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ROLE OF THE COAGULATION SYSTEM IN β CELL LOSS INDUCED BY STREPTOZOTOCIN (STZ): FIBRINOLYTIC FUNCTIONAL INSIGHTS

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder that dysregulates blood glucose levels. This study aims to assess the role of the coagulation system activation in the initiation of type 1 diabetes millets (T1DM) and the potential effect of certain fibrinolytic agents, specifically streptokinase (STK) and alteplase (ALT) against T1DM and insulin secretion. **Methods:** Sixty male Balb/c mice were randomly distributed equally into six groups including normal control, STK, ALT, streptozotocin (STZ), STZ + STK and STZ +ALT. Induction of T1DM was carried out using i.p injection of STZ at a dose of 55 mg/kg body weight for 5 consecutive days in the presence or absence of a single dose of STK (i.v in 8000 IU/kg) or ALT (i.v. in 0.9 mg/kg) on the fifth day of the experiment. Protein expression in the pancreatic tissues for insulin, PAR-2, p-AKT, and PI3K was assessed using immunofluorescence assay and histopathological and nuclear changes were evaluated using hematoxylin and eosin and DAPI stains. Serum insulin level, blood glucose and platelet count were recorded. **Results:** STZ-induced diabetic mice demonstrated increased expression of PAR-2, p-AKT and PI3K along with notable histopathological and nuclear changes, while insulin expression significantly decreased in pancreatic islets compared to control mice. Conversely, STZ-induced diabetic mice treated with STK or ALT improved the protein expression of insulin, PAR-2, p-AKT and PI3K as well as improved histopathological and nuclear changes caused by STZ-induction.

Conclusion: Activation of the coagulation system plays a vital role in the development β -cell death and hyperglycemia in T1DM. In addition, STK and ALT mitigated the adverse effects resulting from STZ-induction with STK demonstrating superior efficacy compared to ALT in preventing the diabetes onset.

Keywords: Coagulation system; Fibrinolytic; Type 1 diabetes mellitus; Pancreatic β cells; Alteplase; Streptokinase.

INTRODUCTION

Diabetes is an increasingly significant health concern in the 21st century, responsible for approximately 1.5 million deaths globally in 2020 [1]. Despite notable improvements in the field of medicine, cardiovascular disease (CVD) continues to be the primary cause of illness and death among individuals with diabetes. Diabetes increases the likelihood of experiencing vascular problems, leading to a substantial decrease in life expectancy [2]. In addition, those with diabetes experience a more negative prognosis after a vascular event, regardless of the therapeutic approach employed during the early phase, compared to those with normal glucose metabolism [3]. Two key factors contribute to the worse vascular prognosis observed in diabetic patients. Firstly, there is a higher incidence of vascular disease among these individuals, which can be attributed to a combination of metabolic dysregulation and endothelial dysfunction. Secondly, diabetic patients exhibit an increased tendency for thrombus formation, leading to a greater prevalence of blood clots [4].

While it is commonly acknowledged that diabetes plays a role in the production of blood clots, methods used to avoid blood clots, which aim to lower the risk of vascular issues, are largely the same for individuals with and without diabetes [2,4]. Furthermore, therapies mostly focus on regulating platelet activation, whereas the management of the fibrin network is typically overlooked until complications arise, such as atrial fibrillation or valvular heart disease. Hence, to minimize the remaining likelihood of vascular complications in diabetes, it is imperative to devise more efficacious approaches for anti-thrombotic therapy [5].

The relationship between diabetes mellitus and coagulation system, a comprehensive meta-analysis of multiple sizable randomized trials, has revealed that thrombolysis treatment resulted in a notable preservation of 71 lives per 1,000 patients diagnosed with complications of diabetes, in contrast to a comparatively of 15 lives per 1,000 patients without complications of diabetes, as observed over a period of 35 days [6]. Persistent hyperglycemia in diabetes mellitus causes coagulopathies due to the glycation of hemoglobin, prothrombin, fibrinogen, and other proteins involved in the clotting mechanism [7,8].
Agents like STK and ALT have demonstrated promising efficacy in managing complications of diabetic patients, with reduced occurrence of adverse events and optimal dosage administration [9,10]. On the other hand, the possible role of fibrinolytic agents in the initiation rather than the complication of T1DM is not clear [11].

Therefore, the current study aims to evaluate the involvement of the activated coagulation cascades on pancreatic β-cell survival, insulin secretion, and thereby influencing the development of T1DM. Additionally, the research will assess the potential effects of fibrinolytic agents, specifically STK and ALT, on the prevention of T1DM initiation and their impact on insulin expression. By elucidating these mechanisms, the study seeks to contribute to the development of targeted therapies that may enhance β-cell function and improve metabolic outcomes in individuals at risk for or diagnosed with T1DM.

METHODOLOGY AND MATERIAL

Animals:

This study employed sixty male Balb/c mice, aged 23 ± 2 weeks, with a body weight of 25 grams. The animals were sourced from the animal facility at Qassim University, located in Qassim, Saudi Arabia. Mice were kept in a controlled environment with a temperature range of 23-25 °C with a humidity level of 55% and unrestricted access to food and water. The practical experiments were conducted by the NIH Guidelines for the Care and Use of Laboratory Animals, as well as the regulations set by Saudi Arabia. The research was approved by the Ethical Committee for the Deanship of Scientific Research at Qassim University (24-84-12).

Drugs and antibodies

Streptozotocin (STZ) was purchased from Sigma-Aldrich (MO, USA) and administered as a single IP 55 mg/kg of BW for consecutive 5 days [12]. Alteplase (Actilyse®, Boehringer Ingelheim Limited, GA, USA) and Streptokinase (Karma®, Karma Pharmatech CmbH, Marburg, Germany) were obtained from the Prince Sultan Military Medical City (Riyadh, KSA), and given as a single i.v. in 0.9 mg.kg-1 [13] or 8000 IU/kg, i.v [14] respectively on the fifth day of the experiment. Mouse monoclonal antibodies against insulin, PAR-2 and p-AKT and rabbit polyclonal antibody against PI3K were purchased from Santa Cruz Biotechnology (TX, USA).

Experimental Design

Mice were divided randomly into six weight-matched groups, each of 10 mice. The first group served as normal control group administered with saline for 15 days, while the second and third groups received a single dose of either STK (8000 IU/kg, i.v.) or ALT (0.9 mg/kg-i.v.) on the 5th day of the experiment. The remaining groups (4-6) received STZ 55mg/kg, i.p. for 5 consecutive days [12] starting from day 1 until day 5. Groups 5^{th} and 6^{th} received a single dose of either STK (8000 IU/kg, i.v.) or ALT (i.p in 0.9 mg/kg-i.v.) on the 5^{th} day of the experiment. All the Mice groups were euthanized on the 15^{th} day of the experiment.

Blood sampling and preparation

Mice were anesthetized, and blood was collected from the retro-orbital plexus, then divided into two portions. The first portion was collected in EDTA tubes for estimating complete blood count (CBC). The second portion was collected in centrifuge tubes and then centrifuged for 20 min at 4000 rpm for serum collection. The serum was stored at −20°C and used for the analysis of biomarkers [15].

Tissue sampling and preparation

At the end of the experiment, mice were anesthetized and sacrificed by cervical decapitation. pancreatic tissues were collected and dissected to be fixed immediately in Davidson's solution and embedded in paraffin blocks. These pancreases were used for histopathological examination and immunofluorescence [16].

Determination of biochemical parameters

Determination of serum insulin, blood glucose levels and platelet count

Blood glucose level was directly measured by single drop in mice blood using a glucometer (Uright, Taiwan). On the other hand, assessment of serum insulin was by using mouse insulin ELISA kit from Biovision Inc. (CA, USA). Platelet count was analyzed by auto blood analyzer (ABX Micros 60 Analyzer, Montpellier, France).

Histopathological study

Pancreatic tissues were fixed in Davidson`s solution for twenty-four hours, was done with tap water, and then serial dilutions of concentrations of ethanol (50–100%) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for twenty-four hours. Paraffin beeswax tissue blocks were prepared for sectioning at 4-5 microns thickness by slide [17]. Sections of samples were collected on glass slides, deparaffinized, and stained by hematoxylin & eosin stain for routine examination. The examination was done through the light electric microscope as described by Bancroft and Steven [18].

Immunofluorescence analysis

Pancreatic tissue samples were collected from all groups of mice and kept in Davidson's for 24 hours followed by 70% alcohol. Samples then were dehydrated through gradient concentrations of alcohol 50%, alcohol 70%, alcohol 95%, and absolute alcohol 100%, then immersed in xylene then paraffin at 56℃. After dipping in paraffin blocks, tissues were cut using microtome into 4 μm thickness

sections and fixed on super frosted slides. Slides containing the tissues was processed according to the following steps [17].
Slides was kept in oven (60°C) for 20 min. to melt paraffin and was deparaffinized by washing with xylene (100%) 2 times, 15 min/each. The slides were gradually hydrated by immersing in absolute ethanol 2 times, 5 min/each, then once with ethanol (90%), (75%), (50%), (30%) and distilled water, 5 min/each. The slides were boiled with Dako® citrate buffer (pH 6.0) in the microwave at 500 Watt for 20 min. to retrieve the antigens, then allowed to cool down to room temperature. The slides were washed with washing solution (0.05% twin in phosphate buffered saline) 3 times, 3 min/each. Tissue sections were fixed by adding methanol for 10 min. using dark humidified chamber. After washing, slides were blocked using blocking solution (1% BSA, 10% horse serum and 1% in PBS) for 1 hour at room temperature, using dark humidified chamber. Slides was washed again with washing buffer 3 times, 3 min/each. Sections was incubated for 3 hours at 37°C followed by overnight at 4°C, with the appropriate primary antibodies (mouse monoclonal antibodies against insulin, PAR-2 and p-AKT and rabbit polyclonal antibody against PI3K). After washing, the slides were incubated at 37ºC for 30 min. with Cyanine red (Cy3) conjugated goat anti-rabbit and goat anti-mouse and Alexa 488 conjugated goat anti-mouse secondary antibodies. The slides were washed again with tween/PBS 2 times, 3 min/each. The slides were incubated for 3 min. at 37ºC with 4',6-diamidino-2-phenylindole (DAPI) for nuclear counterstaining. The slides were washed again with a washing buffer 3 times, 10 min/each, then dried and mounted with coverslips using fluoromount® mounting solution (DAKO, Carpinteria, CA, USA). Tissue sections were analyzed, and the images were captured using Leica fluorescence microscope (Model: Leica DM 5500B, Leica Microsystems, Wetzlar, Germany) using blue, green, and red channels. A minimum of 6 fields of each mice section were used in fluorometric analysis of the results using Image-J software (NIH, USA).

Statistical analysis
The statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer for multiple comparisons. $P<0.05$ was considered statistically significant.

RESULTS

Effect of STZ in the presence or absence of STK and/or ALT on serum insulin level, blood glucose level, and platelets (PLT) count
As shown in **Figure 1A**, the serum insulin levels for groups ALT and STK were comparable while STZ-only ($P<0.001$), STZ+ALT ($P<0.05$), and STZ+STK ($P<0.001$) groups decreased significantly when equated to the normal mice group. On the contrary, the insulin levels for STZ+ALT and STZ+STK increased significantly ($p < 0.05$ and < 0.001 respectively) when compared to the STZ-treated group.

Similarly, the serum glucose levels were comparable for ALT and STK groups when equated to the normal group while the serum glucose levels of STZ-only ($P<0.001$), STZ+ALT ($P<0.001$), and STZ+STK ($P<0.001$) groups increased significantly compared to the normal group **(Figure 1B).** The serum glucose level decreased markedly for the STZ+ALT group (<400 mg/ml, $P<0.01$) but was not significantly decreased for the STZ+STK group compared to the STZ-treated group.

As for the platelet counts, the levels remained insignificant for the ALT, STK, STZ+ALT, and STZ+STK groups while the platelet count increased significantly for the STZ-alone treated mice group compared to the normal group **(Figure 1C).** Additionally, the platelet count for the STZ + ALT and STZ + STK groups decreased significantly, ($P<0.01$ and $P<0.05$ respectively) compared to the STZ-treated group.

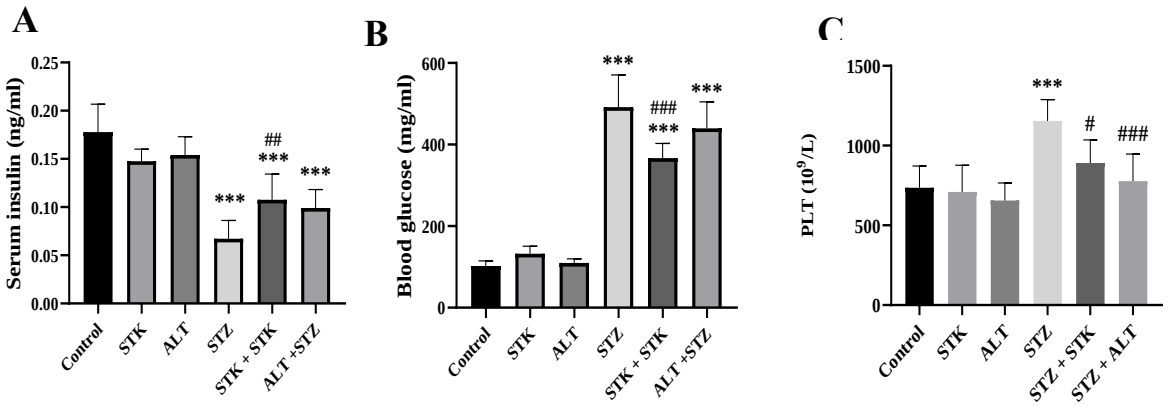


Figure 1. Effect of STZ in the presence or absence of STK and/or ALT serum insulin level (A), blood glucose level (B), and platelets (PLT) count (C). Data in the graph denote as mean ± S.E.M. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer for multiple comparisons where: $^{**}P<0.01$ and $^{***}P<0.001$ considered different significantly when compared to normal mice, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ significantly different considered different significantly in comparison with STZ-treated mice.

Impact of STK or ALT on insulin protein expression in pancreatic β islets of STZ-induced T1DM mice

Fluorescence intensity **(Figure 2A)** was used to assess the expression of insulin protein within pancreatic β islets, the findings revealed a reduction in pancreatic β islet density **(Figure 2B)**, consequently leading to diminished insulin protein expression **(Figure 2C)**. Even though control, STK and ALT-treated mice demonstrated constitutive expression of insulin in pancreatic β islets, nevertheless the insulin expression levels for all the groups ALT (not significant, N.S) and STK ($P<0.05$), STZ-only ($P<0.001$), STZ+ALT ($P<0.001$), and STZ+STK ($P<0.001$) decreased when equated to the normal mice group. STZ-treated mice showed a low number of pancreatic β islets among all the groups and therefore low expression of insulin protein. On the contrary, the expression of insulin markers for STZ-induced mice treated with ALT or STK was enhanced significantly ($P<0.001$) in pancreatic β islets compared to the STZ-alone group.

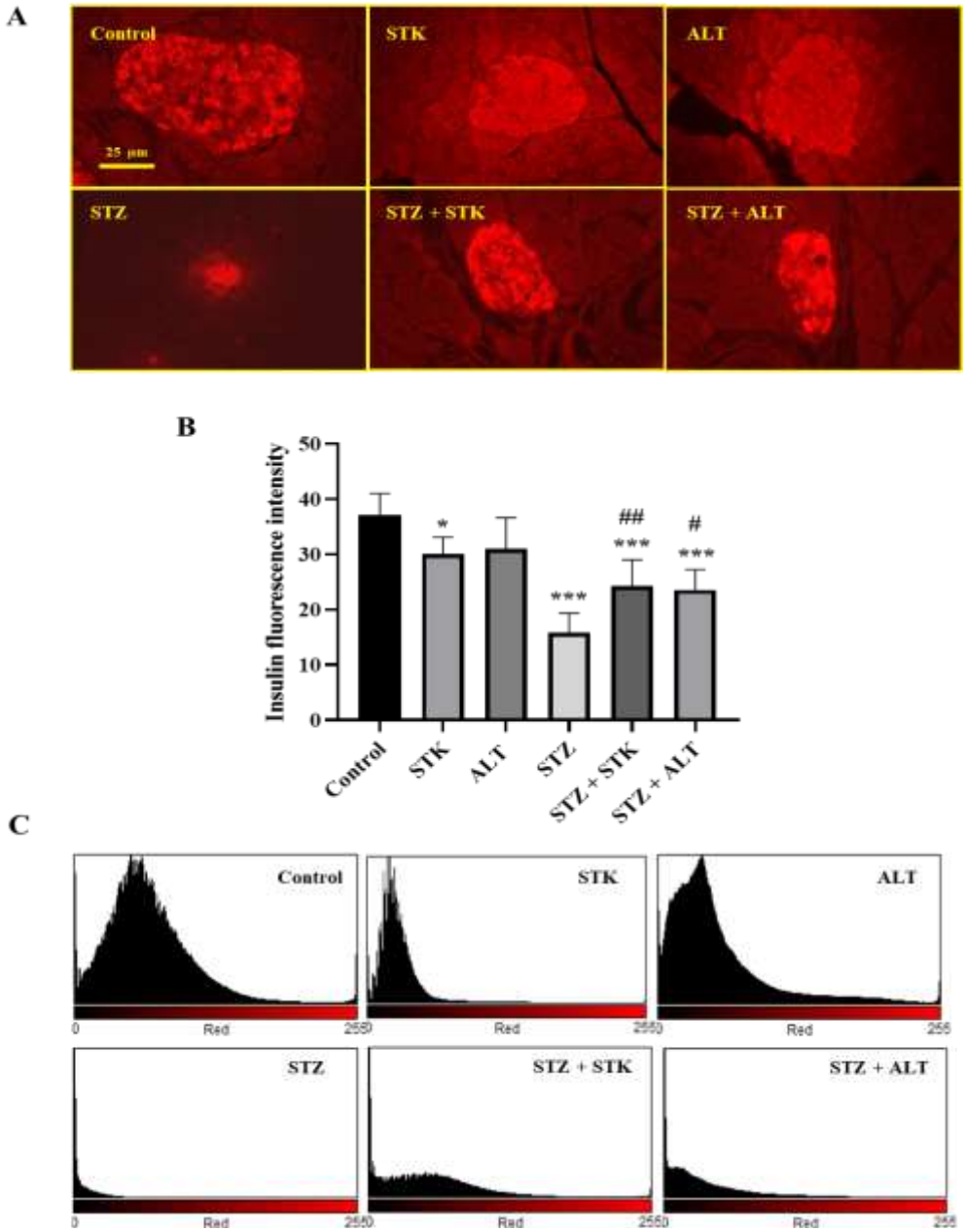


Figure 2. Effect of STK or ALT on the protein expression of a specific marker of pancreatic b islets, insulin, in STZ-induced T1DM in mice as represented by the red immunofluorescence figures **(A)**, fluorescence intensities were calculated and blotted in graph **(B)** or represented as histogram **(C)**. Data in the graph is denoted as mean ± SEM. Statistical analysis was were performed using one-way ANOVA followed by Tukey-Kramer for multiple comparisons where: $^{*}P<0.05$ and $^{***}P<0.001$ considered different significantly when compared to normal mice, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ significantly different considered different significantly in comparison with STZ-treated mice.

Influence of STZ in the presence or absence of STK or ALT on PAR-2 protein expression in pancreatic tissues via immunofluorescence

Pancreatic islets exhibited PAR-2 protein expression across control, STK, ALT, STZ, STZ + STK, and STZ + ALT-treated mice (indicated by yellow arrows, **Figure 3A**) with an increase in intensity for the STZ groups **(Figure 3B)**. The expression of PAR-2 remained insignificant for STK and ALT groups while demonstrating a markedly elevated protein, in STZ ($P < 0.05$), STZ+STK (N.S) and STZ+ALT ($p< 0.001$) compared to the normal group **(Figure 3C)**. The expression of PAR-2 was significantly reduced for STZ-induced mice treated with ALT ($P<0.05$) or STK ($P<0.001$) compared

to the STZ-alone group. Additionally, PAR-2 levels of the STZ+STK group were found to be significantly decreased compared to the STZ+ALT group (P<0.001).

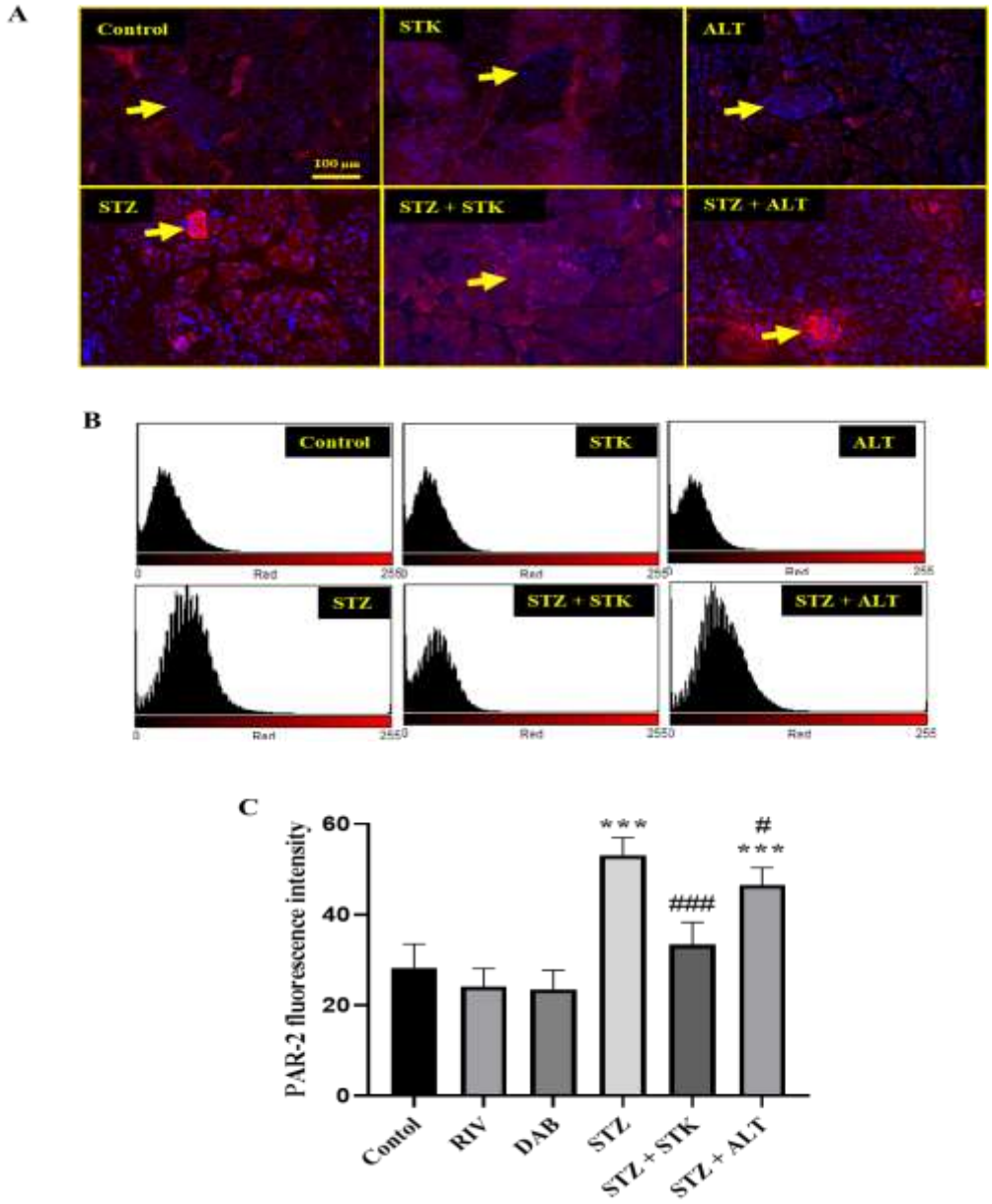
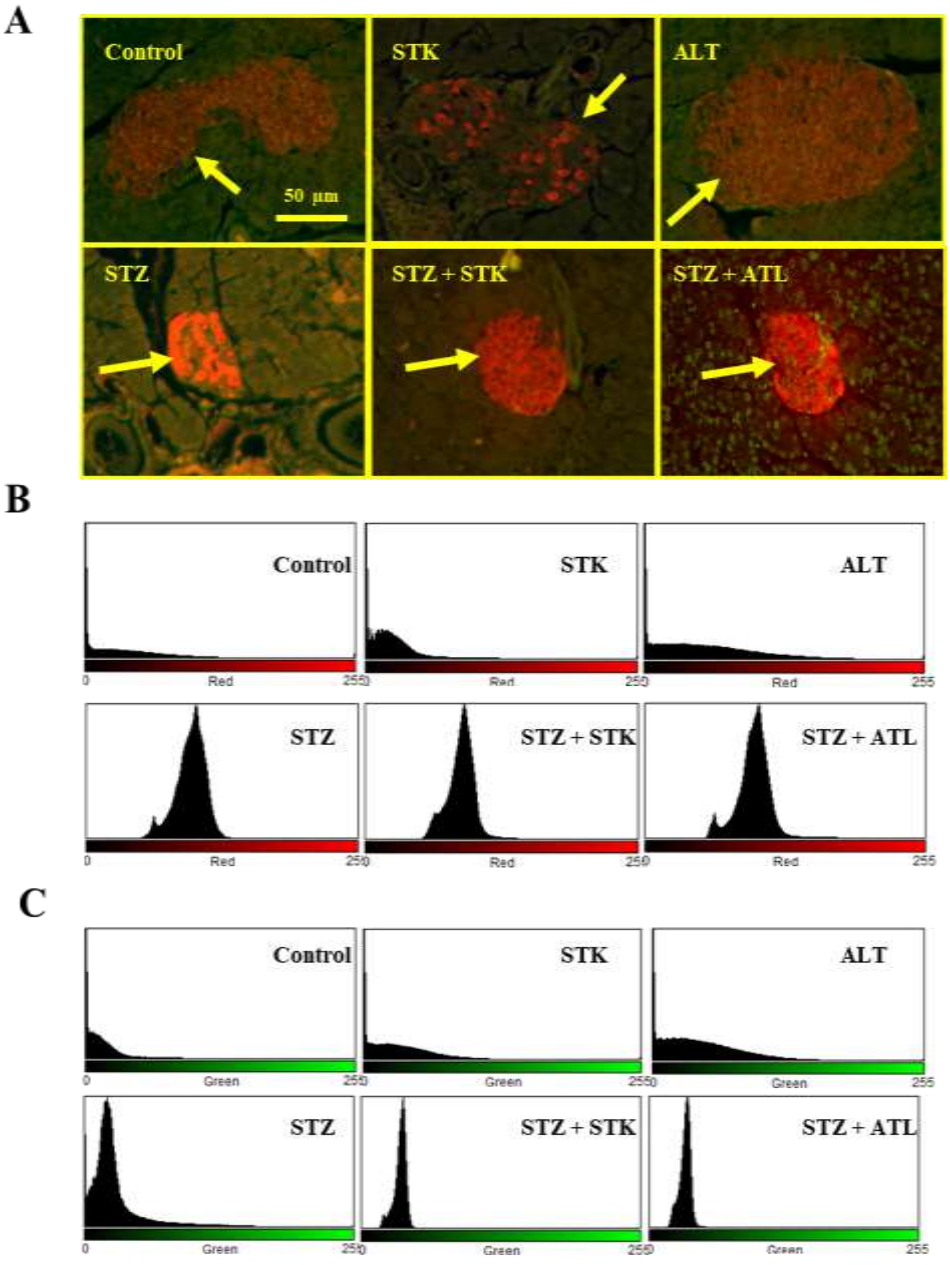


Figure 3: Effect of STZ in the presence or absence of STK or Alt on PAR-2 protein expression in pancreatic mice tissues using immunofluorescence figures **(A)**, represented as histogram **(B)** or fluorescence intensities were calculated and blotted in graph **(C)**. Pancreatic islets showing PAR-2 protein expression in control, STK, ALT, STZ, STZ+ STK and STZ +ALT-treated mice (yellow arrows) with magnification 200 X and scale bar =100 µm (A). Data in the graph is denoted as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer for multiple comparisons where: ***P<0.001 considered different significantly when compared to normal mice, $^{\#}$P<0.05 and $^{\#\#\#}$P<0.001 significantly different considered different significantly in comparison with STZ-treated mice.

Effect of STK or ALT on p-AKT and PI3K protein expressions in the context of STZ treatment in mice

The subsequent figures illustrate the basal expression of p-AKT and PI3K in pancreatic β islets **(Figure 4A)**, denoted by red **(Figure 4C)** and green fluorescence **(Figure 4E)**. The expression levels of p-AKT and PI3K were comparable to the normal group for the STZ and ALT groups **(Figures 4B**

and 4D, respectively). STZ-treated mice and STZ+ALT groups displayed an increased expression of p-AKT (P<0.001) and PI3K (P<0.001) in pancreatic β islets (highlighted by yellow arrows) compared to the normal group. Additionally, the expression of PI3K (P<0.05) for the STZ+SKT group was found to be significantly elevated but was insignificant for the p-AKT expression (NS) compared to the control group. The STZ-induced diabetic group treated with STK exhibited a significant reduction in p-AKT (P<0.001) and PI3K (P<0.001) protein expression compared to the STZ-alone group. Similarly, the STZ+ALT group exhibited a significant reduction in p-AKT (P<0.001) and an insignificant difference in PI3K (NS) protein expression compared to the STZ-alone group.



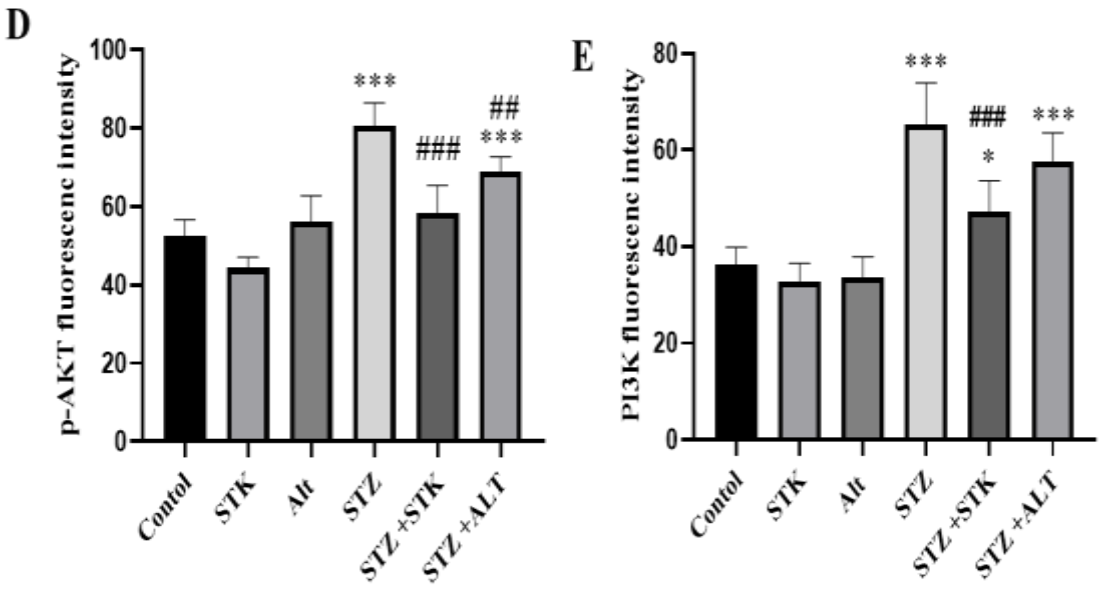


Figure 4: Effect of STK or ALT on p-AKT (red fluorescence) and PI3K (green fluorescence) protein expressions in the presence or absence of STZ treatment in mice as represented by double immunofluorescence figures **(A)**, fluorescence intensities were represented as histogram for p-AKT **(B)** and PI3K **(C)** or calculated and blotted in graph for p-AKT **(D)** or PI3K **(E)**. Control, STK, and ALT-treated mice showing basal expression of p-AKT and PI3K in pancreatic β islets. STZ-treated mice showed increased expression of p-AKT and PI3K in pancreatic β islets (yellow arrows). Mice treated with STK or ALT in the presence of STZ denoted decreased p-AKT and PI3K protein expression in the pancreatic β islets (yellow arrows) as compared to pancreatic sections of STZ-treated mice. Data in the graph denoting mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer for multiple comparisons where: $^{*}P<0.05$ and $^{***}P<0.001$ were considered different significantly when compared to normal mice, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ significantly different and considered different significantly in comparison with STZ-treated mice.

Impact of STK and ALT on the pancreatic architecture in STZ-induced T1DM mice via hematoxylin and eosin staining

Pancreatic tissue sections of control, STK and ALT-treated mice groups stained with H&E **(Figure 5)** displayed average sized islets of Langerhans characterized by predominating β cells and pale blue cytoplasm centrally located (red arrows) interspersed with thin-walled blood capillaries (black arrows) and average exocrine areas (yellow arrows).

Conversely, pancreatic tissues from STZ-induced T1DM mice exhibited few small-sized hypocellular pale staining islets of Langerhans (black arrows), and normal exocrine areas (red arrows) compared to other control groups and groups treated with STK and ALT, with typical intervening capillary structure (blue arrows). Sections from STK or ALT in the presence of STZ displayed islets and exocrine areas of moderate size compared to STZ-induced T1DM mice.

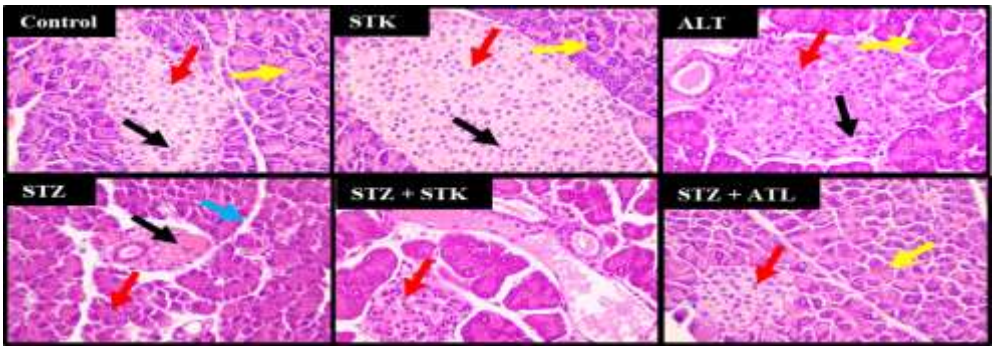


Figure 5: Effect of STK or ALT on pancreatic architecture in STZ-induced T1DM in mice. Arrows indicate average sized islets of Langerhans with predominating β cells with pale blue cytoplasm in the center (red arrows) separated with thin-walled blood capillaries (black arrows) and average exocrine areas (yellow arrows). Tissues from STZ-treated mice showing few small-sized hypocellular pale staining islets of Langerhans (black arrows), normal exocrine areas (red arrows), average intervening capillaries (blue arrows). Magnification 400 X.

Nuclear architecture following treatment with STZ with/without STK or Alt using DAPI staining

There is a notable chromatin condensation for STZ-induced T1DM mice groups treated with STK or ALT signifying elevated levels of mitosis and chromosome division as a consequence of increased expression of pancreatic β islets cells in comparison to the STZ alone group **(Figure 6)**.

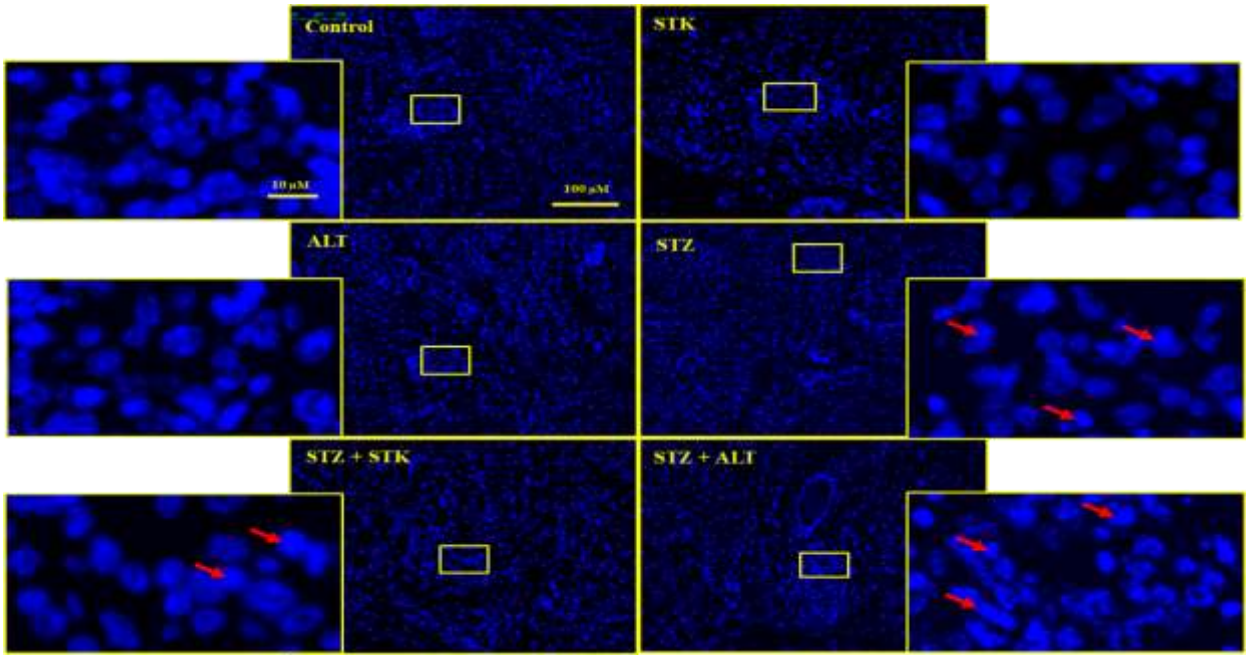


Figure 6: Nuclear architecture subsequent to treatment with STZ with/without STK or ALT as demonstrated by DAPI staining. The yellow box zooms area of the image indicating chromatin condensation. Scale bar =10 μm.

DISCUSSION

Diabetes has emerged as a critical public health challenge in the 21st century, with its prevalence and associated complications contributing significantly to global morbidity and mortality rates. According to Rautio et al. (2017)[1], approximately 1.5 million deaths occurred globally in 2020 due to diabetes alone underscoring it as a major health concern. Despite the recent advancements in medical research, CVD remains the leading cause of illness and death among individuals with diabetes, as highlighted by Dande group [19]. The interplay between diabetes and cardiovascular

complications presents a complex challenge, necessitating the exploration of novel therapeutic strategies to mitigate these risks.

This study aimed to investigate the potential of fibrinolytic agents, specifically STK and ALT, in modulating T1DM and insulin secretion. These fibrinolytics have demonstrated their ability to activate plasminogen, thereby facilitating the breakdown of blood clots. The current study hypothesized the influence of fibrinolytic agents in insulin secretion and glycemic control, offering a dual benefit in managing both thrombotic and diabetic conditions.

In the current experimental setup, mice were injected with STZ to induce T1DM, with subsequent administration of STK, or ALT. The results revealed a notable increase in serum insulin levels in mice treated with these fibrinolytic agents. This finding concurs with the report of Masoomi [20], which reported significantly increased insulin levels among patients with ST-elevation myocardial infarction (STEMI) following STK's administration. In particular, the study demonstrated increased insulin levels in both diabetic (31.6%) and non-diabetic patients (51.0%), indicating a potential link between STK administration and improved insulin secretion [20].

However, on the other hand, another study reported the effects of STZ-induced Type 1 diabetes in male Wistar rats [21]. Rats administered with insulin or saline, followed by tissue plasminogen activator (tPA) or saline, did not contribute significantly to ischemic infarction, suggesting a complex and context-dependent relationship between fibrinolytic therapy and glycemic control [22].

In terms of blood glucose levels, the current study reports decreased glucose levels in STZ-induced diabetic mice treated with STK. Our data is consistent with Wang group [23], which reported hyperglycemia as a significant risk factor in ischemic stroke patients receiving thrombolytic treatment with ALT and STK. The authors also reported the association of elevated glucose levels with a higher likelihood of symptomatic intracerebral hemorrhage (sICH), poor clinical outcomes at 90 days, and increased all-cause mortality, highlighting the critical role of glycemic control in patients undergoing fibrinolytic therapy [23].

The study also examined the impact of fibrinolytic agents on platelet count, revealing a statistically significant difference among the various treatment groups. This finding corroborates the research by Lu group [24], who demonstrated that ALT effectively inhibited platelet aggregation in response to various stimuli, such as adenosine diphosphate (ADP), collagen, ristocetin, arachidonic acid, and epinephrine. The study employed light transmittance aggregometry and flow cytometry to analyze platelet activation and receptor expression, concluding a dose-dependent ALT's anti-platelet observed effects [24].

Moreover, the study explored the effect of STK and ALT on the protein expression of insulin in pancreatic β islets of STZ-induced diabetic mice. The results indicated that treatment with these fibrinolytic agents led to a higher expression of insulin protein compared to STZ-treated mice alone. Our reported data is in agreement with McRedmond [25], who found that STK administration resulted in the upregulation of PAR-2, a receptor involved in platelet thrombin signaling, thereby potentially influencing insulin secretion mechanisms.

Additionally, the study investigated the expression of p-AKT and PI3K proteins, key players in the insulin-signaling pathway, in the presence or absence of STK or ALT. The findings revealed a decrease in the expression of these proteins in pancreatic β islets of mice treated with STZ, with significant differences observed between the STZ-only group and those treated with STK or ALT. These results align with our previous findings demonstrating STK's activation of the coagulation system through PAR-2 and the p-AKT pathway, leading to the induction of apoptotic pathways [13].

Histological analysis of pancreatic tissues using H&E staining further supported these findings. Tissues from STZ-treated mice displayed small, hypo-cellular islets of Langerhans with pale staining, while those treated with STK or ALT showed improved cellular morphology and increased pancreatic cell expression. This observation is consistent with Gupta [26], who reported that STK treatment led to the degradation of the collagenous framework in necrotic pancreatic tissue thereby disintegrating necrotic cells.

Finally, the study assessed the impact of STK and ALT on hematological parameters in STZ-induced diabetic mice, with a particular focus on platelet count. The results indicated a marked decrease in platelet count in STZ-induced diabetic mice treated with STK or ALT, which was consistent with findings by [27] and Abdelouahed [28]. These studies similarly reported significant alterations in platelet count following fibrinolytic treatment, suggesting that these agents may exert broader effects on hematological parameters beyond their thrombolytic activity.

The current study has its limitations in employing the STZ-induced diabetic mice model limiting the generalizability of the findings to human subjects. In addition, the study focused on short-term effects along with a single dose of STK or ALT thus the long-term or varying doses of fibrinolytic implications in fibrinolytic therapy on diabetes management remain unclear. Further studies should delve deeper into the molecular mechanisms by which these agents influence insulin secretion and other metabolic processes along with future clinical trials that can validate the therapeutic potential of STK and ALT in managing diabetes and related complications.

In conclusion, this study provides valuable insights into the potential therapeutic effects of fibrinolytic agents in the context of T1DM and insulin secretion. While the findings are promising, they also underscore the complexity of the interactions between diabetes, cardiovascular disease, and fibrinolytic therapy. Further research is required to fully elucidate the mechanisms underlying these effects and to determine the clinical implications for patients with diabetes who require thrombolytic treatment.

CONCLUSION

This study highlights the potential of fibrinolytic agents, specifically STK and ALT, in modulating insulin secretion and glycemic control in T1DM. The findings suggest that these agents may enhance insulin levels and affect glucose and platelet counts in diabetic mice, offering a dual benefit in managing both thrombotic and diabetic conditions. In addition, STK and ALT improved the deteriorated consequences resulted from STZ. Notably, STK was better than ALT in preventing the development of diabetes. However, the complexity of these interactions warrants further investigation to better understand their mechanisms and clinical relevance.

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Declaration of competing interests

The authors stating that there is no conflict of interests or personal relationships.

Authors' contribution

A.T. A. participated in the design of the study and the practical study, drafting the manuscript and participated in the immunofluorescence imaging and analysis. A.H.A collected data, statistical analysis and revised the manuscript. S.A.A.M. participated in the manuscript editing and overall manuscript revision. M.S.A. collected data, performed *in vivo* studies, performed the mouse model, performed statistical analysis, biochemical assay. All authors approved the final version of the manuscript to be published.

Ethical Approval

The practical experiments were conducted by the NIH Guidelines for the Care and Use of Laboratory Animals, as well as the regulations set by Saudi Arabia. The research was approved by the Ethical Committee for the Deanship of Scientific Research at Qassim University (24-84-12).

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