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Original Article

RAS-Related C3 Botulinum Toxin Substrate 1 Inhibition Attenuates Platelet Chemokine Activation in Diabetes Mellitus

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ABSTRACT

Diabetes mellitus (DM) is a prevalent cause of platelet (PLT) activation. Inflammation-induced dysregulation of PLT function adds to chronic complications. Ras-related C3 botulinum toxin substrate 1 (Rac1), a 21 kDa G-protein, has been shown to modulate many PLT activities. We hypothesized that Rac1 may influence the PLT release of CXCL4 and CCL5, thereby contributing to macrovascular and microvascular problems in DM. The study included Swiss albino male mice pretreated with the Rac1 inhibitor NSC23766 and streptozotocin (STZ) to induce diabetes. A sample of 150 diabetic patients and 50 healthy controls was also analyzed. Statistical analyses were performed using Mann-Whitney tests on one hundred fifty confirmed diabetic patients who visit Layla Qasim health center for diabetes management, and 50 healthy individuals were included in this study. The serum CXCL4 and CCL5 in diabetic patients and healthy volunteer were measured. Swiss albino male mice received pretreatment of 5 mg/kg of the Rac1 inhibitor NSC23766, followed by injection of streptozotocin at a dosage of 45 mg/kg body weight, twice daily for five days. Rac1 activity in the PLT was measured using pulldown assay and Western blot method. Blood chemokine concentrations were also assessed using ELISA, and histological scores for the kidney, liver, pancreas, and lung were evaluated. CXCL4 and CCL5 levels were markedly elevated in DM patients compared to healthy individuals. Our findings indicated that streptozotocin induced diabetes mellitus in mice. GTP-Rac1 was induced in diabetic mice, and pretreatment with NSC23766 was significantly lower compared to vehicle group. Furthermore, diabetic mice showed significantly greater levels of CXCL4 and CCL5 ($P < 0.05$) compared to the sham group. CXCL4 levels were reduced by 80% following Rac1 inhibition ($P < 0.05$), while CCL5 levels decreased by 55.5% ($P < 0.05$). The current study indicates that Rac1 plays a pivotal role in releasing PLT chemokines due to diabetes-induced inflammation in several organs, and inhibiting Rac1 may represent a novel therapeutic approach to managing inflammation in diabetic individuals.

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1. Introduction

Diabetes mellitus (DM) is a metabolic illness characterized by elevated blood glucose (BG) levels, which may significantly impact several organs in the body (1). The global prevalence of diabetes is on the rise, with an estimated 439 million individuals projected to have the condition by 2030 (2). Chronic hyperglycemia, a characteristic of diabetes, exacerbates vascular complications and elevates morbidity and death rates (3). One of the main risk factors for diabetes is cardiovascular disease (CVD), and it has been shown that hyperglycemia increases the generation of reactive oxygen species (ROS), especially in the blood vessel lining (4). Diabetic patients also exhibit a hypercoagulable state, marked by increased platelet (PLT) adhesion and aggregation. Platelet-derived microparticles (PMPs) are formed during PLT activation and have been implicated in various conditions (5). In diabetes, high levels of cell adhesion molecules are observed, and macrophages and monocytes play essential roles in the progression of microvascular complications. Chemokines such as CXCL4 and CCL5, which are small cytokines involved in leukocyte activation and inflammation, are stored in PLT secretory α-granules and attract neutrophils and monocytes (6).

Type 2 diabetes (T2DM) is often accompanied by dyslipidemia, characterized by abnormal lipid profiles, including elevated low-density lipoprotein (LDL) and triglyceride levels, along with decreased high-density lipoprotein (HDL) levels. Dyslipidemia is a significant risk factor for coronary artery disease (CAD) and is likely caused by insulin's effects on liver apoprotein formation, lipoprotein lipase control, and peripheral effects on muscle and adipose tissue. Proper management of lipid profiles is crucial for controlling diabetes and reducing associated complications and mortality rates (7).

The liver is also affected by diabetes. Elevated serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamyltransferase (GGT) are specific markers of hepatic injury, and elevated serum levels of these enzymes are frequently observed in diabetic individuals. T2DM is associated with liver diseases such as cirrhosis, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and ultimately hepatocellular carcinoma (7).

Rac1, a small G protein, plays an essential role in intracellular signaling pathways leading to NADPH oxidase activation, which in turn generates ROS. Rac1 activation is essential for the assembly of NADPH oxidase subunits and subsequent ROS production. However, the activation of Rac1 and its impact on PLT chemokines in diabetes are not yet fully understood. Previous studies have shown the Rac1's involvement of in PLT chemokine secretion and activation in other conditions such as septic lung injury and pancreatitis (8). While the role of PLTs in diabetes is well-documented, the specific contribution of Rac1 to PLT chemokine secretion remains unexplored, representing a significant gap in our current understanding. For the first time, the present study, investigates the role of Rac1 in PLT chemokine secretion in diabetes.

Objectives

This study aims to evaluate PLT chemokines in diabetic patients and investigate the role of Rac1 in DM through the activation of PLT chemokines CXCL4 and CCL5. Furthermore, the current study seeks to evaluate the impact of Rac1 on morphological changes in DM and to elucidate the underlying mechanisms involved in diabetes and its complications.

2. Materials and Methods

2.1. Animals

Swiss albino male mice, aged 8 to 9 weeks and weighing 20 to 25 g, were used in all experiments. The Regional Animal Experimentation Ethical Committee has approved the pharmacy department at Hawler Medical University in Iraq, which complies with laws pertaining to animal welfare standards. Patients with an HbA1c > 6.5% and a verified diagnosis of R2DM were included. The mice were maintained in a pathogen-free setting with a 12-hour light and 12-hour dark cycle. Food and water were supplied twice daily with acess to clean, fresh drinking water via a particular nipple. Following a seven-day acclimatization phase, the trials were conducted. The animals were divided into three groups:

sham (saline injection), vehicle (streptotocin injection), and treatment (NSC+STZ) ,which included pretreatment with NSC23366 and streptozotocin. A maximum of five mice per group were housed in cages with environmental enrichment. Each group contained five mice.

2.2. Materials

The chemical used to induce diabetes mellitus was streptozotocin (STZ; Glentham Life Science, Ltd., U.K.) (9). To make NaOH, the buffer used to prepare streptozotocin, 10.7 g of sodium citrate was dissolved in 200 ml of distilled water, followed by the addition of 9.6 g

of citric acid. Distilled water was then added to reach to 1,000 ml. The solution's pH was adjusted to 4.5 by adding NaOH to the solvent. Chem Cruz, Santa Cruz Biotechnology, California;

NSC23766 (N6-[2-[[4-(Diethylamino) -1-methylbutyl] amino] -6-methyl- 4-pyrimidinyl] -2 methyl-4, 6-quinolinediamine trihydrochloride). The BG meter used was Accu-Chek Active.

2.3. Animal Experiments

An intraperitoneal injection of 5 mg/kg of Rac1 inhibitor (NSC23766) was administered to the animals. This dosage was selected according to previous studies (10). After half an hour, the experimental mice received numerous intraperitoneal injections of STZ (45 mg/kg) to induce diabetes mellitus. Mice received STZ for five consecutive days after it had been dissolved in a buffer containing 0.01 M sodium citrate (pH = 4.5). This STZ dosage was chosen based on earlier research (11).

Subsequent to the injection, the animals were administered a glucose solution (5 percent w/v) for nighttime consumption to mitigate the hypoglycemia induced by STZ. Sham mice received an equal dosage of the vehicle (citrate buffer)mice. Following dosing, the mice were kept in their usual environments for five days and treated with STZ and NSC+STZ. Mice developed diabetes after these times, as shown by fasting BG levels of around 11.1 mmol/L. After a 12-hour fast, blood samples were collected from the tail vein of mice,which were administered STZ and NSC+STZ, to assess blood glucose levels. In diabetic mice, fasting BG levels exceeding 11.1 mmol/L were considered to be indicative of diabetes (12). They were thus chosen for more research. 75 mg of ketamine hydrochloride (HoffmanLa Roche, Basel, Switzerland) and 25 mg of xylazine (Janssen Pharmaceutica, Beerse, Belgium) kg-1 were administered intravenously to induce sedation. Vena Cava's blood was collected ,and the animals were subsequently euthanized. The serum was allowed to coagulate at room temperature for ten to twenty minutes and then centrifuged at 2000–3000 RPM for 20 minutes. The serum was separated, stored at 80°C, and prepared for a subsequent ELISA test. The kidney, liver, lung, and pancreas were preserved in formaldehyde for histological analysis.

2.4. Biochemical Determination

The BG levels of each experimental animal were measured before the process began. Regular fasting BG checks were performed until diabetes was diagnosed. Mice were considered diabetic if their fasting BG level was 11.11 mmol/L or higher. Blood samples (2–3 µL) were collected from the tail veins of all animals. The BG levels were monitored using an Accu-Chek active BG meter.

2.5. ELISA

Serum levels of CXCL4 and CCL5 were successfully measured in all groups, including diabetes patients, healthy controls, and mice. For animal samples, ELISA was performed using the Mouse Platelet Factor 4 ELISA Kit, BT LAB Cat. No E0686Moand a kit from Bender MedSystems, Vienna, Austria. Serum CXCL4 and CCL5 levels in diabetic patients and controls were measured using enzyme-linked immunosorbent assay (ELISA) with the Human Platelet Factor 4 ELISA Kit, BT LAB Cat. No E5885Hu ,and the Human regulated activation in normal expressed and secreted T-cell, RANTES, SUNLOG Cat. No SL1526Hu. Each microplate had a standard curve, generated by diluting a known concentration standard per the manufacturer's guidelines, with absorbance measured at 450 nm. The chemokine concentration of each sample was determined by computing the mean absorbance across wells and applying a logistic curve-fitting method. All absorbance values fell within the linear segment of the standard curve. The data were represented in both ng/ml and pg/ml, respectively.

2.6. Histopathology

Kidney, liver, lung, and pancreatic tissues were fixed overnight in 10% formaldehyde phosphate buffer before dehydration and paraffin embedding. Four-micrometer sections were stained with hematoxylin and eosin. A modified scoring system was used to evaluate renal injury in a blinded manner, including mixed inflammatory cells, necrosis (irreversible damage), apoptosis, fibrosis, vascular congestion, edema and degeneration (irreversible damage). Infiltration was scored on a scale from 0 to 4, where zero indicated absence and four indicated substantial infiltration (12). Histopathological grading used a modified approach that assessed necrosis, inflammation, and fibrosis on a scale from 0 to 4, where 0 denotes no harm and 4 denotes significant injury. The mean value was determined after evaluating five random sites within each tissue sample. The overall histopathological score was obtained by summing the scores of all six criteria.

2.7. Statistics

The data were presented as mean values with standard error of the mean (SEM). Non-parametric tests were selected due to the non-normal distribution of the data. Statistical analyses were conducted using nonparametric testing (Mann-Whitney). N denotes the total count of mice in each group, and a P-value of less than 0.05 was considered significant. Statistical analysis was conducted using SPSS (IBM Corp., Armonk, N.Y., USA).

3. Results

In the study of 150 DM patients, we assessed clinical and laboratory data. 46% (69 individuals) were male, and 54% (81 individuals) were female (Table 1). The age range the diabetic patients were 8–76 years ,with a mean age of 52.185±2.275 years. The BG profile results showed that all patients were hyperglycemic. Table 1 displays the overall HbA1c percentage and fasting blood sugar (FBS) levels for the groups. The control group (CG), consisting of healthy individuals without diabetes, had an age range of age 9-78 years and mean age of 50.560±2.773.

Table 1 shows the means and standard error of all the lipid profile parameters of the participants analyzed. The results indicate significant differences in all parameters between diabetic and non-diabetic individuals. The findings suggest that the mean values of T-CHO (198.4), TG (316.97), and LDL-C (137.04) in diabetes patients were substantially greater than those of non-diabetic participants, which were 160.26, 134.28, and 95.32, respectively, with a P-value <0.05. The mean HDL-C level in diabetes patients was 32.37, considerably lower than the mean value of non-diabetic participants at 40.55 (P<0.05).In clinical practice, liver function tests (LFTs) are frequently used to screen for liver disease, monitor the development of established disease, and keep tabs on the effects of medicines that could be hepatotoxic. The serum aminotransferases and alkaline phosphatase are the two most prevalent LFTs. Aminotransferases, such as ALT and AST, quantify intracellular hepatic enzymes that have entered the bloodstream, serving as indicators of hepatocyte damage. ALP is a marker for cholestasis and biliary function. Both serum ALP and AST levels significantly increased in diabetic patients 137.80±16.68, 32.26±3.86 compared to CG 21.69±2.13 20.671±1.51, respectively (P<0.05) (Table 1).The ALT level in diabetic patients was higher than in healthy individual, however there was no significant increase in patient with diabetes 26.20±3.08 compared to CG 21.69±2.13, representing approximately a 1.2-fold increase.

3.1. Evaluation of Plt CXCL4 Level in DM Patients

Plasma level of CXCL4 levels increased in response to DM in diabetic patients (Figure 1A). Among 200 individuals, plasma level of CXCL4 levels was measured, CXCL4 levels of 150 diabetic patients were significantly higher (15.30±1.59) than in 50 healthy controls (6.76±1.97) (P < 0.05) (Figure 1A), representing more than a 2-folds increase. CXCL4 levels increased by more than 2-fold in diabetic patients compared to controls (P<0.05). Pretreatment with NSC23766 reduced CXCL4 levels by approximately 80% (P < 0.05).

3.2. Evaluation of Plt CCL5 level In DM patients

In response to DM, plasma levels of CCL5 in diabetic individuals increased (Figure 1B). 150 diabetes patients had significantly higher CCL5 levels (15243.56± 1748.32) than 50 healthy controls (8767.16± 232.82) of the 200 subject individuals whose plasma levels of CCL5 were tested (Figure 1B). P-value<0.05 suggests an increase in 42%.

3.3. Streptozotocin- induced DM in mice

The mean FBS for the CG (sham) was 85.00±6.00 mg/dl. When mice were injected with 45 mg/kg body weight with STZ, their FBS levels were considerably higher than those of the sham group (P < 0.05). However, the elevated blood sugar caused by streptozotocin was significantly reduced in the group that received Rac1 inhibitor treatment. The FBS of mice was decreased from 556.2026.65 to 375.2020.7 with 5 mg kg1 of the Rac1 inhibitor (NSC23766), with a P-value of 0.05 after pretreatment. Thus, NSC23766 attenuates Rac1 activation, preventing elevated BG that may trigger PLT activation (Figure 2).

3.4. Role of Rac1 in DM

Rac1 belongs to the Rho family of small GTPases, which regulates cellular growth, differentiation, migration, and inflammation. However, there is still no proof of Rac1 signaling involvement in PLT-dependent inflammation in DM and its consequence. The study demonstrates, for the first time, the role of Rac1 in the release of PLT chemokines associated with DM. In order to understand Rac1's function in vehicle animals that were streptozotocin-induced, we employed the Rac1 inhibitor NSC23766. Serum from control, STZ challenge, and animals treated with the particular Rac 1 inhibitor was collected for a Rac1 activation experiment in order to study how STZ activates the Rac1 GTPase.

Table 1: Basic characteristics of participants.

Variables	Non-Diabetes (n=50)	Diabetes (n=150)	P-Value
	Sex		
Male	22	69	
Female	28	81	
	Age		
Years	9-78	8-76	
Mean±SE	50.560± 2.773	52.185± 2.275	
	FBS		
Mean±SE	92.8±1.24	160.25±59.21	<0.05
	HbA1c		
Mean±SE	5.31±0.04	9.49±0.26	<0.05
	Cholesterol		
Mean±SE	160.26±7.25	262.88±16.61	<0.05
	Triglycerides		
Mean±SE	134.28±17.96	199.14±145.78	<0.05
	LDL cholesterol		
Mean±SE	95.32±5.85	137.04±8.01	<0.05
	HDL cholesterol		
Mean±SE	40.55±1.54	32.37±1.51	<0.05
	AST		
Mean±SE	20.671±1.51	32.26±3.86	<0.05
	ALT		
Mean±SE	21.69±2.13	26.20±3.08	<0.05
	ALP		
Mean±SE	86.125±8.39	137.80±16.68	<0.05

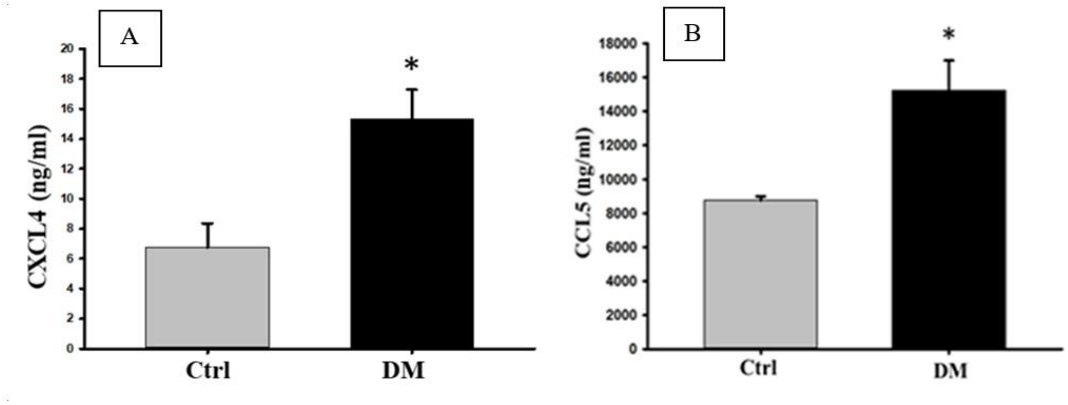

Figure 1. Activated PLTs secrete chemokines in diabetic patients. ELISA was used to quantify the levels. (**A)** CXCL4 in the diabetic patients' serum. (**B)** CCL5 in the diabetic patients' serum. Data represent mean ± SEM (Ctrl=50, DM=150).

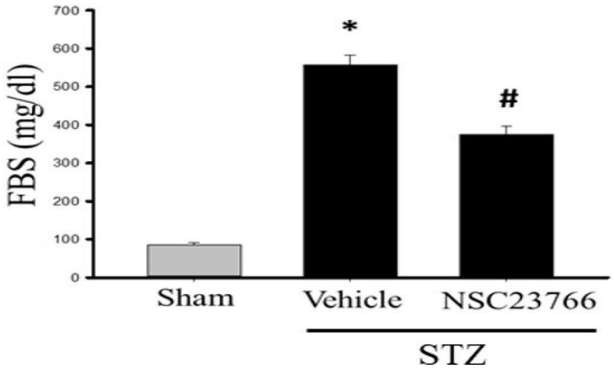

Figure 2. BG levels. Fasting BG levels were assessed on the harvest day after a 5-day induction of mice with STZ (Vehicle) and pretreatment with the Rac1 inhibitor (NSC23766). The data are shown as mean ± SEM (sham=5, vehicle=5).

Rac1 becomes more active (GTP-binding form) after exposure to STZ. Rac1 activation caused by STZ was decreased by pretreatment with the Rac1 inhibitor NSC23766 (Figure 3).

3.5. Rac1 Regulates Plt Secretion of CXCL4 in Diabetic Patients

In sham mice, DM elevated plasma CXCL4 levels from 6.40±0.4 ng/ml to 13.60±1.32 ng/ml, representing a 2.12-fold increase. We induced diabetes mellitus in diabetic mice by administering 45 mg/kg body weight of SZT into the plasma. This suggests that DM stimulates the PLT chemokine production of CXCL4. Notably, DM-induced PLT aggregation and chemokine secretion in PLTs were considerably decreased by NSC23766, a Rac1 inhibitor. This indicates that NSC23766, an efficient inhibitor of Rac1 activation, also reduces the elevated level of PLT chemokines. Pretreatment with NSC23766 reduces CXCL4 serum levels in diabetic mice by more than 80%, from 13.601.32 ng/ml to 7.20±0.8 ng/ml (Figure 4).

3.6. Rac1 regulates PLT secretion of CCL5 in diabetic patients

Plasma CCL5 levels increased from 21.800±1.908 pg/ml in sham mice to 67.400±7.332 pg/ml due to DM, representing a threefold elevation. We found that administering SZT (45 mg/kg body weight) into plasma promotes diabetes mellitus, suggesting that diabetes stimulates the release of the chemokine CCL5 in Plts of diabetic mice. Notably, the Rac1 inhibitor (NSC23766) greatly decreased PLT aggregation and chemokine release induced by DM, demonstrating that NSC23766, an efficient Rac1 activation inhibitor, also inhibits the elevated level of PLT chemokines. In diabetic mice, pretreatment with NSC23766 reduced blood CCL5 levels from 67.4±7.332 pg/ml to 30±2.236 pg/ml, a decrease more than 55.5%. Inhibiting Rac1 may reduce CXCL4 and CCL5 levels, thereby alleviating PLT-driven inflammation, a significant factor in the development of microvascular and macrovascular problems in diabetes (Figure 5).

3.7. Histopathological Alterations in the Kidney

While the treatment group (induced diabetes) showed modest vacuolar degenerations and dispersed chronic inflammatory cell infiltration, the kidney's histopathological alteration in comparison to the normal CG revealed normal kidney histology architecture. Following therapy, there will be a decrease in both degeneration and inflammatory cells (Figure 6 B). When diabetes was induced using streptozotocin, the kidney damage was considerably more severe in diabetic mice than in the sham group (1.13±0.08) ($P<0.05$). Additionally, histology score in these mice decreased to 0.26±0.21 after receiving NSC23766, with a p-value <0.05 (Figure 6A).

3.8. Histopathology Alterations of Liver

The histological examination of the normal control liver revealed intact hepatic architecture. In contrast, the treatment group (induced diabetes) exhibited vascular congestion, degenerative hepatocytes (reversible damage), and dispersed chronic inflammatory cell infiltration (lymphocytes). However, minimal vascular congestion was observedin the group pretreated with NSC23766 (Figure 7 B). Our findings indicated severe liver damage in diabetic mice. Figure 7 shows a significant increase in liver damage caused by streptozotocin injections compared to the CG (1.40.08) ($P < 0.05$). Conversely, NSC23766 decreased the histology score to 0.73 ±0.06 with a P-value of less than 0.05 (Figure 7 A).

3.9. Histopathology Alterations of Lung

The histopathological examination of the normal control lung showed typical histological architecture, whereas the treated group (induced diabetes) exhibited pathological alterations, including moderate destruction of the alveolar structure, loss of normal lung characteristics, and moderate infiltration of mixed inflammatory cells with tissue destruction. After treatment, mild mix inflammatory cell infiltration was observed. A substantial degree of lung injury was demonstrated by our results (Figure 8 B). Streptozotocin injection caused a notable increase in lung damage in diabetic mice compared to the CG 1.26±0.20 ($P<0.05$) (Figure 8). Moreover, NSC23766 treatment decreased the mice's histological score to 0.38 ±0.046 ($P<0.05$) (Figure 8 A).

3.10. Histopathology Alterations of Pancreas

The histopathological examination of the pancreas in the normal CG revealed typical pancreatic histological architecture, while the treated group exhibited vascular congestion, mild degeneration, and reversible injury to the pancreatic beta cell islets, along with scattered inflammatory cells (lymphocytes) and fibrosis (fibroblasts). While after giving treatment there was evidence of regeneration and restoration of the relatively normal architecture of islets of the pancreas, including beta cells (Figure R9 B). Streptozotocin-induced diabetes in mice significantly increased the risk of pancreas injury by 0.83±0.14 ($P<0.05$) (Figure 9).

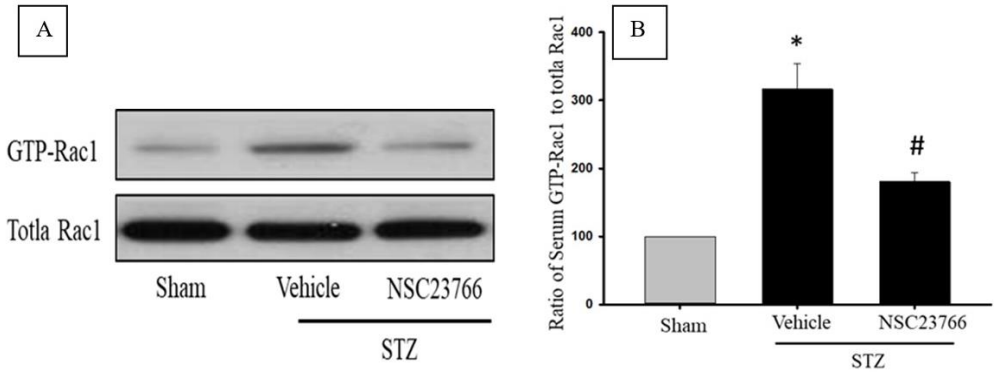

Figure 3. Rac1 activity in serum of DM-induced mice. (A) Rac1-GTP was quantified using western blotting employing GST-PAK pull-down beads upon STZ induction. (B) Band intensities were assessed using densitometry and normalized to the overall Rac1 levels. Western blots exemplify three distinct experiments. Mice received treatment with the Rac1 inhibitor NSC23766 (5 mg/kg) or vehicle administered 30 minutes before STZ induction. Sham mice functioned as negative controls. The bars indicate the mean ± SEM, with n = 3.

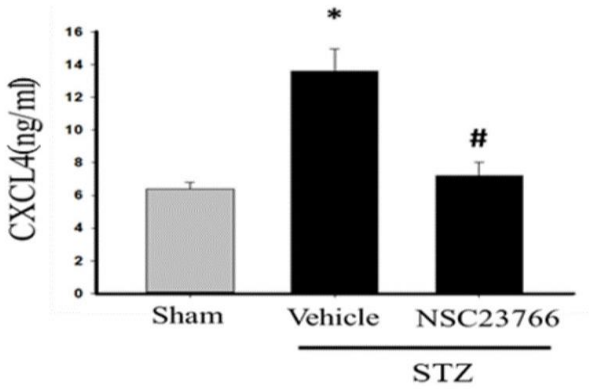

Figure 4. Activated PLTs release chemokines in diabetic mice. ELISA was used to quantify the amounts of CXCL 4 in the serum of diabetic mice. Data are presented as mean ± SEM (sham=5, vehicle=5).

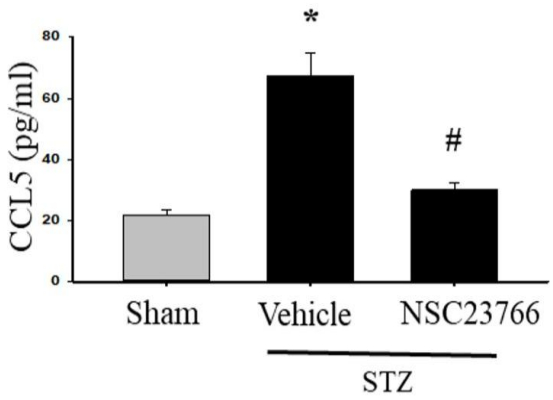

Figure 5. Activated PLTs release chemokines in diabetic mice. ELISA was used to measure the concentrations of CCL5 in the plasma of diabetic mice. The data are shown as mean ± SEM (sham=5, vehicle=5).

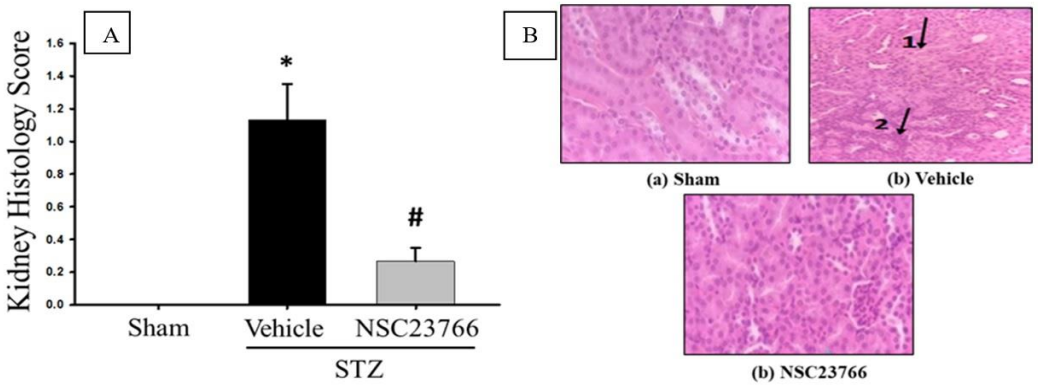

Figure 6. (A): Rac1 regulates kidney damage in DM. The kidney's histopathology score. (B): In DM, Rac1 controls kidney injury. The kidney's representative portions (H&E X100) are shown. (a) The renal histology of the CG is normal. (b) Vacuolar degeneration (arrow 2) and dispersed chronic inflammatory cells (arrow 1). (c) Inflammatory cells and vacuolar degeneration were reduced in the treatment group.

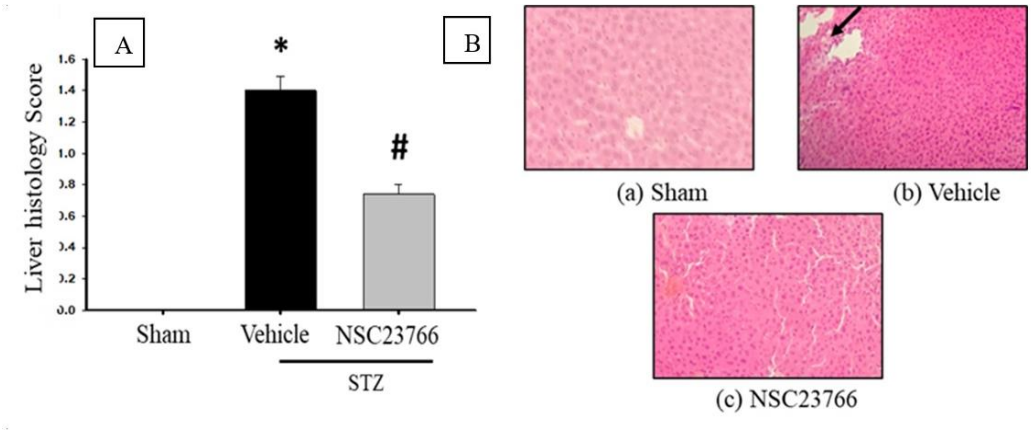

Figure 7. (A): Rac1 regulates liver damage in DM. Histology score in the liver. (B): In DM, Rac1 controls liver damage. The liver's representative H&E X100 slices are shown. (a) Normal liver histology in a healthy CG patient. (b) Dispersed chronic inflammatory cell infiltration, vascular congestion, and degenerative hepatocytes as shown by the vehicle's arrow. (c) Only minimal vascular congestion was seen in the treatment group.

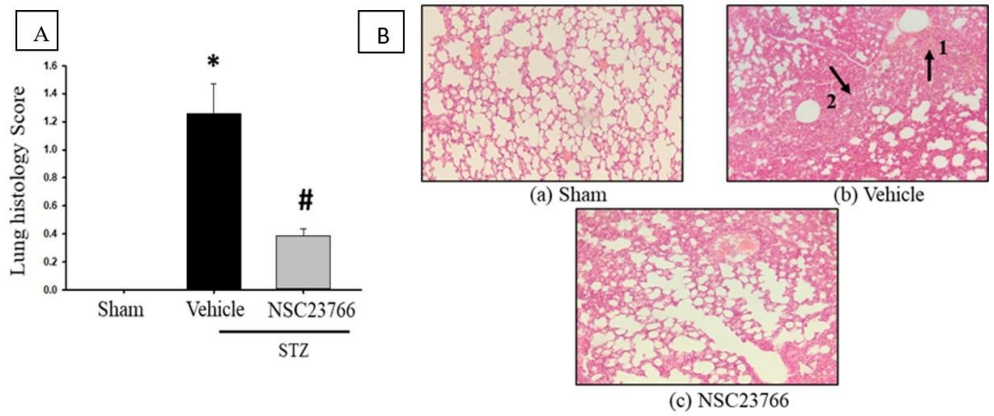

Figure 8. (A): Rac1 regulates lung damage in DM. Histology score in the lung. **(B):** Rac1 regulates lung damage in DM. Representative (H&E X100) sections of the lung are shown. (a) Normal histology of the lung of normal CG. (b) Moderate destruction to the alveolar architecture, loos of lung features and moderate scattered mix inflammatory cells infiltration with tissue destruction as shown in (arrow 1 & 2). (c) Treatment group showed mild mix inflammatory cell infiltration.

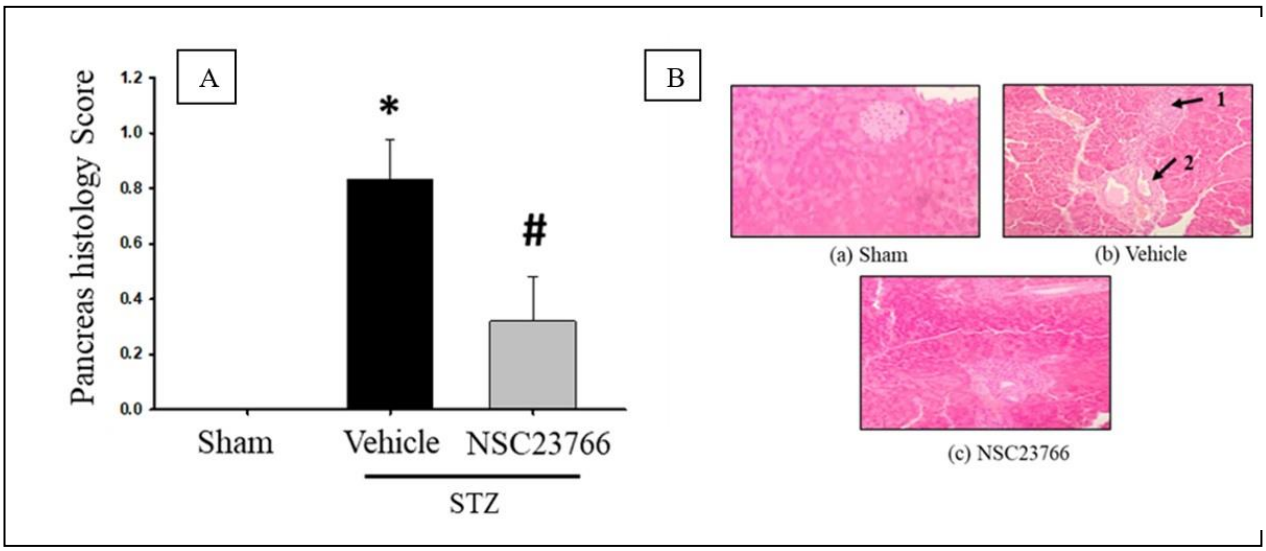

Figure 9. (A): Rac1 regulates pancreas damage in DM. Histology score in the pancreas. (B): Rac1 regulates pancreas damage in DM. Representative (H&E X100) sections of the pancreas are shown. (a) Normal histology of the pancreas of normal CG. (b) vascular congestion, mild degeneration and damage to the islets of pancreas beta cells as shown in (arrow 1), and scattered inflammatory cell (lymphocyte) and fibrosis (fibroblast) (arrow 2). (c) Treatment group regenerative ability with the restoration of the relatively normal architecture of islets of the pancreas including beta cells

4. Discussion

Diabetes mellitus is a chronic metabolic condition marked by elevated blood glucose levels, potentially resulting in significant consequences that impact many organs in the body. The WHO reports a consistent rise in the global prevalence of diabetes. If this trend continues, DM is estimated that the global prevalence of diabetes will reach 439 by 2030. Chronic hyperglycemia, a defining feature of diabetes, is responsible for the development of vascular problems that contribute to increased morbidity and mortality rates (2). Furthermore, DM is a major risk factor for CVD (13). Hyperglycemia, particularly in the endothelial lining of blood vessels, increases the production of ROS, leading to vascular complications (14). In fact, vascular complications associated with diabetes are responsible for more than 75% of deaths among diabetic patients (16). In addition to its role as a risk factor for complications, DM is also associated with a hypercoagulable state, with diabetic patients exhibiting increased PLT adhesion and aggregation (17). PMPs, which are released during PLT activation or physical stimulation, contribute to this process. PMPs consist of membranous micro vesicles and fragments with coagulative activity (18). Diabetic patients, both with type 1 and T2DM, have been found to have elevated levels of cell adhesion molecules, such as PAM-1 and soluble P-selectin, further emphasizing the involvement of PLTs in diabetes-related complications (19).

Additionally, macrophages and monocytes are essential in the development of microvascular problems in diabetes (20). Chemokines, such as CXCL4 (PLT factor 4) and CCL5 (RANTES) are small cytokines that bind to specific G protein-coupled receptors (GPCRs) to regulate leukocyte activation and migrationto inflammation sites. PLT α-granules store chemokines like CXCL4 and CCL5, which attract neutrophils and monocytes (21). These chemokines can form heteromers, such as CXCL4-CCL5 that have a strong effect on the induction of monocytes and neutrophils (22). Dyslipidemia, which is defined as increased LDL cholesterol and triglyceride levels and reduced HDL cholesterol, is common in T2DM and a major risk factor for CAD. Dyslipidemia in T2DM is influenced by insulin resistance and abnormal lipoprotein metabolism, including impaired lipoprotein lipase (LpL) activity and altered peripheral insulin effects on muscle and adipose tissue. Therefore, close monitoring of these parameters is crucial .The liver is significantly affected by diabetes. Specific markers of hepatic injury, such as ALT, AST and GGT, are often elevated in diabetic individuals. Hepatocellular carcinoma and cirrhosis may arise from liver illnesses including NAFLD and NASH, which have been linked to T2DM (7).

Excess free fatty acids, resulting from insulin resistance, can directly damage liver cells through as oxidative stress, mitochondrial malfunction, and cell membrane disruption. The small G protein Rac1 is a crucial signaling molecule associated with diabetes, since it participates in intracellular transduction pathways that activate NADPH oxidase. Rac1 requires isoprenylation to move from the cytosol to the plasma membrane, where it facilitates NADPH oxidase assembly, a vital process in the generation of ROS. Elevated Rac1 activation may exacerbate vascular oxidative stress due to hyperglycemia. While the function of Rac1 in PLT chemokine secretion and activation has been investigated in a number of diseases, including pancreatitis and septic lung damage, research on its activation and effects on PLT chemokines in diabetes is still continuing.

Inhibition of Rac1 signaling with NSC23766 reduces PLT activation and chemokines secretion. Rac1 signaling is elevated in active PLT and controls PLT-derived CXCL4 in DM. Rac1 also controls PLT secretion of CCL5, a powerful stimulator for neutrophil buildup in DM. Finally, Rac1 inhibition attenuates PLT activation by decreasing chemokines secretion. Future studies should investigate the long-term effects of Rac1 inhibition on diabetic complications in clinical settings and explore potential combination therapies targeting multiple inflammatory pathways.

One limitation of the current study was the use of a single animal model, which may limit generalizability to human populations. Further research is needed to explore the precise signaling pathways through which Rac1 modulates chemokine release in diabetic conditions.

In conclusion, diabetes is a complex metabolic disorder affecting multiple organ systems. PLTs, chemokines, dyslipidemia, and liver dysfunction all contribute to the pathogenesis of diabetes and its associated complications. Understanding the molecular pathways that govern diabetes and its complications is essential for formulating successful therapies. Examining the function of Rac1 and PLT chemokines in diabetes may provide significant insights into the disease's causes and facilitate the creation

of tailored therapeutics to prevent or alleviate diabetes-related problems.

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Authors' Contribution
Study concept and design: R. H, H. J, T. S, M.M.
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Acquisition and interpretation data. T. S, M. M.
Drafting of the manuscript: M. M, R. A.
Critical revision of the manuscript for important intellectual content: M. M, R.A.
Statistical analysis: R. H, H. J.
Administrative, technical, and material support: M. M.
Study supervision: M. M.

Ethics
Ethical permission for the human research was obtained from the Institutional Review Board of Layla Qasim Health Center. All individuals provided written signed informed consent prior to participation.

Conflict of Interest
The author declares that there are no conflicts of interest.

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Data Availability
The data supporting the findings of this study are available upon request from the corresponding authors.

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