Turk J Pharm Sci
DOI: 10.4274/tjps.galenos.2025.48482

ORIGINAL ARTICLE

The Effect of Aminoguanidine on Fibrinogen Gene Expression and Oxidative Stress Biomarkers in the Liver Tissue of a Streptozotocin-Induced Diabetic Rat Model

Amir Karbalaee HASANI[1], Hosein Fallahi Kord GHESHLAGHI[1], Mojtaba FATHI[2], Mina HEMMATI[1], Vanoushe Azimi PIRSARAEI[3], Hadi KHODABANDEHLOO[1]*

[1]Zanjan University of Medical Sciences, School of Medicine, Department of Clinical Biochemistry, Zanjan, Iran
[2]Qazvin University of Medical Sciences, Department of Biochemistry and Genetics, Qazvin, Iran
[3]Zanjan University of Medical Sciences, School of Medicine, Student Research Committee, Zanjan, Iran

ABSTRACT

Objectives: Oxidative stress promotes the initiation and progression of diabetic complications. Fibrinogen is a thrombotic factor proposed as a biomarker for prognostic assessment, as a marker for tracking vascular complications, and as a therapeutic target in diabetes. Aminoguanidine, an inhibitor of advanced glycation end products and inducible nitric oxide synthase, has been shown to reduce oxidative stress and related vascular injury. However, the underlying processes still need to be entirely elucidated. This study investigated the effects of aminoguanidine on oxidative stress biomarkers and fibrinogen gene expression in diabetic rats.

Materials and Methods: Twenty-four male rats, matched for body weight and age, were randomly assigned to five groups: (intended n = 6 per group). Due to mortality during the study, the final group sizes were: healthy controls (n = 5), untreated diabetic controls (n = 4), and diabetic rats treated with aminoguanidine at 50, 100, or 200 mg/kg (n = 6, 4, and 5, respectively). Diabetes mellitus was induced by intraperitoneal injection of streptozotocin (50 mg/kg) in rats fasted for 12 h. One week later, diabetic rats received aminoguanidine for 28 days. The liver was selected for analysis due to its key role in oxidative stress and metabolism. Malondialdehyde (MDA) and ferric-reducing ability of plasma (FRAP) were measured in liver tissue as markers of oxidative stress, and fibrinogen gene expression was assessed by real-time polymerase chain reaction.

Results: Aminoguanidine at 50 mg/kg significantly reduced fibrinogen gene expression ($p = 0.047$). Hepatic MDA levels decreased at all doses (50, 100, and 200 mg/kg, $p < 0.05$), while FRAP levels increased significantly at 50 and 100 mg/kg ($p < 0.05$).

Conclusion: Aminoguanidine may attenuate oxidative stress and fibrinogen gene expression in the livers of diabetic rats.

Keywords: Aminoguanidine, fibrinogen, oxidative stress, diabetes mellitus

INTRODUCTION

Hyperglycemia can cause extensive metabolic changes and increase the risk of thrombosis, potentially leading to myocardial infarction, thrombotic stroke, peripheral vascular disease, and venous thromboembolism.[1,2] Prolonged hyperglycemia correlates with higher levels of advanced glycation end products (AGEs), which increase oxidative stress and thrombotic risk by boosting inflammatory responses.[3,4] Oxidative stress can be assessed using various indices such as malondialdehyde (MDA) and ferric reducing ability of plasma (FRAP).[5,6]

Fibrinogen is a plasma-soluble protein produced by the liver and converted by thrombin into fibrin during hemostasis. Fibrinogen levels are independently associated with glycated hemoglobin levels, suggesting that fibrinogen may increase

***Correspondence:** h.khodabandehloo@zums.ac.ir, **ORCID-ID:** orcid.org/0000-0001-9288-3338
Received: 06.04.2024, **Accepted:** 23.11.2025 **Epub:** 09.04.2026

Cite this article as: Hasani AK, Gheshlaghi HFK, Fathi M, Hemmati M, Pirsaraei VA, Khodabandehloo H. The effect of aminoguanidine on the fibrinogen gene expression and oxidative stress biomarkers in diabetic rats. Turk J Pharm Sci.

the risk of cardiovascular events in patients with diabetes. Additionally, AGE levels and fibrinogen have been shown to correlate positively in research,[7-9] suggesting that AGEs and oxidative stress may contribute to thrombosis in diabetic patients.

AGEs can bind to their receptor (RAGE) on hepatocytes, triggering the activation of intracellular signaling pathways, including the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. Activation of these pathways promotes the translocation of transcription factors such as NF-κB into the nucleus, where they enhance the transcription of pro-inflammatory and pro-thrombotic genes, including the fibrinogen gene. Oxidative stress further amplifies this process by increasing reactive oxygen species, which can activate similar signaling cascades and stabilize transcription factors, leading to upregulation of fibrinogen expression.[10,11] Evidence indicates that AGEs can increase production of plasminogen activator inhibitor-1, which arrests fibrinolysis by inhibiting tissue plasminogen activator.[12]

Aminoguanidine (AG), a potential therapeutic agent, could prevent the formation of AGEs by reacting with AGE precursors.[13] In light of the role of oxidative stress in thrombotic complications of diabetes, inhibiting the formation of oxidative stress can reduce fibrinogen expression and the incidence of thrombotic events in diabetic patients. Previous studies have reported protective effects of AG in the context of diabetes and its complications. In streptozotocin (STZ)-induced diabetic rats, AG reduced blood glucose levels and partially reversed diabetes-induced alterations in renal function. It also modulated the expression of angiotensin receptors, which may have contributed to its protective effects on hemodynamic and physiological functions.[14] Another study demonstrated that AG prevented cardiac fibrosis and the development of diabetic cardiomyopathy.[15] In addition, an *in vitro* study using beta-cells showed that exposure to high glucose concentrations for one week significantly reduced insulin secretion and insulin content. Co-treatment with 1 mM AG nearly doubled both basal and glucose-stimulated insulin release, and increased insulin content by 42%, and reduced nitrite accumulation by 33%. These findings suggest that AG may protect beta-cells against glucotoxicity, potentially through modulation of early glycation products and nitric oxide synthase activity.[16] Despite these promising results, some limitations have been noted, including variability in experimental models and dose-dependent effects, which warrant further investigation before translation into clinical practice.

Understanding the effects of AG on oxidative stress and fibrinogen expression is crucial, as it may offer a therapeutic strategy for reducing thrombotic complications in diabetes and provide a rationale for future clinical research. This study assessed the impact of AG on fibrinogen gene expression and on MDA and FRAP levels in the livers of STZ-induced diabetic rats.

MATERIALS AND METHODS

Animal models and study design

The study was conducted on adult male Rattus norvegicus provided by the Experimental Animal Research Center of Zanjan University of Medical Sciences (Zanjan, Iran). The rats (weighing 250–300 g) were kept at room temperature (22–25 °C) with a 12-hour light–dark cycle and unrestricted access to standard laboratory chow (ParsCenter, Iran) and water. Body weight and blood glucose levels were monitored every seven days during the experimental period.

The STZ-induced diabetic rat model was selected because STZ selectively destroys pancreatic beta-cells, reliably inducing hyperglycemia and closely mimicking type 1 diabetes and its associated metabolic disturbances. The liver was chosen as the target organ due to its central role in glucose and lipid metabolism and its susceptibility to diabetes-induced oxidative stress and inflammation. Focusing on the liver allowed assessment of both metabolic and molecular effects of AG in a key organ affected by diabetic complications.[17,18]

The sample size for each group was determined by a priori power analysis (G*Power) with $\alpha = 0.05$ and 80% power, indicating that 4–6 rats per group were required. A total of 24 rats, matched for age and body weight, were randomly assigned to five groups, with an intended size of 6 rats per group. Due to mortality during the study, the final group sizes were: 5 healthy rats on a standard diet (HC group); 4 untreated diabetic rats on a standard diet (DC group); 6 diabetic rats receiving 50 mg/kg of AG (D50 group); 4 diabetic rats receiving 100 mg/kg of AG (D100 group); and 5 diabetic rats receiving 200 mg/kg of AG (D200 group).[19] Randomization was performed using a random number table to minimize allocation bias, and group assignment was conducted by a researcher who was not involved in subsequent interventions or outcome assessments. In addition, the experiments and data analyses were performed by another researcher who was blinded to the group assignments, to further reduce potential bias.

An intraperitoneal injection of 50 mg/kg STZ, dissolved in 0.9% saline, was used to induce diabetes mellitus in rats fasted for 12 hours. Seventy-two hours after the STZ injection, blood glucose levels in fasted rats were measured from tail blood samples using the Accu-Chek Aviva Blood Glucose Monitoring device. Rats classified as diabetics had fasting blood glucose levels above 250 mg/dL. Treatment with intraperitoneal AG (Sigma-Aldrich Co., Cat No. 1068-42-4) began one week after diabetes induction and continued daily for four weeks.[19] Both healthy and untreated diabetic rats received daily saline as a placebo (Table 1). Approval for all procedures conducted was obtained from the Animal Care and Use Committee of Zanjan University of Medical Sciences (approval number: IR.ZUMS.REC.1398.434, dated 14.01.2020).

Liver tissue collection

Ketamine and xylazine were administered intraperitoneally to the rats at the end of the experimental period to induce anesthesia. After removal of the liver, it was rinsed with an

Table 1. Experimental design of the study: animal groups, induction method, and treatments.

Groups	Number of rats	Condition	Induction method	Treatment	Duration
HC	5	Healthy	—	Saline (placebo)	4 weeks
DC	4	Diabetic	STZ (50 mg/kg, i.p.)	Saline (placebo)	4 weeks
D50	6	Diabetic	STZ (50 mg/kg, i.p.)	AG 50 mg/kg (i.p.)	4 weeks
D100	4	Diabetic	STZ (50 mg/kg, i.p.)	AG 100 mg/kg (i.p.)	4 weeks
D200	5	Diabetic	STZ (50 mg/kg, i.p.)	AG 200 mg/kg (i.p.)	4 weeks

AG, aminoguanidine; DC, diabetic control; HC, healthy control; i.p., intraperitoneal; STZ, streptozotocin.

isotonic saline solution and weighed. The tissue was divided into two sections: (a) the first was stored at -80 ˚C for RNA and molecular analyses; (b) the second was homogenized for antioxidant analysis.

FRAP assay

FRAP was measured in the liver using the methods of Benzie and Strain.[20] The intra-assay and inter-assay coefficients of variation were < 1.0% and < 3.0%, respectively, for FRAP values between 100 and 1,000 µmol/L of protein. The FRAP reagent was prepared by incubating a mixture of 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L HCl, 20 mmol/L ferric chloride hexahydrate (FeCl3·6H2O), and 0.3 mol/L acetate buffer (pH 3.6) at a 1:1:10 (*v/v/v*) ratio at 37 ˚C for 10 minutes.

The technique was based on converting the Fe3+-TPTZ complex to the Fe2+-TPTZ complex at low pH, and monitoring the process by detecting the change in absorbance at 593 nm. 300 µL of prepared FRAP reagent and 30 µL of H2O were mixed with 10 µL of the sample solution, incubated at 37 ˚C for 5 minutes, and absorbance was measured using a spectrophotometer. Calibration was conducted using FeSO4·7H2O solutions within the range of 100–1000 µmol/L, and the FRAP value in the tissue was reported as µmol/L of protein.

MDA assay

Lipid peroxidation was assessed by reaction of MDA with thiobarbituric acid according to the methodology outlined by Ohkawa et al.[21] The reaction mixture included 1.5 mL of a 20% acetic acid solution (pH 3.5), 0.1 mL of supernatant, 0.2 mL of sodium dodecyl sulfate (8.1%), and 1.5 mL of a 0.8% aqueous thiobarbituric acid solution. After adding 4 mL of distilled water, the mixture was heated at 95 ˚C for 60 minutes. Distilled water (5 mL) and an n-butanol-pyridine mixture (15:1 *v/v*, 5 mL) were added to the mixture, and it was centrifuged at 4000 r.p.m. for 10 minutes. In the blank, the supernatant was replaced with distilled water. Finally, the results were expressed as µmol L–1 using a spectrophotometer to measure the absorbance of the top layer at 532 nm.

RNA isolation and complementary DNA synthesis

The RNX-Plus kit (SinaClon Co., Tehran, Iran) was used to isolate total RNA from liver tissue. Briefly, 200 µL of chloroform was added to the homogenized liver tissue in 1 mL Trizol, and the mixture was centrifuged at 12,000 r.p.m. and 4 ˚C for 15 minutes. The top aqueous phase was transferred to a new RNase-free tube, combined with an equal volume of isopropanol, vortexed vigorously, and centrifuged at 12,000 rpm at 4 ˚C for 15 minutes. The supernatants were discarded; 1 mL of 75% ethanol was added, and the RNA pellets were centrifuged at 7,500 r.p.m. and 4 ˚C for 8 minutes. After discarding the supernatants and air-drying at room temperature for a few minutes, the pellets were resuspended in 50 µL of DEPC-treated water.

Agarose gel electrophoresis and UV absorbance measurements at 230, 260, and 280 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) were used to assess RNA sample quality and quantity.

Complementary DNA (cDNA) was generated from total RNA using a cDNA synthesis kit (BioFact™ Co., Korea) according to the manufacturer's protocol. After purified total RNA (5 µg) was incubated with oligo dT (1 µL) for 5 minutes at 70˚C, the following components were added to the mixture: 4 µL of 5X reaction buffer, 1 µL of dNTPs (10 mM each), 0.5 µL of RNase inhibitor (40 U/µL), and 1 µL of reverse transcriptase (200 U/µL). The reaction was then incubated at 42 ˚C for 60 minutes, followed by 5 minutes at 70 ˚C.

Real-time polymerase chain reaction

Synthesized cDNA (1 µL) was mixed with forward and reverse primers (0.5 µL each), deionized water, and SYBR Green I master mix. The ABI StepOnePlus real-time polymerase chain reaction (PCR) system was used to perform real-time PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the housekeeping gene due to its widespread use and reported stability in gene expression studies.[22] Ct-value analysis across all experimental groups confirmed that GAPDH expression remained stable under our experimental conditions; therefore, it was used to normalize fibrinogen expression levels.

To ensure the accuracy of the real-time PCR results, both no template control (NTC) and no reverse transcriptase (NRT) reactions were included. The NTC contained all PCR components except cDNA, which was replaced with water; any amplification in this control indicated potential environmental contamination or non-specific priming. The NRT control contained all reaction components; NRT products, generated alongside cDNA synthesis, were added in place of cDNA. Amplification in this control indicated possible contamination with genomic DNA during RNA extraction. NTC reactions were performed in triplicate alongside biological replicates. These procedures ensured that PCR results were not affected by contamination or non-specific amplification.

The PCR cycling conditions involved a 15-minute initial activation step at 95 °C followed by 40 cycles at 95 °C for 15 seconds. The final temperatures were 60 °C and 72 °C, each for 30 seconds. Table 2 displays the primer sequences used in this research. The fold change was calculated using the 2-ΔCt method. To verify product quality, aliquots of real-time PCR reactions that had been dye-stained and subjected to agarose gel electrophoresis were visualized using a UV transilluminator.

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 24.0. The normality of the data was assessed using the Kolmogorov–Smirnov test. The means of two or more independent groups were compared using one-way analysis of variance. GraphPad Prism version 8.0 (GraphPad Software, Inc., CA, USA) was used to generate graphs, and statistical significance was defined as a *p*-value of 0.05 or less. Outliers were identified as values exceeding ±3 standard deviations from the group mean and were excluded from the analysis. No missing data were encountered in this study.

RESULTS

The fibrinogen gene expression

The results demonstrated that the level of fibrinogen gene expression in the liver of untreated diabetic rats was significantly higher than that in healthy rats ($p = 0.001$). Figure 1 shows the comparison of fibrinogen gene expression between the diabetic and healthy groups. In this study, a 28-day treatment with AG at doses of 50, 100, and 200 mg/kg/day decreased the expression of the fibrinogen gene. However, this reduction was statistically significant only at 50 mg/kg/day ($p = 0.047$). Fibrinogen gene expression in treated diabetic rats compared with untreated diabetic rats is presented in Figure 1.

The levels of MDA

Our results suggested that MDA levels in untreated diabetic rats were higher than those in healthy rats ($p < 0.05$). The MDA levels among the untreated rats are illustrated in Figure 2. Measurement of MDA levels, as a marker of oxidative stress, demonstrated that all three doses of AG reduced MDA levels in the livers of diabetic rats ($p < 0.001$). The MDA levels after 28 days of treatment with various doses of AG are shown in Figure 2.

The levels of FRAP

FRAP measurements indicated that the untreated diabetic rats had lower FRAP levels than the healthy group ($p < 0.05$). The FRAP levels in the liver tissue of untreated rats are shown in Figure 3. AG injections increased FRAP levels in diabetic rats, with significant increases at doses of 50 and 100 mg/kg/day compared with untreated diabetic rats ($p < 0.05$). The FRAP levels after treatment with different doses of AG are shown in Figure 3.

Table 2. Detailed information regarding the primers utilized in the study.

Gene	Primer sequence (5′ to 3′)	Length of target
Fibrinogen	Forward: CAAAGGGCAACTGCTTGGAAT Reverse: CGGAGTCAGTAGTTGCAGCTT	132 bp
GAPDH	Forward: CAACTCCCTCAAGATTGTCAGCAA Reverse: GGCATGGACTGTGGTCATGA	118 bp

Bp, base pair; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

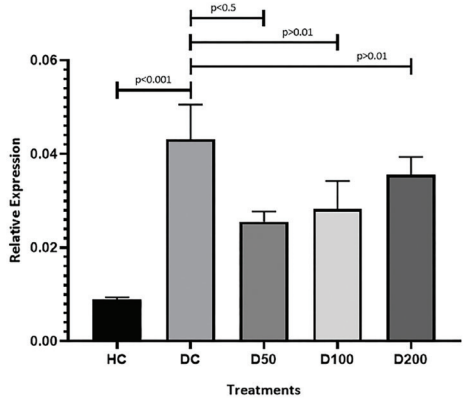

Figure 1. Comparison of fibrinogen gene expression between healthy and diabetic groups and after treatment with aminoguanidine. The data were reported as 2-ΔCt.

D100, diabetic rats administered with 100 mg/kg/day of aminoguanidine; D200, diabetic rats administered with 200 mg/kg/day of aminoguanidine; D50, diabetic rats administered with 50 mg/kg/day of aminoguanidine; DC, diabetic control; HC, healthy control.

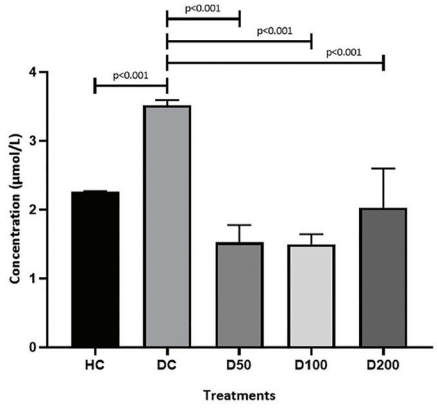

Figure 2. Comparison of malondialdehyde levels between healthy and diabetic groups and after treatment with aminoguanidine. Data were presented as mean ± standard deviation.

D100, diabetic rats administered with 100 mg/kg/day of aminoguanidine; D200, diabetic rats administered with 200 mg/kg/day of aminoguanidine; D50, diabetic rats administered with 50 mg/kg/day of aminoguanidine; DC, diabetic control; HC, healthy control.

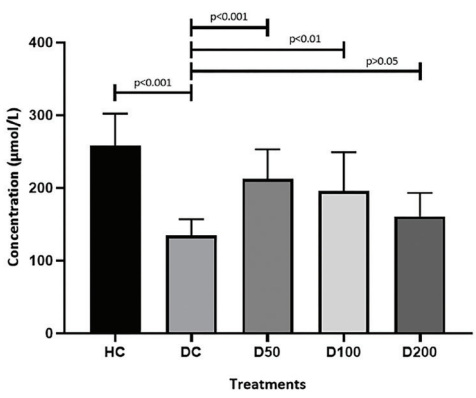

Figure 3. Comparison of FRAP levels between healthy and diabetic groups and after treatment with aminoguanidine. Data were presented as mean ± standard deviation.

D100, diabetic rats administered with 100 mg/kg/day of aminoguanidine; D200, diabetic rats administered with 200 mg/kg/day of aminoguanidine; D50, diabetic rats administered with 50 mg/kg/day of aminoguanidine; DC, diabetic control; FRAP, ferric reducing antioxidant of plasma; HC, healthy control.

DISCUSSION

Cardiovascular diseases account for the majority of deaths among individuals with diabetes mellitus; diabetes is associated with a two- to threefold greater risk of cardiovascular diseases. Evidence suggests that excessive oxidative stress may play a vital role in the development of both diabetes and cardiovascular diseases. Several studies have shown that plasma markers of oxidative stress are elevated in individuals with coronary artery disease or its risk factors.

The present study demonstrated that STZ-induced diabetes mellitus led to oxidative stress and altered MDA and FRAP levels in the livers of diabetic rats. STZ, a toxic glucose analog, selectively damages pancreatic beta cells, leading to oxidative stress.[23] Elevated MDA levels during oxidative stress indicate lipid peroxidation. Preventing oxidative stress can help reduce the severity of diabetes mellitus. Thus, it is crucial to avoid the generation of free radicals by diabetes medications.

The current study assessed the impact of AG on oxidative stress and fibrinogen gene expression in diabetes mellitus. Mechanistically, AG may influence fibrinogen gene expression by modulating oxidative stress–related signaling pathways. Increased oxidative stress in diabetes can activate transcription factors such as NF-κB, which upregulate fibrinogen expression. By reducing reactive oxygen species and enhancing antioxidant defenses, AG may inhibit NF-κB activation, thereby downregulating fibrinogen transcription. Additionally, AG might interact with other signaling pathways involved in inflammation and coagulation, such as the MAPK and JAK/STAT pathways, contributing to its regulatory effect on fibrinogen gene expression.[10,11]

AG contains a nucleophilic hydrazine and a guanidine group, both of which can react with dicarbonyl compounds and biological molecules such as pyridoxal phosphate, pyruvate, glucose, and MDA. AG has been shown to have both antioxidant and pro-oxidant effects.[24]

MDA measurements revealed that all three AG doses significantly reduced MDA levels in STZ-induced diabetic rats. In addition, AG at doses of 50 and 100 mg/kg notably elevated FRAP levels in the livers of diabetic rats. Consistent with these findings, Rastegar-Moghaddam et al.[25] demonstrated that AG administration attenuated hypothyroidism-induced liver injury by significantly lowering MDA, improving liver function tests, restoring antioxidant enzyme activities, and reducing fibrotic changes, particularly at a dose of 30 mg/kg. Similarly, in a model of renal ischemia/reperfusion injury, co-administration of AG mitigated the adverse effects of sumatriptan by reducing serum creatinine and blood urea nitrogen levels, decreasing MDA, and restoring superoxide dismutase activity.[26] In an alloxan-induced rabbit model of type 1 diabetes, AG administration significantly decreased early glycation products, including HbA1c and fructosamine, restored the activity of key antioxidant enzymes, and reduced excessive nitric oxide production.[27] Moreover, Hosseini et al.[28] demonstrated that AG exerted protective effects against endotoxin-induced renal dysfunction in rats, evidenced by reductions in MDA and nitric oxide metabolites, along with restored activities of superoxide dismutase and catalase. Collectively, these studies indicate that the protective effects of AG are largely mediated by inhibition of nitric oxide production from inducible nitric oxide synthase and enhancement of endogenous antioxidant and anti-inflammatory defenses, thereby alleviating oxidative and nitrosative stress-related tissue damage.

Other studies used *in vitro* models of oxidized bovine serum albumin with glucose as a glycating agent to assess antioxidant activity and found that the group treated with AG had higher FRAP levels than the untreated group.[29,30] The FRAP levels in the colon of the irritable bowel syndrome model were not significantly different from those in the AG-treated group (100 mg/kg, intraperitoneally).[31] The models examined in the studies mentioned above could account for the disparity in results. Overall, these findings indicate that the antioxidant effects of AG, demonstrated both *in vitro* and in animal models, may have clinical relevance by helping to mitigate oxidative stress in diabetes and thereby reduce the risk of cardiovascular complications.

The role of fibrinogen in the pathogenesis of atherosclerosis, and its consequences in patients with diabetes mellitus, has been investigated; previous research has confirmed the vital role of fibrinogen in the development of cardiovascular events.[32,33] In addition to modulating oxidative stress and fibrinogen expression, AG may confer cardiovascular protection by improving endothelial function, reducing fibrin thrombus formation, and regulating fibrinolytic factors such as plasminogen activator inhibitor-1 and tissue-type plasminogen activator.[34] However, investigations on the effects of AG on fibrinogen gene expression in diabetes mellitus were limited.

The current study demonstrated that treatment with AG at various doses reduced fibrinogen gene expression in diabetic rats. This reduction was statistically significant only in the 50 mg/kg/day dose. This dose–response relationship suggested

that moderate dosing was optimal for modulating fibrinogen expression, potentially reflecting a balance between inhibition of AGEs and avoidance of compensatory cellular responses that occur at higher doses. Further studies are needed to clarify the underlying molecular mechanisms and to determine the most effective dose range of AG for preventing vascular complications in diabetes.

Su et al.[35] found that plasma fibrinogen levels were higher in patients with cardiovascular disease who died than in participants who survived. No formation of fibrin thrombi was observed in the livers of pigs in the AG group, either before or at the end of the ischemic period. However, the AG group showed reduced fibrin thrombus formation after reperfusion compared with other groups.[36] In another study, treatment with AG (25 mmol/L) during glycation of low-density lipoprotein cholesterol significantly decreased plasminogen activator inhibitor-1 and increased tissue-type plasminogen activator in vascular endothelial cells.[37]

Based on the findings of the current study, future research could explore the long-term effects of AG on oxidative stress and fibrinogen expression in diabetic models. Studies in different animal models, including larger mammals, would help to confirm the generalizability of these results. Furthermore, clinical trials could evaluate the potential translational benefits of AG in managing oxidative stress and reducing cardiovascular risk in patients with diabetes. Additional investigations into the underlying molecular mechanisms and optimal dosing strategies are warranted to maximize therapeutic efficacy and safety.

Study limitations

This study had several limitations that should be noted. First, the sample size was relatively small, which may limit the statistical power and generalizability of the findings. Second, the study duration was short, and the longer-term effects of AG on oxidative stress and fibrinogen expression remain unclear. Third, the use of the STZ-induced diabetic rat model, while widely accepted, may not fully replicate all aspects of human diabetes and its cardiovascular complications. Finally, the study did not assess the potential side effects or toxicity of AG, which should be considered in future investigations. Future studies addressing these limitations would strengthen the evidence base for the therapeutic potential of AG.

CONCLUSION

Diabetes mellitus is a complex metabolic disease in which oxidative stress and hyperfibrinogenemia play central roles in disease progression and the development of cardiovascular complications. In this study, AG, an inhibitor of AGE formation, significantly reduced oxidative damage and fibrinogen gene expression in diabetic rats. These findings suggest that AG may have clinical benefits in mitigating oxidative stress, regulating fibrinogen levels, and lowering cardiovascular risk in patients with diabetes. Future studies are warranted to optimize dosing, evaluate long-term effects, and confirm these protective effects in clinical settings.

Ethics

Ethics Committee Approval: Approval for all procedures conducted was obtained from the Animal Care and Use Committee of Zanjan University of Medical Sciences (approval number: IR.ZUMS.REC.1398.434, dated 14.01.2020).

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Design: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Data Collection or Processing: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Analysis or Interpretation: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Literature Search: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K., Writing: A.K.H., H.F.K.G., M.F., M.H., V.A.P., H.K.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Li X, Weber NC, Cohn DM, Hollmann MW, DeVries JH, Hermanides J, Preckel B. Effects of hyperglycemia and diabetes mellitus on coagulation and hemostasis. J Clin Med. 2021;10:2419.
2. Kwaifa IK, Bahari H, Yong YK, Noor SM. Endothelial dysfunction in obesity-induced inflammation: molecular mechanisms and clinical implications. Biomolecules. 2020;10:291.
3. Fuhr JC, Ramos MEK, Piovesan F, Renner LdO, Siqueira LdO. Relationship of advanced glycation end-products in hypertension in diabetic patients: a systematic review. J Bras Nefrol. 2022;44:557-572.
4. Chen Y, Meng Z, Li Y, Liu S, Hu P, Luo E. Advanced glycation end products and reactive oxygen species: uncovering the potential role of ferroptosis in diabetic complications. Mol Med. 2024;30:141.
5. Alam R, Ahsan H, Khan S. The role of malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP) in patients with hypertension. Molecular and Cellular Biomedical Sciences. 2023;7:58-64.
6. Cordiano R, Di Gioacchino M, Mangifesta R, Panzera C, Gangemi S, Minciullo PL. Malondialdehyde as a potential oxidative stress marker for allergy-oriented diseases: an update. Molecules. 2023;28:5979.
7. Vilar R, Fish RJ, Casini A, Neerman-Arbez M. Fibrin(ogen) in human disease: both friend and foe. Haematologica. 2020;105:284.
8. Abdelmonem M, Abdulla S, Wasim H, Elawamy H. The correlation between plasma fibrinogen levels and glycemic status in type 2 diabetes mellitus. Am J Clin Pathol. 2023;160(Supplement 1):S19-S20.
9. Luzak B, Boncler M, Kosmalski M, Mnich E, Stanczyk L, Przygodzki T, Watala C. Fibrinogen glycation and presence of glucose impair fibrin polymerization—an *in vitro* study of isolated fibrinogen and plasma from patients with diabetes mellitus. Biomolecules. 2020;10:877.
10. Pathomthongtaweechai N, Chutipongtanate S. AGE/RAGE signaling-mediated endoplasmic reticulum stress and future prospects in non-coding RNA therapeutics for diabetic nephropathy. Biomed Pharmacother. 2020;131:110655.
11. Zhang Y, Zhang Z, Tu C, Chen X, He R. Advanced glycation end products in disease development and potential interventions. Antioxidants. 2025;14:492.

12. Khalid M, Petroianu G, Adem A. Advanced glycation end products and diabetes mellitus: mechanisms and perspectives. Biomolecules. 2022;12:542.

13. Song Q, Liu J, Dong L, Wang X, Zhang X. Novel advances in inhibiting advanced glycation end product formation using natural compounds. Biomed Pharmacother. 2021;140:111750.

14. Jogula RMR, Row AT, Siddiqui AH, Row A. The effect of treatment with aminoguanidine, an advanced glycation end product inhibitor, on streptozotocin-induced diabetic rats and its effects on physiological and renal functions. Cureus. 2023;15.

15. Heydari AH, Fathi M, Heydari S, Heidari ME. Advanced glycation end product blocker drugs have a great potential to prevent diabetic cardiomyopathy in an animal model of diabetes mellitus type 2. Cardiovasc Ther. 2022;2022:7014680.

16. Tajiri Y, Grill V. Aminoguanidine exerts a β-cell function-preserving effect in high glucose-cultured β-cells (INS-1). J Diabetes Res. 2000;1:111-119.

17. Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: a practical guide. EXCLI J. 2023;22:274.

18. Vesković M, Šutulović N, Hrnčić D, Stanojlović O, Macut D, Mladenović D. The interconnection between hepatic insulin resistance and metabolic dysfunction-associated steatotic liver disease—the transition from an adipocentric to liver-centric approach. Curr Issues Mol Biol. 2023;45:9084-9102.

19. Alipour M, Adineh F, Mosatafavi H, Aminabadi A, Monirinasab H, Jafari MR. Effect of chronic intraperitoneal aminoguanidine on memory and expression of Bcl-2 family genes in diabetic rats. Can J Physiol Pharmacol. 2016;94:669-675.

20. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70-76.

21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-358.

22. Shahsavarpour S, Shafiei M, Buratti E, Galehdari H. Identification of reliable housekeeping genes for qRT-PCR normalization in neuroblastoma and glioblastoma cell lines infected with lentivirus. Gene, Cell and Tissue. 2025;12.

23. Salazar-García M, Corona JC. The use of natural compounds as a strategy to counteract oxidative stress in animal models of diabetes mellitus. Int J Mol Sci. 2021;22:7009.

24. Köhler ZM, Szepesi Á. More than a diamine oxidase inhibitor: L-aminoguanidine modulates polyamine-related abiotic stress responses of plants. Life. 2023;13:747.

25. Rastegar-Moghaddam SH, Kiumarsi Z, Mirani A, Mortazavi M, Beheshti F, Ebrahimzadeh-Bideskan A, Anaeigoudari A, Hosseini M. Aminoguanidine improved liver function and attenuated oxidative stress in hypothyroid rats by propylthiouracil. Adv Biomed Res. 2025;14:64.

26. Mobasheran P, Rajai N, Kohansal P, Dehpour AR, Shafaroodi H. The effects of acute sumatriptan treatment on renal ischemia/reperfusion injury in rat and the possible involvement of nitric oxide. Can J Physiol Pharmacol. 2020;98:252-258.

27. Arif B, Arif Z, Ahmad J, Perveen K, Bukhari NA, Ashraf JM, Rabbani G. Attenuation of hyperglycemia and amadori products by aminoguanidine in alloxan-diabetic rabbits occurs via enhancement in antioxidant defenses and control of stress. PLoS One. 2022;17:e0262233.

28. Hosseini M, Beheshti F, Anaeigoudari A. Improving effect of aminoguanidine on lipopolysaccharide-caused kidney dysfunction in rats. Saudi J Kidney Dis Transpl. 2020;31:1025-1033.

29. Pawlukianiec C, Gryciuk ME, Mil KM, Żendzian-Piotrowska M, Zalewska A, Maciejczyk M. A new insight into meloxicam: assessment of antioxidant and anti-glycating activity in *in vitro* studies. Pharmaceuticals. 2020;13:240.

30. Mil KM, Gryciuk ME, Pawlukianiec C, Żendzian-Piotrowska M, Ładny JR, Zalewska A, Maciejczyk M. Pleiotropic properties of valsartan: do they result from the antiglycooxidant activity? Literature review and *in vitro* study. Oxid Med Cell Longev. 2021;2021:1-14.

31. Dolatabadi F, Abdolghaffari AH, Farzaei MH, Baeeri M, Ziarani FS, Eslami M, Abdollahi M, Rahimi R. The protective effect of Melissa officinalis L. in visceral hypersensitivity in rat using two models of acid-induced colitis and stress-induced irritable bowel syndrome: a possible role of nitric oxide pathway. J Neurogastroenterol Motil. 2018;24:490.

32. Surma S, Banach M. Fibrinogen and atherosclerotic cardiovascular diseases—review of the literature and clinical studies. Int J Mol Sci. 2021;23:193.

33. Sun J, Han K, Xu M, Li L, Qian J, Li L, Guo Y, Chen Y. Blood viscosity in subjects with type 2 diabetes mellitus: roles of hyperglycemia and elevated plasma fibrinogen. Front Physiol. 2022;13:827428.

34. Beheshti F, Hosseini M, Hashemzehi M, Hadipanah MR, Mahmoudabady M. The cardioprotective effects of aminoguanidine on lipopolysaccharide-induced inflammation in rats. Cardiovasc Toxicol. 2020;20:474-481.

35. Su H, Cao Y, Chen Q, Ye T, Cui C, Chen X, Zhang Y, Lin X, Zhou Y. The association between fibrinogen levels and severity of coronary artery disease and long-term prognosis following percutaneous coronary intervention in patients with type 2 diabetes mellitus. Front Endocrinol. 2023;14:1287855.

36. Isobe M, Katsuramaki T, Hirata K, Kimura H, Nagayama M, Matsuno T. Beneficial effects of inducible nitric oxide synthase inhibitor on reperfusion injury in the pig liver. Transplantation. 1999;68:803-813.

37. Zhang J, Ren S, Sun D, Shen GX. Influence of glycation on LDL-induced generation of fibrinolytic regulators in vascular endothelial cells. Arterioscler Thromb Vasc Biol. 1998;18:1140-1148.