

Upregulation of Apoptotic Markers p53 and Caspase-3 in Cardiac Tissue of Diabetic Rats

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Abstract

Diabetes is a metabolic disorder associated with hyperglycemia. Among the various complications of diabetes, cardiovascular disease remains the leading cause of mortality in diabetic patients. This study aimed to investigate the expression of p53 and caspase-3 in the cardiac tissue of diabetic rats and to assess their association with the progression of diabetes. A single intraperitoneal injection of alloxan induced diabetes at a dose of 120 mg/kg. Rats with blood glucose levels exceeding 250 mg/dL were classified as diabetic. After the experiment, all animals were euthanized, and blood samples and cardiac tissue specimens were collected for further experiments. Biochemical assessments revealed significant increases in blood glucose, cholesterol, and triglyceride levels in the diabetic group compared to the controls. Immunohistochemical analysis showed elevated expression of both p53 and caspase-3 in the hearts of diabetic rats. Notably, the increase in caspase-3 expression was statistically significant ($p = 0.001$), whereas the increase in p53 expression did not reach statistical significance ($p = 0.077$). These findings suggest that diabetes induces cardiac stress and may activate apoptotic pathways, potentially contributing to the development of cardiovascular complications.

Keywords: Diabetes, p53, Caspase 3, Apoptosis, Heart

1. Introduction to Diabetes and Cardiovascular Complications

Diabetes mellitus has emerged as a global health crisis (Molehin et al., 2020). It is estimated that 463 million adults worldwide are affected by diabetes mellitus, with approximately 90% of cases attributed to type 2 diabetes (Srinivas et al., 2021). There is a growing recognition among healthcare professionals that diabetes is closely linked to cardiovascular diseases, which significantly contribute to the increased morbidity and mortality observed in diabetic patients (Setia Santoso et al., 2024). Cardiovascular complications arise from a combination of metabolic disturbances, including insulin resistance, hyperglycemia, hyperlipidemia, hypertension, and chronic inflammation, that collectively exert harmful effects on cardiac function (Nabrdalik et al., 2023). Among these, ischemic heart disease represents the most severe myocardial complication, which may progress to heart failure, depending on the extent of cardiac damage (Chen et al., 2022).

2. The Molecular Mechanisms of p53 and Caspase 3 in Cell Apoptosis

The tumor suppressor protein p53 plays a central role in regulating a wide range of cellular processes, including apoptosis, thereby contributing to the maintenance of cellular homeostasis (Mijit et al., 2020). Its activation is typically triggered in response to cellular stress signals such as genomic instability, DNA damage, and hypoxia, leading to the initiation of apoptotic pathways in compromised cells (Feroz & Sheikh, 2020). Once activated, p53 transcriptionally modulates a variety of target genes involved in critical cellular functions, including cell cycle arrest, senescence, apoptosis, and DNA repair (Lacroix et al., 2020). Emerging evidence suggests that both intrinsic (mitochondrial) and extrinsic (death receptor-mediated) apoptotic pathways may be involved in p53-mediated apoptosis (Capuozzo et al., 2022). In addition to its role in orchestrating apoptosis, recent findings indicate that caspases—key effector proteins in the apoptotic cascade—may also regulate the activation and function of p53, suggesting a bidirectional relationship between p53 signaling and caspase activity (Wang et al., 2023).

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Caspase-3 is recognized as one of the principal executioner enzymes in the apoptotic pathway, playing a pivotal role in the final stages of programmed cell death (Avagimyan et al., 2022). It exerts its effects by cleaving key intracellular substrates, including structural proteins and non-caspase proteases, thereby inducing nuclear condensation, DNA fragmentation, and the formation of apoptotic bodies—hallmarks of apoptotic cell death (Chien et al., 2020). Accumulating evidence suggests a strong association between p53 activation and the induction of caspase-3 activity (Peng et al., 2022). Specifically, p53 upregulates caspase-3 mRNA expression and facilitates its translocation into the nucleus. Conversely, inhibition of p53 via antisense expression vectors has been shown to suppress both the transcriptional and catalytic activity of caspase-3, indicating that p53 may function as an upstream regulator of caspase-3 expression (M. Wang et al., 2022). These findings support the notion that caspase-3 activation is at least partially mediated through p53 signaling pathways (De Geest & Mishra, 2022).

Importantly, dysregulation of apoptosis has been increasingly implicated in the development and progression of diabetic cardiomyopathy. Understanding the molecular interplay between p53 and caspase-3 in the diabetic heart is crucial, as it may reveal novel therapeutic targets. Targeting these apoptotic mediators could offer promising strategies for modulating cardiomyocyte death and mitigating cardiac dysfunction associated with diabetes (Tan et al., 2020).

Recent studies have highlighted the critical role of apoptotic markers, particularly p53 and caspase-3, in the progression of diabetic cardiomyopathy. Tang et al. (2024) demonstrated that irisin ameliorates cardiac injury in diabetic rats by reducing p53 activation and subsequent caspase-3 expression, indicating the central role of p53-mediated apoptosis in diabetic heart damage. Similarly, Khaledi et al. (2023) reported that aerobic exercise reduces cardiac apoptosis by lowering p53 and caspase-3 levels, suggesting physical activity as a protective intervention against diabetes-induced cardiac dysfunction.

Phytochemicals have also been investigated; luteolin was shown to suppress p53 and caspase-3 expression, mitigating metabolic syndrome-related cardiac injury. Mechanistically, Zeng et al. (2021) revealed that SIRT3 reduces p53 acetylation, improving cardiomyocyte metabolism and decreasing apoptosis, highlighting a regulatory pathway involving p53 in diabetic cardiomyopathy. Furthermore, Gu et al. (2018) demonstrated that pharmacological inhibition of p53 reduces early apoptosis and cell senescence in diabetic hearts, underscoring p53 as a potential therapeutic target.

Collectively, these findings emphasize the pivotal involvement of p53 and caspase-3 in diabetic cardiac apoptosis and suggest that targeting these pathways may provide effective strategies to prevent or treat diabetic cardiomyopathy.

3. Study objectives

This study examined the expression of p53 and caspase 3 in the hearts of diabetic rats and their potential correlation with the development of type 1 diabetes.

4. Study methodology

4.1. Induction of Type 1 Diabetes

Following a one-week acclimatization period, the rats were randomly assigned to either the control group (n = 10) or the diabetic group (n = 10). To induce type 1 diabetes (T1D), rats in the diabetic group received a single intraperitoneal injection of alloxan monohydrate (120 mg/kg; Sigma-Aldrich) following a 12-hour fasting period, whereas the control group received an equivalent volume of physiological saline. Blood glucose levels were monitored every day using a Glucocheck glucometer (Homemed Pty Ltd., South Africa). Rats with blood glucose levels ≥ 200 mg/dL were considered diabetic and were subsequently euthanized. Following established protocols, cardiac tissue was immediately harvested post-mortem for histological and immunohistochemical analysis. The methodological procedures employed in this study were adapted from previously published protocols (Alsarhan et al., 2020; Amawi et al., 2019).

4.2. Histopathological and immunohistochemical analysis

The harvested cardiac tissue specimens were fixed in 10% neutral-buffered formalin for 24 hours. Following fixation, the tissues were processed using an automated tissue processor, embedded in paraffin, and sectioned at a thickness of 5 μ m. The sections were deparaffinized in a 65 °C incubator for 60 minutes and subsequently rehydrated through a graded series of alcohols. Endogenous peroxidase activity was blocked by incubating the sections in 1% hydrogen peroxide prepared in absolute methanol for 20 minutes. After rinsing with phosphate-buffered saline (PBS, pH 7.2–7.4) for 5 minutes, the sections were incubated with 1% bovine serum albumin (BSA) for 30 minutes to minimize non-specific binding. During this incubation period, primary antibodies and associated reagents were brought to room temperature. Monoclonal antibodies against p53 and caspase-3 (Santa Cruz Biotechnology) were diluted at a ratio of 1:100 and applied to the sections, which were then incubated in a humidified chamber for one hour. Following primary antibody incubation, the slides were washed with PBS for 5 minutes and subsequently incubated with biotinylated secondary antibodies for 20 minutes. After another 5-minute PBS wash, the sections were treated with streptavidin–horseradish peroxidase conjugate for 20 minutes. Diaminobenzidine (DAB) was used as the chromogen, and color development was monitored until a brown precipitate became visible, indicating positive staining. The reaction was terminated by rinsing the slides with tap water. The slides were then counterstained with hematoxylin for 30 seconds, rinsed with water, dehydrated through graded alcohols, cleared in xylene, and mounted using a suitable mounting medium. All procedures were performed under established protocols previously described by Alsarhan et al. (2020) and Alawneh et al. (2021).

4.3. Results Interpretation

Levels of expression of p53 and caspase-3 proteins were quantified using Adobe Photoshop Software version 7.2, following established protocols (Alsarhan et al., 2020; Amawi et al., 2019). Immunohistochemically stained

tissue sections were photographed at high resolution, and the images were analyzed by pixel segmentation. The number of brown pixels, which represent positive protein expression, was calculated and compared to the total number of pixels within each image. Protein expression was then expressed as the ratio of brown pixels to total pixels, providing a quantitative measure of immunoreactivity.

4.4. Data Analysis

Statistical analyses were conducted utilizing SPSS software (version 21.0, SPSS Inc., Chicago, IL, USA), with statistical significance established at a p -value of less than 0.05.

5. 5. Results

5.1. Biochemical findings

Table 1 presents a comparative analysis of glucose, cholesterol, and triglyceride levels between the control and diabetic groups. The diabetic cohort exhibited a mean glucose concentration of 250 ± 24.3 mg/dL, whereas the control group had a mean glucose level of 97.5 ± 8.4 mg/dL. This difference in glucose levels was statistically

significant ($p < 0.001$). Additionally, the diabetic group showed significantly elevated mean concentrations of both cholesterol and triglycerides compared to the control group, with both differences reaching statistical significance ($p < 0.001$).

Table 1. Biochemical profiles of glucose and lipid levels in study groups

Variable (M±SD)	Control group(M±SD)	Diabetic group(M±SD)	Significance
Glucose (mg/dl)	97.5±8.4	250±24.3	<0.001
Cholesterol (mg/dl)	72.4±7.6	130±15.6	<0.001
Triglyceride (mg/dl)	84.8±8.5	120.4±18.8	<0.001

5.2. Histological studies of the heart

Figure 1 illustrates the histological features of cardiac tissue in the control group. The cardiac muscle fibers are arranged in close apposition, exhibiting a regular and organized structure. The nuclei of the cardiomyocytes are predominantly centrally located, as indicated by the arrow.

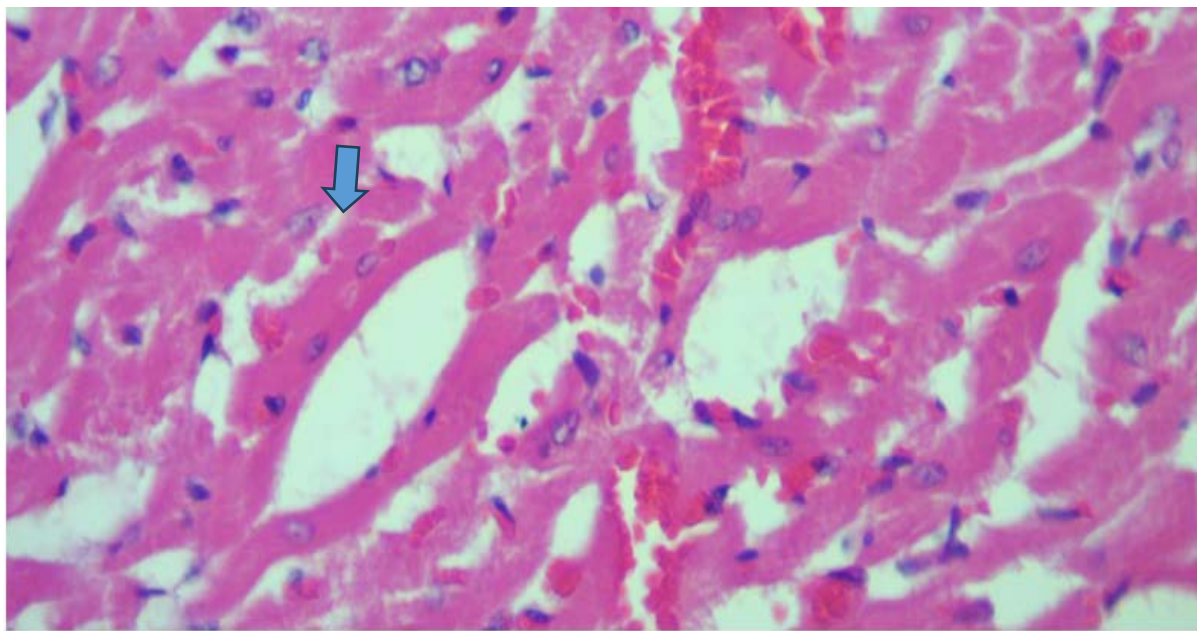


Figure 1. The histological features of heart tissue in the control group, Hematoxylin and Eosin stain, 40X

The histological features of cardiac tissue in the diabetic group are depicted in Figure (2). Three characteristics were exhibited. The eosinophilic cytoplasm of cardiac cells in the diabetic cohort was noted,

accompanied by an enlargement of the cardiac cells. The position of the nucleus appears to have been displaced from the center of the cell to its periphery (indicated by arrows).

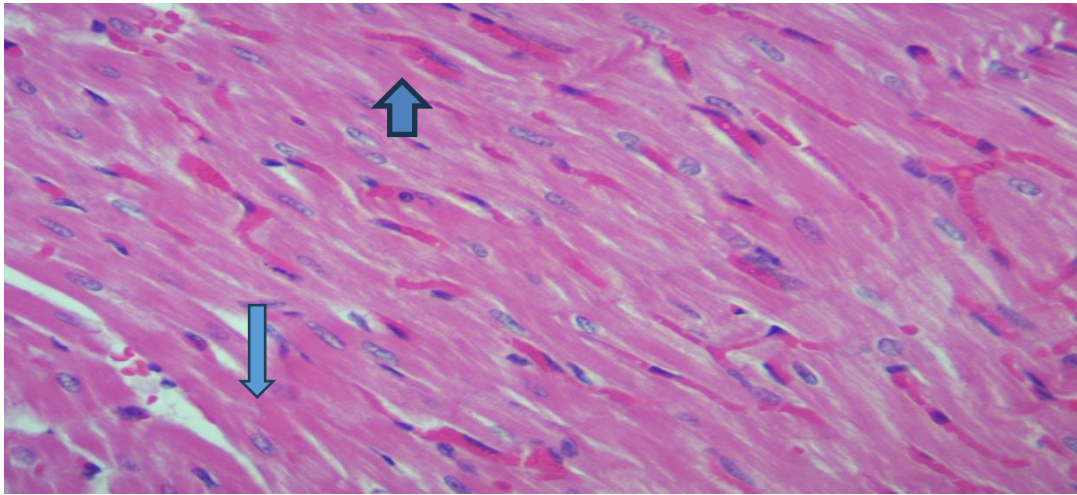


Figure 2. Histological characteristics of the cardiac tissue in the rats with diabetes, Hematoxylin and Eosin stain, 40X

5.3. Expression of Caspase-3

Figure 3 shows the expression of caspase-3 in the cardiac tissue of the non-diabetic (control) group.

Moderate expression of caspase-3 is observed in various regions of the myocardium, indicating a baseline level of apoptotic activity under normal physiological conditions.

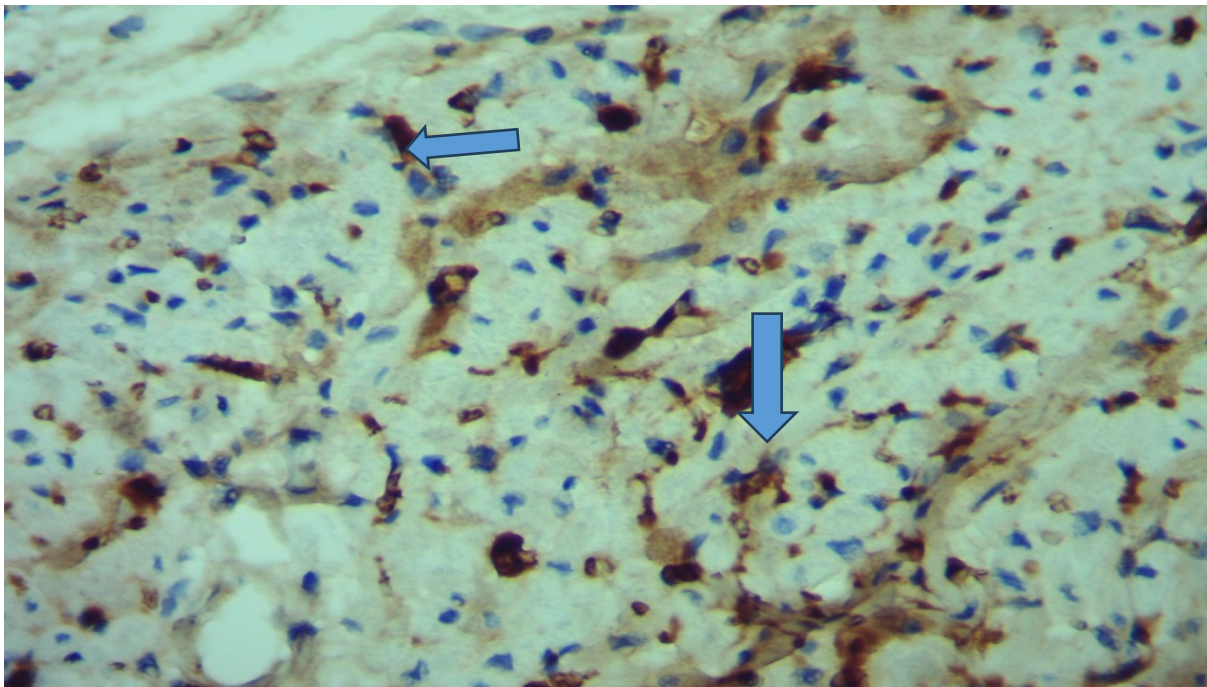


Figure 3. Expression of caspase-3 in the cardiac tissue of the control group. Indirect immunohistochemical staining for caspase-3 at 40× magnification. Moderate expression is evident in myocardial fibers, with localized intense staining indicated by the arrow.

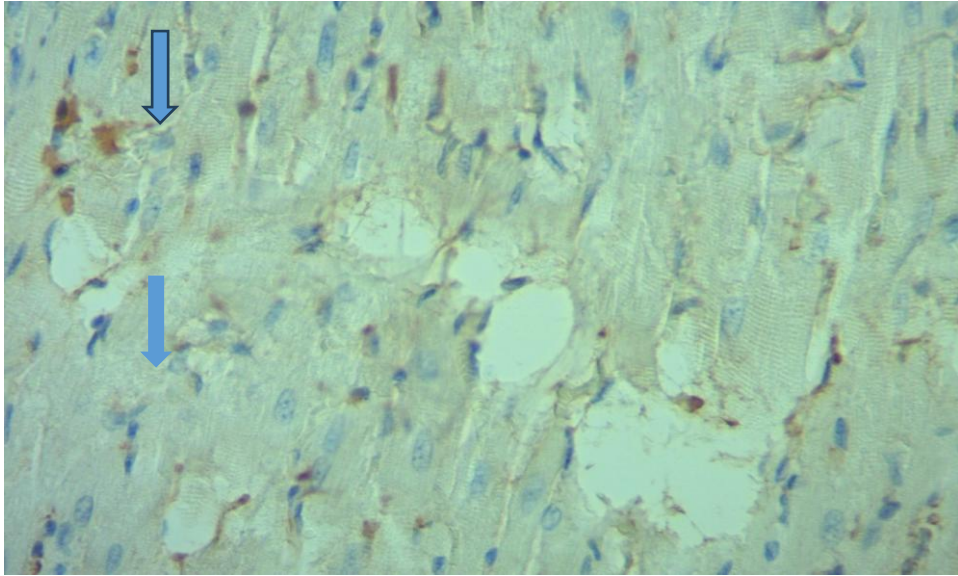


Figure 4. Expression of caspase-3 in the cardiac tissue of diabetic rats. Indirect immunohistochemical staining for caspase-3 at 40× magnification. A noticeable reduction in staining intensity is observed compared to the control group, indicating decreased caspase-3 expression.

5.4. The expression of p53 in the heart tissue

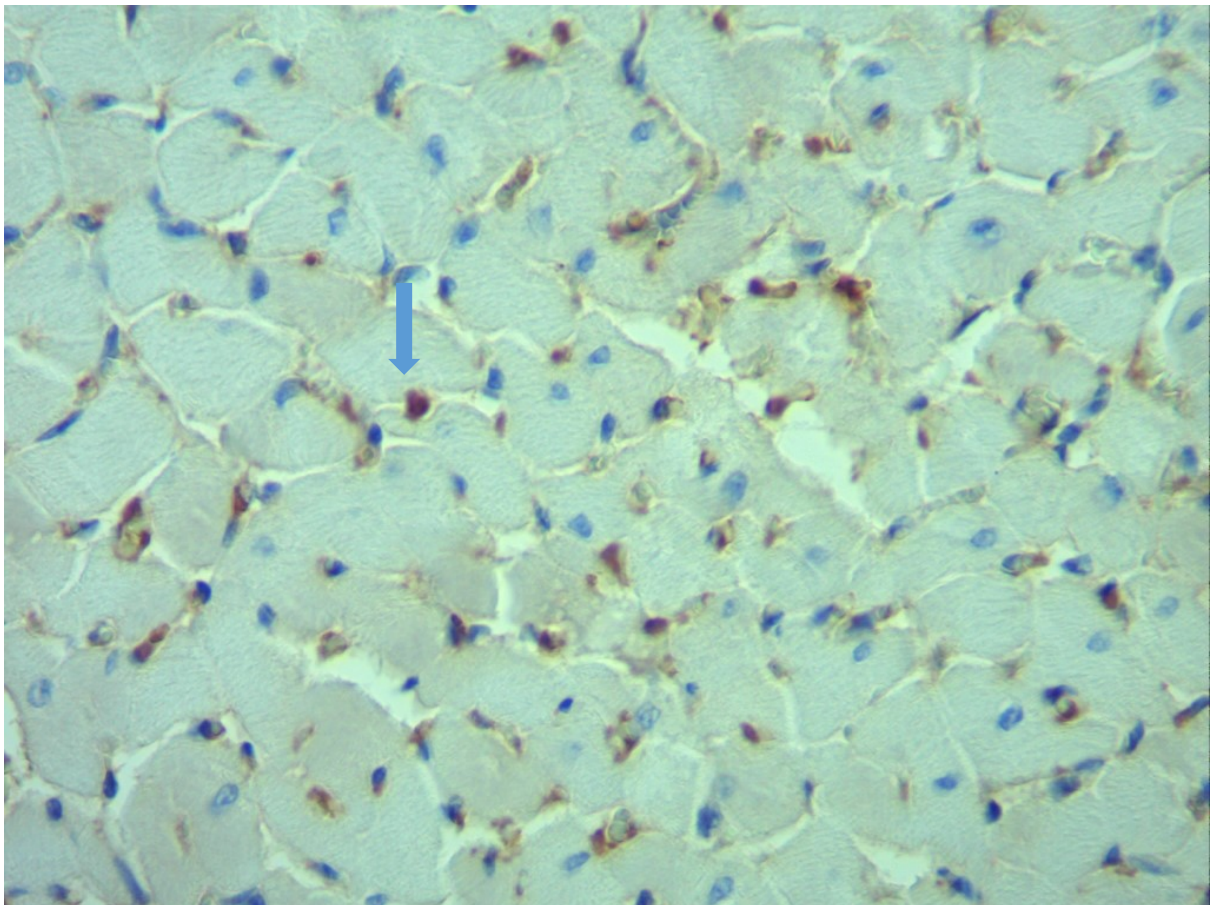


Figure 5. The expression of p53 in the heart tissue of non-diabetic rats, as shown by indirect immunohistochemical staining of p53 at 40X, reveals low levels of p53 expression, with minimal staining observed in myocardial cells.

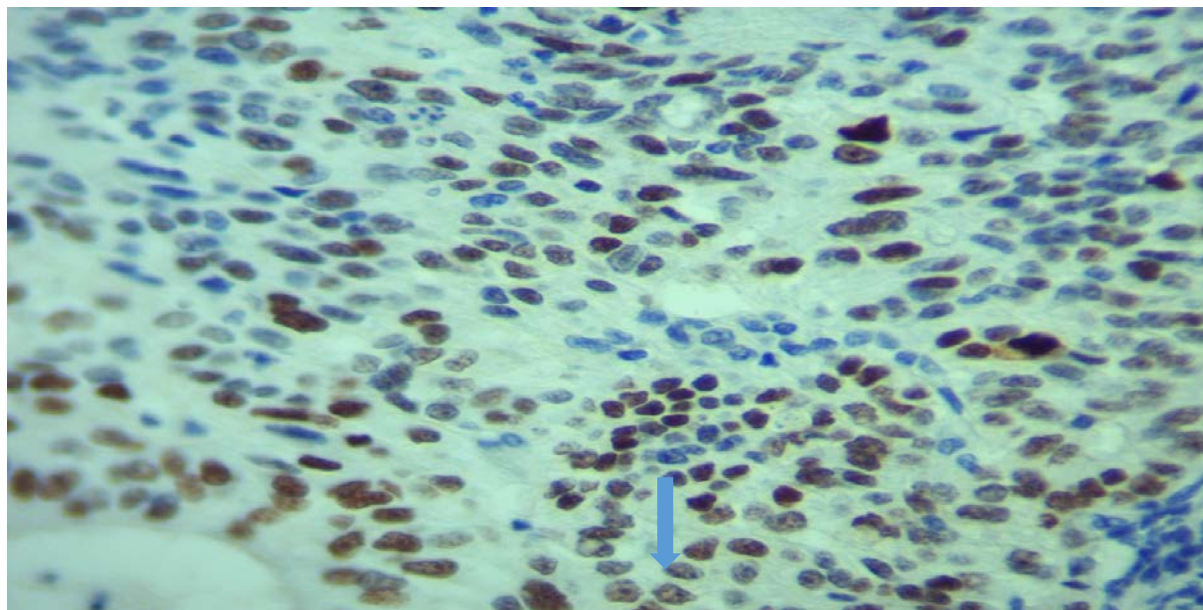


Figure 6. The expression of p53 in the heart tissue of the diabetic rats, indirect immunohistochemical staining of p53, 40X.

5.5. Expression Levels of Caspase-3 and p53

As presented in Table 2, the mean expression levels of caspase-3 and p53 were quantified and compared between the control and diabetic groups. The mean p53 expression in the control group was $10,495.59 \pm 12,150.47$ pixels, which increased to $15,909.37 \pm 21,382.77$ pixels in the diabetic group. However, this difference was not statistically significant ($p = 0.077$). In contrast, caspase-3 expression in the control group averaged $14,703.03 \pm 14,619.24$ pixels and was significantly reduced in the diabetic group, with a mean of $5,533.15 \pm 8,253.87$ pixels ($p = 0.001$).

Table 2. Expression levels of Caspase 3 and p53

Biomarkers	Groups	N	Mean	Std. Deviation	P value
p53	Control	32	10495.5938	12150.47345	0.077
	Diabetes	32	15909.3750	21382.77069	
Caspase 3	Control	32	14703.0313	14619.24565	0.001
	Diabetes	32	5533.1563	8253.87784	

6. Discussion

6.1. Biochemical findings

Biochemical analysis revealed a marked elevation in blood glucose levels in diabetic rats, primarily attributable to impaired glucose utilization, which leads to the disruption of glycemic homeostasis (Sharma et al., 2022). These significant and varied findings underscore the importance of understanding how diabetes alters metabolic processes.

Diabetes alters lipid metabolism, often leading to hypercholesterolemia. Increased cholesterol levels, especially low-density lipoprotein (LDL), are closely linked to a higher risk of cardiovascular disease in individuals with diabetes (Manrique-Acevedo et al., 2024). Notably, higher cholesterol levels are observed in the diabetic group compared to the control group.

Additionally, triglycerides play a critical role in lipid distribution. Previous studies showed that dyslipidemia, characterized by high cholesterol and excessive lipids in the blood, is prevalent among diabetic patients and contributes substantially to the heightened incidence of cardiovascular complications in this population (Bakillah et al., 2023)(Zheng & Lind, 2024).

6.2. Histological studies of the heart

Histological analysis of cardiac tissues from both normoglycemic and diabetic groups reveals pronounced structural alterations associated with diabetes. These observations suggest that diabetes induces significant morphological and functional changes in the myocardium (Ritchie et al., 2020). In the control group, cardiomyocytes exhibit a well-organized, linear alignment with centrally located nuclei. The myocardial architecture remains intact, with no evidence of cellular degeneration or disruption, reflecting the structural integrity characteristic of healthy cardiac tissue (Laura et al., 2020).

Moreover, Cardiac tissue from the diabetic group exhibits three prominent histological features. First, the cytoplasm of cardiomyocytes appears more intensely eosinophilic, which may indicate increased cellular stress or elevated intracellular protein accumulation as previously reported (Shah et al., 2021).

Additionally, the cardiomyocytes exhibit hypertrophy, as evidenced by an increase in cell size—an adaptive response commonly associated with elevated workload or chronic stress on the heart (Ritchie & Abel, 2020). Notably, the nuclei have shifted from their typical central position, as seen in the control group, to a more peripheral location. This nuclear displacement may indicate pathological remodeling or cellular damage, potentially linked to diabetic cardiomyopathy (Calcagno et al., 2022).

6.3. Immunobiological studies

Elevated p53 expression has been linked to cardiovascular conditions, including diabetic cardiomyopathy, due to its role in cellular stress responses. Caspase-3, a key executioner enzyme in the apoptotic

pathway, serves as a reliable marker of apoptosis in cardiac tissue (Yadav et al., 2021a; 2021b). In diabetic rats, a significant reduction in myocardial caspase-3 expression, as shown in Figure 4, suggests impaired apoptotic activity. This disruption may lead to the accumulation of damaged or dysfunctional cells, contributing to cardiac dysfunction (Jia et al., 2020).

p53 is a key protein involved in DNA repair and the regulation of apoptosis, particularly in response to cellular stress (Shi & Dansen, 2020). In non-diabetic rats, its expression in myocardial tissue is minimal, as observed in the control group (Lazzarini et al., 2022).

Figure 6 illustrates increased p53 expression in multiple regions of the myocardium in diabetic rats as previously reported (Chen et al., 2023). Although this increase did not reach statistical significance ($p = 0.077$), it suggests heightened cellular stress, with cells potentially responding to DNA damage to maintain homeostasis (Kung & Murphy, 2016).

6.4. Limitations and future perspectives

This study provides important insights into the role of p53 and caspase-3 in diabetic heart damage, but several limitations exist. Although animal models offer valuable information on the pathophysiological mechanisms of diabetic cardiomyopathy, they do not fully replicate the complex metabolic and cardiovascular disturbances present in human diabetes, which may limit the direct applicability of the findings. Moreover, the study's focus on only two apoptotic markers provides a narrow assessment of the broader apoptotic and cell-death pathways involved in diabetic heart damage. Additionally, no therapeutic agents or interventions were evaluated, restricting the study's ability to determine whether modulation of these pathways could offer potential cardioprotective benefits.

Future studies should explore additional apoptotic and stress-related markers (e.g., Bax, Bcl-2, TNF- α , cytochrome c) to better characterize the mechanisms of cell death in diabetic hearts. Investigating potential therapies that target apoptosis and confirm findings in human or more clinically relevant models will strengthen the transnational value of this research.

7. Conclusions:

This study demonstrates that diabetes significantly impairs metabolic regulation. The pronounced alterations in glucose, cholesterol, and triglyceride levels underscore the necessity for stringent monitoring and management of these metabolic parameters in diabetic patients. Histopathological modifications, coupled with dysregulated expression of apoptotic and stress-related proteins in cardiac tissue, indicate an elevated risk of cardiovascular complications associated with diabetes. These findings warrant further investigation into targeted therapeutic approaches to mitigate such adverse effects. In summary, comprehensive metabolic and histological evaluations are essential for a thorough understanding of diabetes pathophysiology, facilitating the development of improved clinical strategies and enhanced patient outcomes.

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