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Prevention of Diabetic Retinopathy by Melatonin in Rats with STZ Induced Diabetes Mellitus

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Abstract: ***Objectives: This study evaluates effect of melatonin as antioxidant on diabetic retinopathy. The pathophysiology of diabetic retinopathy involves many factors. The pathogenesis of diabetic retinopathy involves oxidative stress and hyperglycemia.Melatonin stimulates endogenous antioxidant enzymes. Methods: Four study groups were included in the study, normal control, diabetic control, melatonin control group, diabetic group treated with melatonin10 mg/kg IP. After sacrifice ,VEGF,MDA, were measured in retina as well as retinal hisopathology. Fasting blood sugar was measured in the study group. Results: diabetic group treated with melatonin showed significant improvement of all parameters measured compared to diabetic group (p value< 0.05).At the end of the study, we concluded that melatonin improved all parameters measured and resulted in improvement of diabetic retinopathy.***

Keywords: Retinopathy, melatonin, VEGF, MDA, Nitrotyrosine

1. Introduction

The etiology of diabetic retinopathy involves many factors. The pathogenesis of diabetic retinopathyinvolves oxidative stress and hyperglycemia[1-2]Oxidative stress, which occur in diabetic retina as a result of hyperglycemia, is caused mainly by thickening of retinal basement membrane, which is the main manifestation of microangiopathy which is seen in diabetic retinal disease. In addition, the retina isconsidered the neurotissue of the eye and is significantly rich with lipid membranes[4]. This character makes it very sensitive to reactive oxygen species and lipid peroxidation. Diabetes mellitus increases oxidative stress and induces vascular leakage and increased retinal vascular permeability, perhaps causing macular edema which is accompanied with vision loss in patients with diabetic retinopathy[3-5].

Diabetes causes upregulation of factors which modulate angiogenesis. Vascular endothelial growth factor (VEGF).The diabetic microvascular complications is mainlyaccompanied by increased VEGF. Vascular dilatation, microaneurysm, and other vascular manifestation which is seen in diabetic retinopathy caused by VEGF. Researches revealed that the disease can be limited by targeting these factor[5] .Antioxidants have been found to have possible protective in diabetic retinopathy. A diurnal hormone, melatonin, is an oxyradical scavenger and stimulates endogenous antioxidant enzymes. Many researcher reported anantioxidative effect of melatonin in several conditions [6-7] . There is a favorable effect on blood glucose levels which was reported with melatonin use[8]. On this background, this study was conducted to evaluate possible protective effect of melatonin on retina in rats with induced diabetes mellitus.

2. Methods

Animals

24 male Wister-albino rats, weighing 160-200 g. The animals were kept in propylene cages under standard laboratory conditions.The animals were kept in standard room conditions and fed with standard rat diet and water ad libitum. All animals received human care according to the criteria outlined in the "Guide the Care and Use of Laboratory Animals". [9] Experimental design and sample collection

Study Groups

24 rats divided into 4 groups consists of 6 rats/group:
Group 1: Normal control group: received IP injection of 2.5 % ethanol
Group2: Diabetic control group
Group3: Normal rats received melatonin IP melatonin 10 mg/kg (dissolved in 2.5% ethanol) in 1 ml daily
Group4: Diabetic rats received melatoninIP melatonin 10 mg/kg (dissolved in 2.5% ethanol) in 1 ml daily

In diabetic group, diabetic rats were followed without any drugs. In melatonin treated diabetes group, diabetic rats were given IP melatonin 10 mg/kg (dissolved in 2.5% ethanol) in 1 ml daily for 4 weeks [9] . At the end of the month, all rats were sacrificed

Induction of diabetes:

Diabetes mellitus was induced by single intraperitoneal (IP) injection of freshly prepared STZ at dose of 60 mg/kg b.w. dissolved in 0.01 M citrate buffer, pH 4.5. After 24 h of STZ injection, blood sample was taken from tail artery of the rats. Animals with FBG level of higher than 250 mg/dl were selected for the diabetic groups(9). Chemicals were purchased from Sigma chemical Co.

Histopathology

After the eye extirpation, tissue (retina) of each rat was examined grossly. The tissue was removed for histologic study, washed with normal saline, and immersion-fixed in 10% buffered formalin immediately upon removal. They were gradually dehydrated, embedded in paraffin, cut into 5-µm sections, and stained with hematoxylin and eosin for histologic examination according to standard procedures

Retinal biochemical analysis

The retinas from the left eyes were dissected right away after killing and kept refrigerated at −70 °C until biochemical analyses were done. For the assays, retina samples were placed in a cold phosphate-buffered saline (diluted ratio

1 : 5; pH 7.2) and homogenized with a mechanic homogenizator as described by Masuzawa et al.10 Aliquots of homogenates were then centrifuged at 10 000 g for 30 min and the supernatants were analyzed for the investigations. Enzyme-linked immunosorbent assay (ELISA) was performed for determinations of retinal VEGF Results are expressed as picogram per milligrams of protein (pg/mg-prot)[10].

Quantification of oxidative damage

Retinas were mechanically lysed in lysis buffer (RayBiotech) containing a protease inhibitor cocktail and the antioxidant butylhydroxytoluene (BHT). Lipid peroxidation was quantified sprectophotometrically at 540 nm (Thermospectronic). MDA level was normalized per mg of protein present in each sample[10].

3. Laboratory Analyses

Blood samples were centrifuged at 3000 × g for 10 min and sera were separated. Serum glucose concentrations were measured by using ACCU-Chek Active at the beginning of the study , after 24 hour from STZ injection and before sacrifice (Roche Diagnostics, Basel, Switzerland).

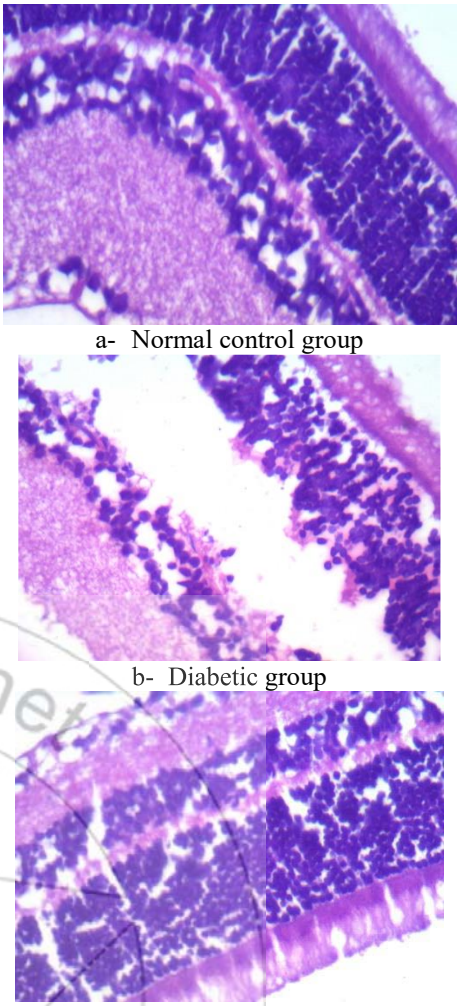
4. Statistical Analysis

Data were analysed by SPSS database (version 16, SPSS Inc., Chicago IL, USA). one-way analysis of variance (one-way ANOVA) was used. The post hoc multiple comparisons among the groups were done with Tukey's significant differences test after the group variances were shown to be equal by Levene's test. The data were expressed as mean±SD (95% confidence interval). A P-value of <0.05 was considered statistically significant.

There is no conflict of interest or funding agency for this work.

5. Results

1-histopathological results:



a- Normal control group

b- Diabetic group

c- Diabetic treated with melatonin

Figure 1: Histopathology of retina in the study groups

Retina was highly organized in the normal control group and melatonin control group with intact layers. Retina was disorganized in the diabetic control group with impaired atrophied layers. In diabetic group treated with melatonin there was improvement in the arrangement of layers (fig1).

2- Retinal VEGF level:

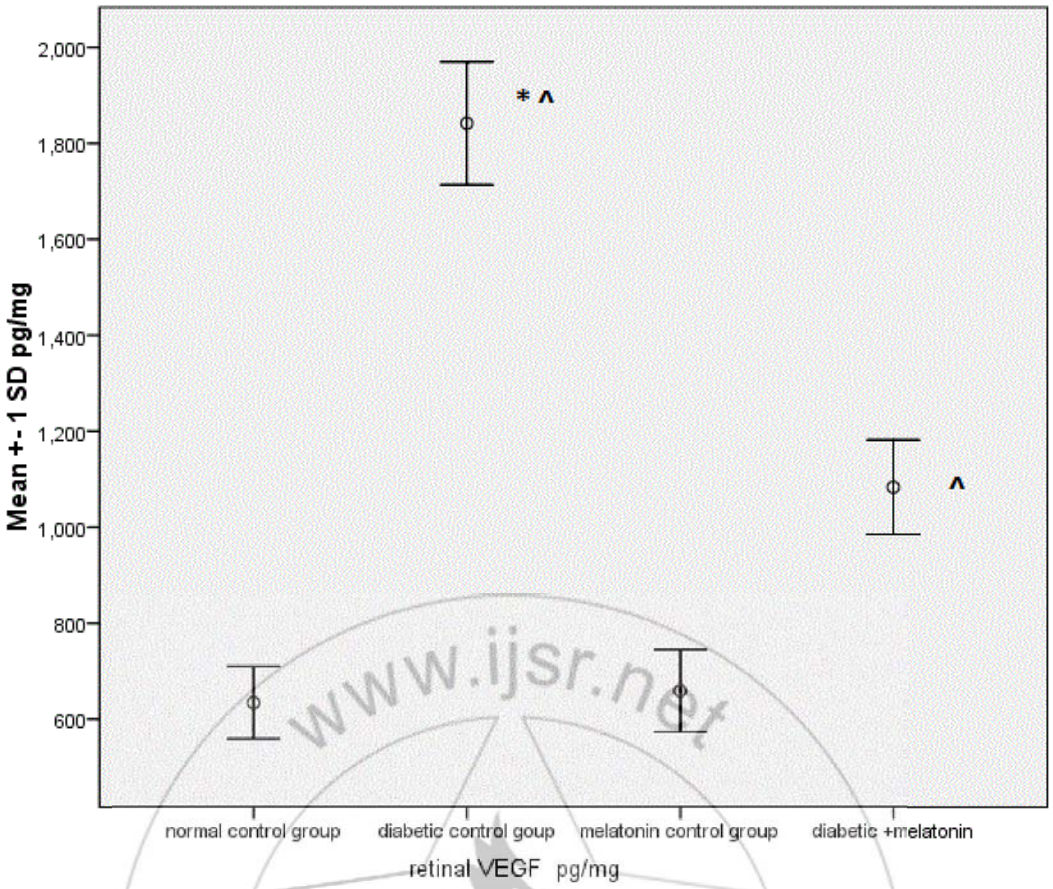


Figure 2: mean ± SD retinal VEGF in the study groups (pg/mg-prot)
^P < 0.05 compared to normal *P <0.05 compared to diabetic group treated with melatonin

Level of VEGF was significantly higher in diabetic control group as compared to normal and melatonin treated group. The level in diabetic group treated with melatonin was significantly lower than diabetic control but higher than normal(fig 2).

3- MDA level in retina:

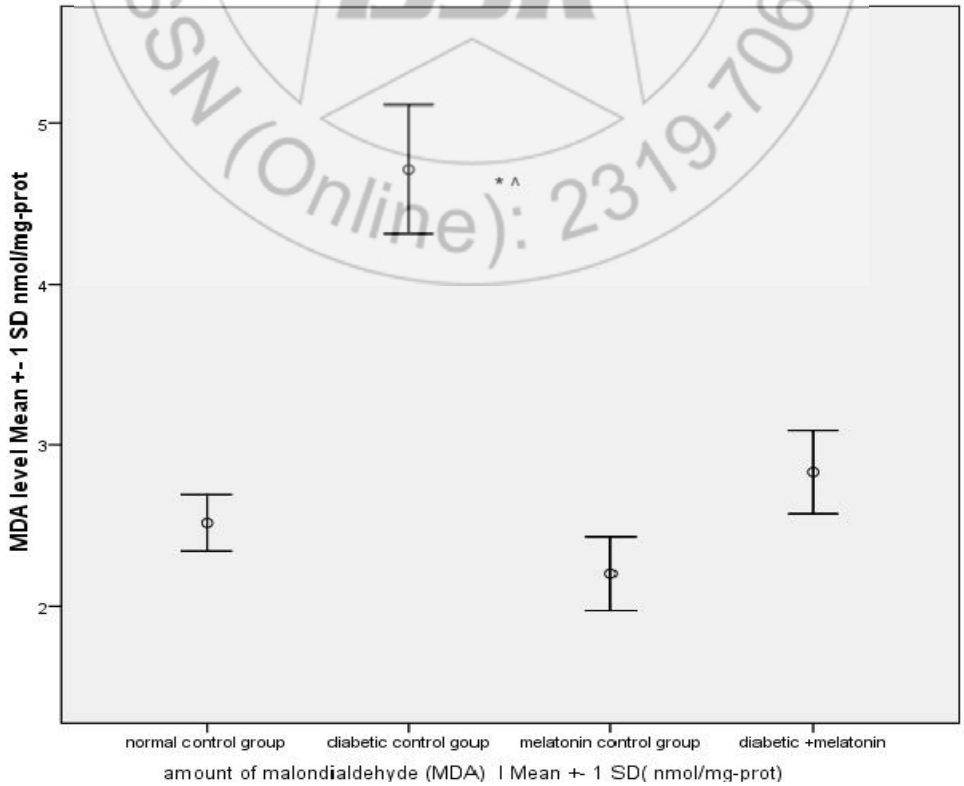


Figure 3: mean ± SD retinal MDA in the study groups (nmol/mg-prot)
^ P < 0.05 compared to normal * P <0.05 compared to diabetic group treated with melatonin

Level of MDA was significantly higher in diabetic control group as compared to normal and melatonin treated diabetic group (fig3).

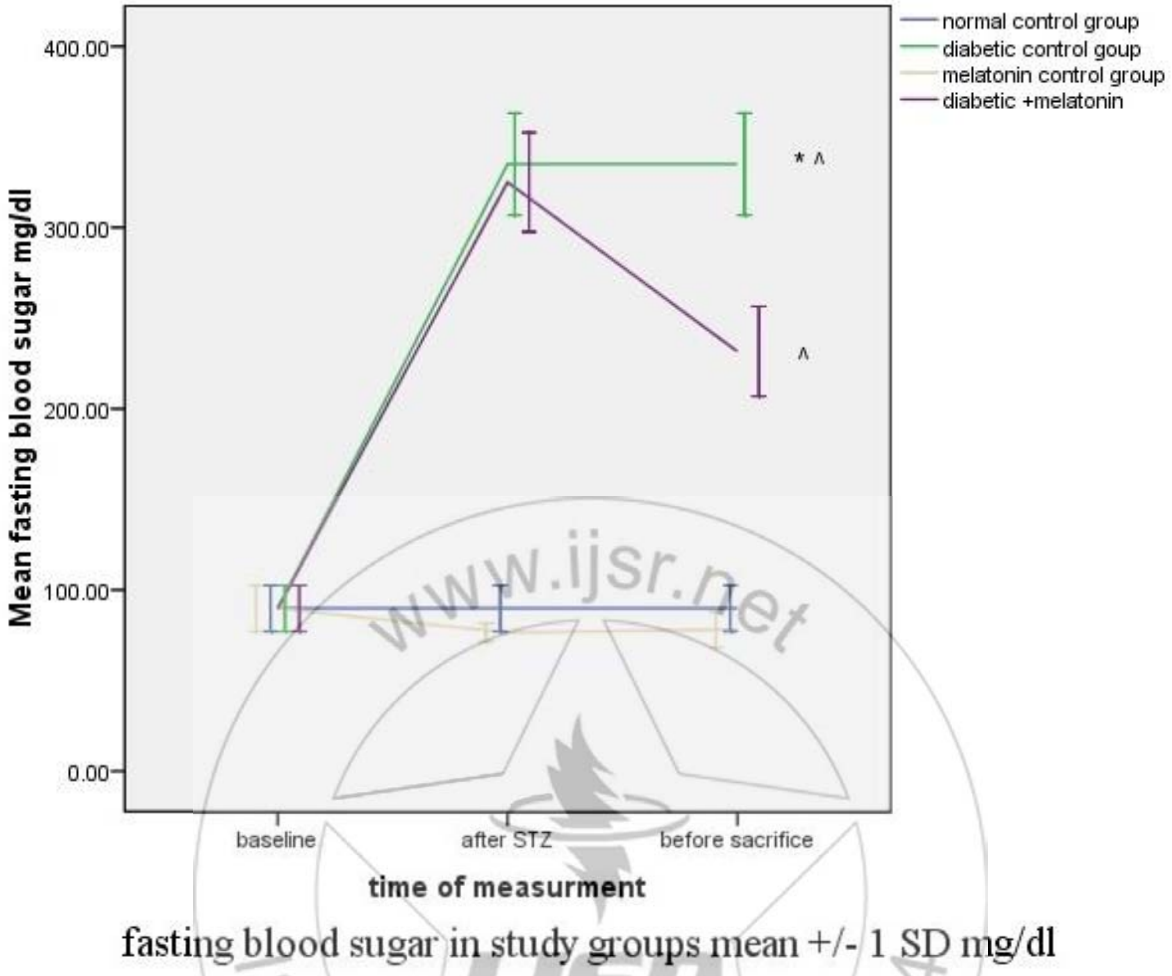


Figure 4: mean ± SD retinal fasting blood sugar in the study groups (nmol/mg-prot)
^ P < 0.05 compared to normal * P <0.05 compared to diabetic group treated with melatonin.

Level of FBS in diabetic control group was significantly higher than other study groups .in diabetic group treated with melatonin level of FBS was significantly lower than diabetic but significantly higher than normal control (fig.4).

6. Discussion

In this study, the retinal histopathology showed disarrangement of retinal layers and showed disorganized cells and vascularity in the diabetic group. By measuring tissue VEGF level ,it was significantly higher in diabetic control group Abnormal vascular changes such as dilatation, microaneurysm, and tortuosity are prominent features of diabetic retinopathy. marked vascular dilatations were reported in diabetic patients elsewhere, mainly created by high levels of VEGF[11]. This vessel active cytokine plays an important role in the development of diabetic complications such as edema and hemorrhages [11].

Melatonin supplementation was successful to reduce diabetic microvascular deterioration in this study and improved histopathological picture.other studies showed that sub cutaneous implantation of melatonin therapy prevented retinal VEGF in diabetic rats [8]. Reduced VEGF secretion by melatonin may be a substantial factor in ameliorating vascular deformation. How melatonin decreases VEGF may be a concern of future investigations.

As showed in other studies[12] , diabetic retinopathy was associated with oxidative and nitrosative stress encountered the same results . In the presence of superoxide, NO forms peroxynitrite and leads to lipid peroxidation and DNA damage. Another study established the elevated nitrotyrosine levels in nerve fiber layer and endothelial cells of diabetic retina [13].These evidence suggest a pivotal role of NO in diabetic retinopathy[14].

Malondialdehyde (MDA) is one of end products formed via the decomposition of lipid peroxidation. In this study, diabetic group had higher MDA levels compared with control. Hyperglycemia oxidized retina and increased MDA content more than twofold. Studies showed that hyperglycemia causes oxidation via induction of several pathways, including protein kinase C, sorbitol, and phoshoinositide 3-kinase pathway.[15]

Free radicals impair cellular membranes and may disrupt endothelial cell integrity, creating both ischemic and

edematous areas. Oxidative stress may be crucial in diabetic retinopathy and clinical implications of diabetes may be carried out by reactive oxygen species [16].

Melatonin can be dissolved in ethanol successfully. Previous studies showed that melatonin membrane receptors were distributed throughout the retina and melatonin administered systemically could reach to the eye, increasing retinal methoxyindole levels.[17]

Another contribution by melatonin is its free radical scavenging property. Melatonin is a known antioxidant and shows antioxidant activity in the retina. Melatonin can remove free radicals directly from the environment effectively and forms stable irreversible metabolites[18]. Therefore, it has been referred to as a terminal or suicidal antioxidant. This aspect might be related to the diminished damage in this study.

Diabetic rat retinas showed increased levels VEGF levels in this experiment, as reported elsewhere. Diabetic retinopathy is associated with an increased level of VEGF even before the development of acellular capillaries[19]

Although melatonin is reported to have decreased blood sugar, in this study it decreased level of FBS but level was still significantly higher than normal. Other researchers reported no effect of melatonin on blood glucose levels.[20] These conflicting results may be related to the strains and the genetic variations of the animals.

This study concluded that melatonin was successful in reducing diabetic retinal damage. Being an ideal antioxidant, melatonin may have the potential for therapeutic applications. Further studies are required to assess effect of melatonin on otherdiabetic complications.

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