurrent administration of melatonin and aluminum [(24)]. A further investigation delineated the oxidative impact of aluminum on rats and the safeguarding function of melatonin on biochemical markers. They linked melatonin's protective function to halting lipid peroxidation [(25)]. Melatonin interacts with opioidergic and GABAergic system receptors to produce a long-lasting and potent antinociceptive impact on neuropathic pain [(28)]. In another study, in comparison to diabetic rats, the melatonin-treated rats showed an improved histological appearance. The endothelium of endoneurial arteries was flat, and the basement membrane was continuous. There were some nerve fibers with a regular outline [(27)]. The observed alleviation of the cerebellar histological alterations may be attributed to melatonin's neuroprotective function. In oxidative liver damage, melatonin was reported to lower lipid peroxidation products and polymorphonuclear leucocytes [(28)]. Furthermore, melatonin demonstrated the ability to shield organelle and cell membranes from damage caused by free radicals [(29)]. In contrast to diabetic rats, alloxan-induced diabetic rats had less GFAP-positive astrocytes after melatonin administration, according to the current study. Free radical production may play a role in the reactive gliosis that develops in diabetes, at least in part [(12,16)]. Reactive gliosis may be avoided by antioxidants by reducing the damaging effects of ROS on the central nervous system. In the current study, melatonin was employed to reduce GFAP expression in the diabetic rat cerebellar cortex based on this theory. GFAP levels in the various brain areas of diabetic rats were shown to be altered by vitamin E as an antioxidant in a prior study because it affects diabetic parameters and has a direct neuroprotective impact by lowering neuroglial damage in the central nervous system. As a result, it may affect and improve CNS astrocyte activities in long-term diabetes [(30)]. Melatonin has been demonstrated in earlier research to have a number of pharmacological actions, such as lowering oxidative stress levels, controlling circadian rhythms, delaying apoptosis, and lowering inflammation[(31)]. Numerous studies reported that melatonin has neuro-protective properties in experimental stroke models and improved the neurological function [(32)]. Recent cases have demonstrated the efficacy of melatonin in reducing ischemia-reperfusion injury, particularly cerebral and cerebellar injury[(33)]. Melatonin may reduce brain damage by reducing cellular oxidative stress, cellular calcium excess, endoplasmic stress, and mitochondrial demise[(34)]. All these research are consistent with our results

Conclusion:

Based on the findings of this study, it can be said that diabetes caused by alloxan caused a range of structural alterations in the rat cerebellum, including an increase in the expression of GFAP, a protein thought to be a sign of reactive Astro cytosis. Melatonin treatment is shown to correct these structural and immunohistochemical alterations. Melatonin can therefore be utilized as an adjuvant medication to prevent and treat diabetic CNS problems.

Availability of data and material:

On reasonable request, the supporting data of this study's findings

can be provided by the corresponding author.

Competing interests:

The authors declare the absence of any conflict of interest.

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التأثير الوقائي المساعد للميلاتونين ضد التغيرات التركيبية السكرية للألوكسان على القشرة المخيخية في الجرذان البيضاء البالغة

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المقدمة: داء السكري هو أضطراب أيضي شائع وله مضاعفات ثانوية خطيرة معروفة. ويرتبط أيضًا بتلف الجهاز العصبي المركزي. يتميز هذا الضرر بضعف وظائف المخيخ مع تشوهات نسجية وكيميائية هيكلية.

الهدف من البحث: سعت هذه الدراسة إلى التحقق من آثار داء السكري الناجم عن الألوكسان على البنية النسيجية للمخيخ لدى الجرذان البيضاء البالغة، بالإضافة إلى أي آثار وقائية محتملة للميلاتونين الذي يتم تناوله عن طريق الفم ضد الضرر الهيكلي للمخيخ لدى الجرذان. المواد والطرق: طوال فترة البحث، تم تقسيم أربعة وعشرين فأراً بالغاً إلى أربع مجموعات متساوية عشوائياً. كانت المجموعة الأولى بمثابة المجموعة الضابطة، في حين تلقت المجموعة الثانية (المجموعة المصابة بالسكري) حقنة واحدة من الألوكسان بجرعة 150 ملغم/كغم/من وزن الجسم، واستهلكت المجموعة الثالثة (المجموعة الضابطة الإيجابية) الميلاتونين عن طريق الفم بجرعة 10 ملغم. /كجم/ من وزن الجسم. يومياً لمدة أربعة أسابيع، تلقت المجموعة الرابعة (مجموعة مرضى السكري المعالجة بالميلاتونين) الميلاتونين عن طريق الفم بجرعة 10 ملغم / كغم / من وزن الجسم. يومياً لمدة أربعة أسابيع بعد تحفيز مرض السكري بواسطة الألوكسان مونوهيدرات داخل الصفاق بجرعة 150 ملغم / كغم من وزن الجسم. في نهاية التجربة تم الحصول على عينات من المخيخ من كل مجموعة وتحضيرها وفحصها تحت المجهر الضوئي.

النتائج: في المجموعة الثانية (المصابة بالسكري) لوحظت تغيرات تنكسية في قشرة المخيخ وخاصة في طبقة خلايا بوركينجي. كان هناك دليل جيد على وجود تليف والذي كان مدعومًا بزيادة كبيرة في عدد الخلايا النجمية. في المجموعة الرابعة (مجموعة مرضى السكري المعالجة بالميلاتونين) كان هناك تحسن في بنية المخيخ بعد تناول الميلاتونين. أدى الميلاتونين إلى تحسين المخيخ، وتقليل كمية الخلايا النجمية مع استعادة عدد خلايا بوركينجي وشكلها.

الخلاصة: يلعب الميلاتونين دورًا في حماية المخيخ من تلف الخلايا العصبية الناجم عن مرض السكري.