Coffee intake (*Coffea arabica L.*) reduces advanced glycation end product (AGEs) formation and platelet aggregation in diabetic rats

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Abstract

Objectives: The study aimed to determine the effect of coffee intake on AGEs formation and platelet aggregation in diabetic Wistar rats. Methods: Coffee powder samples were used to prepare a 10% beverage. Diabetes mellitus was induced in the animals by administering 2% alloxan. All animal experiments were approved by the ethics committee for animal experiments under No. 420/2012 and 536/2013. Diabetic and non-diabetic rats were divided into 6 groups treated and untreated with coffee (7.2 mL/Kg body weight) and aminoguanidine (AGE inhibiting agent) (100 mg/Kg body weight) for 50 days. After 50 days, the animals were fasted for 12 h and anesthetized (40 mg/Kg sodium pentobarbital) intraperitoneally. Blood samples were collected from the abdominal artery puncture. Hematological parameters (red cells, hemoglobin, hematocrit and leukocyte) and glycemic and HbA1c levels were measured. AGEs quantification (spectrofluorometric method) and the platelet aggregation test (aggregation of cuvettes in a four-channel platelet aggregometer) were also conducted. The rats' renal function was evaluated by measuring serum urea and creatinine. Results: Data showed that coffee intake had no effect on the hematological parameters. Fasting glucose and HbA1c dosage were significantly higher in diabetic animals compared to non-diabetic animals (confirmed the effectiveness of inducing and maintaining diabetic status). Results showed that coffee reduced AGE formation and platelet aggregation in our animal model, not altering the animals’ renal function. Conclusions: These results suggest beneficial effects on vasculopathy, a common complication in diabetic patients.

Keywords: *Coffea arabica* L. Diabetes Mellitus. End Products of Advanced Glycation. Aminoguanidine. Platelet Aggregation

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1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia that affects 415 million people worldwide, with an estimate for the coming decades of 642 million new cases. One of its causes may be the resistance of the insulin present in the body or the reduction in the amount of insulin produced (type 2 DM). Another manifestation of this disease occurs when the insulin-producing cells (pancreatic β) are destroyed, hindering hormone secretion (type 1 DM). As the illness progresses, it may trigger disorders such as nephropathy, neuropathy, retinopathy (microvascular complications), coronary heart disease, peripheral vascular disease and heart failure (macrovascular complications).

Complications resulting from chronic hyperglycemia during DM are caused by oxidative stress triggered in different pathways, such as advanced glycation end products (AGEs) formation via polyol, protein kinase C (PKC) and hexosamine biosynthesis. These oxidative changes alter genetic expressions that damage pancreatic β cells and induce insulin resistance. Thus, AGEs formation act as one of the key factors that explain the ability of these compounds to alter the chemical and functional properties of various biological structures. Chronic hyperglycemia contributes significantly to endothelial injury by formation of free radicals, interactions with cellular receptors, or via cross-linking with proteins. The irreversible glycation of subendothelial collagen and other structural proteins changes vascule structure and promotes vascular permeability and synthesis of pro-inflammatory cytokines, leading to diabetic complications such as macrovascular disorders. Platelets, in turn, adhere to subendothelial components and are activated by cellular signaling, resulting in alteration, secretion and aggregation of the cellular form. Hyperglycemia has been reported to increase the propensity for platelets to aggregate and degranulate, leading to hypercoagulability.

Currently, studies consider this chronic metabolic disorder as one of the main causes of increased morbidity and mortality in the world, constituting the main public health issue. Demand for diabetes treatment and prevention measures is imperative, and natural products with bioactive substances are currently a new research trend. Phenolic compounds present in foods and beverages have been associated with a decreased risk of developing several chronic diseases. Coffee, which contains chlorogenic acid as a main component, is the main source of phenolic compounds in the human diet and one of the most appreciated beverages worldwide. Due to its potential protective effects on human health, coffee has gained prominence and has garnered the interest of researchers in the search for a treatment to chronic diseases.

The chemical composition of the coffee bean is quite complex and depends on the variety, farming, processing type, roasting degree and grinding, and the brewing method. Factors and dietary habits of coffee consumers, such as frequency of coffee intake, lifestyle, and genetic predisposition to developing certain diseases, can equally affect the possible effects of coffee on health. Coffee consumption can affect key factors for blood sugar regulation, such as mechanisms of glucose capture in the intestine, hepatic gluconeogenesis and oxidative process in the liver.

Containing a combination of bioactive components, especially phenols, coffee appears as one of the several therapeutic candidates studied in the search for compounds that can prevent complications caused by diabetes. This study aims to determine the effects of coffee on AGEs formation and platelet aggregation in diabetic rats.

2. METHODS

All in vivo experiments followed the ethical principles outlined for animal experimentation adopted by the guidelines of the International Council for Laboratory Animal Science (ICLAS) and were approved by the Ethical Committee for Animal Experimentation of UNIFAL-MG doc. n° 420/2012 and 536/2013.
2.1. Coffee preparation and characterization

The coffee (Coffea arabica L.) samples used in this study came from Fazenda Conquista (865 m altitude), belonging to Ipanema Agricola S.A., located in Alfenas, MG. The natural coffee beans were dried in the sun and resulted in 12% of the unit (gravimetric method - Farmacopeia Brasileira31. They were roasted in a Probat roaster and then ground in a Raiar electric grinder (model RA21) in fine granulometry, vacuum-packed and stored at -20°C, until use.

For study, an aliquot powdered coffee was placed in a paper filter funnel (Whatman Nº3, General Electric Company), and water at 90 °C added over, resulting in a beverage with a 10% concentration. These beverage samples were lyophilized for the chemical analyses28. For the bioassays, drinks were always prepared at the time of administration.

2.2. Coffee administration, diabetes induction and treatment with an AGE inhibitor (aminoguanidine)

All *in vivo* experiments followed the ethical principles outlined for animal experiments adopted by the International Council for Laboratory Animal Science (ICLAS) guidelines and were approved by the Ethics Committee for Animal Experiments of the Federal University of Alfenas, Alfenas, MG, Brazil (UNIFAL-MG) under n° 420/2012 and 536/2013.

Male Wistar rats (22 weeks old, 320 ± 20 g, n = 60) were obtained from the animal house of the UNIFAL-MG and kept under controlled temperature of 23°C, 12 h-light/dark conditions with food and water access *ad libitum*.

Filtered coffee beverages were administered daily to the animals by gavage corresponding to 7.2 mL/kg body weight for a period of 50 days, which is equivalent to the daily human consumption of eight 50-mL cups of coffee. The control group received the same dosage of water.

Diabetes was induced in animals kept fasting for 12 hours by intraperitoneal administration of alloxan (2% solution) at a dose of 150 mg/kg body weight33,34. We considered as diabetic the rats with glycemia above 250 mg/dL verified by the Trinder reaction, seven days after induction35. Blood glucose levels were measured weekly by collecting blood from the rats’ tails to supervise the diabetic process.

The aminoguanidine (AMG) (Sigma Chemical Co, St. Louis-USA), AGE inhibiting agent, treatment was conducted by administering 100 mg/kg body weight by gavage for 50 days, starting seven days after alloxan administration and confirmed diabetic status36,37.

2.3. Experiment design

After a week of acclimatization, the rats were divided into 6 groups (n = 10 rats, group): non-diabetic rats treated with distilled water (ND), non-diabetic rats treated with aminoguanidine (ND + AMG), non-diabetic rats treated with full *Arabica* coffee (ND + CF), diabetic rats treated with distilled water (D), diabetic rats treated with aminoguanidine (D + AMG), and diabetic rats treated with full *Arabica* coffee (D + CF).

On day 51, after treatment, the animals underwent fasting for 12 hours, and then anesthetized and euthanized via deep anesthesia37 after intraperitoneal administration of 40 mg/kg of sodium pentobarbital. Blood samples for biological examinations were collected by abdominal artery puncture with a 19G needle and distributed in: siliconized test tubes without additives, siliconized tubes with ethylenediaminetetraacetic acid (EDTA), and tubes with sodium citrate.
2.4. Biological sample preparation and collection

2.4.1. Obtaining serum

A blood aliquot was distributed in siliconized test tubes without additives containing gel separator. After blood clotting, the samples were centrifuged at 2000 g for 10 minutes. Serum was separated and used for evaluating blood glucose, renal profile and AGEs quantification. 38

2.4.2. Obtaining whole blood

A blood aliquot was distributed in siliconized test tubes containing EDTA. Whole blood was used for determining glycated hemoglobin (HbA1c) and hematological parameters.

2.4.3. Obtaining platelet-rich plasma (PRP)

Blood was collected by abdominal artery puncture and distributed in Falcon type centrifuge tubes containing anticoagulant citric acid 3% w/v, trisodium citrate 4% w/v, glucose 2% w/v, sodium citrate (ADC-C), at a 1:9 (v/v) ratio and homogenized. Then, the whole blood samples were centrifuged at 600 g for 12 minutes at room temperature within four hours after blood collection, to obtain platelet rich plasma (PRP). 39

2.4.4. Obtaining washed platelet

A wash buffer (140 mM NaCl, 0.5 mM KCl, 12 mM trisodium citrate, 10 mM glucose and 12.5 mM sucrose, pH 6) was added to the PRP at a 7:5 (buffer/plasma) ratio and then centrifuged for 13 minutes at 822 g. The platelet pellet was resuspended in Krebs-Ringer solution devoid of calcium with pH precisely adjusted to track 7.2 - 7.4 CO2 39.

2.5. Hematological parameters

Hematological blood samples, collected in tubes containing EDTA, were processed in an Analyzer Counter 19 (Wiener Laboratories) to determine hematological parameters (red cells, hemoglobin, hematocrit, and leukocyte).

2.6. Glycemic and HbA1c levels

Blood glucose was determined by enzymatic method [39]. HbA1c was assessed by ion exchange chromatography (HPLC), using D-10 Hemoglobin A1c Program and Dual Program BIO-RAD® Kit. 37

2.7. AGEs spectrofluorometric measure

To quantify AGE-peptides we measured fluorescence according to Zilin et al. 38. Serum samples (70 μL) were treated with trichloroacetic acid (TCA) and chloroform, under vigorous agitation to precipitate proteins and extract lipids in the organic phase and were then centrifuged. Measurements were performed with a spectrofluorometer adjusted at wavelengths of 350 nm (excitation) and 440 nm (emission) with a gap width of 5 nm. The results were expressed as arbitrary unit divided by the concentration of total proteins (method of Biuret – Layne) in the serum (AU/ mg protein) 37,41.

2.8. Hemostatic parameters

Platelet count in the PRP followed the impedance method proposed by Wallace Counter automated apparatus (Wiener Lab. Counter 19).

To conduct the platelet aggregation test using the aggregation curve we initially performed a platelet count in the PRP, which were adjusted to 250.000 platelets/mm³. After adjustment, the PRP was incubated with 1 mM calcium chloride (CaCl₂), for 5 minutes at 37°C, then added...
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1 μM agent adenosine agonist 5'-diphosphate (ADP), and the variation of light transmission was monitored by standardized turbidimetric method for 5 minutes at 37°C. The platelet aggregation measurements were carried out in bucket aggregations in 4-channel platelet aggregometer (Qualiterm) with ultimate reaction volume of 410 μL.

2.9. Renal function biomarkers

Urea and creatinine were measured by a commercial kit according to the manufacturers’ instructions and using an automatic analyzer (HumanStar, InVitro Diagnóstica).

2.10. Statistical analysis

Three experiments were conducted in Completely Randomized Design (CRD) and analyzed under double factorial scheme (2 levels of diabetes and 3 levels of inhibitors). Tukey test was performed for qualitative factors, when necessary (5% of significance level).

Eventual missing data were estimated by analysis of variance in a linear model, resulting in a balanced scores table.

All analyses were performed in R (R CORE TEAM using ExpDes package).

3. RESULTS

We evaluated the animals’ hematological parameters (red cells, hemoglobin, hematocrit, and leukocyte measurements) to verify whether the diabetes induction or treatments with coffee and AMG modified the erythrocytes and the number of leukocytes in these animals.

For the mean erythrocyte count, we observed no differences between the diabetic and non-diabetic groups (Table 1). AMG treatment and coffee intake effected no change on the rats’ erythrocyte profile and leukocyte counts. The obtained data suggest that coffee consumption does not alter hemoglobin levels or the number of circulating red blood cells and leukocytes when animals have a balanced diet.

### Table 1. Determination of the rats’ hematological parameters in the different treatment groups.

| Parameters       | Hem (x 10^12 L) | Hb (g/dL) | Ht (%) | Leuk (mm^3) |
|------------------|-----------------|-----------|--------|-------------|
| Non-diabetic     | 8.7 (±0.4)      | 16.7 (±0.7)| 46.0 (±1.5)| 5785.0 (±348.4) |
| Diabetic         | 8.8 (±0.4)      | 17.2 (±1.1)| 48.7 (±2.9)| 5522.0 (±243.8) |
| ND + AMG         | 8.6 (±0.6)      | 17.0 (±0.4)| 46.6 (±2.0)| 5825.0 (±436.7) |
| D + AMG          | 8.6 (±0.5)      | 17.1 (±1.1)| 47.9 (±2.8)| 5514.0 (±402.9) |
| ND + CF          | 8.8 (±0.5)      | 17.9 (±0.3)| 47.7 (±1.9)| 5542.0 (±395.2) |
| D + CF           | 8.7 (±0.5)      | 17.0 (±1.0)| 47.8 (±2.9)| 5275.0 (±377.0) |

The results represent the mean ± standard deviation (SD) for five measurements per treatment.

Non-diabetic: non-diabetic animals, Diabetic: diabetic animals, ND + AMG: non-diabetics animals treated with aminoguanidine, D + AMG: diabetic animals treated with aminoguanidine, D + CF: diabetic animals treated with coffee, Hem: red blood cells, Hb: hemoglobin, Ht: hematocrit, Leuk: leucocytes. The data statistical analysis showed no statistically significant difference between the groups and treatments performed.

The fasting glucose (kept above 250 mg/dL throughout the experiment) and HbA1c levels were significantly higher in diabetic animals (D) compared with non-diabetic animals (ND) (Figure 1), showing the effectiveness of alloxan in inducing and maintaining diabetic conditions throughout the experiment. Here, coffee intake had no effect on fasting blood glucose and HbA1c levels for either group (Figure 1). As expected, the HbA1c levels decreased in diabetic animals treated with AMG, a known glycation inhibitor (Figure 1).
Coffee intake (Coffea arabica L.) reduces advanced glycation end product (AGEs) formation and platelet aggregation in diabetic rats. Non-diabetic and diabetic rats were treated with water, aminoguanidine (AMG) or coffee. SD: standard deviation. ***p < 0.001 indicates statistical difference.

Circulating levels of fluorescent AGEs were significantly higher in diabetic animals compared with non-diabetic animals (Figure 2). AGEs serum concentration decreased in both groups treated with AMG (Figure 2). As expected, our results show an efficacy of AMG treatment in preventing AGEs formation in diabetic animals. Coffee was as efficient as AMG in reducing AGEs formation in diabetic animals (Figure 2).

To assess the effects of coffee on primary hemostasis in diabetic rats, we measured the platelet aggregation against the ADP agonist. Platelet aggregation significantly increased in the diabetic animals (Figure 3), suggesting an excess of glucose-induced osmotic effect and reactive oxygen species (ROS) in the serum.
Coffee intake (Coffea arabica L.) reduces advanced glycation end product (AGEs) formation and platelet aggregation in diabetic rats.

We observed that AMG significantly reduced platelet aggregation in non-diabetic and diabetic animals (Figure 3). Our results show that coffee reduced platelet aggregation in diabetic animals to levels comparable to those of the non-diabetic group (Figure 3).

Finally, we evaluated the renal parameters in both animal groups by measuring urea and creatinine levels. Diabetic rats had significantly higher levels of urea in the serum (Figure 4A). Hyperglycemia did not alter the serum creatinine levels, that is, we found no kidney damage due to hyperglycemia in the evaluated period (Figure 4B). The AMG treatment or coffee intake had no effect on these animals’ renal profile.

4. DISCUSSION

The search for therapeutic plants for treating diabetes mellitus has been stimulated by the World Health Organization (WHO) due to low side effects from their prolonged and traditional use, in addition to being a low cost and significantly effective therapeutic alternative.

Figure 3. Evaluation of platelet aggregation percentage in rats. Values are presented as mean ± SD of the three experiments performed in triplicate to measure platelet aggregation in the non-diabetic and diabetic groups. Non-diabetic and diabetic rats were treated with water, aminoguanidine (AMG) or coffee. SD: standard deviation. ***p < 0.001 indicates statistical difference.

Figure 4. Determination of the rats’ renal profile with serum urea (mg/mL) (A) and creatinine (mg/mL) (B) measurements. Values are presented as mean ± SD of the three experiments performed in triplicate to measure serum urea and creatinine in the non-diabetic and diabetic groups. Non-diabetic and diabetic rats were treated with water, aminoguanidine (AMG) or coffee. SD: standard deviation. ***p < 0.001 indicates statistical difference.
Coffee intake (Coffea arabica L.) reduces advanced glycation end product (AGEs) formation and platelet aggregation in diabetic rats

Coffee is a fruit rich in bioactive compounds, such as caffeine, trigonelline, chlorogenic acids, phenolic compounds, diterpenes and melanoidins. Its long-term consumption has shown beneficial effects in chronic diseases such as diabetes. The decreased risk of developing chronic diseases has been related to the presence of phenolic compounds.

Untreated chronic hyperglycemia leads to macro and microvascular diabetic complications. The progression of these complications triggers multiple renal, cardiac and hepatic disorders and, consequently, derangements in the biochemical, hematological and histological parameters that contribute to increase free radicals, associated with the pathophysiology of diabetes.

In the present study, the diabetic status did not alter the number of leukocytes; but this change can occur and is typical of the damage caused by induced diabetes, as reported by. The other parameters (Hem and Hb) also showed no statistical change. Hyperglycemia can modify red blood cell count and cause oxidative stress, hemoglobin glycation and other hematological disorders. Studies have shown the ability of phenolic compounds to form complexes with Fe (II), present in large quantities in the hemoglobin molecule, which could interfere with its absorption in the gastrointestinal tract and consequently lead to iron deficiency. The doses of coffee consumption used in this study, however, were unable to change the hematological parameters in rats with a balanced diet.

Regarding the glycemic profile, diabetes induction and maintenance was confirmed in animals by the increase in fasting glucose and glycated hemoglobin levels. Among the bioactive substances present in coffee, caffeine has controversial effects on blood glucose levels. While short-term metabolic studies performed in humans have shown that caffeine at acute doses reduces insulin sensitivity due to its rapid metabolism, others have observed increased sensitivity in insulin receptors upon caffeine treatment. Long-term coffee consumption can induce glucose tolerance and insulin sensitivity, helping to prevent diabetes symptoms and improving insulin sensitivity.

Several mechanisms have been proposed to explain the beneficial effect of coffee on glucose, insulin homeostasis and diabetes progression. This protective effect can be attributed to other coffee components such as chlorogenic acid and their derivatives – lignans, trigonelline, N-methylpyridinium, minerals and vitamins, proteins and lipids (diterpenes). These compounds can act on harmful free radicals, participate in cell signaling pathways performing, among other functions, anti-glycation activity. Chlorogenic acids and their derivatives represent the most prevalent phenolic compounds in coffee.

The HbA1c level is an important biomolecular marker. It is a chromatographic fraction of hemoglobin that carries an Amadori product in its β chain and that reflects the occurrence of hyperglycemia in the three months prior to its measurement and, indirectly, advanced glycation. After 50 days of treatment, measuring this marker also confirmed the diabetic state induced in the animals.

AMG, an agent commonly used to prevent AGEs formation in animal models, is effective in reducing AGEs and lowering the severity of diabetes-associated structural and functional alterations. The use of a treatment that blocks AGEs or reduces oxidative and nitrosative stress can be effective in controlling diabetic complications.

The contribution of AGEs in the development and progression of diabetic complications is well established. AGEs may act by modifying intracellular functional proteins involved in gene regulation in the nearby extracellular matrix molecules, by interfering in the signaling between the matrix and the cell causing dysfunction in proteins such as albumin. AGE accumulation results in endothelial dysfunction, fibrosis, chronic inflammation and, consequently, in generalized cellular dysfunction.

As AGEs are one of the main factors that could potentially explain how chronic hyperglycemia leads to the cell and tissue damage observed in diabetes, we measured the serum concentration of the well characterized and widely studied fluorescent AGEs pentosidine and argipiridine in the animals. Circulating levels of fluorescent AGE-peptides were significantly higher in diabetic animals compared with the non-diabetic animals. These results were similar to those found by Ferreira et al. Although it has already been
Coffee intake (Coffea arabica L.) reduces advanced glycation end product (AGEs) formation and platelet aggregation in diabetic rats shown that AGE production increases significantly under hyperglycemia or oxidative stress, several of its mechanical details remain unclear. As mentioned above, AGEs serum concentration significantly decreased in aminoguanidine-treated diabetic and non-diabetic rats (Figure 2). Our results show the effectiveness of aminoguanidine treatment in preventing AGE formation in diabetic animals. These data are consistent with the results obtained previously by Ferreira et al. Similarly, coffee efficiently reduced AGE formation in diabetic animals (Figure 2).

There has been significant interest in identifying dietary components that combat or attenuate diabetic complications by reducing AGEs accumulation or inhibiting its effects on the body. Although no food component has been specified as being anti-AGE in vivo, certain coffee components, such as phenolic compounds, have been intensively studied and shown to have the ability to inhibit AGE formation.

Being highly damaging to the integrity and function of blood vessels, AGEs result in the main cause of diabetes complications related to vasculopathy. The interaction of AGEs with components of the vessel wall increases vascular permeability, expression of procoagulant activity and ROS generation, resulting in increased leukocyte adhesion molecules in the endothelial layer. Additionally, platelet-monocyte aggregates and platelet hyperactivity are important factors commonly associated with chronic diabetes complications.

Diabetic patients generally present symptoms of hypercoagulability and hypofibrinolysis, but the degree of hemostatic abnormality in these patients is still poorly understood. To evaluate the effects of coffee on primary hemostasis in diabetic rats, we measured the platelet aggregation against the ADP agonist. Platelet aggregation significantly increased in the diabetic animals (Figure 3), suggesting an excess of glucose-induced osmotic effect and ROS in the serum, leading to platelet aggregation and degranulation and, consequently, decreased endothelial functions. Increased platelet activity and thrombus formation has been suggested to play a role in diabetes, atherosclerosis, cardiac disease, and hypertension.

Several studies have shown increased platelet reactivity in diabetic patients by increased levels of specific markers, such as beta-thromboglobulin and platelet factor 4 in platelet granules in plasma, and increased expression of platelet membrane glycoproteins GPIb and GPIIb/IIIa. Reduced membrane fluidity has also been correlated with glycation of platelet membrane proteins.

We observed that aminoguanidine significantly reduced platelet aggregation in non-diabetic and diabetic animals (Figure 3). This result may be explained by its antioxidant activity, which blocks protein glycation and can reduce reactivity changes in platelets, thus helping ameliorate the endothelial dysfunction that is intrinsic to diabetes.

AGE-induced pathogenesis of diabetic micro- and macroangiopathy is well established in the literature. As kidneys are the major site for AGE clearance, they constitute the main target of deleterious AGE-mediated changes. The severity of vascular damage in renal tissue may result from a decline in soluble RAGE (sRAGE) and the AGEs/sRAGE ratio; associated with urinary albumin/serum creatinine levels, it can measure reno-vascular complications in diabetic patients. Thus, we evaluated the renal function in both animal groups by measuring urea and creatinine levels. Diabetic rats showed significantly higher levels of urea in the serum, and aminoguanidine treatment or coffee intake effected no change in these animals' renal profile (Figure 4A). Urea, the main product of protein catabolism in the liver, is synthesized from CO₂ and ammonia and circulates in the blood before being filtered in the kidneys, where most of it is excreted in urine. Urea levels are sensitive to changes, appear faster in primary renal functions, and is an important marker in conditions that affect primary renal function alterations, like diabetes. DM2 promotes progressive cachexia, which causes skeletal muscle atrophy – result of the imbalance on protein synthesis in favor of its proteolysis. Excessive proteolysis results in an increase in plasma urea, as observed in the study. The atrophy stems from reduced insulin-like growth factor 1 (IGF-1) and hyperglycemic conditions. But as urea concentration is influenced by hydration levels and dietary habits, it is less specific than creatinine for assessing renal function.
In this study, coffee intake or aminoguanidine treatment had no effect on serum creatinine levels in the diabetic animals (Figure 4B). Creatinine, which is a breakdown product of creatine phosphate in muscle, is usually produced at a constant rate and its levels are directly proportional to muscle mass. Interestingly, creatinine levels also reflect the glomerular filtration rate; for its concentration in the blood increases as the renal filtration rate declines. According to Fagbohun et al., plasma creatinine acts as a better indicator of stage 1 kidney toxicity than urea.

Our results showed that aminoguanidine did not alter renal biomarkers. Although not evaluated in this work, recent studies have shown that this AGE-inhibitor can alter liver markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

It is important to highlight that the coffee variety, roasting process, and method of brewing, among other variables, changes the amount of bioactive components, such as chlorogenic acid, caffeine, and melanoids, in the beverage. Such components are the main responsible for the antioxidative effects of coffee and can alter the effects described in other studies.

Thus, it is possible to conclude that the study results showed that coffee intake reduced AGEs formation and platelet aggregation in our experimental model. The protective role of coffee may result from the action of its bioactive components, such as chlorogenic acid. Aminoguanidine was effective in inhibiting AGE formation and platelet aggregation. We also observed that coffee had no effect on the animals’ renal function. These results are especially interesting because diabetes is an important risk factor for thrombosis, characterized by hypercoagulability and hypofibrinolysis, and coffee is a widely consumed beverage in the contemporary world.

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Authors' contributions
ASDS and CSF performed the experiments, collected and analyzed the data; RGFAP, SMSD and MRR designed the study and performed the data evaluation; BCCS and ALMV performed the experiments; FBAP and THA performed the data evaluation; EBF statistical analysis; ASDS and MRR wrote the paper; SAF analysis and evaluation of data and update of article writing.