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DEVELOPMENT, CHARACTERIZATION, AND THERAPEUTIC EVALUATION OF FUNCTIONAL COCOFEE GUMMIES FOR TYPE 2 DIABETES MANAGEMENT IN WISTAR ALBINO RATS

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Abstract

The present study was conducted to develop, characterize, and evaluate the therapeutic potential of functional Cocofee gummies for the management of type 2 diabetes mellitus. The gummies were formulated and analyzed for their proximate composition, revealing that the gummies contained 28.2% moisture, 6.0% crude fat, 3.3% crude protein, 2.8% crude fiber, 2.5% ash, and 42.8% nitrogen-free extract (NFE). Phytochemical evaluation demonstrated appreciable bioactive potential with total phenolic content of 23.6 mg GAE/100 g and total flavonoid content of 4.7 mg CE/100 g. Antioxidant activity, determined by the DPPH radical-scavenging assay, was 21.48%. Color analysis showed L, a*, and b* values of 29.2, 5.7, and 10.5, respectively, with C*, WI, and ΔE values of 26.23, 14.12, and 22. Sensory evaluation indicated good consumer acceptability, with appearance, texture, taste, aroma, and overall acceptability scores of 8.5, 7.8, 6.2, 4.2, and 5.7, respectively. For therapeutic efficacy, diabetes was induced in Wistar albino rats using alloxan, and animals were supplemented with low-dose (1 gummy per day) and high-dose (2 gummies per day) cocofee gummies for 30 days. The high-dose treatment group exhibited the greatest reduction in body weight (6.2%) and random blood glucose levels (11.0%) compared with the diabetic control group. The outcomes suggest that functional Cocofee gummies possess hypoglycemic effects which attributed to the synergistic action of coffee-derived bioactive compounds and dietary fiber from coconut husk that improve glucose metabolism, metabolic regulation, and antidiabetic properties.*

1- Introduction

Persistent hyperglycemia is an early indicator of diabetes mellitus (DM), a chronic metabolic disorder caused by reduced insulin secretion, insulin resistance, or both (Blicher-Hansen et al., 2024; Eyth et al., 2023). Type 2 diabetes mellitus (T2DM) is increasing globally and is strongly linked to obesity (Dagher et al., 2024; Antar et al., 2023). DM is classified into type 1 (immune-mediated, usually developing in childhood) and type 2 (developing later in life and associated with metabolic and aging-related conditions) (Chen et al., 2024; Ceriello et al., 2022). Diabetes places a major socioeconomic, emotional, and financial burden due to its rising prevalence and mortality (Kobayashi & Kadowaki, 2024; Harding et al., 2024).

Globally, 537 million adults (about 1 in 10) were living with diabetes in 2021, with 6.7 million deaths attributed to the disease (Diallo et al., 2024; Eades et al., 2024). Around 50% of people with diabetes remain undiagnosed, and nearly 75% live in low- and middle-income countries (IDF). Overall prevalence among adults aged 20–79 is estimated at 10.5%, with 44.7% still undiagnosed. The Western Pacific region shows the highest prevalence (16.2%), while the Middle East and North Africa has the largest number of cases (205.6 million). Earlier estimates reported 422 million adults living with diabetes in 2014 based on global population studies (DeFronzo et al., 2015). Current projections suggest the global burden may rise to 1.3 billion by 2050 (Sun et al., 2022; Núñez-Baila et al., 2024).

Pakistan has one of the highest diabetes prevalence rates globally, estimated at 26.7% in 2022 (around 33 million cases), ranking among the top countries worldwide (Sodikov et al., 2024; Sun et al., 2022; Núñez-Baila et al., 2024). Reported national prevalence increased from 11.77% in 2016 to 17.1% in 2019, accounts for about 90% of all cases globally (Sodikov et al., 2024). Diabetes also creates a major economic burden, with average medical costs 2.6 times higher than for non-diabetic individuals. Key indirect costs include absenteeism ($35.8 billion), premature death-related productivity loss ($32.4 billion), and disability-related employment loss ($28.3 billion) (Sweeting et al., 2024; Parker et al., 2024). Type 2 diabetes mainly affects adults over 45 years of age (Tripathi et al., 2024; Tokhirovna and Life, 2023).

In recent years, global demand for functional foods and nutraceuticals has increased due to rising health awareness and convenience, driven by socioeconomic factors, population growth, disposable income, life expectancy, and healthcare costs (Kaviya, 2023). Functional foods, a term used since 1984, refer to foods with health benefits beyond basic nutrition (Urooj et al., 2026a), and include natural or processed foods containing bioactive compounds (Efe et al., 2022). These foods are associated with reduced risk of diseases such as diabetes, obesity, cardiovascular disease, autoimmune disorders, and cancer (Gunes et al., 2022).

Caffeine is a widely consumed bioactive food ingredient with significant health effects, found mainly in coffee, tea, cola, energy drinks, chocolate, and some food products like chewing gum (Barrea et al., 2023; Clifford et al., 2024). Coffee is the primary dietary source of caffeine and higher coffee intake has been linked to a reduced risk of T2DM (Coelho et al., 2024). Coffee contains a complex mixture of bioactive compounds, which vary depending on the coffee type, roasting, and processing methods (Aufar et al., 2023; Analianasari et al., 2024).

Coffee beans have a complex chemical composition, including carbohydrates, lipids, acids, minerals, proteins, and nitrogen-containing compounds such as caffeine and trigonelline (Bosso et al., 2023; Domingues et al., 2023). Their composition varies depending on species, cultivation, post-harvest handling, defects, and roasting conditions (Freitas et al., 2020; Halagarda & Obrok, 2023). In general, green Arabica coffee contains about 50–60% carbohydrates, 15–20% lipids, 10–15% proteins, 3–5% minerals, and around 1% caffeine, while Robusta coffee has higher caffeine but lower carbohydrates and lipids (Czarniecka-Skubina et al., 2021; Yeager et al., 2023).

Gummy supplements are an effective and increasingly popular method for delivering medications and nutritional bioactives, especially for children and adults (Herawati et al., 2024; Nascimento et al., 2024). This study focuses on selecting natural vegan ingredients for developing gummy prototypes (Rashmi et al., 2023). With rising consumer interest in health benefits from diet, there is growing demand for functional supplements and innovative delivery systems (Kolb et al., 2020).

Chewable dosage forms are replacing traditional oral administration forms like tablets or capsules due to their flexibility in creating more palatable and easier-to-ingest products. In particular for naturally derived functional ingredients, chewable products offer an appropriate nutritional context and are more naturally associated with food than tablets and capsules (Wang et al., 2024; Misto et al., 2024). When it comes to incorporating water-soluble functional ingredients into food delivery systems, gummy candies can be immensely useful. Meanwhile, a wider distribution and acceptance of these ingredients may be made possible by their growing consumer popularity (Vojvodić Cebin *et al.*, 2024). Study aimed to develop and characterize functional gummies and also investigate the therapeutic effect of functional gummies on diabetes mellitus in the Wistar albino rat model.

2- Materials and Methods

Procurement of raw materials

Raw materials including coffee powder, coconut, black paper, gelatin, oranges, stevia, and lime extract were procured from local market of Faisalabad. All reagents and chemicals needed during the study were of analytical grade and obtained from Merck Germany through authorized scientific chemical suppliers in Faisalabad, Pakistan.

Preparation of coconut husk

Fresh coconuts were thoroughly washed under running tap water. After that, the outer shell and brown testa were manually taken off and the edible white was grated using a sterilized grater to obtain a coarse husk-like texture. The prepared coconut husk was stored at 4 °C until further use.

Preparation of orange juice base

Fresh oranges were washed with potable water, manually peeled, and cut into halves crosswise. Juice extraction was performed by squeezing through hands. Extracted juice was filtered through a sterile muslin cloth and fine mesh sieve to remove seeds and pulp residues.

Formulation and preparation of gelatin-based functional gummies

Formulation of functional cocofee gummies was carried out with slight modifications to previously reported preparation method of functional gummy candies containing natural antioxidants and stevia developed by Roudbari et al. (2024) and gelatin-based functional gummy jellies enriched with oregano oil developed by Ganea et al. (2025). Gelatin (25g) was added in 250 mL fresh orange juice and boiled at 100 °C with continuous stirring until complete dissolution was achieved. Subsequently, coffee powder (500 mg), black pepper powder (100 mg), stevia (2 mg), coconut husk (50 mg), and lime extract were incorporated into the gelatin solution and homogenized thoroughly to ensure uniform distribution of ingredients. Then, mixture was poured into sterile silicone molds and allowed to cool at room temperature for initial gel formation. Thereafter, the gummies were refrigerated at 4°C. The formulated gummies were demolded and stored in airtight containers under refrigerated conditions until further physicochemical, antioxidant, sensory, and therapeutic analyses.

Table 1: Experimental composition of orange juice, coffee, black pepper, stevia and gelatin in coffee gummies

Raw materials	Quantity standardized
Orange juice	250 mL
Coffee	500 mg
Black pepper	100 mg
Stevia	2 mg
Gelatin	25 g
Coconut husk	50 mg

Proximate composition

Gummies were analyzed for proximate composition, including moisture, crude fat, crude protein, crude fiber, ash, and nitrogen-free extract (NFE), following procedures of AOAC (2019) reported in Asif et al. (2023).

Phytochemical composition

Extract preparation

For phytochemical analysis, gummy samples were prepared according to the method described by Górecki & Hallmann (2020) with slight modifications. The samples were diluted 10-fold with distilled water and centrifuged at 5000 rpm for 25 min at 15 °C. The obtained supernatant was carefully collected and used for subsequent analyses (Asif et al., 2025a).

Determination of total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu (FC) colorimetric method as modified by Górecki & Hallmann (2020). Briefly, 200 µL of the methanolic extract was mixed with 100 µL of FC-reagent and 800 µL of distilled water. After 3 minutes, 300 µL of 20% (w/v) sodium carbonate (Na_2CO_3) was added. The mixture was incubated in the dark at 25 °C for 2 hours. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. TPC was quantified using a gallic acid calibration curve and expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/100 g).

Determination of total flavonoid content (TFC)

Total flavonoids of gummies were quantified using the aluminum chloride colorimetric method as outlined by Asif et al. (2025b), with quercetin as the standard. 1 mL of the centrifuged supernatant was further diluted to 5 mL with distilled water. This diluted extract was mixed with 0.3 mL of 5% solution of sodium nitrite ($NaNO_2$) and incubated for 5 min. Following this, the mixture was combined with 0.3 ml of aluminium trichloride ($AlCl_3$) and incubated for an additional 6 min. After incubation, 2 mL of sodium hydroxide (NaOH) was added, and the final volume was adjusted to 10 mL using distilled water. The reaction mixture was then incubated for an additional 5 min. Finally, absorbance was measured at 510 nm using a spectrophotometer at 510 nm. Total flavonoid content was expressed as milligrams quercetin equivalents (mg CE/100 g) of sample.

Determination of anti-oxidant potential (DPPH Assay)

Antioxidant activity of sample was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method as outlined by Górecki & Hallmann (2020) and Shabbir et al. (2026). The sample extract was centrifuged at 5000 rpm for 25 min at 15°C, and the resulting supernatant was separated through filtration. For preparation of the DPPH solution, 4 mg of DPPH was dissolved in 80 mL of methanol. 1 mL of extract was mixed with 3 mL of DPPH solution and stored in the dark for 40 min. The absorbance of the reaction mixture was then measured at 517 nm using a spectrophotometer. A blank solution was also prepared and measured under the same conditions to account for background absorbance. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging (\%)} = \text{X1} - \frac{\text{X2}}{\text{X1}} \times 100$$

X1 = Blank sample's absorbance
X2= Sample's absorbance at 517 nm

Mineral composition

Mineral composition of the gummies was determined according to the method described Adeleke et al. (2024). Mineral analysis was performed using wet digestion followed by atomic absorption spectrophotometry (AAS). In this process, the digested sample was atomized, and the analytes in the ground state absorbed energy of specific wavelengths emitted by a hollow cathode lamp. The absorption intensity, which is directly proportional to the mineral concentration, was measured.

For wet digestion, 3–5 g of the sample was accurately weighed into a conical flask. A mixture of hydrochloric acid (HCl) and nitric acid (HNO_3) in a ratio of 7:3 was added, and the flask was placed on a hot plate. The sample was heated for 3–4 h until the volume was reduced to approximately 1–2 mL and a clear transparent solution was obtained. The flask was then removed from the hot plate and allowed to cool at room temperature. The digested solution was diluted to a final volume of 100–250 mL using distilled water. For quantification, calibration curves were prepared using standard solutions of known concentrations for each mineral. These standard curves were used to determine the mineral content of the samples based on their respective absorbance values obtained through AAS analysis.

Color analysis

Color analysis of gummies samples was conducted using a Hunter Lab colorimeter, as described by Barman *et al.*, (2021) and Iqbal et al. (2026). The product's color was reported by calculating its L* value (positive value indicates lightness, while negative values indicate darkness), a* value (indicating the red and green color difference), and b* value (specifying the yellow and blue color). The change in color (ΔE)), Chroma (C^{*}), and Whitening Index (WI) were designed using formula

$$\Delta E=\sqrt{\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2}}$$

$$C^{*}=\sqrt{(a^{*})^{2}+(b^{*})^{2}}$$

$$WI=\sqrt{(100-L^{*})^{2}+(a^{*})^{2}+(b^{*})^{2}}$$

Sensory evaluation

Sensory features of gummies remained resolute by the technique of Eromo et al. (2020). In a sensory evaluation laboratory, a group of ten adjudicators (scholar and faculty members) over the department of NIFSAT assessed gummies intended for color, taste, overall acceptability a texture on a 9-point hedonic scale (ranging from I strongly dislike to 9 = extremely like). L as mentioned by Urooj et al. (2026b). The sensory review panel assessed the color, taste, texture, and overall acceptability of gummies.

Efficacy plan

Total 21 animals were procured and housed in animal room, UAF. All rats were provided with proper ventilation facility and kept in distinct cage. These rats were classified into groups and each group was containing 3 rats according to the treatment plan. Diabetes was induced in rats from alloxan via intraperitoneally at concentration of 125 mL per kg body weight after fasting. The experimental trial was run for 30 days and above-mentioned biochemical analysis was done weekly basis. Weight of the rats was also observed during this time duration. Functional cocofee gummies were given to the rats with different concentration show in treatment plan and hypoglycemic effect of the functional gummies were checked.

Table 2: Treatment plan for disease induced rats

Groups	Subject status	Disease	Diet
G_0	(-ve) group	Normal	Normal
G1	(+ve) group	Disease	Normal
G_2	(+ve) group	Disease	Normal diet + gummies 1 per day
G_3	(+ve) group	Disease	Normal diet + gummies 2 per day

Biochemical analysis

Body weight

Body weight was recorded weekly using a calibrated digital balance as mentioned by Urooj et al (2026c) to monitor changes during the experimental period and calculated according to the following formula:

$$\text{Body weight gain (\%)} = \frac{\mathit{Final\ body\ weight} - \mathit{Initial\ body\ weight}}{\mathit{Initial\ body\ weight}} \times 100\%$$

Random blood glucose

Random blood sugar (RBS) is evaluated to check the elevation in blood glucose level. In normal individuals the value of RBS ranges between 80-160 mg/dL. In pre-diabetic condition the values increase to 160-200 mg/dl respectively by followed the protocols of American Diabetes Association. (2021).

Statistical analysis

The statistics measured for respective factor remained exposed towards statistical examinations to observe the level of significance as described by Montgomery (2017) using Statistics 8.1. Data was constructed in the form of analysis of variances and comparison of means using factorial design and two-way ANOVA (Analysis of Variance).

3- Results and Discussions

Coffee is among those food items that have potency of hypoglycemic effect and liver protection. Coffee is one of the most widely consumed beverages globally and has been the subject of numerous studies exploring its potential health benefits. The slient purpose of this research was to explain and clarify the significance of coffee for its nutraceutical and therapeutic characteristics. This study is divided into following parts for comprehensive and detailed understanding: Proximate analyses of cocofee gummies and efficacy trials on experimental animals to evaluate anti diabetic potential of coffee.

Proximate composition

Proximate composition of the functional cocofee gummies is presented in Figure 1. The moisture content of the gummies was 28.2±0.12%. The relatively high moisture content may be attributed to the use of orange juice during formulation and gelatin activation. Moisture plays an important role in maintaining the texture, chewiness, appearance, and overall quality of gummies. Similar findings were reported by Ahmad Nasir et al. (2022), who observed that moisture level significantly influences the texture and shelf life of gummy formulations. Crude fat content of cocofee gummies was 6.0±0.15%, comparatively higher due to the incorporation of coconut husk and black pepper, which naturally contain lipids. Fat contributes to the softness, mouthfeel, flavor retention, and stability of gummy products. Similar observations were reported by Vojvodić Cebin et al. (2024), who explained that fats improve the palatability and sensory properties of confectionery products. Coffee beans generally contain 11–18% fat depending on the variety and processing conditions (Makiso et al., 2024). Crude protein of the functional cocofee gummies was 3.3±0.25%. The protein value may be due to the combined contribution of coffee, coconut husk, and black pepper. Protein is an essential nutritional component required for tissue development, enzyme activity, and body functions. Green coffee beans contain approximately 8–12% protein, although roasting reduces the protein level because of thermal degradation and Maillard reactions (Freitas et al., 2024). The protein content obtained in this study is comparable with the findings of Stanek et al. (2021), who reported protein values ranging between 3–5% in functional food products. Crude fiber content of cocofee gummies was 2.8±0.02%. The presence of coconut husk significantly contributed to the fiber content of the gummies. Dietary fiber is important for digestive health and helps in regulating glycemic response. Green coffee beans contain considerable amounts of dietary fiber, ranging from 30–35% (Rosa et al., 2024). Roasting slightly decreases the fiber level due to thermal degradation, but the overall fiber content remains substantial (Le et al., 2024).

Ash content of the functional cocofee gummies was 2.5±0.12%. Ash content represents the total mineral content present in the product. The ash value observed in this study may be due to the addition of coffee and coconut husk, all of which contribute minerals to the formulation. Green coffee beans generally contain 3–5% ash content, mainly consisting of potassium, magnesium, calcium, and phosphorus (Urugo et al., 2024). Similar findings were reported by Lin et al. (2024), who observed that roasting slightly increases ash content because of moisture loss and concentration of minerals. Nitrogen-free extract (NFE) content of cocofee gummies was 42.8±0.4%. NFE represents the digestible carbohydrate fraction of the product, including sugars and soluble carbohydrates that provide energy. The relatively high NFE value indicates that carbohydrates constitute a major portion of the gummies formulation. Carbohydrate level obtained in this study may be associated with the use of sweeteners and fruit juice during formulation.

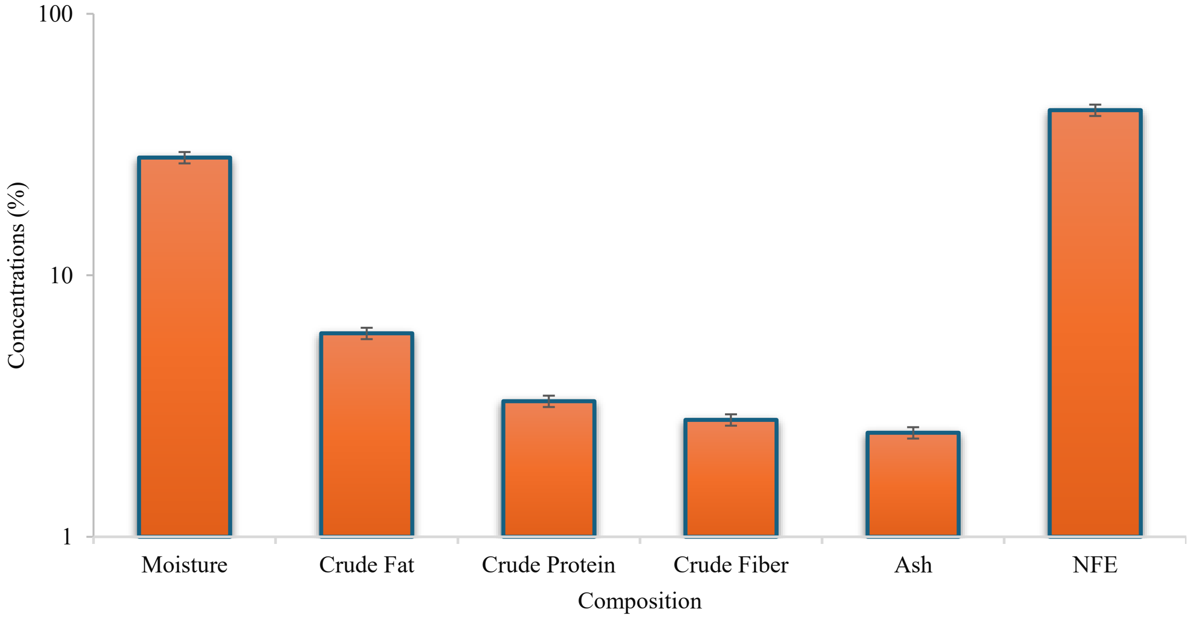

Figure 1. Effect of functional cocofee gummies on proximate composition

Total phenolic contents (TPC)

Phenolic compounds are naturally occurring plant secondary metabolites that possess strong antioxidant properties. These bioactive compounds are widely distributed in plant-based foods and contribute significantly to their nutritional and functional quality. The presence of phenolic compounds in food materials is associated with improved health benefits, including the prevention of chronic diseases and reduction of oxidative stress (Rizvi *et al.*, 2022). A higher concentration of phenolic compounds is also linked to slower oxidative degradation of lipids, which is of great importance in improving food quality and shelf life (El-Sayed and Youssef, 2019). Total phenolic content (TPC) is an important indicator used to evaluate the antioxidant potential of food products. Coffee is particularly rich in phenolic compounds, especially chlorogenic acids (CGAs), which are the dominant phenolics in green coffee beans and are responsible for many of its health-promoting effects (Rigueto et al., 2020). The TPC in coffee varies depending on processing conditions, particularly roasting. Green coffee beans typically contain approximately 40–90 mg GAE/g of phenolic compounds, whereas roasted coffee beans contain relatively lower levels ranging from 30–60 mg GAE/g due to thermal degradation during roasting (Liao et al., 2022; Wu et al., 2022). Brewing methods and formulation types also influence phenolic extraction, with brewed coffee reported to contain approximately 100–300 mg GAE/L depending on preparation conditions (Masek et al., 2020). TPC of functional cocofee gummies was 23.6 mg GAE/100 g. This value indicates the presence of a moderate level of phenolic compounds in the developed product. The observed TPC is mainly attributed to the incorporation of roasted coffee, which retains a portion of its phenolic compounds, particularly chlorogenic acid derivatives and other Maillard reaction products formed during roasting. These compounds contribute to the antioxidant and functional properties of the gummies.

Total flavonoid content (TFC)

Flavonoids are a major group of plant-derived polyphenolic compounds widely distributed in foods, where they contribute significantly to antioxidant, anti-inflammatory, and other health-promoting activities. These bioactive compounds play an important role in neutralizing free radicals and protecting biological systems against oxidative stress (Pratyusha, 2022). In coffee-based products, flavonoids are among the key contributors to antioxidant potential, flavor development, and overall functional properties. Total flavonoid content (TFC) in coffee is influenced by several factors, including the type of coffee bean, roasting conditions, and preparation method. Arabica and Robusta coffee varieties differ in their flavonoid profiles, with Arabica generally exhibiting a distinct phytochemical composition (de Sousa et al., 2021). Roasting also significantly affects flavonoid levels, as thermal processing may lead to degradation or transformation of flavonoid compounds. Light roasting tends to preserve more flavonoids compared to dark roasting (Tsai and Jioe, 2021). Similarly, brewing or extraction conditions such as temperature and pressure influence flavonoid yield, with higher extraction conditions generally increasing flavonoid release. Green coffee beans contain higher flavonoid levels (approximately 2–4 mg catechin equivalents/g) due to the absence of heat-induced degradation (Bobková et al., 2020). TFC of functional cocofee gummies was 4.7 mg catechin equivalents/g. This value indicates the presence of appreciable flavonoid compounds in the developed product, contributing to its antioxidant potential. The observed TFC may be attributed to the incorporation of roasted coffee extract, which retains flavonoid derivatives along with melanoidins formed during roasting. The presence of black pepper and orange juice may have further contributed to the overall flavonoid content, as both are known sources of bioactive antioxidant compounds.

DPPH activity

Antioxidants are natural compounds present in food materials that help neutralize free radicals generated in the body through normal metabolic processes. These compounds play an important role in reducing oxidative stress and preventing cellular damage. The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay is widely used to evaluate the free radical scavenging ability of food samples due to its simplicity, stability, and reliability in measuring antioxidant capacity (Munteanu and Apetrei, 2021). DPPH radical scavenging activity of cocofee gummies was 21.48%. This value indicates a moderate antioxidant capacity of the developed product. The observed DPPH activity suggests that the formulation has the ability to donate hydrogen or electrons to stabilize free radicals, thereby reducing oxidative reactions. The antioxidant potential of the gummies is mainly attributed to the presence of roasted coffee, which contains bioactive compounds such as polyphenols, caffeine, and chlorogenic acid derivatives. Coffee is known to exhibit strong antioxidant activity, which varies depending on the type of coffee bean, roasting level, and extraction method (Jaiswal, 2020). Roasting plays an important role in enhancing antioxidant activity due to the formation of Maillard reaction products and the transformation of chlorogenic acids into more active phenolic compounds (Xiao et al., 2020). These compounds contribute significantly to free radical scavenging ability. Similar findings were reported by Muzykiewicz-Szymańska et al. (2021), who observed that roasting can enhance antioxidant activity through the formation of melanoidins, despite partial degradation of some polyphenols. The antioxidant activity of coffee-based products is also influenced by the type of coffee used. Arabica coffee generally shows higher DPPH scavenging activity compared to Robusta due to its higher phenolic content (Bibi et al., 2022). However, processing conditions and formulation ingredients may also affect the final antioxidant capacity of the product.

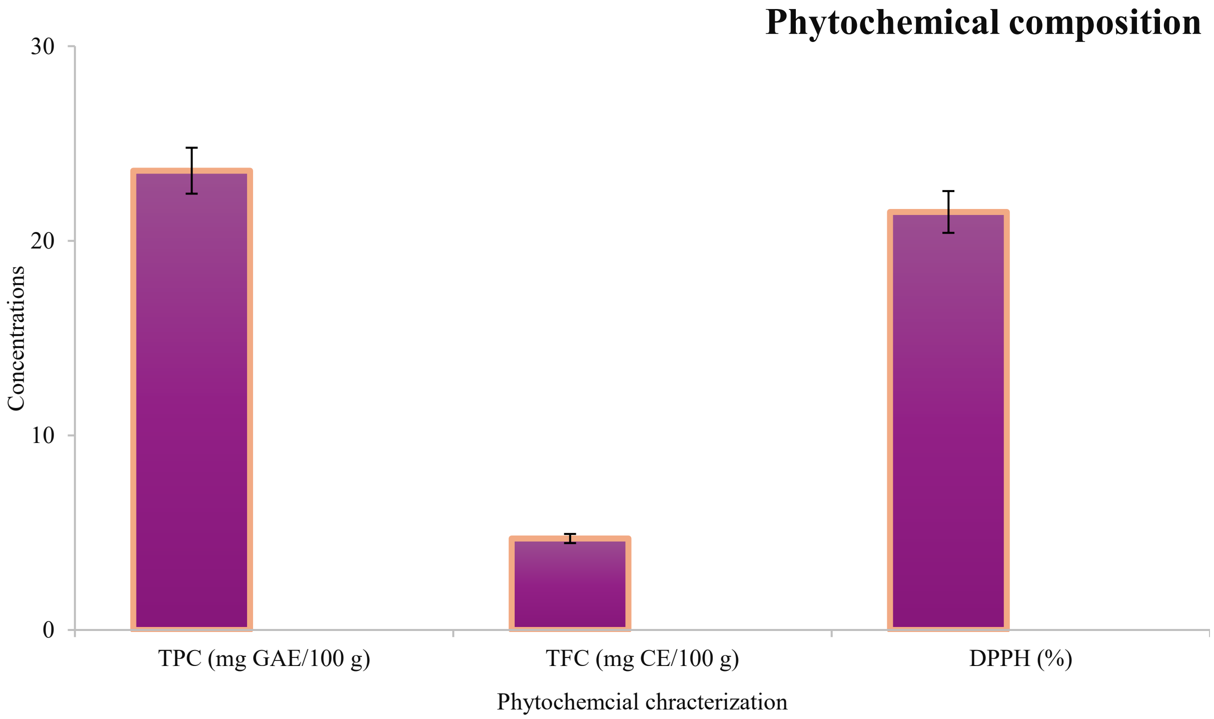

Figure 2. Effect of functional cocofee gummies on phytochemical and antioxidant composition

Color characteristics

Color characteristics of functional cocofee gummies are presented in Figure 3. Color is an important quality attribute of food products and greatly influences consumer acceptance and preference. The color parameters of cocofee gummies were measured in terms of lightness (L*), redness (a*), yellowness (b*), chroma (C*), whitening index (WI), and Delta E (ΔE). The cocofee gummies exhibited L*, a*, and b* values of 29.2, 5.7, and 10.5, respectively. The low L* value indicated a dark appearance of the gummies, while the positive a* and b* values indicated slight redness and yellowness in the formulation. The dark brown color of the cocofee gummies may be attributed to the presence of coffee and the cooking process during preparation. Coffee contains melanoidins and other brown-colored compounds formed during roasting, which contribute to the characteristic dark color of coffee-based products. Maillard reaction and caramelization during heating at 80°C may also have contributed to the darker color of the gummies. Similar observations were reported by Iriondo-DeHond et al. (2021) who found that higher cooking temperatures negatively affected color parameters and caused darkening in food products.

Chroma (C*) value of cocofee gummies was 26.23±1.3, indicating moderate color intensity. Chroma represents the saturation or vividness of color perceived by consumers. The comparatively lower chroma value may be due to the coating of coconut husk on the gummies, which reduced the brightness and intensity of the product color. Similar trends were reported by

Kingwascharapong et al. (2020), who observed a reduction in chroma values in coffee products due to processing and environmental conditions. Whitening index (WI) of cocofee gummies was 14.12±1.6, indicating a low degree of whiteness. The low WI value was expected because of the naturally dark brown color imparted by roasted coffee compounds. Unlike products formulated with whitening agents, coffee-based products generally retain their dark pigmentation due to melanoidin formation during roasting. The presence of coconut husk and black pepper may have also contributed to the darker appearance of the gummies. (ΔE) value of cocofee gummies was 22, indicating a noticeable overall color difference in the product. Delta E values represent the extent of color variation perceived by the human eye. The observed color difference may be associated with ingredient composition, roasting characteristics of coffee, and thermal processing during gummy preparation. Similar color changes due to enzymatic and non-enzymatic browning reactions have also been reported in previous studies on processed food products.

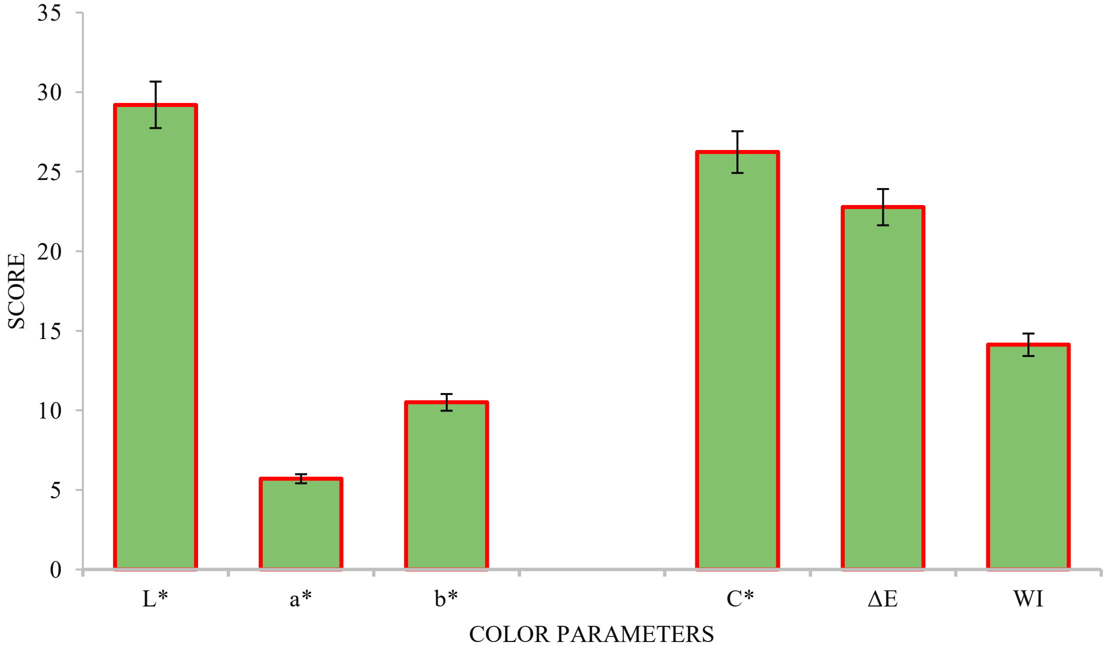

Figure 3. Effect of functional cocofee gummies on color characteristics

Sensory evaluation

Hedonic response is crucial to judge the product for acceptance and marketability. Good sensory response ensures consumer acceptance and confidence on the developed product. Cocofee gummies are generally dark brown in color, resembling the hue of brewed coffee varies depending on factors such as the type of coffee used, concentration of coffee extract and other additional ingredients like black pepper, gelatin powder and coconut husk (Makiso *et al.*, 2024). The appearance of cocofee gummies has a direct impact on their commercial and economic value of cocofee gummies were 8.5±0.2. Cocofee gummies were appealing due to their color and coconut coating. Texture of gummies, including coffee, is a key aspect of their appeal and enjoyment. Gummies are known for their chewy texture, which is achieved through the use of gelatin or pectin. Gelatin, derived from collagen in animal bones and skin, is the traditional gelling agent and provides a firm yet yielding chewiness. Gummies have a smooth surface texture, which contributes to their pleasant mouthfeel. This smoothness is achieved during the manufacturing process, where the gummy mixture is poured into molds and allowed to set (Roudbari *et al.*, 2024). The differences in texture could also be due to of fruit use, rate of water absorption from the surroundings and fiber contents of the fruits and sugar addition as well (Shende *et al.*, 2020). Texture analysis of functional cocofee gummies were 7.8±0.4, which indicated that texture of cocofee gummies showed significant results and become slightly soft may be due to temperature and concentration of gelatin. These results are in agreement with a literature in which they study that the texture of gummies becomes soft due to temperature (Santos *et al.*, 2022). For many years, taste analysis has been used to assist develop new products, understand the nature of developed items, explore shelf life and maintain the quality of food. The taste of the gummies can be affected by the level of the raw materials and by added ingredients to the optimum level (Shende *et al.*, 2020). Taste plays a vital role in quality and acceptance of gummies. It is frequently defined as the "olfactory component of taste" that is perceived retro nasally since many consumers generally associate taste with what they smell (Shende *et al.*, 2020). Sensory outcomes of cocofee gummies regarding taste were observed, in which mean values 6.2±0.42. The primary taste profile of cocofee gummies was the distinct taste of coffee. Cocofee gummies often have a balance of sweetness to complement the bitterness of coffee. Aroma, in the context of food and beverages, refers to the characteristic smell or fragrance that emanates from a substance, often influencing its overall flavor perception. It's the smell or odor characteristic of a substance or ingredient, especially as distinct from its taste. In food science and sensory evaluation, aroma is an essential aspect of how we perceive and enjoy foods and beverages. It contributes significantly to the overall sensory experience, influencing taste perception and enhancing the enjoyment of flavors. In the case of cocofee gummies, aroma plays a vital role in freshly brewed coffee, contributing to their appeal to consumers according to (Adebowale and Adeyanju, 2022). The mean value for the aroma of cocofee gummies was 4.2±0.2. Coffee aroma includes a complex blend of fragrances such as fruity, floral, nutty, spicy and caramelized tones, depending on factors like roast level, bean origin, and brewing method. Bitter notes can be present in coffee due to compounds like caffeine and chlorogenic acids, which contribute to its characteristic taste profile (Zakidou *et al.*, 2021). Overall acceptability refers to the extent in which a product or service meets the expectations and preferences of consumers or users. Consumers evaluate how well these sensory qualities align with their expectations and enjoyment. The perceived quality of the product based on factors such as freshness, ingredients used, and consistency. The overall acceptability was 5.7±0.23 which should be chewy yet firm, with a smooth and pleasant mouthfeel that enhances the overall sensory experience. Aroma contributes to the appeal of cocofee gummies.

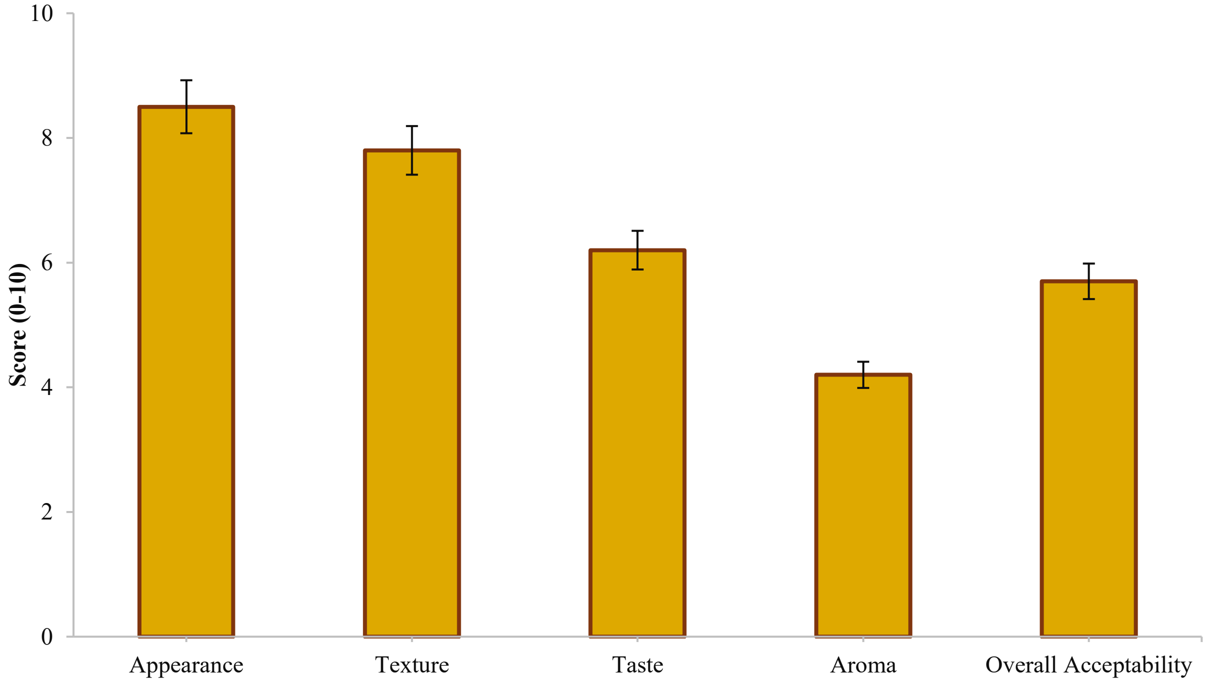

Figure 4. Effect of functional cocofee gummies on sensory attributes

Efficacy trials

Effect of cocofee gummies on body weight

Body weight is an important indicator of metabolic health and is closely associated with the progression and management of diabetes. The effects of cocofee gummies supplementation on body weight of diabetic rats are presented in Figure 5. At the beginning of the study (day 0), no significant differences ($p > 0.05$) were observed among the experimental groups, indicating a comparable baseline body weight. During the experimental period, the disease control group (G1) exhibited a slight increase in body weight from 177.64 ± 0.302 g on day 0 to 178.74 ± 0.722 g on day 30 (+0.9%), suggesting that diabetes-induced metabolic disturbances were not effectively controlled in untreated animals. In contrast, rats receiving cocofee gummies showed a progressive reduction in body weight throughout the study period. The G2 group, supplemented with a lower dose of cocofee gummies, demonstrated a reduction in body weight from 173.64 ± 0.020 g at baseline to 170.56 ± 0.032 g on day 15 and 169.11 ± 0.854 g on day 30, corresponding to an overall decrease of 2.4%. Similarly, the G3 group, receiving the higher dose of cocofee gummies, exhibited the greatest reduction in body weight, decreasing from 176.42 ± 0.893 g at day 0 to 170.92 ± 0.833 g at day 15 and 167.22 ± 0.853 g at day 30, representing a total reduction of 6.2%.

Compared with the disease control group (G1), both cocofee gummies-treated groups displayed improved body weight management, with the highest dose (G2) producing the most pronounced effect. The reduction observed in G3 was greater than that recorded for G2, indicating a dose-dependent response of cocofee gummies. The beneficial effect may be attributed to the combined action of caffeine from coffee powder, dietary fiber from coconut husk, and gelatin, which are reported to promote satiety, regulate energy metabolism, and support weight management. The normal control group (G0) also showed a slight decline in body weight from 174.74 ± 0.032 g to 170.32 ± 0.302 g (1.3%) over the study period. However, the magnitude of reduction was substantially lower than that observed in cocofee gummies-treated groups, particularly G3. Analysis revealed that treatment had a significant effect on body weight ($p < 0.05$), indicating that different levels of cocofee gummies supplementation significantly influenced body weight changes among the experimental groups. The effect of study duration was highly significant ($p < 0.01$), demonstrating that body weight responses became more evident with increasing treatment time. Furthermore, the interaction between treatment and days was significant ($p < 0.05$), suggesting that the influence of cocofee gummies on body weight varied according to the duration of supplementation. Similar observations have been reported by Lafontant et al. (2024), who demonstrated that caffeine-containing functional foods can enhance weight control through increased energy expenditure and appetite regulation.

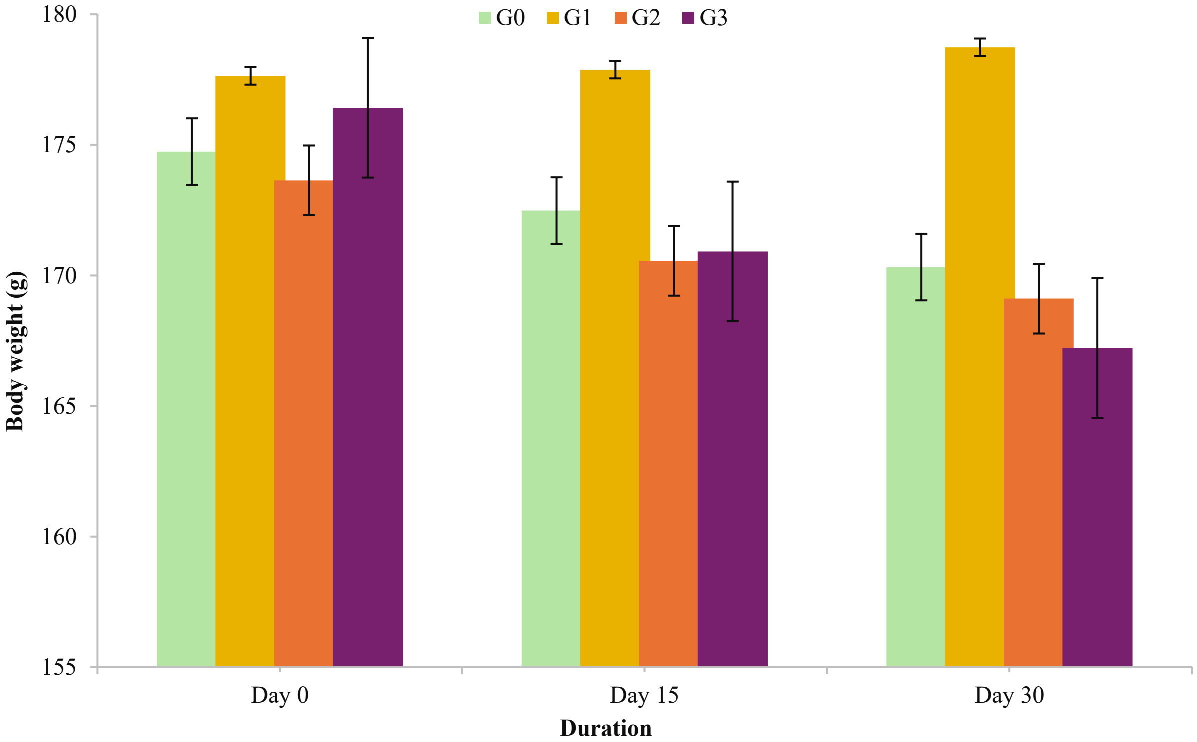

Figure 5. Effect of functional cocofee gummies on body weight

Note:
G_0: normal control group (no disease, standard diet)
G_1: diabetic control group (disease induced, no treatment)
G_2: diabetic group treated with low-dose (1 gummy per day)
G_3: diabetic group treated with high-dose (2 gummy per day)

Effect of cocofee gummies on random blood glucose

Random blood glucose level is an important indicator for evaluating glycemic status and the effectiveness of antidiabetic interventions. The effects of cocofee gummies supplementation on blood glucose levels of diabetic rats are presented in Figure 6. At baseline (day 0), no significant differences ($p > 0.05$) were observed among the experimental groups, indicating that all animals had comparable glucose levels before treatment. During the experimental period, a gradual reduction in blood glucose levels was observed in all groups; however, the magnitude of reduction varied according to treatment. The disease control group (G1) showed a decrease in blood glucose level from 180.00 ± 0.833 mg/dL at day 0 to 173.00 ± 0.633 mg/dL at day 15 and 165.00 ± 0.393 mg/dL at day 30, corresponding to an overall reduction of 4.2%. Although some improvement was observed, glucose levels remained higher compared with cocofee gummies-treated groups. Administration of cocofee gummies resulted in a greater reduction in blood glucose levels. The G2 group exhibited a decrease from 178.86 ± 0.853 mg/dL at baseline to 171.00 ± 0.873 mg/dL on day 15 and 163.00 ± 0.482 mg/dL on day 30, representing an overall reduction of 6.0%. The G3 group, receiving the higher dose of cocofee gummies, demonstrated the greatest glucose-lowering effect, with blood glucose levels decreasing from 180.83 ± 1.302 mg/dL at day 0 to 172.63 ± 0.722 mg/dL on day 15 and 160.90 ± 0.854 mg/dL on day 30. This corresponded to the highest reduction among all groups (11.0%). Compared with G2, the higher-dose group (G3) achieved superior glycemic control, indicating a dose-dependent hypoglycemic effect of cocofee gummies. The improved glucose regulation may be attributed to the synergistic effects of coffee-derived bioactive compounds, dietary fiber from coconut husk, and gelatin. Dietary fiber is known to slow glucose absorption and improve insulin response, while coffee phytochemicals have been reported to enhance glucose metabolism and insulin sensitivity. The normal control group (G0) also showed a slight reduction in blood glucose level from 179.53 ± 0.893 mg/dL to 173.00 ± 0.783 mg/dL, representing a 3.0% decrease during the study period. However, the reduction was less pronounced than that observed in the cocofee gummies-treated groups. Treatment had a highly significant effect on blood glucose levels ($p < 0.01$), indicating that different levels of cocofee gummies supplementation significantly influenced glycemic control. The effect of treatment duration was also significant ($p < 0.05$), demonstrating that glucose regulation improved progressively with time. Furthermore, the interaction between treatment and days was significant ($p < 0.05$), suggesting that the glucose-lowering effect of cocofee gummies depended on both dosage and duration of administration. These findings indicate that prolonged supplementation with cocofee gummies enhances glycemic control and may serve as a promising functional food approach for diabetes management. Similar findings have been reported by Rawat et al. (2024), who demonstrated that caffeine and other coffee bioactive compounds can improve glucose tolerance, enhance insulin sensitivity, and contribute to better regulation of blood glucose levels. The present results therefore support the potential application of cocofee gummies as a dietary strategy for improving glucose homeostasis in diabetic conditions.

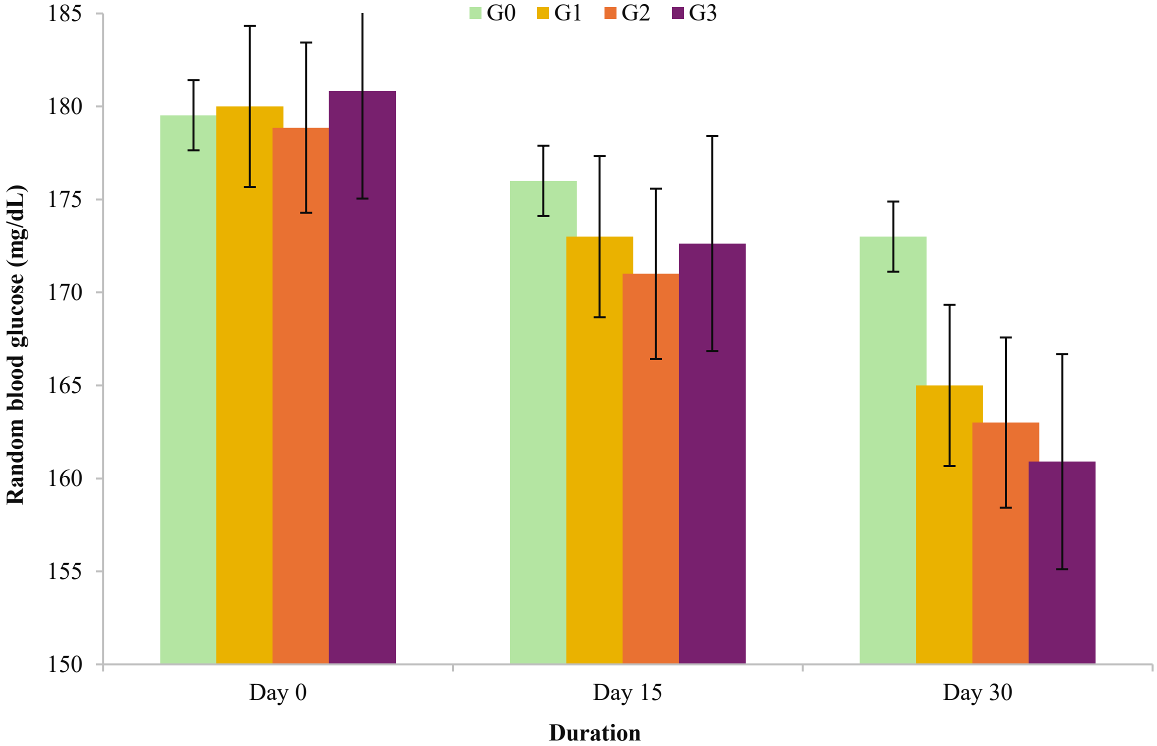

Figure 6. Effect of functional cocofee gummies on random blood glucose

Note:
G_0: normal control group (no disease, standard diet)
G_1: diabetic control group (disease induced, no treatment)
G_2: diabetic group treated with low-dose (1 gummy per day)
G_3: diabetic group treated with high-dose (2 gummy per day)

4- Conclusion

The present study demonstrated that coffee-enriched functional gummies possess significant anti-diabetic potential due to the presence of bioactive compounds derived from coffee and black pepper. The formulated gummies exhibited appreciable nutritional composition along with considerable antioxidant activity, total phenolic contents, and total flavonoid contents, indicating their potential as a functional food product for diabetes management. The efficacy study revealed that administration of functional cocofee gummies significantly reduced blood glucose levels in diabetic groups compared to the diabetic control group. Both treatment doses (1 gummy and 2 gummy) showed promising hypoglycemic effects; however, the higher dose exhibited greater effectiveness in improving glycemic response. The observed anti-diabetic activity may be attributed to the antioxidant and anti-inflammatory properties of chlorogenic acids, caffeic acid, melanoidins, and other phenolic compounds present in coffee, along with the bioactive constituents of black pepper. Furthermore, sensory evaluation confirmed the acceptability of the formulated gummies, highlighting their potential as a convenient and palatable nutraceutical delivery system. Overall, the findings suggest that coffee-enriched functional gummies may serve as a promising dietary intervention for the management of type 2 diabetes and could be incorporated into routine diets to support glycemic control and metabolic health.

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