Original Article

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Effect of Ticagrelor on Glycaemic Parameters and Inflammatory Mediators in High Fat Diet and Streptozotocin Induced Diabetic Rats: An Experimental Study

Pharmacology Section

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ABSTRACT

Introduction: Type 2 Diabetes Mellitus (T2DM) is a growing global health concern, often associated with chronic inflammation and cardiovascular complications such as Acute Coronary Syndrome (ACS). Emerging evidence suggests that antiplatelet agents may exert beneficial effects beyond their cardiovascular indications, including the modulation of glycaemic control and inflammatory responses. Ticagrelor, a P2Y12 receptor antagonist primarily used for managing ACS, has been proposed to influence metabolic and inflammatory pathways.

Aim: To evaluate the effect of ticagrelor on glycaemic parameters in a High Fat Diet (HFD) and Streptozotocin (STZ)-induced diabetes mellitus in male Wistar rats.

Materials and Methods: The present experimental study was conducted in the Department of Pharmacology at Jawaharlal Nehru Medical College, Belagavi, Karnataka, India, from March 2021 to February 2022. A total of 36 rats were utilised in the study. Among these, six were designated as the normal control group. The remaining 30 rats were subjected to diabetes induction through a combination of HFD administration for two weeks and a single intraperitoneal injection of STZ at a dose of 35 mg/kg. Following the induction of diabetes, one group served as the diabetic control with no treatment, while other groups received oral treatment either with metformin, ticagrelor (16.2 mg/kg), or ticagrelor (35 mg/kg) for six weeks. Body weights and Fasting Blood Glucose (FBG) levels were measured at baseline, 14 days of HFD, three weeks following treatment, and at the end of the study. Glycated Haemoglobin A1c (HbA1c) was measured at baseline and at the end of the study. Inflammatory markers Interleukin (IL)-1β, Tumour Necrosis Factor (TNF)-α, and IL-6 were assessed at the end of the study. Data were presented as Mean±Standard Error of the Mean (SEM). A p-value ≤0.05 was considered statistically significant. One-way Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test was used for the analysis of study variables, and paired data were analysed using the paired t-test.

Results: All three treatments significantly reduced FBG ($p<0.0001$) and HbA1c ($p<0.0001$) compared to the untreated rats. Additionally, compared to the ticagrelor (16.2 mg/kg) group, the ticagrelor (35 mg/kg) group significantly decreased these values ($p<0.0001$). The inflammatory markers (IL-1β, IL-6, TNF-α) were significantly reduced ($p<0.0001$) in all treatment groups compared to untreated rats.

Conclusion: The treatment of diabetic rats with oral ticagrelor improved the HFD and STZ-induced elevations in Fasting Blood Sugar (FBS), glycosylated hemoglobin (HbA1c), and inflammatory mediators. Furthermore, ticagrelor (35 mg/kg) was found to be more efficacious across all trial variables compared to Ticagrelor (16.2 mg/kg).

Keywords: Acute coronary syndrome, Coronary heart disease, Chronic inflammation, Type 2 diabetes mellitus

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic disorders characterised by hyperglycaemia due to defects in insulin secretion, insulin action, or both. These disorders cause secondary pathophysiological changes in multiple organ systems, imposing a tremendous burden on the healthcare system. The International Diabetes Federation predicts that 642 million people will have diabetes by 2040. Type 2 diabetes is rising rapidly due to obesity and inactivity, with cardiovascular mortality increasing twofold in men and fourfold in women [1].

The pathophysiology of T2DM involves impaired insulin secretion and reduced tissue sensitivity to insulin, driven by multiple mechanisms such as glucotoxicity, lipotoxicity, oxidative stress, endoplasmic reticulum stress, alterations in gut microbiota, and amyloid deposits in pancreatic islets. Importantly, these processes are all linked to hyperglycaemia-induced inflammation, which plays a key role in the progression of diabetes [2]. Various mechanisms have been proposed to explain the role of inflammation in the initiation and progression of diabetes. Hyperglycaemia associated with diabetes increases pancreatic islet cell metabolism, producing Reactive Oxygen Species (ROS) that activate inflammasomes like NLRP3 (Nucleotide Binding Oligomerisation Domain, Leucine-Rich Repeat, and Pyrin Domain-3) and Caspase-1. These, in turn, stimulate the release of IL-1β, which triggers the secretion of cytokines such as IL-6, IL-8, and TNF-α. This leads to an accumulation of immune cells in the islets, contributing to insulin resistance [3].

NLRP3 inflammasome-induced release of IL-1β is associated with β-cell dysfunction and apoptosis, leading to insulin deficiency and the progression of T2DM [4]. Inappropriate activation of NLRP3 is also linked to related conditions such as atherosclerosis and acute myocardial infarction [5]. Since diabetes increases both the risk and mortality of Cardiovascular Diseases (CVD), NLRP3 appears to be a potential target for investigation in the management of diabetes and its associated co-morbid cardiovascular conditions [4,5]. Interestingly, the anti-platelet drug ticagrelor has been found to indirectly inhibit the NLRP3 inflammasome [5].

Ticagrelor, a P2Y12 receptor antagonist, is used to prevent vascular events in CVD and ACS. Beyond its antiplatelet role, it reduces inflammation by inhibiting IL-1β and TNF-α, both key to the pathogenesis of T2DM [5]. Studies have reported that ticagrelor decreases Caspase-1 maturation, IL-1β, and pro-inflammatory

cytokines in various models, including Lipopolysaccharide (LPS)-primed macrophages, murine peritonitis, and sepsis, independent of P2Y12 signaling. In ACS patients, it suppressed IL-1β and TNF-α production in Peripheral Blood Mononuclear Cells (PBMCs) [1,3,5]. In diabetic nephropathy, ticagrelor effectively prevented diabetes-induced mesangial matrix expansion, podocyte damage, and glomerular endothelial injury, significantly reducing both plasma and kidney mRNA levels of TNF-α and Caspase-3 [6].

It could therefore be hypothesised that ticagrelor may demonstrate therapeutic potential in NLRP3-associated diseases like diabetes mellitus. However, information is scarce regarding the effect of ticagrelor on glycaemic parameters in diabetes per se. Therefore, the present study was planned primarily to investigate the effect of the commonly used anti-platelet drug ticagrelor on glycaemic parameters in a HFD and STZ-induced rodent model of diabetes. Secondly, the study evaluated the effect of ticagrelor on inflammatory markers, including IL-1β, TNF-α, and IL-6.

MATERIALS AND METHODS

The present experimental study was conducted in the Department of Pharmacology at Jawaharlal Nehru Medical College, Belagavi, Karnataka, India, from March 2021 to February 2022. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), bearing registration number: 627/PO/Re/S/02/CPCSEA, via resolution no. 14/4 dated 05/02/2021. The study was conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Animals were housed with free access to standard rat chow and water, and they were acclimatised to a 12:12-hour light-dark cycle for 10 days before starting the experiment.

Inclusion criteria: Healthy, adult, male Wistar rats weighing 180±20 g were selected for the study. Animals with Fasting Blood Glucose (FBG) >200 mg/dL upon induction of diabetes were included for further investigation.

Exclusion criteria: Animals not meeting the required weight range of 180±20 g were excluded from the study. Of the selected animals, those with FBG <200 mg/dL upon induction of diabetes were not included.

Study Procedure

A total of 36 animals were included in the study. A day prior to the initiation of the study, the rats were fasted overnight. On day one, 2 mL of blood was collected from each rat via the tail vein for baseline estimation of FBG and HbA1c. The minimum number of animals required to achieve statistical significance is five per group for preclinical animal studies [7]. Given the anticipated higher mortality associated with diabetes induction, the sample size for the diabetic groups was increased per group. Accordingly, six animals were included in the normal control group, while diabetes was induced in the remaining animals. Blinding was implemented to eliminate observer bias and was achieved through coding.

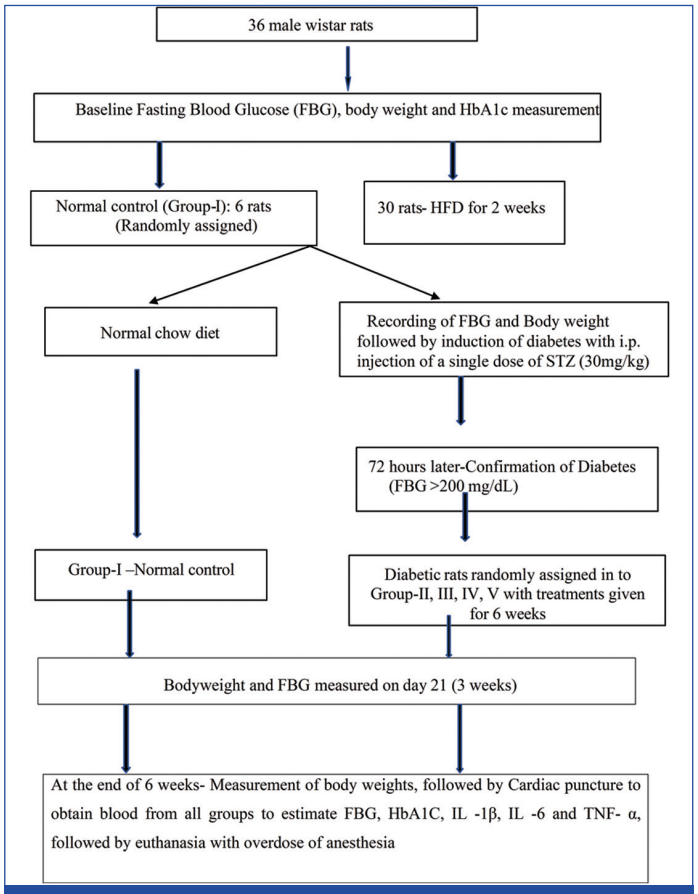
Induction of diabetes: The remaining rats were fed a HFD for 14 days. The composition of the HFD is shown in [Table/Fig-1] [8]. STZ (Cayman Chemical Company, catalogue no. 13104) was procured from Everon Lifesciences, New Delhi. On day 15, diabetes was induced by intraperitoneal injection of STZ at a dose of 35 mg/kg of rat, dissolved in 0.05M citrate buffer. An equal volume of citrate buffer was injected intraperitoneally into the normal control rats. Following the injection of STZ, all the rats were given 5% glucose instead of water for 24 hours to minimise STZ-induced hypoglycaemic shock-related mortality [9,10].

Confirmation of the diabetes: Seventy-two hours following STZ administration, blood samples were collected from the tail vein, and FBG levels were measured using a glucometer. Diabetes was confirmed in animals with FBG levels exceeding 200 mg/dL.

Ingredients	Diet (g/kg)
Powdered Normal Pellet diet (NPD)	365
Lard	300
Casein	280
Soya oil	50
Vitamin and mineral mix	50
Starch	260
Yeast powder	01
Cholesterol	10

[Table/Fig-1]: Composition of HFD.

Subsequently, these animals were randomly allocated into various treatment groups, as detailed in [Table/Fig-2]. All the drugs were administered orally as a single daily dose for six weeks. The doses of the drugs used were calculated using the multiplication factor proposed by Paget and Barnes [11].



[Table/Fig-2]: Schematic diagram illustrating the experimental design.

Metformin, being the first-line anti-diabetic agent, was selected as a standard. The maximum daily human dose of metformin, which is 2 g, was selected for the study and was equivalent to 180 mg/kg in rats. Similarly, the maximum recommended human dose of ticagrelor (180 mg/day) corresponds to an equivalent dose of approximately 16.2 mg/kg in rats. A previous study has reported that a dose of 35 mg/kg/day of ticagrelor in rats is the minimum required to exhibit a significant inhibitory effect on the NLRP3 inflammasome, and hence this dose was selected for the study [5].

At the end of six weeks of treatment, 5 mL of blood was withdrawn by cardiac puncture for the estimation of FBS, HbA1c, and inflammatory mediators, and the animals were sacrificed using an overdose of Thiopentone anesthesia, as per CPCSEA guidelines [Table/Fig-3].

Groups	Treatment	Dose
Group I: Normal Control (NC) (n=6)	Vehicle only	1 mL
Group II: Diabetic Control (DC) (n=8)	Vehicle only	1 mL

Group III: Diabetic Rats + Metformin (MF) (n=7)	Metformin(standard)	180 mg/kg (around 1 mL)
Group IV: Diabetic Rats + ticagrelor -16.2 mg/kg (TCG-16.2) (n=8)	Ticagrelor	16.2 mg/kg (around 1 mL)
Group V: Diabetic Rats ticagrelor -35 mg/kg (TCG-35) (n=7)	Ticagrelor	35 mg/kg (around 1 mL)

[Table/Fig-3]: Number of rats per group with treatment schedule.

Outcome Measures

The various parameters assessed were body weight, FBS, HbA1c, and inflammatory markers, namely IL-1β, TNF-α, and IL-6. Body weights and FBS were recorded at baseline, 14 days of HFD, three weeks following treatment, and at the end of the study after six weeks of treatment. HbA1c was measured at baseline and at the end of the study, while the inflammatory markers were measured using Enzyme Linked Immuno Sorbent Assay (ELISA) kits upon completion of the study.

STATISTICAL ANALYSIS

All results were expressed as mean±Standard Error of Mean (SEM). The data were analysed using GraphPad Prism Version 10.4. A p-value of <0.05 was considered statistically significant. The study variables were analysed using one-way ANOVA followed by post-hoc Bonferroni's test. Paired data were analysed using the paired t-test.

RESULTS

Effects on body weight at different time intervals: The mean body weights of all the groups were comparable at baseline, and a one-way ANOVA revealed no significant difference between the various groups. After 21 days of treatment and at the end of the study, there was a statistically significant reduction in weight in the Diabetic Control (DC) group compared to the normal control and treatment groups (p<0.0001). In comparison to the standard metformin, the Ticagrelor (TCG) 16.2 group showed a statistically significant weight reduction, while no significant difference was reported in the Ticagrelor 35 (TCG 35) group [Table/Fig-4].

Groups	Baseline	After 14 days of HFD	After 21 days of induction	End of study
Normal Control (NC)	183.5±0.7491	204.6±0.5648	245.3±0.619	295.4±0.3323
Diabetic Control (DC)	184.2±0.354	209.2±0.6412	204.8±0.4214 p<0.0001*	165.8±0.3899 p<0.0001*
Metformin (MF)	191.5±0.6068	212.2±0.6024	242±0.412 p<0.0001* p<0.0001#	263.3±0.6274 p<0.0001* p<0.0001#
Ticagrelor (16.2)	189.2±0.8093	213.9±0.9348	221±0.4729 p<0.0001* p<0.0001# p=0.0029$	243.6±0.5252 p<0.0001* p<0.0001# p<0.0001$
Ticagrelor (35)	184.7±0.4402	211.3±0.4214	251.1±0.5256 p<0.0001* p<0.0001#	272.8±0.3885 p<0.0001* p<0.0001#

[Table/Fig-4]: Effect on Bodyweight at different time intervals.
Data are presented as Mean±SEM. Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparison test. p<0.05 was considered as significant. Significance levels are indicated as follows: *comparison of normal control with other groups; #Comparison of diabetic control with other treatment groups; $comparison of metformin with TCG 16.2 and TCG 35; Bodyweight measured in grams

Effect on Fasting Blood Glucose (FBS) at various intervals: The FBG was measured at baseline, following 14 days of HFD, 72 hours after STZ injection (only for confirmation of diabetes), 21 days after treatment, and at the end of the study. After 14 days of HFD, a statistically significant rise in FBG was reported in all diabetic groups compared to the NC group (p<0.0001). The FBG values after 72 hours of STZ increased compared to their respective baseline values, suggesting hyperglycaemia and confirming Diabetes Mellitus (DM). Following 21 days of treatment and at the end of the study, FBG levels in the DC group were significantly elevated compared to all other experimental groups (p<0.0001). Bonferroni's multiple comparison test revealed that the FBG levels in both the MF and TCG-35 groups were significantly lower (p<0.0001) than those in the TCG-16.2 group. Furthermore, there was no significant difference in FBG levels between the MF and TCG-35 groups, indicating comparable glycaemic control [Table/Fig-5].

Within each group, a Student's paired t-test was used to compare FBG levels before and after treatment. FBG values at the end of the study in the MF, TCG-16.2, and TCG-35 groups were significantly reduced (p<0.0001) compared to FBG values after induction [Table/Fig-6].

Effect on HbA1c: By the end of the study, the DC group exhibited a significantly higher mean HbA1c level compared to the normal group (p<0.0001). MF, TCG-16.2, and TCG-35 showed a statistically significant reduction in HbA1c levels compared to the DC group (p<0.0001). Notably, compared to the MF group, HbA1c levels in the TCG-16.2 group remained significantly higher (p<0.0001), while those in the TCG-35 group were comparable [Table/Fig-7].

Effect on inflammatory markers: ANOVA followed by Bonferroni's post-hoc test revealed a significant increase in the levels of these inflammatory markers in the DC group (p<0.0001) compared to the normal control group. In contrast, treatment with metformin, ticagrelor 16.2 mg/kg, and ticagrelor 35 mg/kg resulted in a significant reduction in all inflammatory markers compared to the DC group (p<0.0001). Among the treatment groups, the TCG-16.2 group exhibited significantly higher levels (p<0.0001) of IL-1β, TNF-α, and IL-6 than both the MF and TCG-35 groups, whereas no significant differences were observed between the MF and TCG-35 groups [Table/Fig-8].

DISCUSSION

As the HFD-STZ model mimics the natural pathology of T2DM, it was used for the induction of diabetes in the current study. The HFD used is known to induce insulin resistance and/or glucose intolerance in rats, similar to the condition observed in humans. STZ is known to cause destruction of pancreatic β-cells through DNA alkylation, Nitric Oxide (NO) release, and ROS generation. STZ is considered a better chemical for inducing diabetes due to its selective action via GLUT-2 receptors, lower toxicity, and reduced mortality rate. A state of permanent hyperglycaemia develops in the rats 48 hours after STZ injection, which is considered the onset of the disease. Additionally, 72 hours post injection is regarded as the best time point for measuring blood glucose levels to confirm the diagnosis of diabetes [12,13].

Groups	Baseline	After 14 days of HFD	After 72 hours of STZ (Confirmation of DM)	After 21 days of induction	End of study
Normal Control (NC)	103.4±0.2587	102.7±0.4564	-	103±0.3768	102.5±0.3313
Diabetic Control (DC)	105.6±0.3528	117.9±0.2963 p<0.0001*	302.6±0.4729	370.2±0.5167 p<0.0001*	389.2±0.8819 p<0.0001*
Metformin (MF)	107.3±0.4619	119.1±0.5913 p<0.0001*	327.2±0.5066	298.5±0.4472 p<0.0001* p<0.0001#	257.4±1.417 p<0.0001* p<0.0001#
Ticagrelor (16.2)	102.7±0.3756	124.7±p0.628 p<0.0001*	311.7±0.3962	318.4±0.5974 p<0.0001* p<0.0001# p<0.0001$ p<0.0001@	283.6±0.4199 p<0.0001* p<0.0001# p<0.0001$ p<0.0001@

Ticagrelor (35)	105±0.6152	130.2±0.8328 p<0.0001*	326.7±0.7265	301.7±0.693 p<0.0001* p<0.0001#	265.2±0.8002 p<0.0001* p<0.0001#

[Table/Fig-5]: Effect on fasting blood glucose levels at different time intervals.
Data are presented as Mean±SEM. Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparison test. p<0.05 was considered as significant. Significance levels are indicated as follows: *Comparison of normal control with other groups; #Comparison of diabetic control with other treatment groups; #Comparison of metformin with TCG 16.2 and TCG 35; @Comparison between two groups of ticagrelor; FBS measured in mg/dL

Timepoint	Normal Control (NC)	Diabetic Control (DC)	Metformin (MF)	Ticagrelor (16.2)	Ticagrelor (35)
72 hours of STZ	–	302.6±0.4729	327.2±0.5066	311.7±0.3962	326.7±0.7265
21 days of induction	103±0.3768	370.2±0.5167 p<0.0001*	298.5±0.4472 p<0.0001*	318.4±0.5974 p=0.3734* ns	301.7±0.693 p<0.0001*
End of study	102.5±0.3313	389.2±0.8819 p<0.0001#	257.4±1.417 p<0.0001#	283.6±0.4199 p<0.0001#	265.2±0.8002 p<0.0001#

[Table/Fig-6]: Effect of various treatments on fasting blood glucose levels at different time intervals (Results of Paired t-test).
Data are presented as Mean±SEM. Statistical analysis was performed using Paired t-test. p<0.05 was considered as significant. Significance levels are indicated as follows: *Comparison of FBS at 21 days of treatment with that at 72 hours after induction; #Comparison of FBS at the end of the study period with that at 72 hours after induction; "ns" indicates Non-significant values; FBS measured in mg/dL

Groups	Baseline	End of study
Normal Control (NC)	4.497±0.06642	4.543±0.03252
Diabetic Control (DC)	4.423±0.04573	9.752±0.04324 p<0.0001*
Metformin (MF)	4.383±0.04944	7.242±0.04902 p<0.0001#
Ticagrelor (16.2)	4.32±0.07024	8.217±0.09804 p<0.0001# p<0.0001$ p<0.0001@
Ticagrelor (35)	4.3±0.1342	7.2±0.2082 p<0.0001# p>0.9999$ ns

[Table/Fig-7]: Effect of various treatments on HbA1c levels.
Data are presented as Mean±SEM. Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparison test. p<0.05 was considered as significant. Significance levels are indicated as follows: * comparison of normal control with diabetic control; #Comparison of diabetic control with other treatment groups; $Comparison of metformin with TCG 16.2 and TCG 35; @Comparison between two groups of ticagrelor; "ns" indicates Non-significant values

Groups	IL-1β (pg/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)
Normal Control (NC)	0.3683±0.03311	28.67±0.4432	26.88±0.342
Diabetic Control (DC)	2.37±0.03958 p<0.0001*	93.95±0.303 p<0.0001*	113.1±0.4917 p<0.0001*
Metformin (MF)	0.625±0.03981 p<0.0001#	67.38±0.2561 p<0.0001#	56.13±0.8065 p<0.0001#
Ticagrelor (16.2)	1.067±0.08819 p<0.0001# p<0.0001$ p<0.0001@	75.5±0.4282 p<0.0001# p<0.0001$ p<0.0001@	96.12±0.327 p<0.0001# p<0.0001$ p<0.0001@
Ticagrelor (35)	0.65±0.04344 p<0.0001# p>0.9999$	61.03±0.8061 p<0.0001# p=0.5231$ ns	65.6±0.5164 p<0.0001# p>0.6432$ ns

[Table/Fig-8]: Effect of various treatments on inflammatory markers.
Data are presented as Mean±SEM. Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparison test; p<0.05 was considered as significant. Significance levels are indicated as follows: *Comparison of normal control with diabetic control; #Comparison of diabetic control with other treatment groups; #Comparison of metformin with TCG 16.2 and TCG 35; @comparison between two groups of ticagrelor; "ns" indicates Non-significant values

In the present study, at the end of 14 days on the HFD, the body weights of all the groups were higher compared to the normal control group, though not statistically significant. These findings were consistent with the results of a study by Marques C et al., which reported no statistically significant weight gain with HFD in Wistar rats at the end of two weeks, but a statistically significant weight gain after four weeks of HFD [14]. This weight gain in Wistar rats fed an HFD could be attributed to higher caloric intake, primarily due to an increase in adipose tissue mass, in comparison to the normal control rats fed a chow diet [15-17]. With the progression of diabetes, there was significant weight reduction in the untreated diabetic rats compared to the non diabetic animals. Such results have been reported earlier and can be explained by the fact that STZ-induced diabetes is accompanied by a substantial decrease in body weight due to hyperglycaemia, hypoinsulinaemia, muscle wasting, and protein loss [18-20].

Fat metabolites and Free Fatty Acids (FFAs) that enter the liver are known to impair insulin sensitivity in liver cells and disrupt glucose metabolism, leading to elevated glucose levels [21]. A state of hyperglycaemia was successfully generated following a single dose of STZ in this study. Treatment with ticagrelor significantly reduced FBG levels, indicating its anti-hyperglycaemic effect. A previous study by Chen H et al., demonstrated that while ticagrelor alone did not significantly lower blood glucose, its combination with Dapagliflozin improved glycaemic control in diabetic mice. Both agents contributed to cardioprotection by mitigating diabetic cardiomyopathy through inhibition of the NLRP3 inflammasome and its downstream pathways [22].

At the end of the study, the HbA1c of the untreated diabetic rats was significantly higher than that of the normal rats. All three treatment groups (metformin, ticagrelor-16.2, and ticagrelor-35) significantly lowered HbA1c compared to the diabetic group, which indicates that all three treatments were effective in controlling the rise in HbA1c. The beneficial effect of ticagrelor on HbA1c seen in the present study was consistent with prior animal and human studies. The THEMIS-PCI trial found that ticagrelor produced a favorable net clinical benefit across different durations of diabetes and HbA1c levels [23]. A sub-study from the PLATelet Inhibition And Patient Outcomes (PLATO) trial by James S et al., reported that ticagrelor reduced ischemic events in Acute Coronary Syndrome (ACS) patients and decreased HbA1c levels without an increase in major bleeding events [24]. An experimental animal study by Chen H et al., postulated that ticagrelor reduced pancreatic cell inflammation and increased insulin production [22]. However, there is no evidence that the improvement in HbA1c is based on interference with inflammation, emphasising the importance of further exploring ticagrelor as a promising anti-inflammatory agent. This gap has been addressed by the present study, which evaluated the effect of ticagrelor on inflammatory markers like IL-1β, IL-6, and TNF-α to confirm our findings. Although a reduction in HbA1c was observed in all treatment groups, the ticagrelor 16.2 mg/kg group had higher HbA1c levels than the metformin and ticagrelor 35 mg/kg groups. However, the ticagrelor 35 mg/kg dose maintained HbA1c levels within American Diabetes Association (ADA)-recommended targets, while the ticagrelor 16.2 mg/kg group did not. Given the ADA's recommendation to add second-line therapy if HbA1c remains uncontrolled on metformin, ticagrelor may be a promising adjunctive treatment for diabetes and beneficial for patients with both ACS and diabetes [25].

In the present study, all three treatments significantly lowered serum IL-1β, IL-6, and TNF-α levels compared to untreated diabetic rats, confirming their anti-inflammatory effects. Previous studies have

demonstrated that metformin reduces these markers in diabetic models, including reductions in TNF-α, IL-6, IFN-γ, and IL-1, which is consistent with the findings of the present study [26-28]. The current study demonstrated that ticagrelor at both doses effectively reduced inflammatory markers, with a more pronounced effect observed at the higher dose. This suggested that ticagrelor's superior efficacy at 35 mg/kg may result from its stronger anti-inflammatory properties, contributing to improved glycaemic control via enhanced insulin sensitivity. Ticagrelor's anti-inflammatory effects-including inhibition of IL-6, IL-1, TNF-α, NF-κB, and Caspase 3- are well documented [29]. Previous studies, including one by Uil M et al., reported that ticagrelor alleviates diabetic nephropathy and systemic inflammation by downregulating pro-inflammatory cytokines and improving endothelial function [6]. A 2020 review highlights ticagrelor's potential in reducing the risk of various diseases through its anti-inflammatory mechanisms [30]. An extensive animal study by Huang B et al., demonstrated that ticagrelor enhances insulin signaling in liver and adipose tissues while reducing NLRP3 inflammasome activation in macrophages [5]. Similarly, Chen D et al., reported that ticagrelor effectively reversed chronic inflammation, suggesting its potential as an alternative therapy for insulin resistance [22]. Wang J et al., proposed that beyond its role as a platelet P2Y12 receptor antagonist, ticagrelor also acts on endothelial cells, reducing Reactive Oxygen Species (ROS) generation and endothelial Nitric Oxide Synthase (eNOS) phosphorylation, which contributes to its anti-inflammatory effects [31]. Furthermore, a randomised controlled trial by Husted S et al., found that ticagrelor significantly reduced inflammatory biomarkers such as C-reactive Protein (CRP) and IL-6 [32]. Existing evidence indicates that targeting inflammatory mediators like IL-1β and TNF-α not only improves insulin sensitivity but also benefits glycaemic control.

Limitation(s)

Two doses of ticagrelor were employed in the present study: a lower dose based on the clinically used human dose for anti-platelet effect and a minimum dose reported to exhibit anti-inflammatory effects. The present study has reported the effects of ticagrelor alone on glycaemic parameters of diabetes. Several potential confounders may have influenced the study outcomes. Variability in diabetes induction, lack of insulin measurements, and absence of pharmacokinetic data in rodents limit the interpretation of the glycaemic effects of ticagrelor. Circadian rhythms, animal handling stress, and dietary intake differences may have contributed to intra-group variability. Additionally, ticagrelor's off-target effects on adenosine reuptake may have contributed to the observed metabolic outcomes independent of its anti-platelet action. Future studies incorporating mechanistic analyses and combination therapies with standard anti-diabetic drugs are recommended to validate and expand upon these findings.

CONCLUSION(S)

Ticagrelor exerts beneficial effects on glycaemic parameters of diabetes, which could be due to its effect on inflammatory markers. However, further studies are required to establish the effect of ticagrelor in combination with standard anti-diabetic drugs like metformin to support its inclusion in the treatment of diabetes with comorbid conditions like ACS.

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