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Potential antidiabetic activity of probiotic and *Garcinia kola*-yoghurt and its role in regulation of male fertility-stimulating hormones in high-fat diet/low dose streptozotocin-treated rats

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Abstract

Garcinia kola Heckel seed is widely used in the African traditional medicine as a aphrodisiac and male fertility enhancer. Probiotics can reestablish glucose homeostasis and improve blood lipid profiles by altering the composition of the intestinal flora. The study was planned to assess the efficacy of co-administration of *Garcinia kola* seed aqueous infusion and probiotic yoghurt in the management of diabetes and associated male fertility-stimulating hormones abnormalities. *G. kola* seed infusion was prepared, assessed for radical scavenging capacity, total phenolic content and phenolic profile using HPLC. Fermented yoghurt was prepared and inoculated with probiotic mixture. Rats were given a high-fat diet for four weeks and received an intraperitoneal injection of streptozotocin (30 mg/kg) to induce type 2 diabetes. Diabetic rats were received 200 mg/kg freeze-dried infusion of *G. kola* seed, probiotics yoghurt, and probiotic yoghurt mixed with *G. kola* once a day for four weeks. The levels of glucose, insulin, testosterone, follicle-stimulating hormone, luteinizing hormone, inflammatory indicators, oxidative markers, lipid profiles and liver as well as kidney biochemical indicators were measured. The administration of *G. kola* seed, probiotic yoghurt, or their combination to diabetic rats demonstrated potential anti-diabetic effects as evidenced by the down-regulation in glucose, insulin, lipid profile, oxidative markers, and inflammatory markers simultaneously with an up-regulation in testosterone, FSH, and LH levels compared to diabetic rats. *G. kola* seed, probiotic yoghurt, or their combination increased testosterone, FSH, and LH levels and are thought to have therapeutic promise for T2DM and its related oxidative stress.

Keywords *Garcinia kola*, Probiotic, Type 2 diabetes, Male fertility-stimulating hormones

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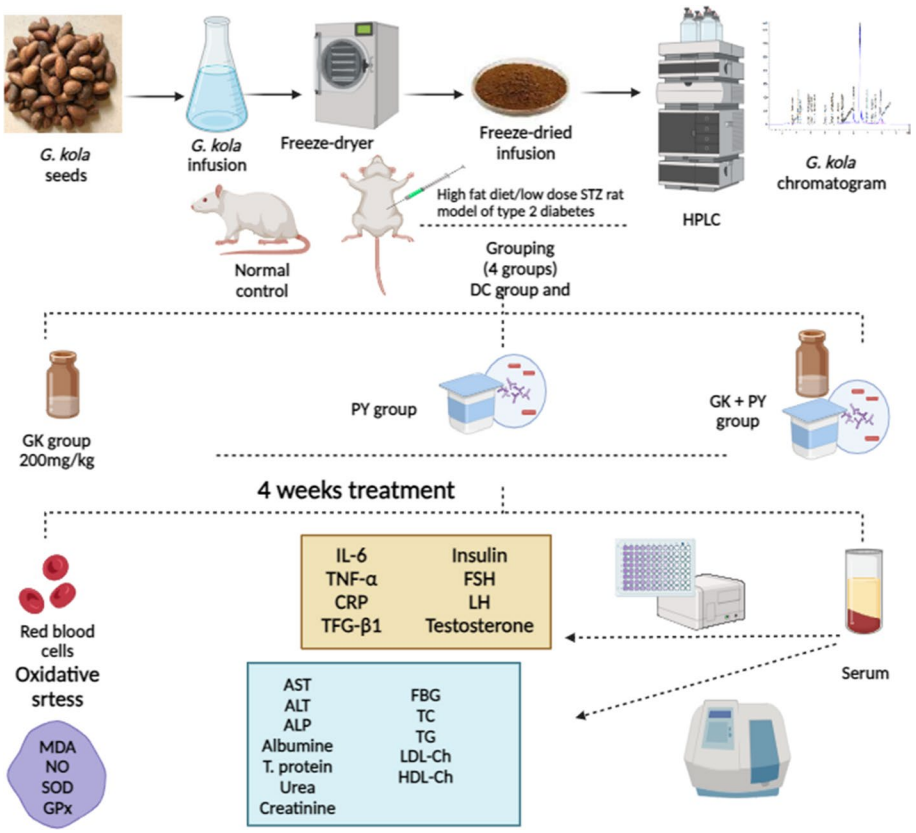
Graphical Abstract



Introduction

By 2045, there will be 629 million cases of type 2 diabetes mellitus (T2DM) worldwide according to the prediction of the International Diabetes Federation (Cho et al. 2018). T2DM, a complex disorder that affects the host's homeostasis, is characterized by high fasting blood glucose, decreased insulin efficacy, abnormal carbohydrate and lipid metabolism, and mild gut microbial dysbiosis (Al Bataineh et al. 2023). The risk of vascular inflammation, major micro- and macro-vascular changes, and multiorgan dysfunction which can result in a wide range of diabetes complications, including neuropathy, nephropathy, retinopathy, and cardiovascular disorders is, in fact, increased by persistent hyperglycemia)Mansour et al. 2023). It has been determined that 25% to 50% of men with T2DM have low testosterone (T) levels, resulting in male hypogonadism or erectile dysfunction (Van Cauwenberghe et al. 2022). Testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are the major fertility-stimulating hormones in males. Testosterone stimulates and maintains spermatogenesis and fertility in conjunction with FSH. LH regulates testosterone production by Leydig cells, which are found in the interstitium of the testis (Oduwole et al. 2021). Insulin resistance or insufficient insulin levels can have an adverse effect on the brain, pituitary gland, gonads, and perigonads. As a result, T2DM is associated with the reduced secretion of testosterone, FSH, LH, and gonadal steroids such gonadotropin-releasing hormone (Andlib et al. 2023). The astonishing rise in the prevalence of type 2 diabetes around the world, as well as its economic cost, highlights the need for new strategies to halt the disease's onset and progression, especially since currently available diabetes reducers have side effects such as hypoglycemia and digestive tract problems (Mohamed & Abdel-Salam 2021). Additionally, there hasn't been medications that addresses diabetics' reproductive health in male up to this point (Andlib et al. 2023).

Human health is impacted by the gut microbiota's balance. Diabetes may be facilitated by an imbalance in the intestinal flora. However, dietary interventions can prevent diabetes by restoring normal intestinal flora (Wang

et al. 2023). Probiotics, live microorganisms that have health benefits when consumed in appropriate amounts, can improve reestablish glucose homeostasis and blood lipid profiles by altering the composition of the intestinal flora. In addition, probiotics can generate short-chain fatty acids (SCFAs), which can improve gut flora and suppress pathogenic ones, lowering the incidence of type 2 diabetes mellitus (Luo et al. 2023).

Garcinia kola Heckel seeds from the African rain forest (Malpighiales order, Clasiaceae family, Bull. Soc. Bot. France 30 (Rev. Bibliogr.): 150 (1883), theplantlist.org, website accessed on August 29, 2023) are known as "Onie" (in the Fang-Beti languages of Cameroon, Gabon, and Equatorial Guinea), "Orogbo" (in the Yoruba language of Nigeria), or even male kola or bitter-kola (Farahna et al. 2017) because of their bitter flavour. In West African traditional medicine, these seeds are used to treat a variety of ailments, such as diabetes mellitus (Adedara et al. 2015) and malaria (Ogunkunle et al. 2014). A growing number of research (Adedara et al. 2015; Farahna et al. 2017; Idris et al. 2020) indicate that *G. kola* seeds have anti-inflammatory and anti-diabetic properties. The seed is used in folk medicine to treat fever, digestive, inflammatory, brain, and liver issues. It is also used as an aphrodisiac and a fertility-boosting substance. The major phytochemicals present in *G. kola* seeds are flavonoids, saponins, tannins, phenols, glycosides, and alkaloids (Kareem et al. 2022).

It has been determined that probiotics hypoglycemic mechanism is connected to the control of the gut microbiota. Consequently, it is thought that taking the aphrodisiac and fertility enhancer *G. kola* with probiotics would be more efficient in management of diabetes and its associated problems, particularly the decrease in male fertility-stimulating hormones. Therefore, the study was planned to assess the role of supplementation with *G. kola,* probiotic yoghurt or their mixture in regulation of blood glucose and male fertility-stimulating hormones (testosterone, FSH and LH) levels in a T2DM rat model.

Materials and methods

Materials

Garcinia kola Heckel seeds were purchased from a market in Saudi Arabia. Botanists from Qassim University College of Agriculture verified the seeds. *G. kola* seeds were peeled, dried, grounded and stored until use. Fresh cow milk samples was obtained from healthy lactating animals in local farm, Cairo, Egypt. Cow milk is available in Arab countries, and *G. kola* seeds can be imported from the Kingdom of Saudi Arabia or from Nigeria. Probiotic starter cultures of DVS-ABT2 (containing *Streptococcus thermophilus, Lactobacillus acidophilus* and *Bifidobacterium bifidum*) were imported from Chr. Hansen's Lab., Copenhagen Denmark. Chemicals and pure reagents were imported from Sigma (Sigma-Aldrich, St. Louis, MO, USA). Elisa kits were imported from Sunlong (Sunlong Co., Ltd. China).

Animals

Adult male Sprague Dawley rats that were 4 weeks old and weighed 111.36 ± 8.01 g as Mean ± SD were used. The National Research Centre's animal house in Cairo, Egypt, supplied the animals. Individually housed in stainless steel metabolic cages with free access to food and water, the animals were maintained at room temperature (24 ± 2 °C and 40–60% relative humidity) with 12-h light and dark cycles. The study was approved by the National Research Center's Medical Research Ethics Committee (MREC), in accordance with the requirements of the pertinent Egyptian legal framework, the Helsinki Declaration, the institutional Animal Care and Use Committee's (IACUC) guidelines and recommendations, and WHO regulations governing the ethical conduct of scientific research. The No of Ethical Approval Certificate is 24911122022.

Methods

Preparation of aqueous infusion of *G. kola* (GK)

According to Farahna et al. (2017) and Abdel-salam et al. (2009 and 2018), fifty grams of *G. kola* seeds powder were placed in a beaker, 500 ml of distilled water (70 °C) was poured over the plant material, then the beaker was closed and the material infused for 6 h, then the mixture was filtered twice, first through cheese-cloth (50% cotton, 50% polyester) and then through filter paper (Whatman No.2). The amount of the obtained infusion was preserved in sterile dark bottles in a cool environment (4° C) until further use. The infusion of *G. kola* was freeze dried by freeze drier (Crest Alpha 1–4 LSC plus Germany). Later, the freeze-dried powder was sieved by 140 meshes then kept, in polyethylene pouch bags, in refrigerator until used.

High-performance liquid chromatography (HPLC) fingerprinting of GK aqueous infusion

An Agilent 1260 series was used for the HPLC analysis. 4.6 mm × 250 mm i.d., 5 µm, Eclipse C18 column was used for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were the components of the mobile phase, which had a flow rate of 0.9 ml/min. The linear gradient was sequentially programmed into the mobile phase as follows:0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A); 15–16 min (82% A) and 16–20 (82%A). At 280 nm, the multi-wavelength detector was monitored. For each of the sample solutions, 5 l of injection volume was used. The column was kept at a

constant temperature of 40 °C. The retention times of the identified compounds of interest were measured. To create a curve showing peak area and retention time in a chromatogram, the extract was injected into a high-performance liquid chromatographic device. The concentration of the sample is then determined by comparing the peak area of the sample with that of the standard relative to the standard's concentration.

Determination of total phenolic content of GK aqueous infusion

Using the Folin-Ciocalteu reagent, the total phenolic content of freeze-dried GK infusion was colorimetrically assessed (Singleton et al. 1999). Gallic acid equivalents (GAE), reported as mg/g of sample, were used to measure total phenolic content. The assay was performed in triplicate, and the mean and standard deviation are shown for the outcomes.

Determination of antioxidant activity of GK aqueous infusion

GK infusion's capacity to scavenge free radicals was assessed using three methods DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical cation assay and FRAP (ferric ion reducing antioxidant power). The DPPH radical scavenging activity was assessed according to the method of Nguyen et al. (2017). To 1 ml of the freeze-dried GK infusion in ethanol with varied concentrations (25, 50, and 75 µg/ml), 0.1 mM of DPPH was added. After fully vortexing, the reaction mixture was kept at room temperature for 30 min. At 517 nm, the absorbance was spectrophotometrically quantified. The assay was performed in triplicate. The following equation was used to calculate the % DPPH scavenging activity:

Percent inhibition (%) = $(A_0 - A_1/A_0)$ x 100, where A_0 represented the absorbance of the control response and A_1 the absorbance while the test material was present.

The ABTS radical cation assay was performed as described by Prior et al., (2005). The $ABTS^+$ cation radical was generated by mixing 10 mg of ABTS and 2 mg potassium persulfate in water and storing it in the dark at room temperature for 12–16 h before use. After diluting the $ABTS^+$ solution (1 ml) with methanol (60 ml), the absorbance was measured at 734 nm, 30 min after the addition of 5 µl of each freeze-dried GK infusion concentration (25, 50, and 75 µg/ml) to 3.995 ml of diluted $ABTS^+$ solution. The assay was performed in triplicate. The following equation was used to calculate the % ABTS scavenging activity:

ABTS scavenging (%) = (A0−A1/A0) x 100, where A0 and A1 are the absorbance without the GK and the absorbance with the GK, respectively.

The FRAP assay was performed as described by Gomez et al. (2023). The components 300 mM acetate buffer, 10 ml tripiridyltriazine in 40 mM hydrochloric acid, and 20 mM ferric chloride ($FeCl_36H_2O$) were combined in a ratio of 10:1:1 at 37°C to create the FRAP reagent. 5 µl of each freeze-dried GK infusion concentration (25, 50, and 75 µg/ml) was combined with 3.995 ml of FRAP reagent, and thoroughly mixed. The reduction of ferric tripiridyltriazine to ferrous tripiridyltriazine form in the reaction mixture produced a complex with a strong blue colour. After 30 min of incubation at 37°C, the absorbance was measured at 593 nm against a reagent blank made up of 3.995 ml of FRAP reagent and 5 µl of distilled water. The assay was performed in triplicate. Plotting the absorbance at 593 nm allowed for the creation of a calibration curve using various $FeSO_4$ concentrations.

Preparation of fermented probiotics-yoghurt (Zabady)

According to the procedures outlined by Tamime and Robinson (1999) and Abdel-Salam et al. (2018), probiotic fermented yoghurt was prepared. Whey protein powder was added to milk samples to standardize them, and then the milk was pasteurized for 15 min at 85 °C before being cooled to 40 °C. *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* probiotic cultures were inoculated and incubated for 4 to 8 h at 42 °C.

Preparation of diets for animals

A regular, balanced diet with a total kcal value of 412 kcal/kg (12% protein, 10% corn oil, 10% sucrose, 58.5% maize starch, 5% fibre, 3.5% AIN-93 salt mixture, and 1% AIN-93 vitamin mixture) was prepared according to Reeves et al. (1993). A high-fat diet (HFD) with a total kcal value of 528 kcal/kg (12% protein source, 30% lard, 1% cholesterol, 10% sucrose, 42.5% maize starch, 3.5% salt mixture, and 1% vitamin mixture) was prepared according to Zhu et al. (2017). Each diet, with a known weight, was added to crockery inside the metabolite cages on a daily basis.

Type 2 diabetes induction

According to the method of Srinivasan et al. (2005) and Qian et al. (2015), type 2 diabetes was induced in

rats. Thirty rats were divided into normal group ($n=6$) and HFD group ($n=24$). Rats in the normal group were given the balanced diet, while rats in the HFD group were given the HFD for four weeks until the abdominal circumference increased by 7 cm compared to the normal group rats (Novelli et al. 2007), then the HFD group's rats were injected with streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO). Each rat received an intraperitoneal injection of a single low dosage of STZ (30 mg/kg, dissolved in 0.1 M sodium citrate buffer at pH 4.4). After 72 h, a blood glucose metre (One Touch, LifeScan, Zug, Switzerland) was used to measure the animal's fasting blood glucose (FBG). The high fasting blood glucose level (over 200 mg/dl) in rats who had diabetes was evidence that the disease had started.

Groups and administrations

The non-diabetic group was considered as the normal control (NC) group and maintained on the balanced diet. The twenty four diabetic rats were continued to feed on the HFD for further 4 weeks after dividing them into four groups (6 rats in each group depending on Mead's resource equation which is often used for estimating sample sizes of laboratory animal experiments) as follows; diabetic control (DC) group where diabetic rats were untreated, *G. kola* (GK) group where diabetic rats orally administrated with freeze-dried *G. kola* aqueous infusion (200 mg/kg, dissolved in distilled water) for 4 weeks, probiotics-yoghurt (PY) group where diabetic rats orally administrated 1 ml of probiotic yoghurt containing 10^8 CFU of probiotics for 4 weeks, and Gk + PY group where diabetic rats orally administrated 1 ml of probiotic yoghurt (containing 10^8 CFU of probiotics) mixed with freeze-dried *G. kola* aqueous infusion (200 mg/kg rat body wight) for 4 weeks.

The oral dose of 200 mg/kg of *G. kola* infusion was used in this study based on studies that estimated the daily doses of *G. kola* used in traditional medicine to be between approximately 100 and 300 mg/kg (orally) (Adedara et al. 2015; Ogunkunle et al. 2014), as well as taking into account the outcomes of Ajayi et al. (2011) study who reported that 200 mg/kg of *G. kola* aqueous extract exhibited neuro-protective effect in mice while Cetkovic-Cvrlje et al. (2022) found that 100 mg/kg of *G. kola* aqueous extract had no improvement effect on type 1 diabetes in mice.

Thus, the experiment lasted approximately 8 weeks, during which time the total food intake, body weight gain and feed efficiency ratio (Body weight gain/total food intake) were calculated. Blood samples were collected from slightly anaesthetized rats after overnight fast over heparin and without heparin. The blood samples were centrifuged at 3500 rpm for 15 min to separate plasma and serum which stored at -80 °C till analysis. Finally, rats in the study were euthanized by decapitation and dissected to obtain kidney and liver.

Biochemical analysis

Serum of each rat was analyzed for insulin, testosterone (T), luteinizing hormone (LH), C-reactive protein (CRP), interlukin-6 (IL-6), tumor necrosis factor (TNF-α) and transforming growth factor β1 (TGF-β1) using sandwich ELISA detection kits (SinoGeneclon Biotech Co., Ltd.), Fasting blood glucose levels was determined according to Trinder (1969). The activities of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were determined according to Rheinhold and Seligron (1953) and Reitman and Frankel (1957), respectively. The levels of albumin, creatinine and urea and the activity of lactate dehydrogenase (LDH) were determined depending on Doumas et al. (1997), Larsen (1972) and Fawcett and Scott (1960) and Zimmerman and Weinstein (1956), respectively. Plasma total cholesterol (T-Ch), high-density lipoprotein cholesterol (HDL-Ch), low-density lipoprotein cholesterol (LDL-Ch), and triglycerides (TG) were determined according to Watson (1960), Burstein et al., (1970), Schriewer et al., (1984) and Megraw et al. (1979), respectively. Cholesterol/HDL-C ratio was also calculated. Red blood cells lipid peroxidation (MDA) glutathione peoxidase (GPx), nitric oxide (NO) and superoxide dismutase (SOD) activity were determined according to Ohkawa et al. (1979), Paglia and Valentine (1967), Montgomery and Dymock (1961) and Nishikimi et al. (1972), respectively.

Statistical analysis

Statistical analyses were done using SPSS version 21. The results were expressed as mean ± standard error (SE) and analyzed statistically using one-way analysis of variance (ANOVA) Duncan test was used to analyze the statistical differences between groups. The results of body weight gain of normal and diabetic rats post feeding on the high fat diet for 4 weeks were statistically analyzed using T-test. The statistical significance of difference was taken at $P \leq 0.05$.

Results

HPLC fingerprinting analysis of GK aqueous infusion

The HPLC detected 18 known phenolic compounds in the freeze-dried *G. kola* infusion. The presence of different flavonoids and phenolic components in the GK freeze-dried infusion was demonstrated by the HPLC chromatogram (Fig. 1). These phytochemicals were examined from the chromatogram in significant amounts and are shown in Table 1. The most prevalent phenolic component in GK was quercetin (103955.7 g/g), which was followed by hesperetin

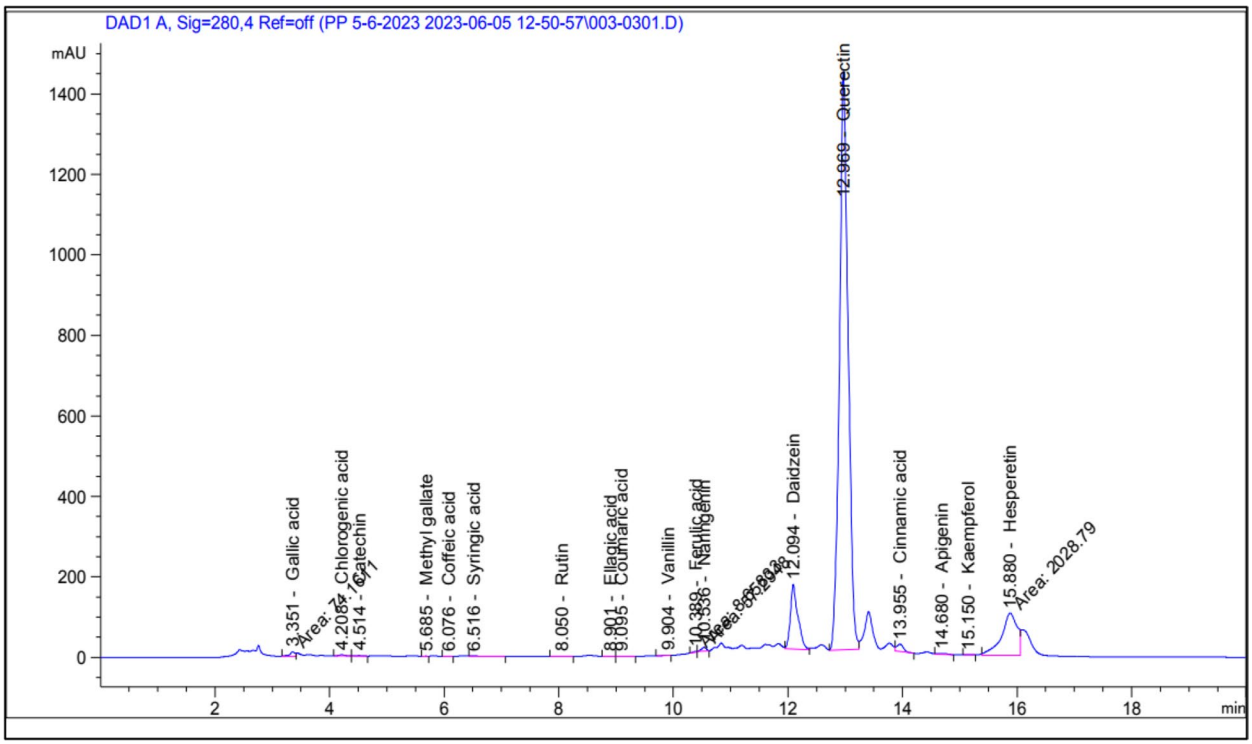


Fig. 1 The HPLC chromatogram of GK aqueous infusion

Table 1 Polyphenolic profile of GK aqueous infusion

Compound	Area	Conc. (µg/g)
Gallic acid	74.16	320.20
Chlorogenic acid	23.27	159.30
Catechin	12.49	154.67
Methyl gallate	1.67	4.57
Coffeic acid	0.97	3.71
Syringic acid	12.33	41.80
Rutin	3.59	20.84
Ellagic acid	11.09	102.80
Coumaric acid	12.72	20.06
Vanillin	4.56	9.97
Ferulic acid	8.66	29.58
Naringenin	57.29	345.45
Daidzein	1458.38	4504.99
Querectin	15094.60	103955.70
Cinnamic acid	147.84	136.17
Apigenin	23.19	88.27
Kaempferol	3.35	13.00
Hesperetin	2028.79	5913.21

(5913.21 µg/g), daidzein (4504.99 µg/g), and naringenin (345.45 µg/g). Coffeic acid (3.71 µg/g) was the least prevalent substance.

Total phenolic content and antioxidant activity of GK aqueous infusion

Table 2 displays the total phenolic content (146.4 mg/g) of the freeze-dried GK infusion. Owing to antioxidant molecules in GK namely phenolic compounds, it exhibited radical scavenging activity as revealed in Table 2. The DPPH radical scavenging, ABTS radical cation and FRAP assays were used to determined the antioxidant activity of the freeze-dried GK infusion. At low concentration (25 µl/ml), GK scavenged 52.93 and 75.53% of the DPPH and ABTS radicals, respectively and recorded 78.00% FRAP.

Bioassays in a T2DM rat model

Effects of GK, probiotics-yoghurt and their combine on the growth performance

Figure 2 depicts the body weight gain of HFD and normal rats after four weeks of consuming a high-fat diet. Compared to the NC group, the high fat diet considerably accelerated the body's weight gain. Table 3 displays the effects of *G. kola*, fermented probiotics, and their combination on the development and performance indices of diabetic rats. Despite the fact that there was no discernible difference in the food intake among the various groups after the 4-week experiment, diabetic rats showed a significant ($p < 0.05$) weight loss as compared to the NC

Table 2 Total phenolic content and antioxidant activity of GK aqueous infusion

Total phenolic content (mg/g)	146.40 ± 0.62
DPPH scavenging activity (%)	
25 (µl/ml)	52.93 ± 0.42
50 (µl/ml)	66.00 ± 0.62
75 (µl/ml)	73.40 ± 0.20
ABTS scavenging activity (%)	
25 (µl/ml)	75.53 ± 0.60
50 (µl/ml)	86.43 ± 0.51
75 (µl/ml)	94.77 ± 0.68
Ferric ion reducing antioxidant power (%)	
25 (µl/ml)	78.00 ± 0.80
50 (µl/ml)	86.10 ± 0.85
75 (µl/ml)	97.50 ± 1.32

Values are presented as mean ± S.D. ($n = 3$)

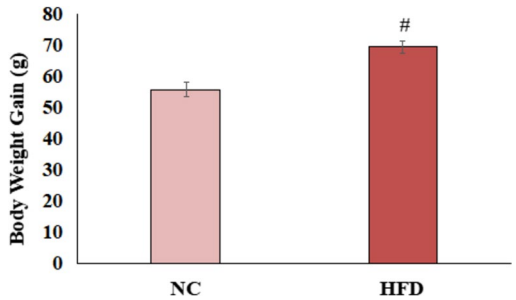


Fig. 2 Body weight gain of normal ($n = 6$) and HFD ($n = 24$) rats post feeding on HFD (4 weeks). #, significantly different from the normal rats at $P < 0.05$

group. In comparison to the DC group, final body weight of the *G. kola*, fermented probiotics-yoghurt, and their combined groups significantly ($p < 0.05$) increased.

Effects of GK, probiotics-yoghurt and their combine on glucose, insulin and inflammatory markers

Figure 3 shows serum FBG, insulin and inflammatory markers levels of experimental rats. FBG of DM group (231.17 mg/dl) was significantly higher than NC group (94.07 mg/dl). Compared to NC group (4.82 mU/L), DM group was at significantly higher insulin concentration (7.83 mU/L). Inflammatory markers (TGF-β1, TNF-α, CRP and IL-6) of DM group (67.27 ng/ml, 17.87 pg/ml, 5.57 ng/ml and 75.68 pg/ml, respectively) were significantly higher than NC group (24.42 ng/ml, 6.45 pg/ml, 2.63 ng/ml and 25.92 pg/ml, respectively). However, FBG, insulin and inflammatory markers of *G. kola*, fermented probiotics-yoghurt and their combine groups were significantly ($p < 0.05$) less than DM group. The co-administration with *G. kola* and fermented probiotics-yoghurt revealed the highest improvement in FBG, TGF-β1, TNF-α, CRP and IL-6 levels (138.34 mg/dl, 35.98 ng/ml, 8.95 pg/ml, 3.05 ng/ml and 28.66 pg/ml, respectively).

Effects of GK, probiotics-yoghurt and their combine on the male fertility-stimulating hormones and oxidative markers

Compared to NC group, all diabetic rats (Fig. 4) showed significant decrease in testosterone, LH and FSH levels along with significant increase in RBCs MDA and NO and drop in RBCs antioxidant enzymes (SOD and GPx). Contrary to the DC group, significant increase in testosterone, LH and FSH levels accompanied with significant decline in RBCs MDA and NO levels in addition to increase in the activity of SOD and GPx were observed in *G. kola*, fermented probiotics-yoghurt and their combine groups. Among the experimental treatment groups, the combine of *G. kola* and fermented probiotics-yoghurt showed the most significantly increase in testosterone, LH and FSH levels (1.82, 4.13 and 11.93 ng/ml, respectively), the less MDA and NO levels (9.5 and 1.93 nmol/g Hb, respectively) and the highest activity of SOD and GPx (4.5 and 32.18 U/g Hb, respectively).

Effects of GK, probiotics-yoghurt and their combine on the lipid profile and dyslipidemia

Regarding the lipid profile and dyslipidemia parameters (Fig. 5), significant elevation in serum cholesterol,

Table 3 Effects of GK, probiotics-yoghurt and their combine on the growth performance

	NC	DC	GK	PY	GK + PY
Initial body weight (g)	$167.00^{a} \pm 2.11$	$180.00^{b} \pm 2.05$	$181.67^{b} \pm 1.71$	$182.50^{b} \pm 3.34$	$179.50^{b} \pm 2.56$
Final body weight (g)	$231.83^{c} \pm 1.89$	$210.00^{a} \pm 6.44$	$216.50^{ab} \pm 2.32$	$227.50^{bc} \pm 3.44$	$224.00^{bc} \pm 3.85$
Body weight gain (g)	$64.83^{c} \pm 2.75$	$30.00^{a} \pm 6.39$	$34.83^{ab} \pm 2.09$	$45.00^{b} \pm 5.02$	$44.50^{b} \pm 3.55$
Total food intake (g)	$530.83^{a} \pm 4.90$	$524.17^{a} \pm 5.07$	$529.50^{a} \pm 3.45$	$527.33^{a} \pm 4.80$	$518.50^{a} \pm 4.55$
Food efficiency ratio	$0.12^{c} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.07^{ab} \pm 0.01$	$0.08^{b} \pm 0.01$	$0.09^{b} \pm 0.01$
Liver weight (g)	$8.90^{b} \pm 0.23$	$7.88^{a} \pm 0.27$	$7.92^{a} \pm 0.26$	$8.15^{ab} \pm 0.30$	$7.95^{a} \pm 0.20$
Kidney weight (g)	$1.79^{c} \pm 0.02$	$1.52^{a} \pm 0.04$	$1.59^{ab} \pm 0.05$	$1.69^{bc} \pm 0.02$	$1.67^{b} \pm 0.04$

Values are tabulated as mean ± SE ($n = 6$). At a statistical significance level of $P < 0.05$, various superscript letters (a, b, c, ab and bc) were added to represent group significant differences

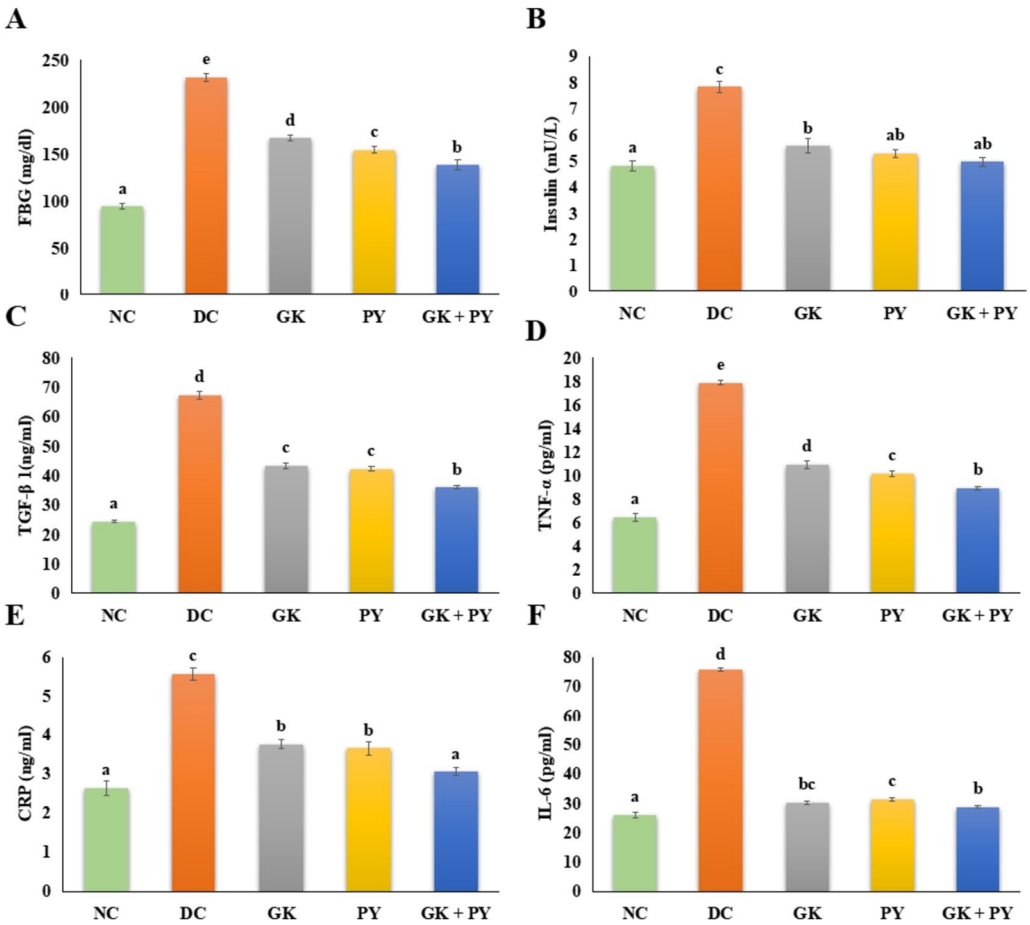


Fig. 3 Effects of GK, probiotics-yoghurt and their combine on FBG (**A**), insulin (**B**), TGF-β1 (**C**), TNF-α (**D**), CRP (**E**) and IL-6 (**F**). Values are set as mean ± SE ($n = 6$). At a statistical significance level of $P < 0.05$, various superscript letters were added to represent group differences

TG, LDL, non-HDL-Ch, Ch/HDL ratio and VLDL levels accompanied with significant decrease in HDL level was observed in high-fat diet/low STZ-treated rats versus the NC group. Meanwhile, the administration of *G. kola*, fermented probiotics-yoghurt and their combine caused a significant ($p < 0.05$) decline in serum cholesterol, TG, LDL, non-HDL-Ch, Ch/HDL ratio and VLDL levels and significant elevation in HDL level as compared with the DC group. Notably, the combine of *G. kola* and fermented probiotics-yoghurt significantly showed the most reduction in lipid parameters recording 109.67, 86.23, 46.98 and 38.15 mg/dl for TC, TG, LDL and HDL, respectively and the less atherogenic indices (non-HDL-Ch, VLDL and Ch/HDL ratio) at 71.52, 17.25 mg/dl and 2.88, respectively.

Effects of GK, probiotics-yoghurt and their combine on the liver and kidney biochemical indicators

Concerning liver an kidney biochemical indicators, Fig. 6 illustrates that the activity of AST, ALT, ALP, LDH and the levels of urea and creatinine were significantly ($p < 0.05$) higher in DC group than NC group while, albumin and total protein levels were significantly lower in DC group than NC group. Compared to DC group, the administration of *G. kola*, fermented probiotics-yoghurt and their combine to the diabetic rats led to suppression in the dysfunction of liver and kidney. Despite the converging between the different treatments in their effects on liver and kidney functions, the co-administration of *G. kola* and fermented probiotics-yoghurt significantly showed the less activity of AST, ALP and LDH and the highest level of total protein.

Discussion

T2DM is a dangerous chronic disease that poses a threat to the public's health. It is an endocrine and metabolic disorder primarily defined by high blood glucose levels. Additionally connected to it include obesity, liver damage, insulin resistance, and malfunction of the pancreatic islet cells (Ouyang et al. 2022). In addition to combating

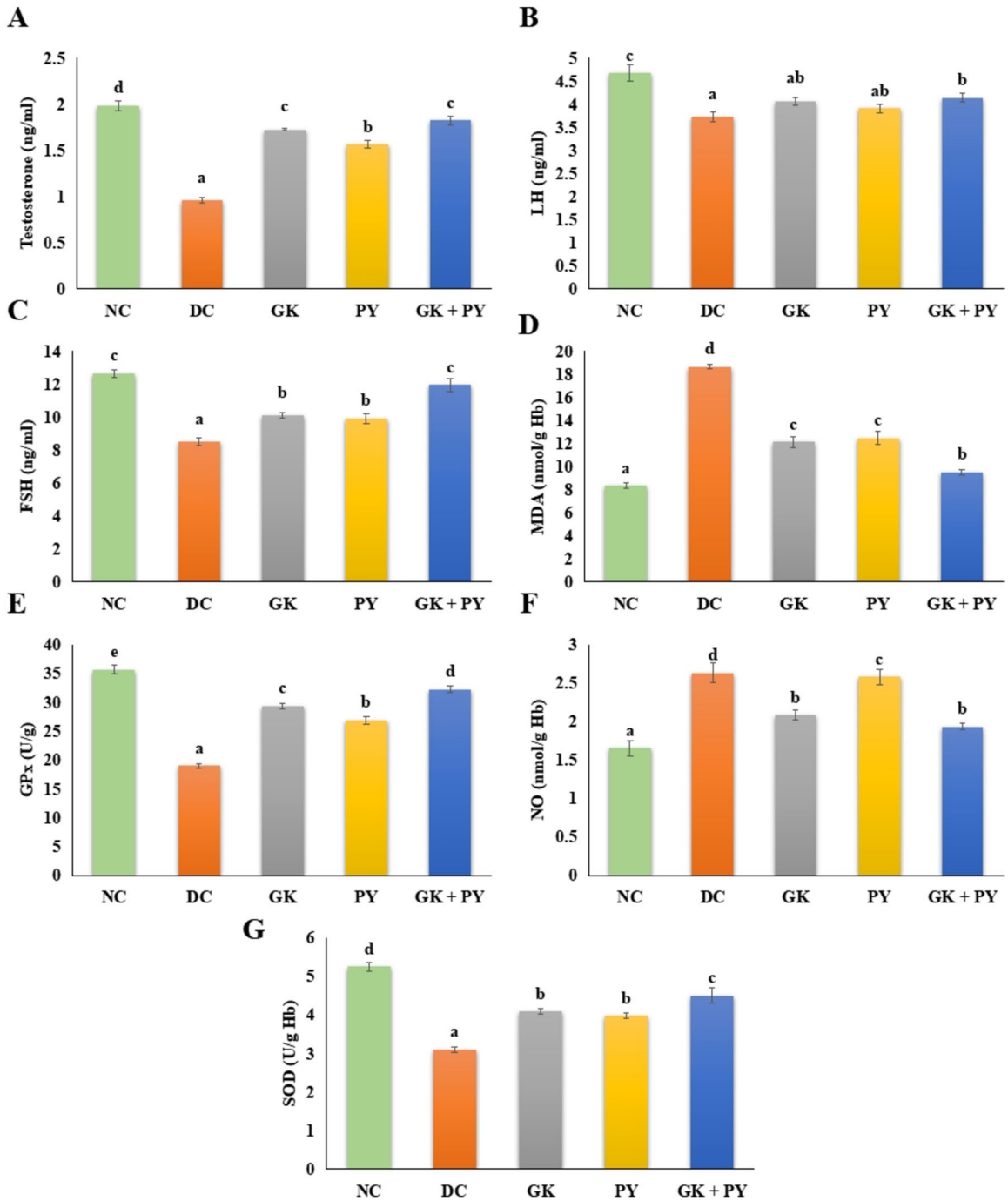


Fig. 4 Effects of GK, probiotics-yoghurt and their combine on testosterone (**A**), LH (**B**), FSH (**C**), MDA (**D**), GPx (**E**), NO (**F**) and SOD (**G**). Values are set as mean ± SE ($n = 6$). At a statistical significance level of $P < 0.05$, various superscript letters were added to represent group differences

diabetes through a number of mechanisms, including glucose utilization in extra-hepatic tissues, a direct diminution in macrophage infiltration, an enhancement in beta cell function, and an increase in the functional activity of glucose transporters, the bioactive phytochemicals of *G. kola* seeds demonstrated several beneficial effects, including antioxidant and anti-inflammatory (Oyenihi et al. 2022).

Studies using animal models suggest that probiotics may lower blood glucose levels by reducing inflammation and preventing the breakdown of β-pancreatic cells. Consuming probiotics is thought to protect the

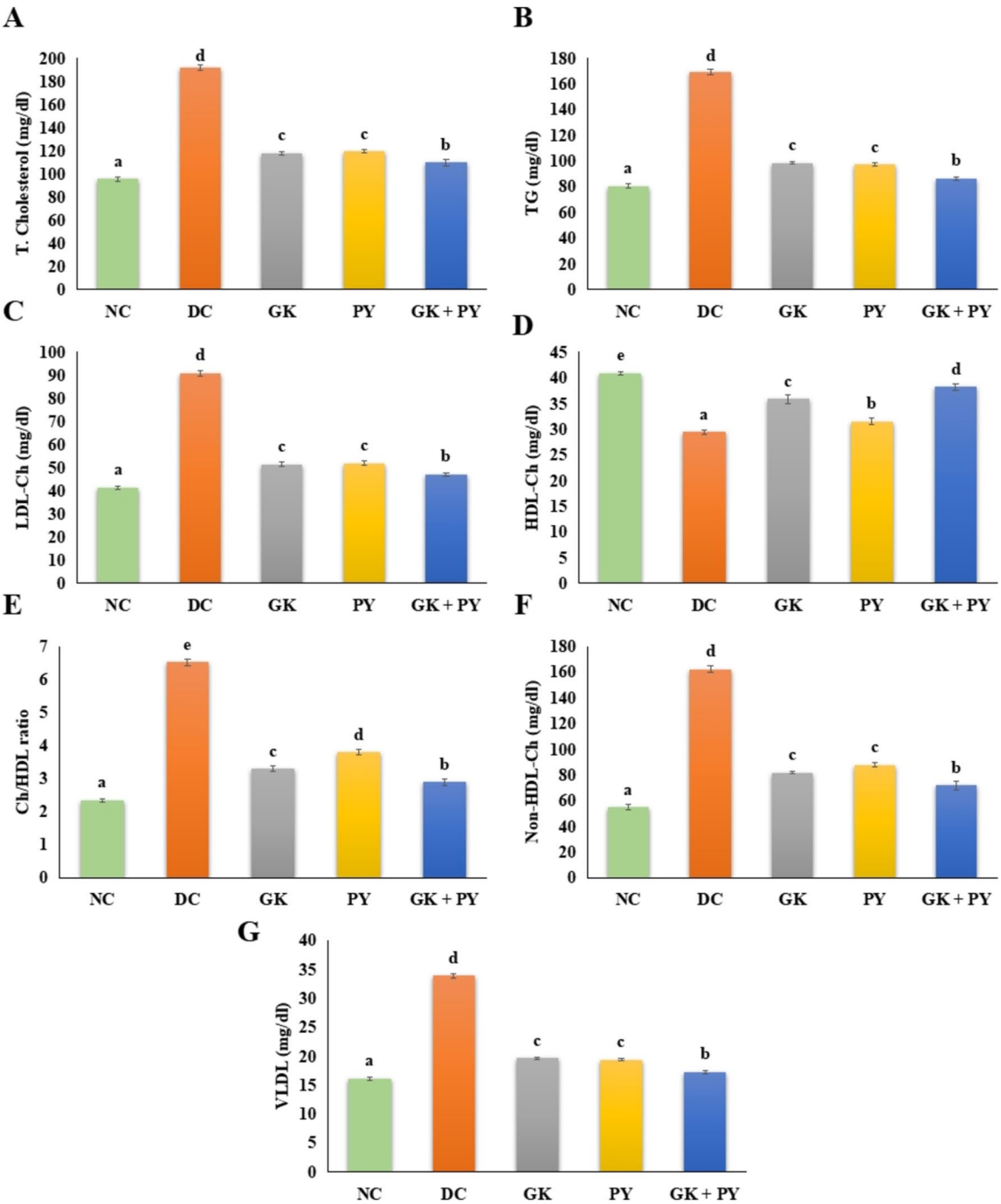


Fig. 5 Effects of GK, probiotics-yoghurt and their combine on total cholesterol (**A**), TG (**B**), LDL-Ch (**C**), HDL-Ch (**D**), Ch/HDL ratio (**E**), non HDL-Ch (**F**) and VLDL (**G**). Values are set as mean ± SE ($n = 6$). At a statistical significance level of $P < 0.05$, various superscript letters were added to represent group differences

β-pancreatic cells from oxidative damage by reducing lipid peroxidation and elevating antioxidants like glutathione and peroxide dismutase. Additionally, probiotics have been found to affect insulin resistance and inflammation by boosting natural killer cells and controlling the expression of TNF-α. The increase in gliclazide bioavailability, the blockage or delay of intestinal glucose absorption, and changes to the gut flora are other potential pathways that could have an impact on glucose metabolism (Yanni et al. 2020).

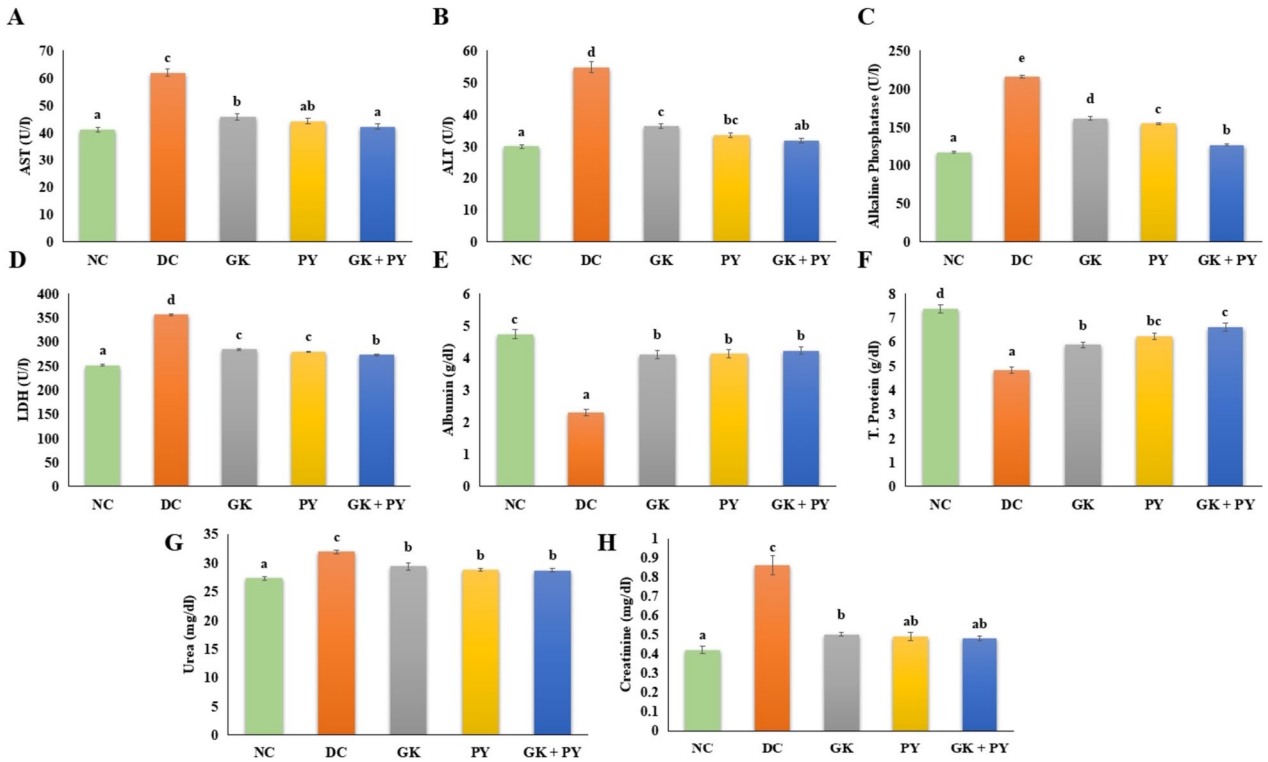


Fig. 6 Effects of GK, probiotics-yoghurt and their combine on AST (**A**), ALT (**B**), ALP (**C**), LDH (**D**), albumin (**E**), total protein (**F**), urea (**G**) and creatinine (**H**). Values are set as mean ± SE ($n = 6$). At a statistical significance level of $P < 0.05$, various superscript letters were added to represent group differences

Thus, the freeze-dried aqueous infusion of *G. kola* seeds, fermented probiotics-yoghurt and both together were evaluated for their potential therapeutic effect of type 2 diabetes and its problems included the dysfunction of male fertility-stimulating hormones. Among the most prevalent secondary metabolites in nature are phenolic compounds. Because they include phenolic groups that donate electrons to other molecules, polyphenols have the ability to act as antioxidants. Few polyphenols are hydrolyzed or absorbed in the stomach because they are resistant to the stomach's acid. Large amounts of ingested polyphenols interact with the gut microbiota, the indigenous bacteria, in the large intestine. Epidemiological studies demonstrate that a long-term, moderate diet of foods high in polyphenols can halt the progression of chronic illnesses like type 2 diabetes, cardiovascular disease, and neurodegenerative diseases as well as obesity (Bié et al. 2023). Thus, the phenolic profile of the aqueous infusion of *G. kola* was evaluated in this study and 18 known phenolic compounds in the freeze-dried *G. kola* infusion were identified. The radical scavenging activity of *G. kola* is most probably attributed to the phenolic compounds. The greatest way for phenolic compounds to act as antioxidants is via directly scavenging free radicals. A hydrogen atom transport mechanism and an electron transfer mechanism are both involved in this process (Kostić et al. 2023).

Because the high fat diet and STZ treatments are designed to resemble the stages and transitions in the progression of type 2 diabetes (Andonova et al. 2023), the high fat diet and STZ-induced type 2 diabetic rats model was used in this study. The high fat diet significantly increased the body weight gain compared with the NC group. While, diabetic rats were found to have significant weight loss, compared with NC group. Andonova et al. (2023) also found that feeding rats on a diet high in fat, mainly lard both saturated and monounsaturated fatty acids, led to an increase in the weight of rats compared to normal rats, while the weight of those fed on fat decreased after being injected with STZ for ten days as a result of the development of type 2 diabetes. T2DM can display a number of symptoms, including hyperglycemia, hyperlipidaemia, and loss of body weight. High blood glucose levels prevent the absorption of energy sources and eliminated through urination to cause weight reduction (El-Newary et al. 2021). In this study, the different therapies converged greatly in their efficacy on improving diabetes-related weight loss. Data indicate that the

administration of *G. kola*, fermented probiotics-yoghurt or their combine could effectively improve weight loss in T2DM rats. The amalgamation of *G. kola* and probiotics-yoghurt had the most promising effect on diabetic and its complications, which can be strongly attributed to the richness of seeds in phenolic compounds, as confirmed by the results of the current study since the effective role of phenolic compounds as prebiotics has been indicated, and it has been shown that the combination of phenolic compounds and probiotics may have health-promoting effects (Plamada & Vodnar 2021). The synergistic effect between flavonoids and Bifidobacterium spp was reported by Pan et al. (2023), in 10 healthy Chinese volunteers, an in vitro induced fermentation method was utilized to assess the effect of flavonoids included hesperetin-7-O-glucoside, hesperetin, naringin, naringenin, rutin, isoquercitrin, and quercetin. The results showed that hesperetin-7-O-glucoside and quercetin supplementation greatly increased the abundance of Bifidobacterium spp.

In this study,through the antioxidant, anti-inflammatory, nephroprotective, hepatoprotective, and cardioprotective properties of probiotic strains and the phytochemicals of *G. kola* seeds, the probiotic yoghurt and garcina infusion represented their ameliorative effect against type 2 diabetes and its complications. FBG level, which represents the blood glucose level in right now, is a crucial reference point in the diagnosis of T2DM. The phytochemicals found in *G. kola*, mainly flavonoids, saponins, tannins, phenols, glycosides, and alkaloids are involved in the hypoglycemic effect of *G. kola* infusion. Furthermore, the detected phenolics, namely daidzein and quercetin, exert hypoglycemic effect and suppress inflammation and oxidative stress resulting in attenuating of cardiac, hepatic and renal injury (Laddha & Kulkarni 2021 and Kareem et al. 2022). In this study, the hypoglycemic effect of the probiotic yoghurt strongly attributed to the selected strains, as *L. acidophilus* could relieve T2DM via control hepatic glucose, lipid metabolism, and gut microbiota in mice (Yan et al. 2019). The combined of *L. acidophilus and B. bifidum* positively correlated to SCFAs and type 2 diabetes management (Meng et al. 2023).

There is a correlation between blood glucose and serum insulin, reflecting the balance between glucose metabolism, glycogenolysis, and insulin secretion (Li et al. 2022). The results demonstrated that *G. kola* and probiotic yoghurt decreased the insulin resistant. Such effect might be duo to flavonoids compounds in *G. kola* (Li et al. 2023) and *L. acidophilus* along with bifidbacterium in the fermented yoghurt as confirmed by Rezazadeh et al. (2021) who found that probiotic yogurt containing *L. acidophilus* and *B. lactis* exhibited antioxidant activity and improved insulin sensitivity in metabolic syndrome-patients.

Inflammatory processes are implicated in diabetes mellitus. However, inflammatory responses could be modulated by pharmacological interventions, to prevent and treat complications of diabetes (Li et al. 2023). The anti-inflammatory effect of garcina infusion might be attributed to flavonoids, namely quercetin, hesperidin, naringenin and rutin detected in garcina seeds and suppress inflammatory mediators such as TNF-α and IL-6, thus controlling NO generation as reported by Li et al. (2023). The anti-inflammatory effect of probiotic yoghurt may correlated to *L. acidophilus*, Bifidobacterium as these probiotic strains apply a potent anti-inflammatory effect through adjusting toll like receptor 2-mediated NF-κB and MAPK signaling pathways in inflammatory cells (Li et al. 2019).

As diabetes worsens, hyperglycemia induces mitochondrial dysfunction and the production of reactive oxygen species (ROS), which causes oxidative stress in multiple organs, including blood vessels, pancreatic beta cells, liver and kidney (Mohamed & Abdel-Salam 2021). Oxidative stress originates as a result of uncontrollable generation of ROS and decreased the activity of glutathion and SOD as well as mediated lipid peroxidation and protein carbonylation (Singh et al. 2019). This was evident in diabetic rats in this study. The dysfunctional anti-oxidative system was restored by treatment with *G kola* and probiotic yoghurt. Such effect might be duo to polyphenols and alkaloids in *G kola* (Kareem et al. 2022) and *L. acidophilus* along with bifidbacterium in the fermented yoghurt (Rezazadeh et al. 2021).

Pancreatic cells normally release insulin. The intracellular protein tyrosine kinase (PTK) is then activated once insulin connects to their receptors. To activate phosphoinositide 3-kinase (PI3K), activated PTK is able to phosphorylate and activate insulin receptor substrates (IRS). The PI3K signalling cascade is triggered, which increases gonadotropin-releasing hormone (GnRH) production in the hypothalamus. GnRH then drives pituitary secretion of LH and FSH, which in turn triggers the release of sex hormones by the gonads. Accordingly, increased insulin resistance followed by a defect in the production of testosterone, FSH and LH (Liu et al. 2023). Furthermore, in adipose tissue, modified sex hormone binding globulin (SHBG) levels, elevated levels of oxidative stress, and elevated levels of aromatase (CYP19) activity are exist during diabetes. This causes testosterone and androstenedione to be converted into estradiol and estrogen, respectively, which lowers the levels of testosterone in the blood in diabetic males (Liu et al. 2023). The antioxidant and insulin sensitivity stimulating effects of garcinia may be implicated in its effect on improving testosterone, FSH and LH hormone imbalances associated with type 2 diabetes. Additionally, *G. kola* is one of the plants that have

a role against infertility and reproductive dysfunction of males through pretesticular effect by increasing testosterone, FSH and LH levels (Farombi et al. 2013).

The occurrence and progression of diabetic vascular problems are caused by a low-grade inflammatory reaction of the blood vessel wall caused by hyperglycemia. Inflammatory mediators continually activate vascular endothelial cells, and the integrity of the connection between endothelial cells, as well as cell gaps, is damaged as a result of increased vascular permeability and macro-molecular material leakage. When these leaky macro-molecular compounds accumulate in the blood artery wall, they cause tissue edema and accelerate atherosclerosis (Li et al. 2023). Dyslipidemia accompanied by impaired liver and kidney functions were evident in diabetic rats in this study. The disturbance in lipid metabolism and impaired liver and kidney functions were attenuated by treatment with *G kola* and probiotic yoghurt. The anti-inflammatory effect of *G kola* phytochemicals and probiotic strains might be contributed to their improvement effect against dyslipidemia and impaired liver and kidney biochemical indicators. The activation of the AMP-activated protein kinase (AMPK) has frequently been linked to the molecular mechanisms behind the beneficial effects of flavonoids. The control of lipid metabolism and adipogenesis depends heavily on the enzyme AMPK. Catabolic activities like glucose absorption and glycolysis are enhanced by AMPK phosphorylation and activation, while anabolic processes like fatty acid synthesis and gluconeogenesis are inhibited (Sandoval et al. 2020). Bifidobacterium, as a probiotic strain, has the potential to reduce blood TC and LDL-C levels in hyperlipidemic patients via gut microbiota modulation. The greater abundance of anti-obesity-related genera and fecal metabolites could be the underlying mechanism (Chu et al. 2023).

Conclusion

The results of the HPLC analysis of freeze-dried aqueous infusion of *G. kola* demonstrated the presence of several polyphenols such as querectin and hesperetin. Owing to these antioxidant compounds, the freeze-dried aqueous infusion of *G. kola* exhibited potent radical scavenging activity. By analyzing the results, it was possible to draw the conclusion that the co-administration of the freeze-dried aqueous infusion of *G. kola* and the fermented probiotics-yoghurt was promising in regulation of glucose, insulin and the male fertility-stimulating hormones in diabetic rats. Additionally, this co-administration resulted in down-regulation of inflammatory markers (CRP, TNF-α, IL-6 and TGF-1β) and oxidative markers (MDA and NO), up-regulation of antioxidant enzymes (SOD and GPx) and reduction of dyslipidemia in type 2 diabetic rats.

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Authors' contributions
R.S.M. Conceptualization, animal experiment, analysis, sampling, methodology, writing- original draft preparation. K.F. Conceptualization, animal experiment, analysis, sampling, methodology, writing- original draft preparation. A.H.Z. Conceptualization, sampling, methodology, writing- original draft preparation. A.M.A. Conceptualization, sampling, methodology, writing- original draft preparation.

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Availability of data and materials
The datasets used and/ or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study has been approved by the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt (No.24911122022) and all the procedures on animals were carried out according to guidelines and recommendations of the above committee.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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