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Ethiopian coffee (*Coffea arabica*) improves glucose uptake and modulates metabolic enzyme activities linked to hyperglycemia-induced infertility in isolated rats' testes

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Abstract

The present study evaluated the inhibitory effect of Ethiopian coffee (*Coffea arabica*) on carbohydrate digestive enzymes and its protective effect against glucose-induced testicular dysfunction using in vitro and in silico study models. Testicular oxidative stress was initiated by co-incubating testocular tissue collected from male Sprague-Dawley rats in glucose solution with different concentrations of Ethiopian coffee aqueous extracts (hot and cold) for 2 h at 37ºC. Glucose-mediated oxidative stress significantly ($p < 0.05$) depleted reduced glutathione and total glycogen levels while it lowered catalase and superoxide dismutase (SOD) activities in the testicular tissue. Concomitantly, this led to elevated malondialdehyde and nitric oxide levels while it also increased glycogen phosphorylase, fructose-1,6-bisphosphatase, ATPase, and acetylcholinesterase activities. Treatment with different concentrations of coffee aqueous extracts restored the enzymes' and markers' levels and activities. Although both the cold and hot coffee extracts strongly inhibited α-glucosidase and α-amylase enzymes, the former showed better activities. The subjection of the coffee extracts to LC-MS analysis indicated the presence of several compounds, including chlorogenic acid, caffeic acid, cafestol, kahweol, caffeine, quinic acid, ferulic acid, and catechol which were further docked with the carbohydrate digestive enzymes. The in silico results displayed that among the various metabolites, chlorogenic acid strongly interacted and had the best binding affinity with α-glucosidase and α-amylase. Our findings implied that Ethiopian coffee may have a preventive effect against glucose-induced testicular damage. These are evidenced by the capacity of the plant product to decrease oxidative stress and protect against testicular dysfunction.

Keywords *Coffea arabica*, Testicular dysfunction, Antioxidant, Purigenic enzyme, Glycogenesis

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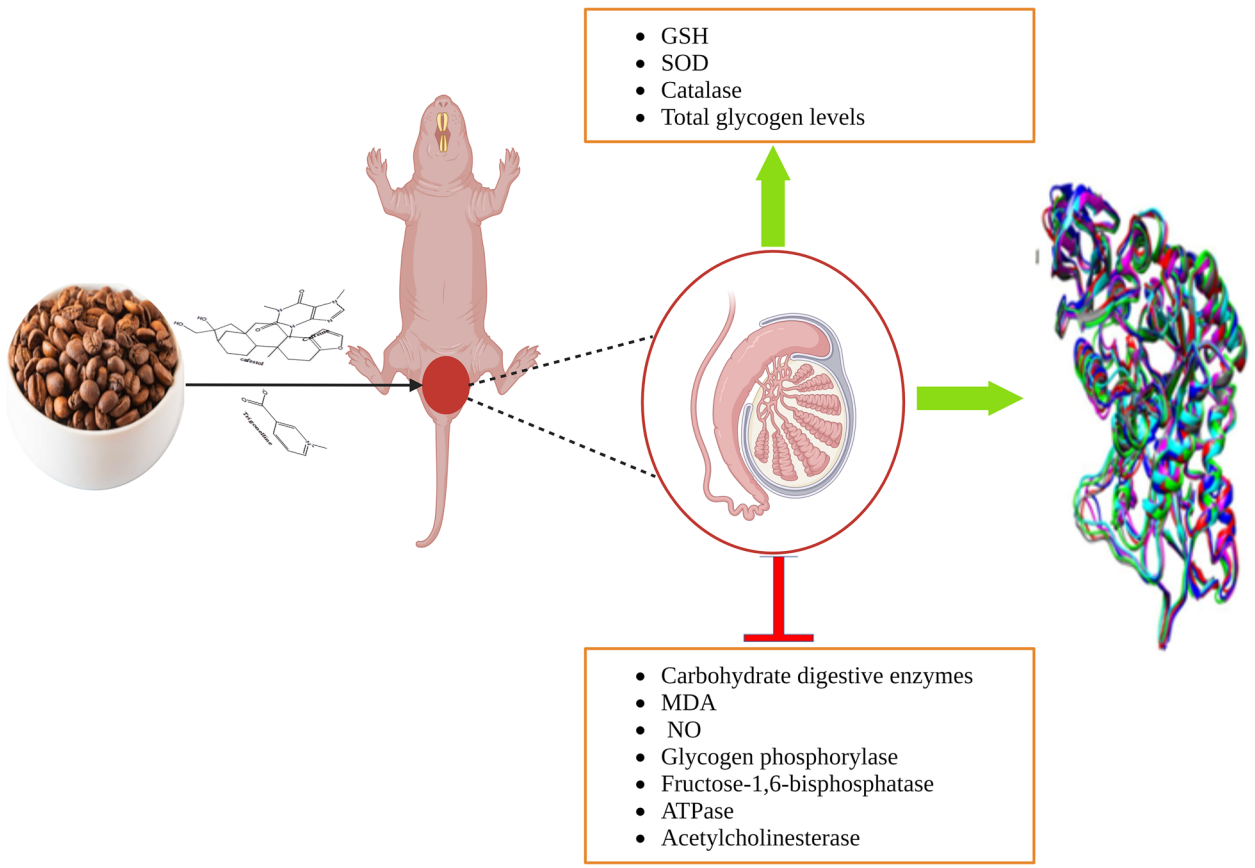
Graphical Abstract



Introduction

Diabetes complications have been described to affect the male reproductive system (Ding et al. 2015). According to previous studies, 59% of diabetic men have reproductive issues (Castela & Costa 2016; He et al. 2021), such as erectile dysfunction, structural changes in reproductive organs, premature ejaculation, and modifications in the content of their sperm (Castela & Costa 2016). Diabetes complications have been reported to reduce sperm count and testosterone levels (Maresch et al. 2019). It is a metabolic disorder that is absolutely related to insulin resistance and progresses in the presence of oxidative stress (Barbagallo & Dominguez 2007; Rani et al. 2016). Oxidative stress plays a crucial role in male infertility due to the extraordinarily high rate of cell reproduction and mitochondrial oxygen consumption in testicular tissue and the significant amounts of unsaturated fatty acids in this tissue compared to other tissues (Asadi et al. 2017).

Long-term hyperglycemia resulting from uncurbed blood glucose levels is one of the links between diabetes and its complications. Hyperglycemia-induced excessive generation of reactive oxygen species (ROS), especially superoxide radical (O_2-), is a linkage between high blood glucose and pathways involved in hyperglycemic tissue damage (Rolo & Palmeira 2006). Moreover, an increase in ROS and their interaction with nitric oxide (NO) stimulates the release of peroxynitrite anion ($ONOO^-$), which causes DNA damage, glucose oxidation, and ATP generation (Kassab & Piwowar 2012; Pacher et al. 2007). Hyperglycemia-induced oxidative stress is a key contributor to endothelial erectile dysfunction as increased ROS alters endothelial NO bioavailability and cavernosal smooth muscle reactivity. The deleterious effect of hyperglycemia and excess oxidative stress can lead to eventual endothelial cell apoptosis that causes disturbances in testicular homeostasis (Castela & Costa 2016).

Furthermore, diabetes results in glycogen deposition inside the lysosomes due to metabolic alterations in sorbitol metabolism bypass, arteriosclerosis, and disruption in neurotrophic supply (He et al. 2021). Glycogen appears

to have an essential function in the testicles, especially when it regulates germ cell survival (Villarroel-Espíndola et al. 2013).

Diabetes-related male reproductive dysfunction can be challenging to treat if hyperglycemia persists (He et al. 2021). In recent years, considerable effort has been devoted to researching therapeutic strategies for treating diabetes-related male infertility (Pereira et al. 2022). Despite their extensive therapeutic benefits, pharmacological medicines are sometimes inaccessible, high in cost, and may have unintended side effects (Mutabingwa 2005). However, medicinal plants and their bioactive phytochemicals have been used to effectively treat chronic conditions such as diabetes and its complications (Teodoro 2019). One of these medicinal plants is coffee beans, which are prescribed to manage diabetes issues related to male reproduction (Abdel Mohsen et al. 2021; Al-Megrin et al. 2020).

Coffee is one of the world's most widely consumed beverages (Liang & Kitts 2014). Coffee is rich in bioactive chemicals (Franca & Oliveira 2016), such as caffeine (de Mejia & Ramirez-Mares 2014) and chlorogenic acid (Tajik et al. 2017), which are linked to various health benefits (Ludwig et al. 2014; Tajik et al. 2017). Numerous studies have examined the relationship between coffee consumption and diabetes related to male reproduction (Maresch et al. 2018; Santos & Lima 2016): some investigations, including clinical studies, have consistently indicated a correlation between coffee consumption and managing sexual dysfunction (Bu et al. 2020; Ricci et al. 2017).

Considering the reported therapeutic properties of coffee beans, there is a scarcity of research on the influence of coffee processing on their bioactivity content, including roasting temperature and boiling methods (Bolka & Emire, 2020). Hence, the current study was designed to evaluate the protective effect of Ethiopian coffee (hot and cold extracts) on hyperglycemia-mediated redox imbalance and glucose metabolism related dysfunction in the testes of normal rats.

Material and methods

Chemicals and reagents

There are various chemicals and reagents have been used to conduct this study, such as, α-glucosidase, α-amylase, sodium phosphate buffer, p-nitrophenyl-α-D-glucopyranoside (pNBG), starch, dinitrosalicylate, isoform, kerbs buffer, CO_2, glucose, acarbose, metformin, Ellman's reagent, TCA, DETAPAC solvent, 6-hydroxydopamine, SOD, hydrogen peroxide (H_2O_2), acetic acid, SDS, Milli-Q water, thiobarbituric acid (TBA), ammonium molybdate, Griess reagent, KOH, glycogen, alcohol, H_2SO_4, glucose-1-phosphate, KCl, Fructose, Tris-HCl buffer, EDTA, $MgCl_2$, ATP, acetylcholine iodide.

Plant sample

Ethiopian coffee beans (*Coffee arabica*) were collected from the Harari state coffee farm in eastern Ethiopia in April 2023.

Preparation of coffee bean extracts

Coffee beans were roasted at 150°C for 15 min, then ground to a fine powder, according to the traditional technique of coffee preparation previously documented (Caprioli et al. 2015; Illy 2002). Coffee bean aqueous extracts (hot and cold) were prepared. The hot extract was made by mixing the coffee powder with boiling water at 100°C for 5 min, while the cold extract was made by adding the powder to cold water (10°C). Both extracts were macerated for 24 h before the coffee bean extracts were filtered and freeze-dried.

In vitro antidiabetic inhibitory assays

Assessment of α-glucosidase inhibition

The α-glucosidase inhibitory effect was determined in accordance with Ademiluyi and Oboh (2013) with minor changes. Briefly, various concentrations of Ethiopian coffee extracts (30–240 µg/mL) were added to 100 (1.0 U/mL) α -glucosidase enzyme solution and incubated at ambient temperature for 15 min. After adding 50 µl of pNPG mixture (5 mM) in 100 mM phosphate buffer (pH 6.8), the combined product was incubated at 37°C for 30 min. The absorbance of the generated nitrophenol was measured at 405 nm, and its inhibitory effect was represented as a proportion of the inhibitor-free reference sample. The experiment was performed in triplicate, and the enzyme's inhibitory activity was estimated by employing the following equation:

$$\%\,\text{Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

Assessment of α-amylase inhibition

The α- amylase inhibitory capacity was measured using the method described by Shai et al. (2010), with slight changes. A 250 µL of the Ethiopian coffee crude (cold and hot) extract or acarbose (standard) at various concentrations (30–240 µg/mL) were allowed to incubate for 20 min at 37°C with 500 µL of porcine pancreatic amylase (2 U/mL) in phosphate buffer (100 mM, pH 6.8). The resulting solution was incubated further with 250 µL of 1% starch dissolved in 100 mM phosphate buffer (pH 6.8) at 37°C for 1 h. Then, 1 mL of dinitrosalicylate colour reagent was added, and the entire mixture was boiled

for 10 min. The absorbance of the final combination was measured at 540 nm, and the inhibition capacity of the enzyme was represented as the percentage of the control with no inhibitors using the equation below;

$$\%\,\text{Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

Animals protocol

Ten male Sprague-Dawley rats weighing between 180 and 220 g were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal, South Africa. The animals were subjected to 12 h fast before sacrifice. They were euthanized humanely with isoform, and their testes tissues were immediately collected for biochemical assays.

The animal handling procedure adhered to the Animal Research Ethics Committee of the University of KwaZulu-Natal, South Africa (the ethical approval number: AREC/00002325/2021).

Assessment of glucose uptake in rat testes

Using the protocol developed by Chukwuma and Islam (2015), the effect of coffee bean extracts on glucose uptake in freshly excised testes was assessed. The harvested testicular tissue was collected and subsequently chopped into 0.5 g pieces. Each testicular piece was incubated for 2 h at 37°C and 5% CO_2 in 8 mL of Krebs buffer containing 11.1 mM glucose and different concentrations of coffee bean (cold and hot) extracts (30–240 µg/mL). The negative control was a Krebs buffer containing glucose but no coffee extract. Metformin (240 µg/mL) is utilized as the standard anti-diabetic medication. The normal control experiment contained testicular tissue in a solution lacking glucose or coffee bean extracts. Following incubation, aliquots of the incubating buffer were collected, and the glucose concentration was determined using an Automated Chemistry Analyzer (Labmax Plenno, Labtest Inc., Lagoa Santa, Brazil). The following formula was used to calculate testicular glucose uptake: Glucose uptake per g of testis = (GC1-GC2)/Weight of testicular tissue (g) Where GC1 and GC2 are glucose concentrations (mg/dL) before and after incubation, respectively.

Preparation of tissue homogenates

After glucose uptake determination, the testicular tissues were homogenized using an electronic homogenizer in 5 mL of 50 mM sodium phosphate buffer (pH 7.5). After homogenization, the homogenates were centrifuged at 15,000 rpm using a microcentrifuge set-up at 4°C for 15 min. For further ex vivo analysis, 2 mL of the supernatant was transferred into microtubes and subsequently stored at -80°C.

Antioxidants assay

Assessment of reduced glutathione (GSH) level

Ellman's method (1959) was used to quantify GSH levels in the incubated tissues. The mixtures were precipitated with 10% TCA then centrifuged at 2000 rpm for 10 min at 25°C. Following this step, 100 µL of the resulting supernatant was mixed with 50 mL of Ellman's reagent in 96 microwell plates and incubated for 10 min. The absorbance was consequently recorded at 415 nm after 15 min of incubation at 25°C.

Assessment of superoxide dismutase (SOD) activity

Using the protocol as established by Gee and Davison (1989), 0.1 mM DETAPAC solvent (170 µL) was transferred into a 96-well plate, to which 15 µL of a sample or SOD test buffer was previously added. All samples were instantly mixed by gently tapping the plate before 15 µL of 1.6 mM 6-hydroxydopamine (6-HD) buffer was added. The absorbance was measured every 1-min period for 5 min at 492 nm.

Assessment of catalase activity

The collected samples were examined according to a previously reported protocol for detecting lower absorbance of tissues in testing induced by H_2O_2 decomposition (Aebi, 1984). 0.1 mL of testicular supernatant was combined with 1 mL of hydrogen peroxide H_2O_2 (0.065 mM) and subjected to a 2-min incubation at 37°C. The reaction was stopped by mixing in 100 µL of a solution containing 32.4 mM ammonium molybdate. The absorbance was recorded at 240 nm for 3 min at 1-min intervals.

Assessment of malondialdehyde (MDA) equivalent level

According to the previous method, MDA concentration equivalent was measured to estimate the extent of lipid peroxidation in the testes tissue as thiobarbituric acid reactive substance (TBARS) (Chowdhury & Soulsby 2002). Briefly, 200 µL of tissue supernatant was boiled with a combination of 750 µL of acetic acid (20%), 200 µL of SDS (8.1%), 2 mL of thiobarbituric acid (TBA) (0.25%), and 850 µL Milli-Q water for 1 h. The absorbance of a 200 µL aliquot of the cooled liquid was recorded at 532 nm. The MDA equivalent level in the tissue was determined by extrapolating from a standard curve of different malondialdehyde (MDA) concentrations.

Assessment of nitric oxide (NO) level

The nitric oxide level of the tissues was determined by utilizing Griess reagent, as previously reported (Erukainure et al. 2019). Concisely, the tissue supernatant (100 µL) or the blank (distilled water) was incubated for 30 min with an equivalent volume of Griess reagent

in the dark at 25°C. Then, the absorbance at 548 nm for each sample was determined.

Glycolytic enzymes activities

Assessment of total glycogen concentration

With minor modifications, the glycogen content of the testicular sample was calculated using a previously reported approach (Oyebode et al. 2022). A total of 0.3 g of tissue from the testes was liquefied in 300 µL of 30% KOH saturated in Na_3PO_4. This mixture was then heated for 30 min before being totally submerged in ice. To guarantee sufficient glycogen precipitation, the mixture was treated with 670 µL of 95% alcohol and centrifuged twice for 30 min. After draining the supernatant, the collected precipitate was dissolved in 1 mL of Milli-Q H_2O. Subsequently, an aliquot of 20 µL of the dissolved glycogen was mixed with 180 µL purified H_2O, to which 200 µL of phenol (5%) and 1 mL of highly concentrated H_2SO_4 were added carefully. Then, the mixture was heated for 10 min before the absorbance of the cooled sample was read at 490 nm.

Assessment of glycogen phosphorylase activity

Homogenate samples (100 µL) were incubated for 10 min at 30°C with 4% glycogen and 64 mM glucose-1-phosphate. This reaction was stopped by adding 20% ammonium molybdate in concentration H_2SO_4. Subsequently, Elon reducer and distilled water were added to the reaction mixture, followed by incubation at 30°C for 45 min. Finally, the absorbance was measured at 340 nm.

Assessment of fructose 1,6 bisphosphatase activity

A mixture of the tissue supernatant (100 µL) and the equivalent amount of 0.1 M KCl and 0.05 M fructose, 1200 µL of 0.1 M Tris–HCl buffer, pH 7.0, 250 µL 1 mM EDTA, and 250 µL of 0.1 M $MgCl_2$, were incubated for 15 min at 37°C. After stopping the enzyme action with 10% TCA, the mixture was then centrifuged at 3000 rpm (4°C) for 10 min. Then, to 100 µL of the supernatant, 50 µL of 1.25% ammonium molybdate and a freshly prepared 9% ascorbic acid were added. This solution was left to equilibrate for 20 min before absorbance was determined at 680 nm.

Assessment of ATPase level

A 20 µL of tissue supernatant and solution of 5 mM KCl, 1.3 mL of 0.1 M Tris-HCl buffer, and 0.04 mL of 50 mM ATP were incubated at 37 °C for 30 min. Consequently, the reaction was stopped by adding 1 mL of Milli-Q H_2O and ammonium molybdate. The resultant solution was allowed to cool down on ice for 10 min after being mixed with freshly prepared 9% ascorbic acid. Then, the samples' absorbance was recorded at 660 nm.

Assessment of acetylcholinesterase activity

The acetylcholinesterase activity of the supernatants was determined using Ellman's method (Ellman 1959). In brief, 0.02 mL of the supernatant was combined with 0.01 mL of 3.3 mM Ellman's reagent (pH 7.0) and allowed to equilibrate for 20 min at 25°C with 0.05 mL of 100 mM phosphate buffer (pH 8). After adding 0.01 mL of 50 mM acetylcholine iodide to the mixture, the absorbance was measured at 3-min intervals at 412 nm.

Determination of phytochemical contents of Ethiopian coffee bean extracts

The phytochemical composition of hot and cold extracts of Ethiopian coffee beans was evaluated using a Shimadzu LCMS-2020 Single Quadrupole liquid chromatography-mass spectrophotometer (LC-MS) as described by Erukainure et al (2017). A loop was used to inject the sample directly. The LC timer was set at 4.00 min. The sampling frequency of the PAD was 1.5625 Hz, and the operating parameters were set at a low-pressure gradient. Other operational parameters were as follows: LC-2030 Pump, Flow rate was set at 0.200 mL/minutes, A Conc: 95.0%, B Conc: 0.0%, C Conc: 0.0%; A: water and B: methanol were utilized as the mobile phase; Detector wavelength start at: 190-nm; Wavelength end at 800 nm; cell temperature: 40°C, start time: 0.00 min, end time: 4.00 min, acquisition mode: Scan polarity is positive, event duration is 1.00 s, detector voltage is +1.00 kV, threshold is 0, start m/z is 50.00; End m/z is 1,700.00, and scan speed is 1.667 u/s. The compounds were identified by comparing their retention times and mass spectra in the NIST library.

Molecular docking

For the in silico study, Molecular Operating Environment (MOE 2015.10) software was employed. Based on the LC-MS data, 9 compounds representing the phytochemical obtained from the Ethiopian coffee bean extracts represent the ligands. The Protein Data Bank website (PDB) (https://www.rcsb.org/) was used to obtain 3D models of α-glucosidase (PDB code 5NN5) (Roig-Zamboni et al. 2017) and α-amylase (PDB code 1OSE) (Desseaux et al. 2002) proteins. The active ligands were created using 3D protonation, partial charge calculation, and energy reduction using Force Field MMFF94x. Moreover, proteins were created without adding repeat chains and H_2O. MOE Quick Prep was used to fix structural defects, perform 3D protonation, and compute partial charge. MOE was utilized to generate the best binding pocket under specific conditions, using a triangle matcher as the placement method and London dG as the major scoring function. An extra refinement step was performed utilizing the rigid receptor technique with the GBVI/WSA dG score function to retain poses with the protein's largest

hydrophobic, ionic, and hydrogen-bond interactions. Then, the compound-enzyme complex was visually analyzed using Maestro Schrodinger software (2018-4).

Statistics

All results were displayed as mean ± SD. A one-way analysis of variance (ANOVA), with a significant difference established at $p < 0.05$, was utilized to analyze the results obtained using the IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 27.0 (IBM Corp., Armonk, NY, USA).

Results

The impact of Ethiopian coffee on carbohydrate digestive enzymes

As depicted in Fig. 1A, a dose-dependent and significant α-glucosidase inhibitory activity of Ethiopian coffee bean extracts was observed in most concentrations ($P < 0.05$). Moreover, the IC_{50} value of the cold extract (4 µg/mL) is comparable to that of acarbose (3.95 µg/mL). In terms of α-amylase inhibitory activity, cold extract was more effective than hot extract and acarbose Fig. 1B, the IC_{50} values for cold extract, hot extract, and acarbose were (62.76 µg/mL, 64.07 µg/mL, and 64.33 µg/mL), respectively.

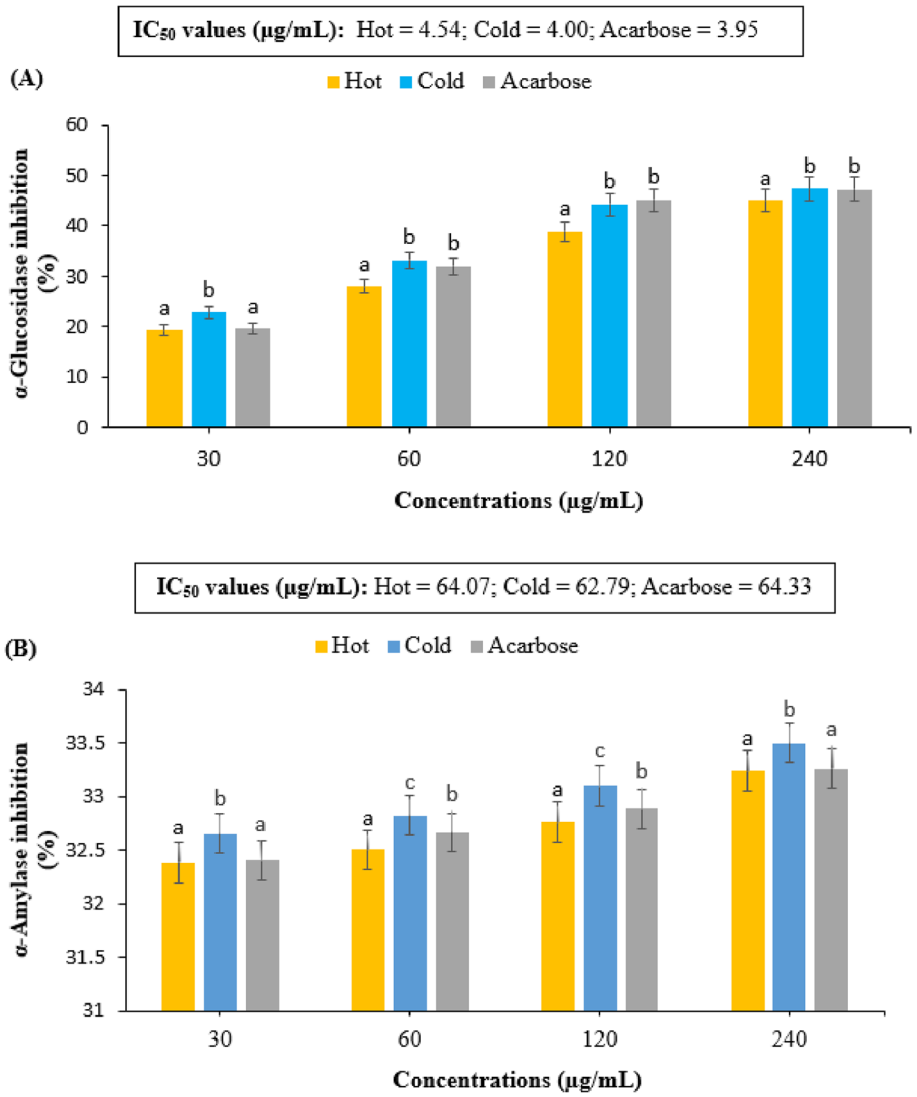


Fig. 1 **A** α-Glucosidase inhibitory activity and **B** α-Amylase inhibitory activity of Ethiopian coffee beans. Values = mean ± SD; $n = 3$. Bars with different alphabet a-c at each concentration are significantly different from each other at $p < 0.05$

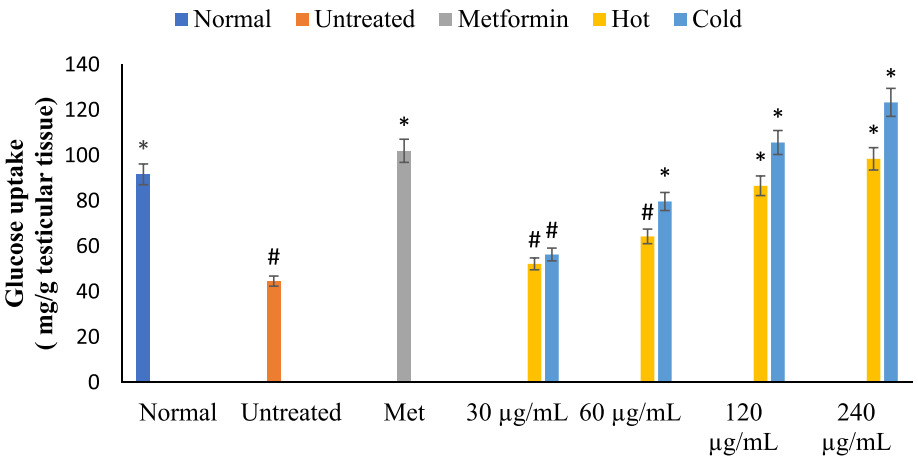


Fig. 2 Glucose uptake activity of Ethiopian coffee beans in rat testes. Values = mean ± SD; *n* = 3. *Significantly different from untreated and # Significantly different from normal ($p < 0.05$, Tukey's HSD-multiple range post-hoc test, IBM SPSS for Windows)

The impact of Ethiopian coffee on glucose uptake in testes

In testes incubated with coffee extracts, glucose uptake was enhanced significantly ($P < 0.05$), as illustrated in Fig. 2. Interestingly, the uptake was higher in cold extract-treated testes than in metformin-treated testes.

The impact of Ethiopian coffee on oxidative stress biomarkers

As illustrated in Fig. 3A-D, the incubation of testes tissue with glucose only resulted in a significant ($p < 0.05$) decrease in the level of GSH, SOD, and catalase activities, while MDA equivalent level concomitantly increased. The treatment with both coffee extracts (hot and cold) led to a significantly ($p < 0.05$) increased GSH level and activities of SOD and catalase, with a decreased MDA equivalent level. The coffee extract with high concentration showed a remarkable boost in the level and activities as compared with metformin performance.

As displayed in Fig. 4, testicular NO level increased significantly ($p < 0.05$) upon incubation with only glucose.

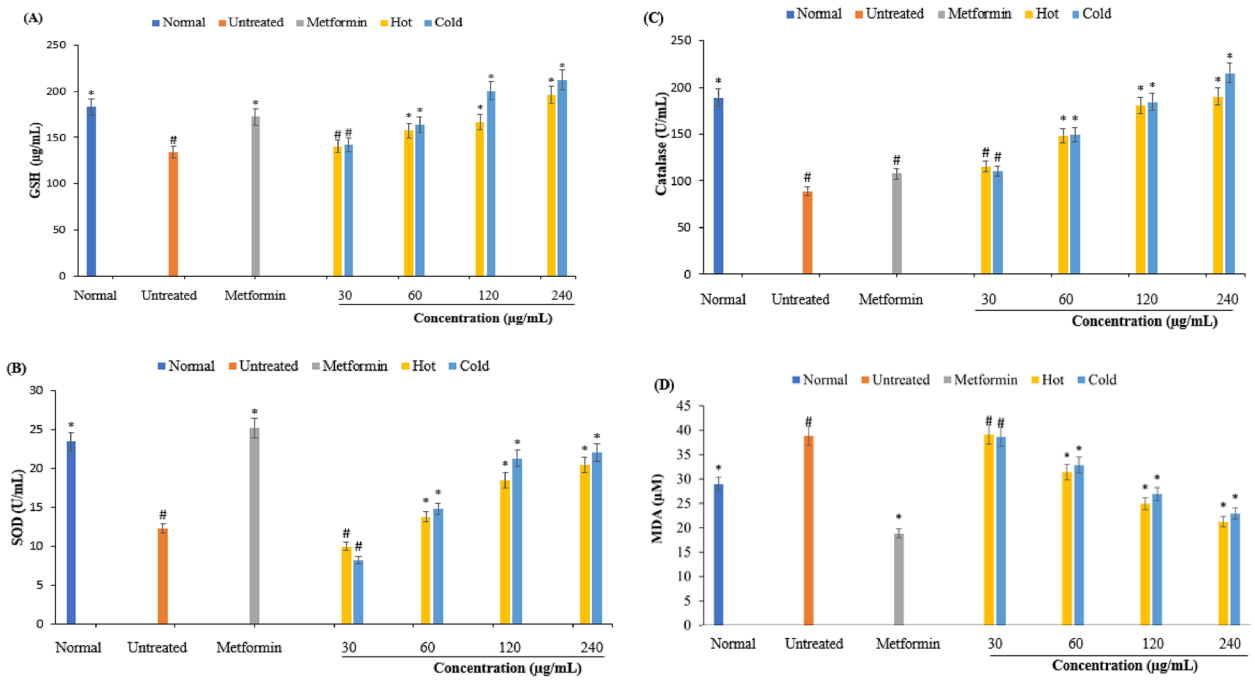


Fig. 3 Effect of Ethiopian coffee beans on antioxidant status in oxidative testicular injury. Values = mean ± SD; *n* = 3. *Significantly different from untreated and # Significantly different from normal ($p < 0.05$, Tukey's HSD-multiple range post-hoc test, IBM SPSS for Windows). GSH, reduced glutathione; SOD, superoxide dismutase; MDS, malondialdehyde

NO level decreased after the coffee extract treatment in all concentrations (30–240 µg/mL). Moreover, in the concentrations 120 µg/mL to 240 µg/mL, the cold extract dropped NO levels to normal tissue levels. In comparison, the hot extract decreased the NO level from 60 µg/mL to 240 µg/mL.

The impact of Ethiopian coffee on glycolytic enzyme activity

Following incubation with glucose only, the testicular activities of fructose-1,6- bisphosphatase and glycogen phosphorylase increased significantly ($p<0.05$), whereas glycogen content decreased, as shown in Fig. 5A-C. After treatment with the coffee, the levels of fructose-1,6- bisphosphatase and glycogen phosphorylase were significantly ($p<0.05$) reduced to a level comparable to metformin, while the content of total glycogen was significantly ($p<0.05$) increased.

The impact of Ethiopian coffee on purinergic enzyme activity

As shown in Fig. 6, incubation with glucose only significantly ($p<0.05$) elevated the purinergic activity of the injured testicular tissue as represented by the elevated ATPase activity. However, this level was significantly ($p<0.05$) decreased dose-dependently after treatment with Ethiopian coffee. In high concentrations of coffee, the ATPase level is reduced to the same extent as with metformin.

The impact of Ethiopian coffee on acetylcholinesterase activity

Following the incubation of testicular tissue with glucose only, the activity of acetylcholinesterase enzyme was significantly ($p<0.05$) elevated, as shown in Fig. 7. In the treatment with Ethiopian coffee, acetylcholinesterase activity was significantly ($p<0.05$) depleted in the injured testes tissue. Ethiopian coffee at a high concentration significantly ($p<0.05$) decreased testicular acetylcholinesterase activity to levels comparable with the normal and metformin.

LC–MS analysis

LCMS screening revealed various bioactive compounds from cold and hot coffee extracts (Fig. 8 and Table 1). Compounds identified from the cold extract include chlorogenic acid, caffeic acid, cafestol, kahweol, caffeine, and quinic acid. Although ferulic acid and catechol were detected in the hot extract, others, including caffeine and quinic acid, present in the cold extract, were also found in the hot extract.

Molecular docking

A docking study assessed the interaction of hot and cold extract compounds from Ethiopian coffee beans with α-glucosidase and α-amylase digestive enzymes. Their free binding energy is displayed in Table 2. The best binding energy based on the molecular interaction is illustrated in Table 3. Among the phytochemicals in the coffee beans, Chlorogenic acid had the lowest bind affinity with α-glucosidase and α-amylase by having -7.5 kCal/mol and -6.87 kCal/mol binding energies, respectively. In comparison, acarbose (standard) scored the highest activity with α-glucosidase (-9.82 kCal/mol) and α-amylase (-8.24 kCal/mol). The chlorogenic acid shows 3 H-donor and H-acceptor links to ASP26, GLU271, ASP202, and ARG400 with α-glucosidase, as shown in Fig. 9A. On the other hand, chlorogenic acid formed H-donor and H-acceptor

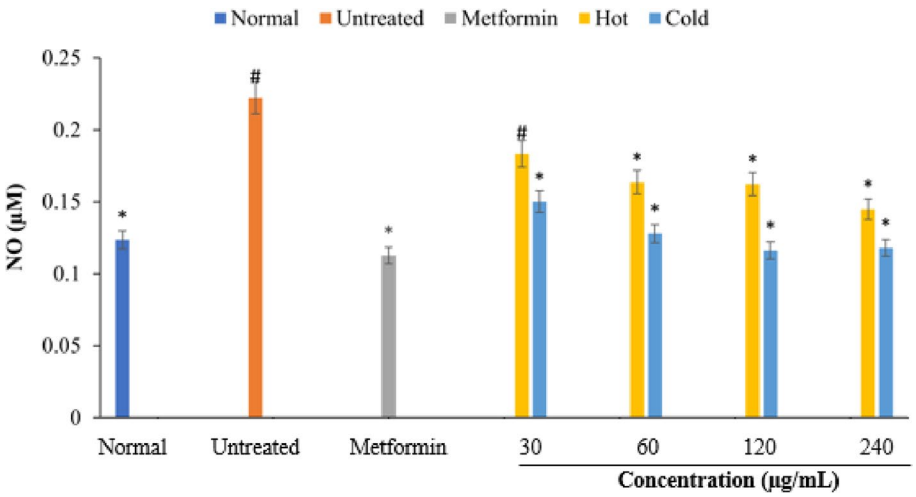


Fig. 4 Effect of Ethiopian coffee beans on nitric oxide (NO) activity level in oxidative testicular injury. Values = mean ± SD; $n=3$. *Significantly different from untreated and # Significantly different from normal ($p<0.05$, Tukey's HSD-multiple range post-hoc test, IBM SPSS for Windows)

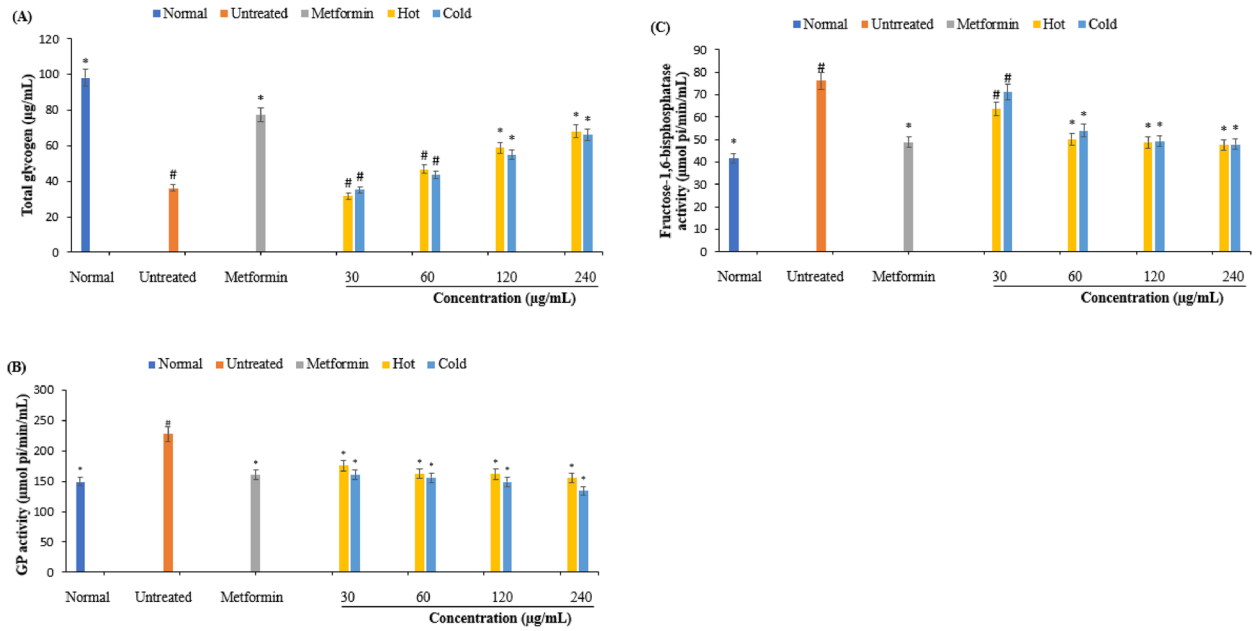


Fig. 5 Effect of Ethiopian coffee beans on (**A**) Total glycogen, (**B**) Glycogen Phosphorylase, and (**C**) Fructose -1,6-bisphosphate activity level in oxidative testicular injury. Values = mean ± SD; $n = 3$. *Significantly different from untreated and # Significantly different from normal ($p < 0.05$, Tukey's HSD-multiple range post-hoc test, IBM SPSS for Windows)

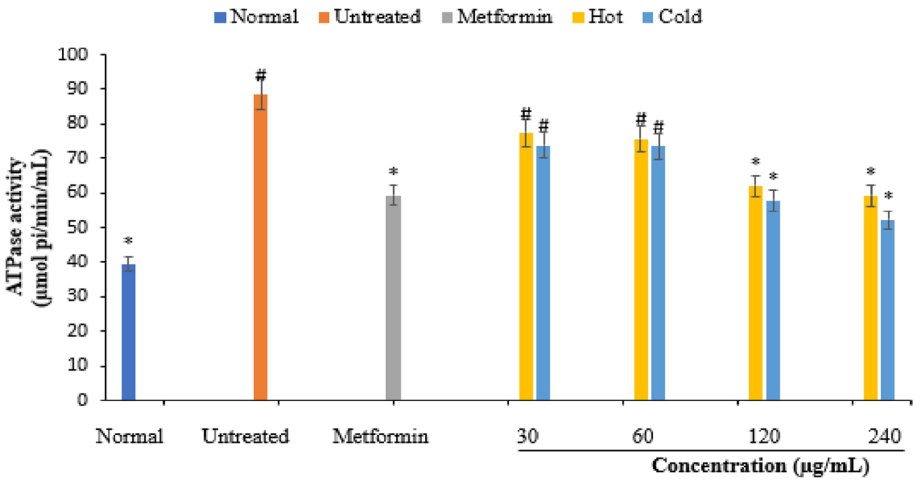


Fig. 6 Effect of Ethiopian coffee beans on ATPase activity level in oxidative testicular injury. Values = mean ± SD; $n = 3$. *Significantly different from untreated and # Significantly different from normal ($p < 0.05$, Tukey's HSD-multiple range post-hoc test, IBM SPSS for Windows)

integrated to ASP166 and GLY110 in the active site of α-amylase Fig. 9b.

Discussion

According to statistical reports from the international diabetes federation organization (IDF), 537 million people live with diabetes worldwide (IDF 2021). This figure is expected to increase to around 642 million by 2040 (Colagiuri 2010). Lifestyle factors and genetics could affect the rise in this statistic. Chronic hyperglycemia imposes long-term damage and failures to several body organs, including the male reproductive system (Ding et al. 2015). Glucose metabolism is critical for spermatogenesis, and many studies have reported

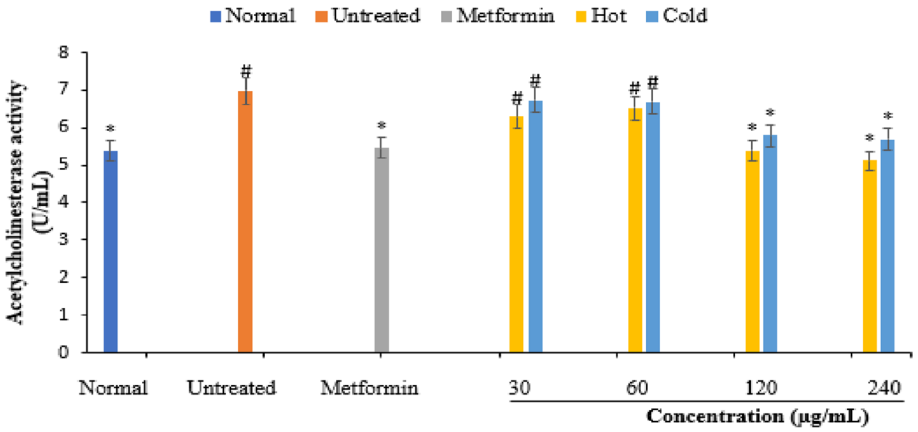


Fig. 7 Effect of Ethiopian coffee beans on acetylcholinesterase activity level in oxidative testicular injury. Values = mean ± SD; $n = 3$. *Significantly different from untreated and # Significantly different from normal ($p < 0.05$, Tukey's HSD-multiple range post-hoc test, IBM SPSS for Windows)

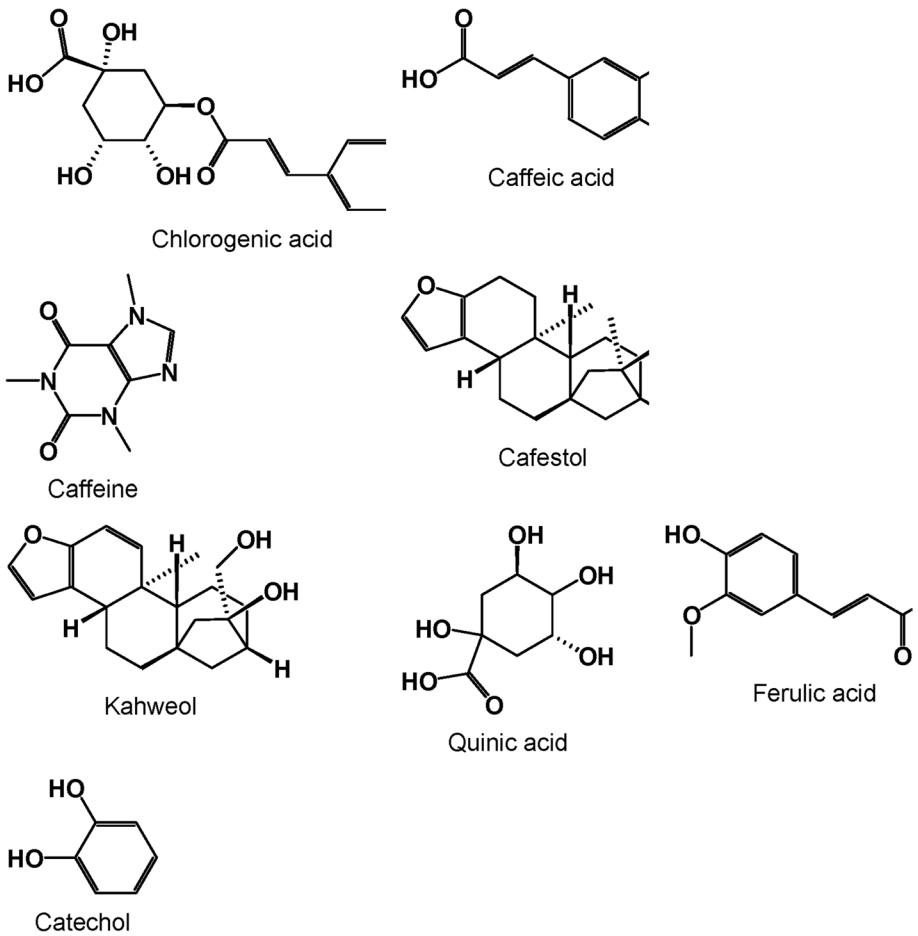


Fig. 8 Different coffee bean compounds identified by LC-MS analysis

the damaging effect of diabetes on male reproductive homeostasis, with oxidative stress playing a vital role (Agbaje et al. 2007; Kilarkaje et al. 2014). Ingestion of coffee has been associated with improved glucose tolerance and reduced risk of type 2 diabetes (T2D) (Agardh et al. 2004; Van Dam & Feskens 2002), and coffee has

Table 1 Chemical analysis data of compounds identified from the Ethiopian coffee beans

	Retention time (Min)	Relative abundance (%)	Ionization mode	Molecular formular
Catechol	6.50	0.309	Positive	$C_6H_6O_2$
Caffeic acid	6.60	1.416	Positive	$C_9H_8O_4$
Caffeine	6.90	1.544	Positive	$C_8H_{10}N_4O_2$
Ferulic acid	7.50	0.296	Positive	$C_{10}H_{10}O_4$
Cafestol	8.80	0.261	Positive	$C_{20}H_{28}O_3$
Kahweol	9.90	0.859	Positive	$C_{20}H_{26}O_3$
Styrene	6.50	0.423	Negative	C_8H_8
Qunic acid	7.90	1.209	Negative	$C_7H_{12}O_6$
Chlorogenic acid	9.00	2.503	Negative	$C_{16}H_{18}O_9$

been reported to play a significant role in many oxidative stress biomarkers (Al-Brakati et al. 2020; Naveed et al. 2018). The current study assessed the potential protective activity of different aqueous extracts (cold and hot) of Ethiopian coffee beans (*Coffee arabica)* against glucose-induced rat testicular damage.

α -Glucosidase and α -amylase are the primary carbohydrate digestive enzymes. Regulation of the activities of these enzymes is vital for glucose homeostasis by effectively delaying and slowing the absorption of glucose and managing the rise in blood sugar levels (Huneif et al. 2022). As determined by anti-diabetic enzyme assays, the dose-dependent inhibition of α -glucosidase and α -amylase by coffee beans observed in Fig. 1 illustrates its capacity to delay carbohydrate digestion, thereby decreasing the level of glucose absorption. Due to the existence of numerous bioactive compounds in coffee beans, it has been shown to exert a similar effect on α-amylase and α-glucosidase as acarbose. The α-glucosidase and α-amylase inhibitory activities of coffee may be ascribed to the presented strong binding affinity between the LC-MS-identified coffee compounds, especially as found in chlorogenic acid when docked with α-amylase and α-glucosidase enzymes Fig. 9A & C and Tables 2 and 3. Chlorogenic acid, one of the prominent compounds present in coffee, has been shown to reduce postprandial hyperglycemia by suppressing α-glucosidase and α -amylase activities (Meng et al. 2013).

Table 2 Free binding energy of phenolic metabolites of Ethiopian coffee with the target proteins

Ligand	Free energy of binding (kCal/mol) Target proteins	
	α- Glucosidase	α- Amylase
Chlorogenic acid	-7.5	-6.87
Caffeic acid	-6.00	-5.30
Caffeine	-5.43	-5.20
Cafestol	-4.22	-6.00
Kahweol	-4.08	-5.93
Styrene	-4.18	-4.33
Quinic aicd	-5.28	-5.05
Ferulic acid	-5.62	-5.25
Catechol	-4.27	-4.26
Acarbose	-9.82	-8.24

Previous research investigating cellular mechanisms beyond the transportation of glucose in testicular cells indicates new perspectives on the effects of diabetes on the fertility of men (Alves et al. 2013). Testicular tissues have a unique glucose-sensing system that responds to hormonal fluctuations and numerous pathways that mitigate hyperglycemia (Alves et al. 2013). In the present study, the ability of coffee bean extracts to effectively facilitate testicular glucose uptake Fig. 2 may be associated with the bio-active compounds, including chlorogenic acid present in coffee beans. In a study reported by Ihara et al. (2023), chlorogenic acid has been demonstrated to enhance insulin secretion and glucose uptake in INS-1 cells, a rat pancreatic β cell line, in a KATP channel-independent manner. However, considering potential chemical variations between hot and cold coffee, it has previously been stated that a reduction in chlorogenic acid content is associated with increased coffee temperature (Fuller & Rao, 2017).

Aerobic cells have created an antioxidant defensive mechanism consisting of enzymatic and non-enzymatic components to control the fluctuation of ROS (Michiels et al. 1994; Sachdev et al. 2021). Low molecular weight antioxidant molecules and numerous antioxidant enzymes constitute the antioxidant system (Taysi et al. 2002). However, some hyperglycemia consequences, including testicular issues, are attributed to the development of the oxidative stress phenomena. GSH, SOD, and catalase are antioxidant enzymes that function as the primary defensive mechanism against the production of ROS in the body by scavenging the damaging intermediate

Table 3 Summary of Molecular Operating Environment (MOE) docking results for the different metabolites with targeted proteins

Ligands	Protein	*Hydrogen bonds between atoms of ligands and amino acids of receptor*						S- score (binding energy) (kCal/mol)
		ligands Atoms	receptor		Type	Distance (Å)	Energy (kCal/mol)	
			Atoms	Residues				
Chlorogenic acid	α-glucosidase	10	OD2	ASP26	H-donor	2.89	-3.7	-7.50
		24	OE2	GLU271	H-donor	2.60	-2.5	
		25	OD1	ASP202	H-donor	2.73	-3.7	
		10	NH1	ARG400	H-acceptor	3.01	-3.9	
		24	NH2	ARG200	H-acceptor	2.98	-2.9	
		25	NE2	HIS332	H-acceptor	3.06	-2.3	
	α-amylase	O24	DO1	ASP166	H-donor	2.69	-2.6	-6.87
		O8	N	GLY110	H-acceptor	3.02	-3.0	
Acarbose	α-glucosidase	O26	SD	MET302	H-donor	3.28	-0.9	-9.82
		O31	O	GLY228	H-donor	2.83	-3.3	
		N33	OE2	GLU271	H-donor	3.01	-14.1	
		N33	OD2	ASP333	H-donor	3.08	-3.8	
		C38	OD2	ASP202	H-donor	3.37	-0.8	
		O42	OD2	ASP62	H-donor	2.93	-0.8	
		O44	OD1	ASP333	H-donor	2.92	-3.3	
		O40	NH2	ARG200	H-acceptor	3.20	-1.8	
		N33	OE2	GLU271	Ionic	3.01	-4.4	
		N33	OD1	ASP333	Ionic	3.97	-0.6	
		N33	OD2	ASP333	Ionic	3.08	-3.9	
	α-amylase	O16	OD1	ASP166	H-donor	2.93	-3.2	-8.24
		O20	N	GLY175	H-acceptor	3.02	-2.7	
		O41	N	ALA111	H-acceptor	3.44	-0.6	
		O44	N	ASP166	H-acceptor	3.47	-0.5	
		O33	5-ring	TRP140	Cation-pi	3.62	-4.8	

of incomplete oxidation (Yoon & Park 2014). In normal circumstances, these enzymes are used to eliminate the excessive creation of ROS and to mitigate the damage caused by oxidants (Uttara et al. 2009). In hyperglycemia, however, the antioxidant enzymes are vulnerable to glycosylation, impairing activity and causing oxidative damage to organs (Babizhayev et al. 2015). In addition, according to studies, the reduction of intrinsic antioxidant enzymes, including GSH, SOD, and catalase, upon exposure to high levels of glucose might trigger lipid peroxidation, resulting in testes damage induced by oxidative stress (Olofinsan et al. 2021). MDA is generated in several diabetic tissues, and these characteristics have often been linked to the pathogenesis found in diabetes patients (Singh et al. 2015). Based on our results, the depletion of GSH level, SOD, and catalase activity and increased MDA equivalent level, following incubation with glucose Fig. 3A–D, demonstrates the presence of lipid peroxidation and oxidative stress in testicular tissues. T2D has been extensively researched for its capacity to cause oxidative stress in various tissues. Hyperglycemia generates an increase in mitochondrial glucose oxidation, which releases a large quantity of superoxide and other free radicals into the cytoplasm. However, the increased level of GSH, SOD, and catalase activities with concomitant suppression of MDA equivalent level following treatment with the extract may indicate the anti-oxidative and protective effect of coffee.

Nitric oxide (NO) is a gaseous free radical with a biological half-life of a few seconds (Bryan & Grisham 2007). Diabetes increases nitric oxide synthase activity and nitric oxide-mediated relaxations, causing changes in several intracellular molecules of protein, lipid, and carbohydrate membrane components, damaging tissues, including testes (Sönmez et al. 2016). Excessive superoxide ions freely react with NO to generate peroxynitrite (ONOO-), a reactive radical that induces proinflammation (Ncume et al. 2023). The increased NO level in testicular tissues after incubation with glucose Fig. 4, as well as the observed reduction in SOD activity Fig. 2B, could result in the generation of elevated ONOO-, thus insinuating proinflammation. The lower level of NO in

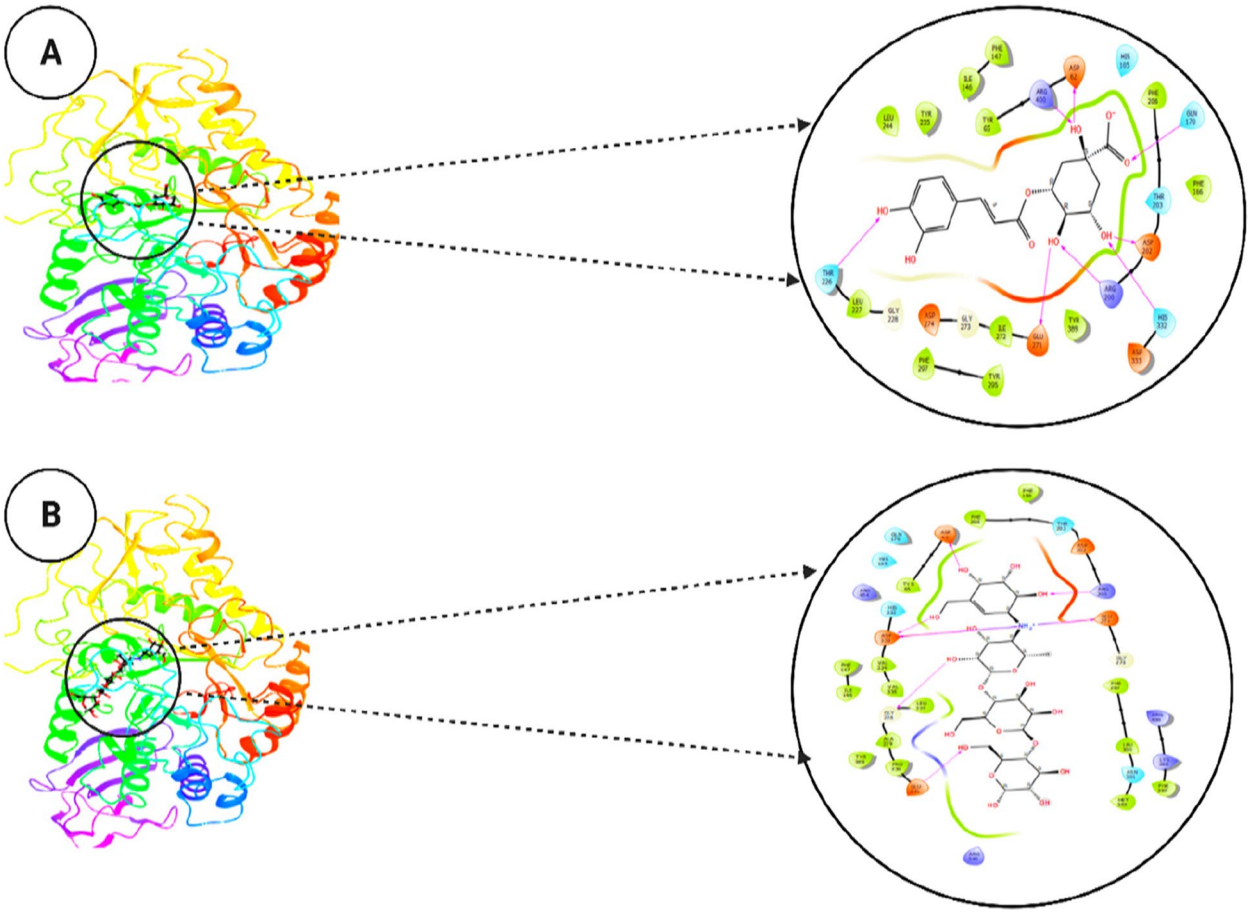


Fig. 9 Images showing chlorogenic acid (**A**) and acarbose (**B**) interactions with the α-glucosidase protein target

the tissues co-incubated with Ethiopian coffee beans may suggest an anti-inflammatory effect, which is corroborated by the decreased SOD activity Fig. 2B.

Identifying abnormalities in hypothalamic hormone- and nutrient-sensing pathways that control glucose homeostasis provides insight into an essential component contributing to diabetes persistence (He et al. 2006; Lam 2010). Regulated glucose metabolism is critical for spermatogenesis and testicular functions. Impaired glucose metabolism in the testes has been reported in testicular dysfunction in T2D, leading to male infertility (Salau et al. 2021). Maintenance of glucose homeostasis in the testes has been recognized as an essential factor of testicular health. Glycolysis represents one of the primary metabolic pathways that provide energy to the cell while also providing material for several kinds of biosynthesis (Erukainure et al. 2021a, b). Every cell undergoes glycolytic processes catalyzed by enzymes (Rizzieri et al. 2019). Glycogen and glycogen phosphorylase are essential for glycogenolysis, whereas fructose 1,6-bisphospahatase is essential for gluconeogenesis (Salau et al. 2020). However, inhibiting these enzymes has been shown to be effective in treating and controlling T2D as well as T2D-induced male infertility (Hayes et al. 2014; Salau et al. 2021). In the present study, the increased activity of glycogen phosphorylase and fructose 1,6-bisphosphatase in testicular tissues incubated with only glucose Fig. 5B & C represents a breakdown of testicular glycogen to glucose and inhibition of glycolysis, respectively. The increased activities of these enzymes, thus, imply exacerbated cellular levels of glucose. This is corroborated by the decreased level of glycogen content in Fig. 5A. Elevated glucose concentration has been reported to be harmful to testicular tissue functions and health, as hyperglycemia may contribute to the generation of ROS, leading to oxidative stress damage in the testes (Erukainure et al. 2021a, b). The reduced activity of these enzymes following treatment with Ethiopian coffee beans may imply the activation of glycogenesis and glycolysis, which is further supported by the increased level of testicular glycogen content. As a result, the glucose concentration is depleted, and the production of free radicals

is reduced, thus suggesting that coffee can modulate altered testicular glucose metabolism.

Purinergic enzymes are identified as a significant regulator of male spermatogenesis and impotence (Olofinsan et al. 2021). The high energy requirement for spermatogenesis and sperm motility has been related to the relevance of ATP and adenosine in male fertility (Poongothai et al. 2009). Figure 6 shows that the elevated ATPase activity in tissue treated with glucose implies a decreased ATP and adenosine level in the testes. As a result, lowering these enzyme activities may help manage/treat male infertility (Shpakov 2021). The reversed actions in testes tissues treated with Ethiopian coffee extracts show a higher ATP and adenosine level, suggesting that energy is available for regular physiological activity of the testes. This indicates that coffee can modulate testicular purinergic dysfunction.

Growing data from experimental modules suggests that oxidative stress may play a crucial role in elevating the levels of acetylcholinesterase in various mammalian tissues (Greig et al. 2002). Acetylcholinesterase plays an essential role in the pathogenesis of testicular dysfunction diseases by influencing the oxidative stress of pathological patients (Aboul Ezz et al. 2015). The deficiency of acetylcholinesterase transmission may play an essential role in reduced sperm counts, decreased seminal gland weight, and impaired sperm motility compared with age-matched nontransgenic controls (Mor et al. 2001). These enzymes have been recognized as primary therapeutic targets in treating and managing several diseases (Erukainure et al. 2021a, b; Lazarevic-Pasti et al. 2017). However, increased acetylcholinesterase levels following incubation with glucose indicate acetylcholinesterase dysfunctional activity (Fig. 7). Interestingly, Erukainure et al. (2021a, b) reported an increase in acetylcholinesterase in the testes due to oxidative stress. Thus, reductions in the enzyme activity to near normal levels at 240 µg/ml of the coffee bean extracts could indicate the beneficial effect of the plant's bioactive compounds in mitigating the biochemical insult that could emerge from dysfunction in the enzyme's activity.

Conclusion

From our results, it can be concluded that Ethiopian coffee beans can protect against hyperglycemia-induced testicular oxidative damage, as portrayed by their ability to inhibit carbohydrate enzyme activities, mitigate testicular oxidative damage, and modulate purinergic and cholinergic dysfunctions while improving altered glucose metabolism in testicular tissues. Intriguingly, the cold coffee extract had a higher concentration of bioactive compounds and exhibited better activities than the hot coffee extract. Despite promising results, the present work is still limited, being an in vitro study. However, additional in vivo and molecular studies are required to understand the molecular mechanisms involved in T2D-induced male reproductive dysfunction to aid in facilitating the development of novel solutions for tackling sexual dysfunctions in diabetic men.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
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Availability of data and materials
No new data were generated for this study.

Declarations

Ethics approval and consent to participate
The animal handling procedure adhered to the criteria of the University of KwaZulu-Natal Animal Research Ethics Committee of the University of KwaZulu-Natal, South Africa (the ethical approval number: AREC/00002325/2021).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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