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EXPLORING THE LINK BETWEEN OXIDATIVE STRESS AND DIABETES: BIOCHEMICAL INSIGHTS

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ABSTRACT

BACKGROUND: The study was aimed to evaluate the link between Oxidative Stress and diabetes and to determine the possible levels of antioxidant enzymes and levels of gene expression between control and diabetic induced male albino wistar rats. **METHODOLOGY:** Diabetes was induced by injecting single low-dose streptozotocin along with providing high fats diets in the experimental group whereas in the control group standard feeding protocol was employed. The analyses of the outcome measures were performed twice at baseline and after 6 weeks of intervention. **RESULTS:** Diabetic group had shown higher levels of gene expression of an oxidative damage marker, indicating increased OS and cellular damage. The discoveries uncovered that the outflow of qualities encoding cell reinforcement catalysts was downregulated in the diabetic gathering, demonstrating a misfortune in cancer prevention agent safeguard. These outcomes gives proof at the degree of sub-atomic pathways that make sense of diabetes' irregularity between cell reinforcement safeguards and oxidative harm, stressing the basic job of operating system in illness movement. **CONCLUSION:** Taking everything into account, the research center based exploratory review utilizing a creature model of diabetes has given new data on the association among operating system and diabetes mellitus. The discoveries feature operating system's essential capability in the beginning and movement of diabetes, particularly when cell reinforcement safeguard components are powerless. The intricate link between OS and diabetes inflammation is made clear by the fact that diabetics have downregulated genes for antioxidant enzymes and increased inflammatory markers.

KEYWORDS: Diabetes Mellitus, mouse, Romo1 protein, Antioxidant Response Elements

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INTRODUCTION

Millions of people worldwide suffer from diabetes, a common chronic metabolic disease with a significant number of cases in Asia alone. Type 2 diabetes mellitus (T2DM) is portrayed by the pancreas' inability to create sufficient insulin to enough oversee glucose levels, inferable from expanding insulin request brought about by insulin resistance1-2. T2DM is firmly connected to beta cell glitch, dedifferentiation, and diminished beta cell mass. Diabetes's beta cell degeneration is largely caused by inflammation, hyperlipidemia, and hyperglycemia. These circumstances cause beta cell damage and dedifferentiation largely by inducing ER stress, mitochondrial malfunction, and Oxidative Stress (OS)[3-4]. While ER stress and mitochondrial malfunction have both been linked to beta cell dysfunction and death, new research has focused on how they work together to exacerbate beta cell OS. Because of their high generation of reactive oxygen species (ROS) and limited antioxidant capability, pancreatic beta cells are especially sensitive to OS[5]. This flaw suggests that OS plays a significant role in beta cell failure. OS OS has been linked to the development of a number of

illnesses, including cardiovascular disease and cancer. As a result, substantial research has been performed to investigate the therapeutic potential of pharmacological drugs targeting OS[6]. OS is defined as an imbalance between the generation of free radicals and the body's antioxidant defenses, plays a crucial role in the development and progression of diabetes. Free radicals, such as ROS and reactive nitrogen species (RNS), are generally produced during metabolic processes and immunological responses, where they perform important physiological activities in a variety of cellular pathways. OS develops when free radical generation surpasses the body's natural antioxidant capability. Excess OS can induce cellular damage and dysfunction in diabetics[7-8]. Biologic cells contain built-in defense systems against free radicals, including enzymes such as Superoxide Dismutase (SOD) and glutathione (GLT)[9]. Diabetes, on the other hand, may overwhelm the antioxidant defense system, resulting in increased OS. Free radicals are particularly reactive due to their unpaired electrons and may interact with biomolecules like as proteins, lipids, and nucleic acids, producing structural and functional alterations. Oxidative damage has an impact on cell signaling, gene expression, and cell survival[10]. Diabetes complications are linked to OS through lipid peroxidation, DNA damage, and mitochondrial dysfunction[11-12]. These mechanisms all lead to insulin resistance, beta cell malfunction, and overall glycemic control impairment[13]. To address the negative consequences of OS in diabetes, it is critical to understand the underlying molecular pathways involved. Researchers can create possible treatment techniques to protect beta cells, maintain glycemic control, and perhaps enhance overall disease management by identifying critical biomarkers and pathways associated to OS in diabetes patients. The use of an animal model of diabetes in a laboratory-based experimental investigation allows for a full examination of OS indicators, antioxidant enzyme activities and oxidative damage markers. This study's findings have the potential to greatly influence the development of individualized treatments, which could ultimately lead to improved outcomes for people with diabetes and related illnesses.

METHODOLOGY

STUDY DESIGN

A lab based exploratory procedure was directed at Suleman Roshan Clinical School Research center, to look at the connection among operating system and diabetes utilizing a creature model. A control group and a diabetes-induction group comprised the study's two groups.

Study Setting:

The Suleman Roshan Medical College provided the necessary facilities and resources for the study.

Sample Size:

The review utilized a sum of 20 male pale skinned person Wistar rodents as its example size. The rodents were haphazardly designated to one of two gatherings: control or diabetes-acceptance.

Animal Housing:

The male pale skinned person Wistar rodents were housed in a controlled climate to guarantee their prosperity and consistence with moral standards. For the duration of the study, significant attempts were taken to maintain adequate temperature, humidity, and lighting conditions, giving the rats with a suitable home.

Diabetic Induction:

Diabetes was induced by injecting single low-dose streptozotocin along with providing high fats diets in the experimental group whereas in the control group standard feeding protocol was employed.

Study Duration:

The study was conducted for the duration of six weeks.

Data collection:

Data was collected at baseline and after six weeks of intervention. The levels of ROS, SOD and GLT were monitored at baseline and after six weeks. Moreover levels of lipid peroxidation were also determined at baseline and compared with the values after six weeks of intervention. In addition to that relative gene expression was also determined for three genes representing SOD, GLT and lipid peroxidation.

Ethical Considerations:

Ethical standard governing the animal as a subject for study was maintained. The research had included lowest possible number of animals that could have provided significant findings and moreover researcher had shown all possible respect for animals regardless of their

utility value. Study was ethically approved from Suleman Roshan Medical College

RESULTS

Baseline Characteristics: The baseline characteristics had revealed that at day one the mean weight of rats in the control group was 220±3.5gm and in the experimental group was 218±2.1gm that had remained non significantly different p>0.05 in the control group, whereas in the experimental group the value found to be significantly raised to 235.2±4.2gm. The demographic characteristics were illustrated in table 1

Table 1: Demographic Characteristics of Albino Wistar Rats

Variables	Average weight in grams ± SD (Baseline)	Average weight in grams ± SD (After 6 weeks)	Mean Difference	Level of Significance p-value
Control Group	220±3.5	220.58±2.3	0.58	p=0.08
Experimental Group	218±2.1	235.2±4.2	17.2	p=0.001

Further reactive oxygen species levels (ROS) were identified in both the group and the analyses of the findings had revealed that from baseline the values found to be significantly raised p<0.05 in the experimental group with an increase from 6.8±1.2 units to 11.42±3.4 units, whereas in control no significant difference was observed (table 2). In addition to that the levels of antioxidants enzymes were also estimated including SOD and GLT peroxidase and the findings revealed that from baseline till week six the values of both the enzymes were significantly reduced in the experimental group p<0.001 whereas in control group the values were turned out to be relatively stable. Lipid peroxidation levels were also identified and it was found that the levels were significantly elevated p<0.05 in the diabetic induced group where the baseline values were 2.25±1.4nmol/mg that had increased to 5.58±1.58nmol/mg after six weeks. The detailed analyses was illustrated in table 2 as under:

Table 2 Pre –Post analyses of values of ROS, antioxidant enzymes and lipid peroxidation

Reactive Oxygen Species Levels (ROS Units))				
Variables	**Baseline ± SD**	**After six weeks ± SD**	**MD**	**Level of Significance**
Experimental Group	6.8±1.2	11.42±3.4	4.62	p<0.01
Control Group	6.45±0.89	6.51±1.2	0.06	p=0.08
Antioxidant Enzymes levels (SOD µmol/g Hb)				
Experimental Group	46.28±1.25	29.85±1.47	16.43	p<0.001
Control Group	45.22±2.14	44.98±2.09	0.24	p=0.07
Antioxidant Enzymes levels (GLT Peroxidase µmol/g Hb)				
Experimental Group	35.92±3.56	22.56±4.58	13.36	p<0.001
Control Group	38.56±2.12	39.47±2.01	0.91	p=0.25
Marker of Oxidative Damage (Lipid peroxidation levels nmol/mg)				
Experimental Group	2.25±1.4	5.58±1.58	3.33	p<0.01
Control Group	2.01±0.85	1.98±0.98	0.03	p=0.086

Table 3 shows the gene expression profiles of major antioxidant enzymes such as SOD and GLT peroxidase, as well as an oxidative damage marker, in the diabetic-induced and control groups. The results showed that in the diabetic group, the expression of genes encoding antioxidant enzymes was downregulated, indicating a reduction in antioxidant defense. On the other hand, the diabetic group had significantly higher levels of an oxidative damage marker's gene expression, indicating greater OS and cellular damage. The significance of OS in the progression of diabetes is highlighted by these findings, which shed light on the molecular processes that are responsible for the imbalance between antioxidant defenses and oxidative damage in the disease.

Table 3 Gene Expression of major antioxidant enzyme and marker of oxidative Damage

Variables	Expression value	Level of significance p-value
SOD		
Experimental Group	0.98±0.54	p<0.05
Control Group	1.56±0.23	
GLT Peroxidase		
Experimental Group	0.76±0.23	p<0.05
Control Group	1.85±1.01	
Marker of Oxidative Damage (Lipid peroxidation)		
Experimental Group	1.97±0.85	p<0.05
Control Group	0.48±0.2	

DISCUSSION

The creature based concentrate on utilized an exploratory model to assess the relationship among operating system and diabetes. In terms of the rats' baseline parameters, there was initially no significant weight difference between the control and experimental groups. In any case, following a month and a half, the rodents in the exploratory gathering had put on

weight essentially more than the benchmark group. All through the examination, the degrees of ROS were surveyed. From baseline to week six, the experimental group's ROS levels significantly increased, indicating an increase in OS. ROS levels, on the other hand, did not significantly rise in the control group. We measured the antioxidant enzymes' activity, particularly SOD and GLT peroxidase. The action of the two compounds in the exploratory gathering declined decisively during a six-week time frame, showing debilitated cell reinforcement safeguard components. The control group, on the other hand, maintained rather steady levels of antioxidant enzyme activity. Levels of lipid peroxidation, a sign of oxidative damage, were also measured. Lipid peroxidation rose considerably from baseline to week six in the diabetic-induced group, indicating greater cellular damage owing to OS. In addition, gene expression study of important antioxidant enzymes such as SOD and GLT peroxidase, as well as an oxidative damage marker, was carried out. The findings demonstrated that the expression of genes encoding antioxidant enzymes was downregulated in the diabetic group, while the expression of the oxidative damage marker was dramatically elevated. These results shed light on the molecular pathways that underpin OS in diabetes. The study emphasizes the crucial role of OS in the development of diabetes by emphasizing the imbalance between antioxidant defenses and oxidative damage. According to the evidences Diabetes mellitus is associated with long-term innate immune activation that results in chronic inflammation[14]. Inflammatory mediators such as interleukin-1-beta, 6 and tumor necrosis factor-alpha have been associated to type 2 diabetes and contribute to the production of ROS[15]. When combined with these inflammatory variables, hyperglycemia increases the generation of ROS. Furthermore, a faulty antioxidant defense system, whether caused by endogenous changes or external deficiencies, can tip the balance in favor of free radicals, resulting in OS. Numerous experimental and clinical research have found a link between OS and diabetes mellitus[16-17]. This relationship is demonstrated by oxidative indicators tested in diabetic and non-diabetic animals and people. The data shows that OS plays a significant role in chronic hyperglycemic-induced insulin resistance[18]. Furthermore, hyperglycemia causes the release of inflammatory mediators that are mediated by OS, confirming the link between diabetes, OS, and inflammation. In another such evidence authors had established the effects of antioxidants and antioxidants enzyme system in improving wound healing process among Diabetes mellitus patient and it was found that diabetic wounds present considerable problems in the healing process due to variables such as hyperglycemia, neuropathy, decreased blood flow, altered matrix turnover, wound contraction, and microbiota disruptions. Through integrated signaling pathways, OS plays a critical role in wound healing regulation. As a result, controlling reactive oxygen species (ROS) levels using antioxidants and antioxidative enzyme systems has the potential to reduce OS-induced damage and improve wound healing in diabetics[19]. Researchers want to investigate new treatment options for enhancing wound healing outcomes and minimizing complications associated with diabetic wounds by addressing the complex link between OS and diabetes mellitus. The strength of the animal-based study is its controlled experimental approach, which allows for a direct evaluation of the relationship between OS and diabetes in a controlled setting. Increasing scientific rigor and providing crucial insights into molecular pathways are made possible by the utilization of an animal diabetes model, which enables manipulations and evaluations that would not be possible in human trials. Besides, utilizing quantitative biochemical tests to measure operating system markers and cancer prevention agent protein action adds objectivity to the discoveries, supporting their validity. However, the study's translation of data from an animal model to human patients may be problematic due to species-specific biological reactions. In addition, despite the work's useful mechanistic insights, further clinical validation is required to ensure that it accurately depicts the complexity of diabetes mellitus and the problems it causes in actual populations.

CONCLUSION

In conclusion, the animal diabetes model used in the laboratory-based research provided fresh perspectives on the connection between OS and diabetes mellitus. The findings emphasize that OS plays a crucial role in diabetes development

and progression, particularly when antioxidant defense systems are compromised. The noticed downregulation of cell reinforcement compound qualities and overexpression of provocative markers in diabetics features the convoluted cooperation among operating system and irritation in diabetes. The significance of comprehending the molecular mechanisms underlying insulin resistance and beta cell damage caused by OS is emphasized by these findings. The examination adds to the growing group of data focused on at further developing diabetes the board and limiting diabetic inconveniences by recognizing conceivable treatment choices to diminish operating system and speed up injury mending. However, additional research is required to verify these findings in clinical settings and investigate the translational implications of OS-related therapies in human diabetic patients. Generally speaking, our examination gives light on atomic experiences into the connection among operating system and diabetes, giving the structure to future exploration and maybe innovative strategies to further develop diabetes treatment and patient results.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

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