

# MELATONIN PRESERVES THE MORPHOLOGY OF PAROTID GLAND DAMAGED BY STREPTOZOTOCIN INDUCED DIABETES

Mariyah Hidayat<sup>1</sup>, Muhammad Tahir<sup>2</sup>, Amir Ali Shoro<sup>3</sup>

<sup>1</sup> Assistant Professor, Department of Anatomy, Rahbar Medical College, Lahore - Pakistan.

<sup>2</sup> Professor, Department of Anatomy, University of Health Sciences, Lahore - Pakistan.

<sup>3</sup> Professor, Department of Anatomy, Liaquat National Medical College, Karachi - Pakistan.

**Address for correspondence:**  
**Dr. Mariyah Hidayat**

Assistant Professor, Department of Anatomy, Rahbar Medical College, Lahore - Pakistan.

E-mail: drmariyah.hidayat@gmail.com

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## ABSTRACT

**Objectives:** This study investigated the injurious effects of Streptozotocin (STZ) induced diabetes on histology of rat salivary gland along with amelioration of these effects by the antioxidant melatonin.

**Methodology:** This prospective experimental study was conducted in the department of Anatomy, Basic Medical Sciences Institute, JPMC, Karachi for a period of 6 weeks from November to December, 2012. 45 male albino rats were divided into 3 groups, containing 15 animals each. Group A was treated as control, group B and C received 37 mg/kg STZ Intraperitoneally (i/p) once at the start of experiment, whereas group C additionally received 10mg/100 ml of melatonin (MEL) 3-days prior to STZ administration. After sacrificing the animals, the parotid glands were processed for histological examination and viewed under the light microscope.

**Results:** The histological findings demonstrated changes in the parotid gland morphology of group B animals, such as the presence of lipid vacuoles in the serous acini and degeneration of the acinar cells. There was a significant reduction in the severity of these changes in melatonin treated group C. Serum glucose was significantly increased in both group B and C as compared to control. Total serum cholesterol was significantly increased in group B as compared to group A and C.

**Conclusions:** STZ altered the histology of parotid gland by lipid infiltration and degeneration of acini. Melatonin suppressed the progression of damage induced by STZ due to its antioxidant properties and also reduced total serum cholesterol levels, but it could not decrease STZ induced hyperglycemia.

**Key Words:** Streptozotocin, Melatonin, Serous acini, Oxidative stress, Hyperglycemia, Parotid Gland, Antioxidant.

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## INTRODUCTION

Saliva is a major determinant of the oral environment and serves as an easily available diagnostic tool of systemic conditions<sup>1</sup>. Parotid gland parenchyma is subject to changes in its histological characteristics because of local or systemic conditions<sup>2</sup>. Any alterations in the integrity and activity of the salivary glands can change salivary flow and its composition, thus affecting patient's nutritional intake<sup>3</sup>.

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction or failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels<sup>4</sup>. Symptoms of hyperglycemia include polyuria, polydipsia, polyphagia and weight loss.

Polyphagia is associated with increased mastication frequency, which, in turn influences salivary gland activity<sup>5</sup>.

Diabetes causes damage in the parotid gland which could compromise the salivary functions<sup>6</sup>. Experimental animal models of diabetes have been used to study aspects of pathophysiology of this disease in the parotid gland. These studies showed a reduction of glandular weight, changes in the morphology and secretory functions as well as alterations in antioxidant enzymes glutathione peroxidase and catalase<sup>7</sup>.

Persistent hyperglycemia in the diabetic state may cause considerable production of free radicals in many tissues<sup>8</sup>. Melatonin is an indole amine produced in various parts of the body, mainly in the pineal gland. This

gland produces melatonin in a circadian manner, synchronizing a number of biologic processes in a 24-hour, day–night rhythm<sup>9</sup>. Melatonin has strong antioxidant effects that can protect cells against inflammatory processes and oxidative damage<sup>10</sup>.

After release of melatonin into the blood stream, it diffuses into the saliva. The proportion of plasma melatonin passing into the mouth via salivary glands appears to be relatively stable, ranging from 24% to 33%<sup>11</sup>. Melatonin was first discovered in rat salivary gland using immunohistology. Salivary melatonin may protect periodontal tissues, as seen in human diabetics<sup>12</sup>.

Taking into account the protective role of melatonin as an antioxidant and free radical scavenger, the aim of this study was to observe the effect of this hormone on the morphology of parotid gland damaged by STZ.

## METHODOLOGY

This study was conducted in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Post Graduate Medical Center, Karachi for a period of 6 weeks from November to December, 2012. In this study, 45 healthy male albino rats, 90-120 days old were obtained from the animal house of BMSI and divided into 3 groups, each containing 15 animals. Serum glucose of all the animals was determined by one touch glucometer (Roche Diagnostics) from the tail vein.

The duration of experiment was 42 days (6 weeks). On the first day, group A was taken as control. The animals of group B were fasted overnight and administered STZ i/p in a dose of 37 mg/kg dissolved in 1 ml of citrate buffer at 4 PH, only on the first day of the experiment. STZ was administered only once throughout the period of study. Group C received 10 mg/100 ml of melatonin (obtained from Sigma Aldrich, Germany). dissolved in drinking water each day, starting from the first day of the experiment. The rats were administered STZ i/p in the same dose as in group B only once on the 4<sup>th</sup> day of the experiment.

Clean water bottles and freshly prepared melatonin solutions were provided.. A stock solution of melatonin was prepared freshly every 3-4 days containing 10 mg

of melatonin dissolved in 100 ml of distilled water<sup>10</sup>. Melatonin is water soluble at this concentration. The water bottles were wrapped in aluminum foil to avoid degradation of melatonin by sunlight.

All animal procedures were in accordance to the standards set forth in guidelines for the care and use of experimental animals by Committee for Purpose of Supervision of Experiments on Animals (CPSEA), BMSI, Karachi. The study protocol was approved by Animal Ethics Committee, BMSI, Karachi.

The animals were sacrificed at the end of their respective treatment. The total serum cholesterol level was measured by ELISA method at the beginning and end of experiment, whereas serum glucose was measured weekly by a glucometer in all the 3 groups After sacrificing the rats on 43<sup>rd</sup> day of experiment, the parotid glands were exposed and dissected. After washing with normal saline, they were fixed in buffered neutral formalin for 24 hours and then kept in 70% alcohol overnight. Dehydration of the tissues was done with ascending strengths of alcohol, cleared in xylene and infiltrated with paraffin. Paraffin blocks of tissue were made and 5microns thick longitudinal sections were cut by a Leica RM2235 rotatory microtome, mounted on labeled glass slides and stained with Haematoxylin and Eosin for a detailed morphological examination of the parotid gland under the light microscope.

## RESULTS

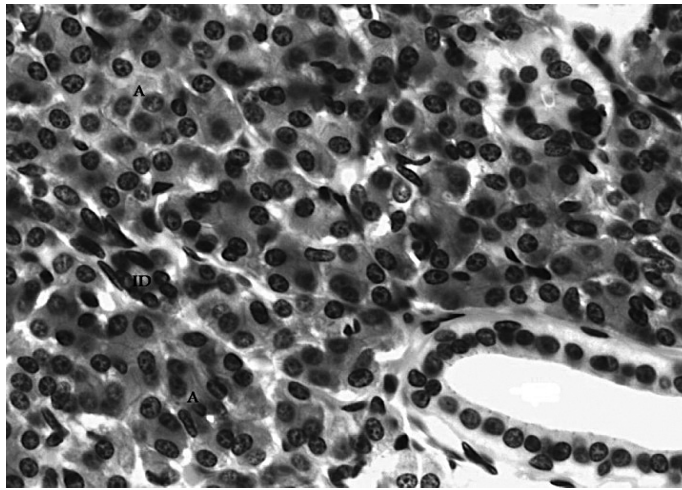
The results are shown in Table 1 and Figures 1, 2 and 3. The serum glucose levels were significantly increased in group B as compared to group A and C, highlighting the insignificant effect of melatonin on glucose levels (Table 1). Total serum cholesterol levels were within the normal range in melatonin treated group C, but significantly elevated in STZ treated group B, as compared to control group A (Table-1). All calculations were done using SPSS version 11 and p-values < 0.05 were considered significant.

A normal architecture of the parotid gland was observed in H and E stained slides in group A under the light microscope at 400 times magnification (figure 1). According to histological findings, lipid infiltration of

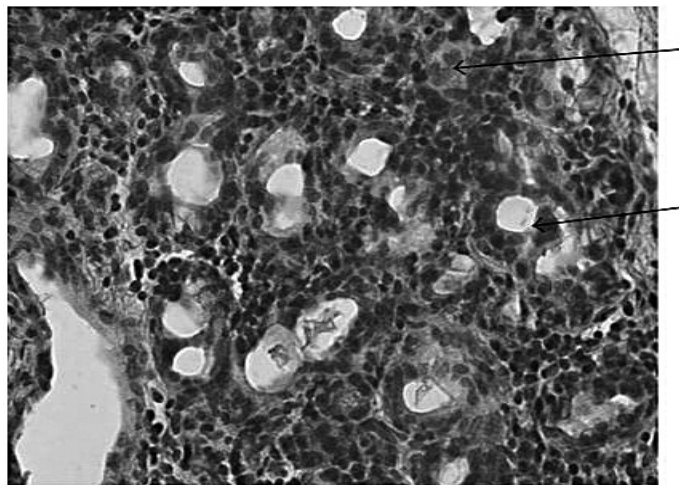
**Table 1: Mean values of serum glucose and Total Serum Cholesterol at 6 weeks.**

Groups	Treatment received	Serum Glucose(gm/dl)	Total Serum Cholesterol (mg/dl)
Group A	-	88± 5	59.95± 0.07
Group B	STZ	392**± 15	81.16±0.24**
Group C	STZ + MELATONIN	378**± 10	64.20±0.07*

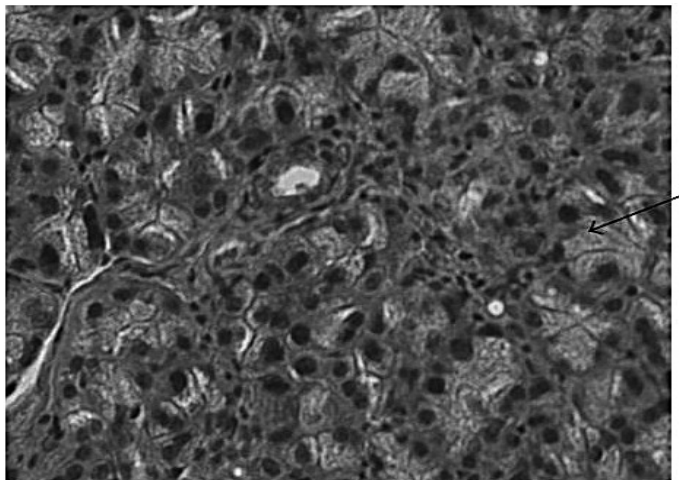
**Figure 1: Photomicrograph of 5 microns thick H and E stained section from parotid gland of group A (Control) rat showing normal architecture of serous acini. Photomicrograph x 400.**



**Figure 2: Photomicrograph of 5 microns thick H and E stained section from parotid gland of group B (STZ treated) rat showing distorted architecture of serous acini and presence of lipid vacuoles. Photomicrograph x 400.**



**Figure 3: Photomicrograph of 5 microns thick H and E stained section from parotid gland of group C (STZ + MEL) treated rat showing preserved architecture of serous acini and no sign of lipid vacuoles. Photomicrograph x 400.**



the parotid gland in the form of lipid vacuoles, along with degeneration of the serous acini in STZ treated animals in group B was observed (figure.2). A significant decrease in the lipid vacuoles was observed in group C and there was no distortion of serous acini seen (figure.3), indicating the significant role of melatonin in preserving architecture of parotid gland.

## DISCUSSION

Persistent hyperglycemia in the diabetic state may cause considerable production of free radicals in many tissues. Diabetes mellitus can elicit changes in the morphology, secretory function and acyl fatty acid quantity in the isolated rat parotid gland<sup>13</sup>.

STZ induced hyperglycemia is a well-documented experimental model of both type 1 and type 2 diabetes. In a study conducted by Hidayat et al, STZ at the dose of 37mg/kg for 6 weeks produced marked hyperglycemia in albino rats which is in agreement with our results. In the present study, STZ resulted in significant hyperglycemia and melatonin supplementation did not affect this parameter. In a similar study conducted by Sudnikovich et al<sup>14</sup>, STZ administration to albino rats for 25 days resulted in significant hyperglycemia and retarded growth of animals, whereas melatonin administration did not affect these parameters.

During metabolism of STZ, a variety of toxic intermediates are produced. Besides alkylating agents like methylcations and methyl radicals, it has been shown that reactive oxygen species (ROS) are produced by STZ as well<sup>15</sup>. Baydas et al<sup>16</sup> stated that in the serum of animals with STZ-induced diabetes, melatonin remarkably reduces the degree of both lipid peroxidation and protein glycosylation, decreases the levels of cholesterol, triglyceride and low-density lipoprotein, which is in agreement to the present study. This result is consistent with melatonin's ability to increase leptin expression by adipocytes<sup>17</sup>.

The most pronounced effect of melatonin administration is the prevention of an increase in nitric oxide (NO) levels in blood plasma during STZ-induced diabetes, which implies that melatonin may operate as a NO scavenger and carrier<sup>14</sup>. Despite this fact, another investigator concluded that the protective effects of melatonin against STZ induced  $\beta$ -cell damage may be related to interference with DNA damage rather than through effects on NO pathways. On the other hand, STZ induced diabetes reduced the nocturnal pineal melatonin content in Syrian hamster, but not in rats, and the plasma and saliva melatonin levels in type 1 and type 2 diabetic patients<sup>18</sup>.

Moreover, Stebelova et al<sup>19</sup> observed in a study that

STZ-induced diabetes resulted in lower melatonin levels in the pancreas, kidney and duodenum compared to the control, thus suggesting that the lower amplitude of melatonin in target organs induced by STZ might contribute to the desynchronization of daily rhythms and might also weaken the antioxidant capacity of tissues.

In a study conducted by Bellavia et al<sup>20</sup>, melatonin was found to enhance serum amylase secretion in the parotid gland. Similarly, in a study conducted by Ashour<sup>21</sup> on submandibular salivary gland, melatonin increased the cellular activity and retarded the process of degeneration in the secretory acini in aged rats.

Taken together, STZ induced diabetes increases oxidative stress through generation of free radicals, liberation of nitric oxide, lipid peroxidation, protein glycosylation, decreased levels of catalase and glutathione peroxidase, as well as DNA single-strand breaks.

The results of this investigation show that the morphology of parotid salivary gland is impaired by STZ induced oxidative damage and melatonin as an antioxidant, can combat the free radicals and preserve the morphology of parotid salivary glands, without affecting serum glucose levels.

## CONCLUSION

Diabetes causes an accumulation of lipids in the parotid glands of rats, with an increase in the synthesis of total serum cholesterol. Melatonin, as an antioxidant, preserves the architecture of the parotid glands, prevents elevation of serum cholesterol and inhibits accumulation of lipids inside the parenchyma of this gland. Moreover, it also decreases the serum cholesterol levels, without having any significant effect on STZ induced hyperglycemia.

STZ induced hyperglycemia caused an accumulation of lipids in the parotid glands of rats, with an increase in the synthesis of total serum cholesterol. Melatonin preserved the architecture of the gland by inhibiting lipid accumulation and preventing elevation of serum cholesterol. However, it could not significantly alter serum glucose levels, indicating that damage to the architecture of the parotid gland was mainly due a rise in free radicals due to hyperglycemia.

It may be beneficial to add melatonin to the treatment regime of diabetic patients in order to combat the free radicals produced due to a rise in serum glucose. Melatonin, therefore, has a promising role as an antioxidant in the treatment regime of diabetes.

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## CONTRIBUTORS

MH planned the study, did data analysis and wrote manuscript. MT and AAS helped in manuscript writing and supervising the study. All authors contributed significantly to the final manuscript.