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IMPACT OF INSULIN AND MELATONIN ON HEPATIC ANATOMY, CELLULAR BIOCHEMISTRY, AND RECEPTOR EXPRESSION IN DIABETIC HEPATIC DAMAGE

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Abstract:
Objectives:
To examine the influence of exogenous melatonin and insulin on the biochemical, serological, histological, and receptor expression patterns within liver tissues following hepatic damage.

Materials and Methods: This observational study was conducted at Ghulam Muhammad Mahar Medical college Sukkur, Pakistan. We have enrolled 74 patients with DM. The study was conducted from July, 2023 to December, 2023. All patients enrolled underwent liver function test and lipid profile. The patients received a combination therapy involving melatonin and insulin. Blood samples were obtained from each participant and forwarded for laboratory analysis. The treatment's efficacy was documented by observing outcomes across all patients. Data collection utilized a predetermined questionnaire.

Results: The mean age and BMI of the entire group of 74 patients were 49.17±9.42 years and 27.31±4.08 (kg/m²) respectively. Our analysis revealed elevated levels of ALT (765.14±154.8), AST (694.59±158.4), ALP (705.70±149.3), serum cholesterol (179.98±8.27), LDL (96.68±3.8), and VLDL (83.37±7.37), accompanied by a reduction in HDL levels (45.22±3.51). Based on Hepatic Anatomy, cirrhosis was observed in 16(21.6%), and fatty liver was observed in 16(21.6%) patients. Hepatic injury recovery was observed in 69 out of 74 cases (93.2%). Remarkable enhancements were detected in the biochemical profile, cellular structure of liver cells, and the expression pattern of MT1, MT2, and IR receptors, suggesting substantial recovery and restoration.

Conclusion: It was concluded that the combined administration of melatonin and insulin among diabetic patients with hepatic injury yielded notably favorable outcomes in terms of effectiveness and disease recuperation.

Key words: Insulin, melatonin, DM, Hepatic injury

INTRODUCTION:

Obesity is indeed a significant global health concern.(1) It is associated with numerous health complications, including type 2 diabetes, cardiovascular diseases, certain types of cancer, and musculoskeletal disorders.(2) The prevalence of obesity has been steadily increasing worldwide over the past few decades, driven by various factors such as changes in dietary habits, decreased physical activity, urbanization, and socioeconomic factors.(3) The number of individuals worldwide who are classified as overweight or obese exceeds 2 billion.(4)

Melatonin is indeed involved in a wide range of physiological functions in the body. Melatonin is synthesized and released by the pineal gland in response to darkness.(5) Melatonin exerts its physiological effects through interaction with specific receptors known as melatonin receptors 1 (MT1) and 2 (MT2), which are members of the G protein-coupled receptor (GPCR) family.(6) These receptors are widely distributed throughout the body and are found in various tissues and organs, allowing melatonin to modulate numerous physiological functions.(7)

Insulin resistance, a hallmark of type 2 diabetes, is associated with a chronic low-grade inflammation throughout the body.(8) This inflammatory state contributes to the development of various complications associated with diabetes.

Insulin and melatonin can influence hepatic histology, cellular biochemistry, and receptor expression in diabetics and may have therapeutic implications for the management of hepatic damage associated with diabetes.(4) Their effects are multifaceted and involve complex interactions with various cellular pathways involved in hepatic metabolism and homeostasis. Melatonin, renowned for its antioxidant capabilities, is not only produced by the pineal gland but also synthesized in the liver.(9) In goldfish, enzymes essential for melatonin production, namely AANAT (arylalkylamine N-acetyltransferase) and ASMT (acetylserotonin O-methyltransferase), are found in the liver.(10) In the liver and gastrointestinal system, melatonin acts by binding to its receptors, MT1 and MT2, which are G protein-coupled receptors (GPCRs).(11, 12) This interaction influences processes such as digestion, metabolism, and immune function, highlighting the widespread effects of melatonin beyond its role in sleep regulation. Melatonin has been found to influence pancreatic beta-cell function, which is responsible for insulin production.(13) Studies have suggested that disruptions in the normal circadian rhythm, which can occur due to factors such as shift work or irregular sleep patterns, may lead to alterations in melatonin secretion.(14) These disruptions can potentially impact insulin sensitivity and glucose metabolism, contributing to the development or progression of type 2 diabetes.

Objective:

To examine the influence of exogenous melatonin and insulin on the biochemical, serological, histological, and receptor expression patterns within liver tissues following hepatic damage.

MATERIALS AND METHODS:

Study Design: Observational study

Study setting: Ghulam Muhammad Mahar Medical college Sukkur, Pakistan

Duration of the study: The study duration was 6 month from (from July, 2023 to December, 2023).

Inclusion Criteria:

- Individuals with Diabetes Mellitus of age 18 to 70 year.
- Participants suffering from hepatic damage.
- Participants currently undergoing treatment with insulin, melatonin, or a combination of both for their diabetes and/or hepatic condition.
- Both gender.

Exclusion Criteria:

- Patients having history of cardiac surgery.
- Individuals who have undergone liver transplantation.

- Pregnant or breastfeeding individuals.
- Individuals with severe renal dysfunction or end-stage renal disease.
- Individuals taking medications known to significantly affect liver function, insulin sensitivity, or melatonin metabolism.

Methods:

This observational study was conducted at Ghulam Muhammad Mahar Medical college Sukkur, Pakistan after the approval of hospital ethical committee. We enrolled a total of 74 patients with diabetes and unique number were given to each and every patients after obtaining an inform consent. Besides the Glucose level, all enrolled patients underwent liver function tests, including assessments for ALT, AST, ALP, serum cholesterol, LDL, VLDL, and HDL. Patients were administered a combination therapy of melatonin and insulin. Blood samples were collected from all participants and sent for laboratory analysis. The effectiveness of the treatment was recorded based on outcomes observed among all patients. All patients underwent hepatic ultrasound examination. A predesign questionere were used to collect data.

RESULTS:

The mean age and mean BMI of all 74 patients was 49.17±9.42 years and 27.31±4.08 (kg/m2) respectively (Table 1). We have found an increase in ALT (765.14±154.8), AST (694.59±158.4), ALP (705.70±149.3), serum cholesterol (179.98±8.27), LDL (96.68±3.8), VLD (83.37±7.37) but decrease in HDL level (45.22±3.51) (Table 1). Demographic characteristics of the patients was given in table 2, in which it was shown that 14(18.9%) of the patients were Illiterate, 29(39.2%) had primary level of education, 26(35.1%) had intermediate level and 5(6.8%) had university level education. Out of total about 68(91.9%) were married and 29(39.2%) were smoker. 40(54.1%) of the patients were male and the remaining 34(45.9%) were female patients (Table 2). 16(21.6%) of patients were suffering from T1DM and the remaining 58(78.4%) were suffering from T2DM. Based on Hepatic Anatomy, cirrhosis was observed in 16(21.6%), and fatty liver was observed in 16(21.6%) patients. We observed hepatic injury recovery in 69(93.2%) cases. Significant improvements were noted in the biochemical profile, cellular structure of liver cells, and the expression pattern of MT1, MT2, and IR receptors, indicating substantial recovery and restoration (Table 3, Fig 3).

Table 1: Demographic and clinical characteristics of all enrolled Patient *(n=74)*

Variables	**Mean±SD**
Age (Years)	49.17±9.42
BMI (kg/m2)	27.31±4.08
Liver function test	
ALT (U/L)	765.14±154.8
AST (U/L)	694.59±158.4
ALP (U/L)	705.70±149.3
lipid profiles	
Serum cholesterol mg/dl	179.98±8.27
LDL mg/dl	96.68±3.8
VLD	83.37±7.37
HDL mg/dl	45.22±3.51
Hepatic Anatomy	
Fatty liver	22(29.7%)
cirrhosis	16(21.6%)

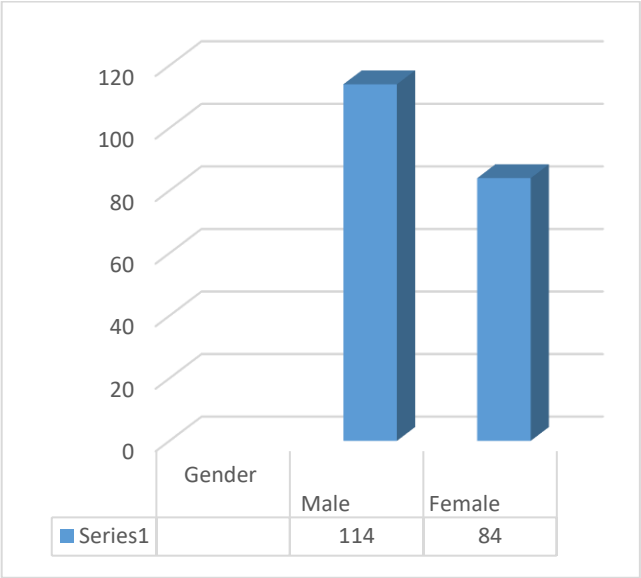

Fig 1: *Bar graph showing gender distribution*

Table 2: characteristic of all the enrolled patients *(n=74)*

Variables	Frequency (Percentage)
Gender	
Male	40(54.1%)
Female	34(45.9%)
Education Status	
Illiterate	14(18.9%)
Primary	29(39.2%)
Intermediate	26(35.1%)
University	5(6.8%)
Married	68(91.9%)
Smokers	29(39.2%)

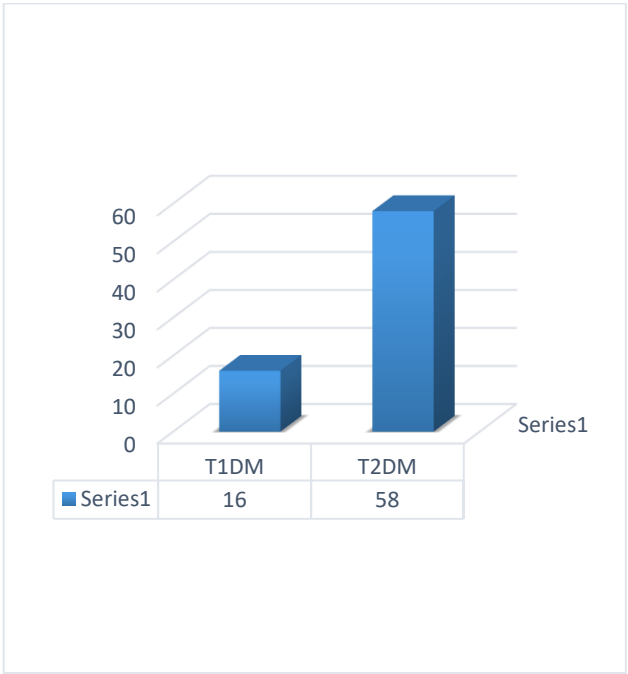

Fig 2: *Frequency of type of DM*

Table 3: Efficacy among all enrolled patients (*n=74*)

Variables	Frequency (Percentage)
Effectiveness	
Yes	69(93.2%)
No	5(6.8%)

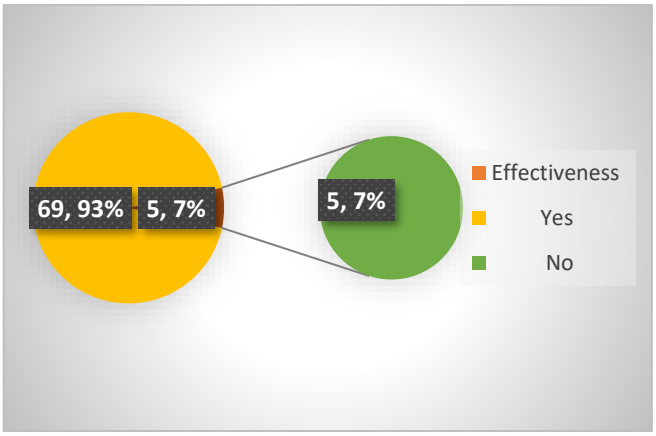

Fig 3: *Frequency of effectiveness*

Discussion: The present study aims to investigate the effects of insulin and melatonin on hepatic damage in diabetic individuals. It involves assessing how these treatments influence three key aspects of liver health: histological structure, cellular biochemistry, and receptor expression.

In the present study we observed hepatic injury recovery in 69(93.2%) cases. Significant improvements were noted in the biochemical profile, cellular structure of liver cells, and the expression pattern of MT1, MT2, and IR receptors, indicating substantial recovery and restoration. That highlights positive outcomes in the recovery of hepatic injury, emphasizing improvements in various aspects of liver function and structure, which collectively signify a meaningful restoration of liver health.

Individuals with diabetes were found to have significantly higher blood glucose levels.(15) Melatonin's involvement in glucose metabolism is a growing research focus.(16, 17) Although famed for its role in sleep regulation, melatonin also affects insulin secretion and peripheral tissue glucose uptake.(16) Conversely, insulin is the principal hormone governing blood glucose levels by facilitating glucose uptake into cells.(18) Previous studies have shown that melatonin positively impacts the proliferation/regeneration of beta cells and inhibits apoptosis.(19) The current study's results clearly support and validate these previous findings regarding melatonin's effects on beta cell proliferation/regeneration and apoptosis inhibition. Pancreatic injury is closely linked to diabetes onset.(20) Damage to pancreatic islet cells results in decreased insulin production and higher blood glucose levels, key features of diabetes.(21)

High blood sugar levels can induce oxidative stress, affecting various physiological processes like reducing red blood cell count, glycosylating hemoglobin, and causing other hematological abnormalities.(22) Melatonin has demonstrated its capability to prevent beta-cell damage and enhance their function in response to such stress.(23) In individuals with diabetes, both melatonin and insulin levels can decrease, but supplementation with exogenous melatonin and insulin may correct this imbalance.(24) This suggests that counteracting the stress induced by diabetes may provide protection, reducing cellular damage from oxidative stress and safeguarding against functional overload.(25) Explored the role of melatonin in influencing glucagon secretion through receptor-mediated mechanisms. By utilizing both non-specific melatonin antagonists such as luzindole and MT2 receptor-specific antagonists like 4P-PDOT in incubation studies with TC1.9 cells, they were able to pinpoint that melatonin indeed acts through its receptors to modulate glucagon release. This

finding sheds light on the intricate interactions between melatonin and pancreatic hormone regulation, opening avenues for further exploration into the physiological implications of these interactions.(26) PI3K regulation is another pathway influencing melatonin's actions in pancreatic β-cells. The PI3K pathway serves as a signaling mechanism for melatonin's impact on beta-cell function. Activation of PI3K results in downstream target phosphorylation, affecting processes like insulin secretion. Treating TC1.9 cells with the PI3K antagonist wortmannin (inhibitor of the PI3K family) suppressed melatonin's glucagon-increasing effect, as demonstrated in incubation experiments.(26) Melatonin's effects on pancreatic alpha-cells, responsible for glucagon secretion, are less understood compared to its impact on beta-cells responsible for insulin secretion. While some research suggests melatonin may influence alpha-cell function, the mechanisms and effects are not as well-defined as in beta-cells. The interplay between phospholipase C (PLC) and PI3K signaling pathways in pancreatic cells is crucial for understanding cellular responses to stimuli like melatonin. Studies, such as Batty et al.'s work in glioma cells, demonstrate these pathways can interact and influence each other's activity.(27) Various types of white blood cells, such as basophils, eosinophils, and neutrophils, are crucial for the body's immune defenses. Neutrophils, acting as the primary leukocytes and the body's initial defense line, are vital for initiating immune responses. A reduction in their numbers may impair their ability to combat infections, potentially slowing down wound healing processes. Diabetic individuals often encounter challenges in wound recovery, increasing the risk of complications like amputation. This heightened susceptibility to infections can result in significant suffering and even mortality. Additionally, notable alterations were observed in lipid profiles (including serum cholesterol, LDL, and VLDL) and liver enzymes (ALT, AST, and ALP), including reduced HDL levels. The pattern of increased ALT, AST, ALP, serum cholesterol, LDL, and VLDL, along with decreased HDL levels, suggests liver injury or dysfunction, along with dyslipidemia. These findings may indicate a need for further evaluation and management to address liver health and lipid metabolism issues. It's essential to consider the underlying causes of these changes and develop appropriate interventions to promote liver health and reduce cardiovascular risk.

Conclusion: The results of this study concluded that using both melatonin and insulin together helped diabetic patients with hepatic injury get better and recover from their illness more effectively.

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