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Effect of Caffeine Adsorption by Activated Carbon from Green Coffee Extract on Blood Glucose Reduction in Rats

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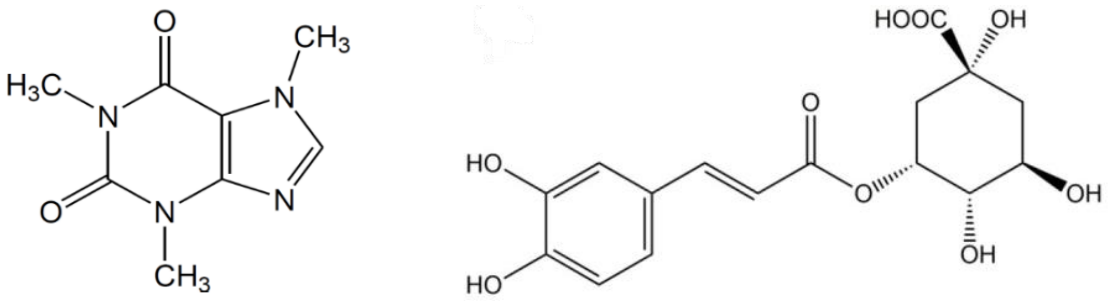
ABSTRACT. Green coffee beans (*Coffea Canephora*) contain chlorogenic acid, an active compound known to aid in lowering blood glucose levels. However, the caffeine content in green coffee beans can reduce insulin sensitivity, thereby diminishing glucose tolerance. This study aims to improve the hypoglycemic potential of green coffee by removing caffeine through adsorption using activated carbon. Green coffee beans were extracted in water at 60–70°C, and caffeine adsorption was carried out with activated carbon. FTIR analysis was performed on the activated carbon to confirm caffeine adsorption, while HPLC analysis was conducted on the green coffee extract before and after adsorption to determine caffeine and chlorogenic acid contents. Hypoglycemic activity was evaluated in alloxan-induced albino Wistar rats (150–250 g). Statistical analysis using the t-test was employed to assess the effectiveness of caffeine adsorption in lowering blood glucose levels. Activated carbon reduced caffeine content by 23.71%, as confirmed by FTIR spectra showing hydrogen bonding interactions between the –OH groups of activated carbon and the C=O or N atoms of caffeine. Rats receiving the caffeine-reduced green coffee extract reached normal blood glucose levels faster than untreated diabetic rats. The effectiveness of caffeine adsorption was supported by the t-test results, which showed a significant reduction in mean blood glucose levels compared with the untreated diabetic group ($p = 0.002$) and from baseline values ($p = 0.004$). Although the treated diabetic group still differed significantly from the normal control ($p = 0.021$), the glucose level approached the normal range, indicating substantial recovery toward normoglycemia.

1. INTRODUCTION

Diabetes mellitus (DM) is a disease that is common among adults today. Based on data from the International Diabetes Federation (IDF), the number of people with diabetes in 2019 worldwide was 463 million [1] and is estimated to reach 592 million by 2035 [2]. This disease is very difficult to control because it is influenced by factors such as food intake, activity, stress, and medication [3]. Poorly controlled diabetes can lead to severe complications, including retinopathy, nephropathy, cardiovascular disease, and even death [4]. Moreover, the high cost of long-term treatment remains a major burden for patients and healthcare systems [5]. Diabetes is characterized by impaired pancreatic function, making it unable to synthesize insulin, which plays a role in lowering blood glucose [6]. These challenges highlight the urgent need for the development of alternative, affordable, and effective antidiabetic agents.

Green coffee beans (*Coffea canephora*) contain two main compounds, namely caffeine and chlorogenic acid [7]. The chemical structures of these two compounds are shown in Figure 1. Chlorogenic acid is considered capable of preventing insulin resistance [8]. Therefore, the consumption of chlorogenic acid is expected to lower blood glucose levels more quickly because of increased insulin sensitivity. However, caffeine causes a decrease in insulin sensitivity, which results in low glucose tolerance [9]. Although caffeine provides temporary stimulatory effects—such as increased alertness and improved mental performance—in healthy individuals, these effects are short-lived and do not contribute to long-term glucose regulation. This physiological contrast between chlorogenic acid (hypoglycemic) and caffeine (hyperglycemic effect) highlights the antagonistic interaction within green coffee that may limit its hypoglycemic potential [10]. Therefore, the separation of caffeine and chlorogenic acid in coffee solutions needs to be carried out to increase the effectiveness of lowering blood glucose levels.



a b

Figure 1. Compounds in coffee : (a) caffeine [11]; (b) chlorogenic acid [12]

The use of green coffee is based on the instability of chlorogenic acid during the roasting process to obtain roasted coffee. Coffee roasting is carried out at temperatures of 155–165°C (light roasting), 175–185°C (medium roasting), and 205–215°C (dark roasting) [13]. The increase in roasting temperature is proportional to the decrease in chlorogenic acid content in coffee beans [13].

The coffee extraction process aims to dissolve the compounds in the coffee beans. Caffeine has a solubility of 20 g/L in water at room temperature [14]. Meanwhile, chlorogenic acid has a solubility of 20 g/L in water at room temperature [15]. The coffee extraction process using water allows both of these compounds to dissolve in the solvent. Therefore, coffee extracts need to be separated so that chlorogenic acid with high purity can be obtained.

Activated carbon derived from coconut shell contains functional groups such as hydroxyl (–OH), carboxyl (–COOH), amine (–NH), ester (–C=O), aldehyde (–C=O–H), and alkane (–CH) groups [15]. These groups participate in non-covalent interactions during adsorption, primarily through hydrogen bonding [16,17]. Furthermore, SEM analysis confirmed that the activated carbon exhibits a well-developed porous morphology, which enhances its effectiveness as an adsorbent [18].

Previous studies have mainly focused on the relationship between coffee consumption and blood glucose levels, but few have attempted to isolate the role of chlorogenic acid by selectively removing caffeine from green coffee extracts. Various decaffeination methods—such as solvent extraction, supercritical CO_2, and adsorption—have been explored [19,20], yet maintaining chlorogenic acid content during caffeine removal remains a major challenge [21]. Thus, a research gap exists regarding how selective adsorption using activated carbon can effectively remove caffeine without significantly reducing chlorogenic acid levels, and how this chemical modification influences the overall hypoglycemic activity of the extract.

Accordingly, this study aims to obtain a decaffeinated green coffee extract through adsorption using activated carbon (AC) and to evaluate its hypoglycemic activity in alloxan-induced diabetic rats. This study aims to answer the research question: *Does the removal of caffeine from green coffee extract through adsorption using activated carbon enhance its ability to reduce blood glucose levels in diabetic rats?* The novelty of this research lies in the selective optimization of green coffee's hypoglycemic potential through a combination of adsorption and in vivo biological testing on alloxan-induced diabetic rats. The findings are expected to provide a scientific basis for developing low-caffeine functional coffee products that are both safe and effective as supportive therapy for blood glucose regulation.

2. Materials and Methods

2.1 Materials

This study aimed to obtain a decaffeinated green coffee extract through adsorption using activated carbon (AC) and to evaluate its hypoglycemic activity in diabetic rats. The materials used included green and roasted Aceh Gayo Fullwash coffee beans obtained from a certified coffee producer in Central Aceh, Indonesia. Activated carbon (coconut-shell based, food-grade, particle size 100–200 mesh) was purchased from CV Indococo Pasific, Indonesia, with halal certification number LPPOM MUI 15170044730220. Other materials included distilled water, analytical-grade ethanol, and alloxan monohydrate (Sigma-Aldrich, USA) for diabetes induction.

2.2 Ethics Approval

This study was conducted after obtaining ethical approval from the Health Research Ethics Committee of Dr. Moewardi General Hospital, Surakarta, Indonesia, under approval number 498/IV/HREC/2021.

2.3 Caffeine Adsorption and Extraction Procedure

The extraction and adsorption process was designed to selectively remove caffeine from the green coffee extract while preserving chlorogenic acid. A total of 4 g of green coffee powder was extracted using 100 mL of distilled water at 60–70 °C for 15 minutes with continuous stirring at 150 rpm. The temperature range was selected based on the optimal solubility of caffeine and chlorogenic acid in water at moderate conditions [22]. After filtration, the resulting extract (100 mL) was mixed with 4 g of activated carbon and stirred at 300 rpm for 1 hour at a constant temperature of 60–70 °C. The ratio of 4 g AC per 100 mL extract (4% w/v) was chosen based on prior adsorption studies [23,24], where similar ratios achieved effective caffeine removal while minimizing chlorogenic acid loss. This mass-to-volume ratio ensured sufficient surface area for adsorption while preventing oversaturation of the adsorbent pores. The mixture was then filtered through Whatman No. 1 filter paper, and the filtrate (decaffeinated extract) was stored at 4 °C before further analysis. To ensure replicability, all adsorption experiments were performed in triplicate (n = 3) under identical conditions. The resulting filtrates from each replicate were analyzed independently by HPLC to quantify caffeine and chlorogenic acid levels.

2.4 FTIR and HPLC Analyses

FTIR analysis (Shimadzu IRTracer-100, Japan) was conducted on activated carbon before and after adsorption to identify changes in functional groups indicating caffeine binding. Spectra were recorded in the range 4000–400 cm^{-1} using a resolution of 4 cm^{-1} and 32 scans per sample. HPLC analysis (Shimadzu LC-20AT, Japan) was used to determine caffeine and chlorogenic acid concentrations in the extracts before and after adsorption. Separation was performed using a C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase of methanol : water : acetic acid (30 : 69 : 1, v/v/v) at a flow rate of 1 mL/min, detection wavelength at 272 nm, and injection volume 20 µL. Calibration curves were prepared using analytical standards of caffeine and chlorogenic acid (Sigma-Aldrich, USA).

2.5 Blood Glucose Reduction Test in Rats

Male albino Wistar rats (150–250 g) were fasted for 16 hours before diabetes induction using alloxan monohydrate (150 mg/kg body weight, intraperitoneally). Diabetes was confirmed when fasting blood glucose levels exceeded 200 mg/dL.

The rats were divided into four groups (n = 7 per group):

- Group A: Normal control (non-induced)
- Group B: Diabetic control (no treatment)
- Group C: Diabetic rats treated with non-decaffeinated green coffee extract
- Group D: Diabetic rats treated with decaffeinated green coffee extract (after AC adsorption)

Each treatment was administered orally (1 mL/day) for 7 days, and blood glucose levels were measured daily using a GlucoDr auto glucose analyzer (All Medicus Co., Ltd., Korea).

2.6 Statistical Analysis

Data were analyzed using Minitab 18 software (Minitab Inc., USA). Normality of data was tested using the Shapiro–Wilk test. Homogeneity of variances was verified with Levene's test. Differences among groups were assessed using independent-sample t-tests for pairwise comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Result and Discussion

3.1 Adsorption by Activated Carbon

The extraction of coffee aims to dissolve the compounds contained in the coffee beans, one of which is caffeine. Adsorption using activated carbon was chosen due to the presence of pores and functional groups that facilitate the adsorption process of caffeine [23]. Based on Table 1, the caffeine content decreased by 23.71%. Meanwhile, chlorogenic acid content before and after adsorption showed an insignificant decrease of 0.7%. This result is supported by the caffeine adsorption selectivity value of 92%, indicating that activated carbon tends to adsorb caffeine rather than chlorogenic acid. Chlorogenic acid has a more complex molecular structure than caffeine. Caffeine has a relatively simple structure with a fused pyrimidine–imidazole ring system and methyl groups, which allows it to adopt a relatively flat conformation [25]. On the other hand, chlorogenic acid is a larger molecule with

a non-planar conformation. This molecule consists of caffeic acid and quinic acid, with several hydroxyl (–OH) groups on both the aromatic and cyclic structures. The presence of hydroxyl groups and the larger quinic acid ring make chlorogenic acid more sterically hindered than caffeine [26]. Greater steric hindrance reduces the likelihood of adsorption into the pores of the adsorbent [27].

Table 1. Caffeine and chlorogenic acid content before and after adsorption

Compound	Before Adsorption (ppm)	After Adsorption (ppm)	Percentage Reduction (%)	Caffeine Adsorption Selectivity (%)
Caffeine	214.46 ± 4.28	163.61 ± 3.97	23.71 ± 1.12	92.32 ± 0.85
Chlorogenic Acid	600.54 ± 7.36	596.31 ± 6.89	0.70 ± 0.15	

FTIR analysis was used to examine the interactions occurring between activated carbon and caffeine after the decaffeination process in roasted and green coffee extracts. FTIR analysis was performed on activated carbon before (a) and after adsorption (b). Figure 2 shows several FTIR absorption peaks that indicate the chemical bonds present in activated carbon. The absorption at 3440 cm^{-1} indicates O–H stretching vibration [28,29]. The absorption at 1589 cm^{-1} indicates C=C stretching vibration in aromatic rings [29]. The absorption at 1440 cm^{-1} corresponds to C–H bending vibration [30]. The absorptions at 1027 cm^{-1} and 871 cm^{-1} indicate C–O stretching and C–H bending vibrations in aromatic rings, respectively [29]. Based on FTIR analysis, activated carbon is composed of aromatic ring structures with hydroxyl (–OH) and carboxylate (–COOH) groups around them. This is consistent with the structural description of activated carbon proposed by Kurzweil (2009), which consists of polyaromatic compounds with surrounding –OH groups [31].

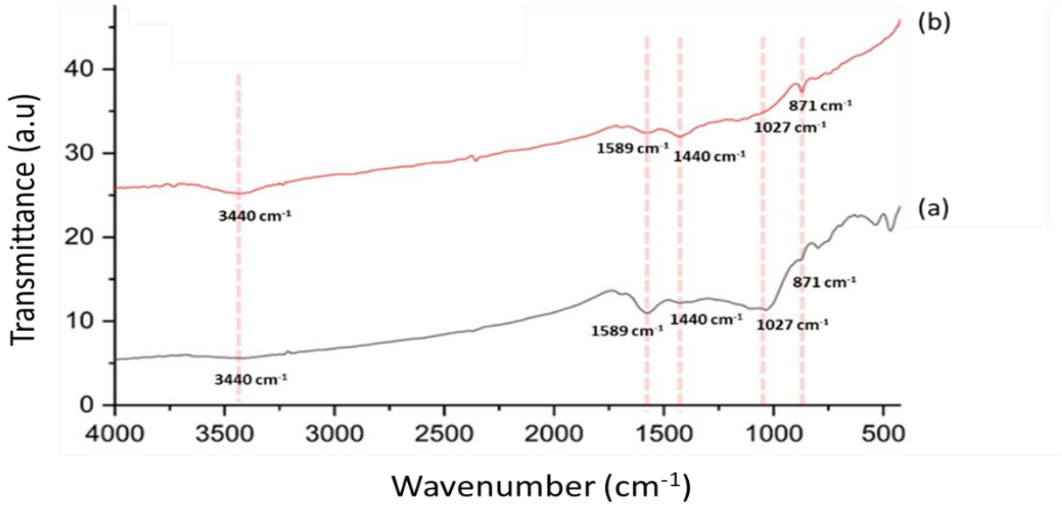


Figure 2. FTIR analysis: (a) before adsorption and (b) after adsorption

The absorption peaks in activated carbon before the decaffeination process tend to be sharper than after decaffeination. This result indicates that an interaction occurred between activated carbon and caffeine. The interaction is non-covalent in nature, as evidenced by the absence of new absorption peaks in the IR spectra. The possible interaction is hydrogen bonding between the –OH groups in activated carbon and the C=O group or nitrogen atoms in caffeine [23]. A similar phenomenon was observed in the adsorption of lead by activated carbon conducted by Moyo et al. (2014), where non-covalent interactions caused a reduction in the FTIR absorption peaks of activated carbon [28]. Activated carbon has also been used in the adsorption of caffeine, ibuprofen, and triclosan by Kaur et al. (2017). Their results showed non-covalent interactions between caffeine and each of these three compounds, resulting in no new absorption peaks and a decrease in the FTIR absorption of each functional group in activated carbon [24].

3.2 Blood Glucose Reduction Test

Figure 3 shows the rat groups exhibited an increase in blood glucose levels after alloxan induction on day 1 (Groups B, C, and D). Meanwhile, the group without alloxan induction (Group A) maintained stable blood glucose

levels over the 7-day observation period. Alloxan selectively damages pancreatic β-cells, which are responsible for insulin production [32]. As a result, blood glucose levels increase when insulin function becomes impaired.

The results showed that a significant reduction in blood glucose occurred after 7 days. The most substantial decrease was observed in the group treated with green coffee extract in which caffeine had been removed using activated carbon (Group D). According to the literature, caffeine negatively affects glucose tolerance by reducing insulin sensitivity[9]. Therefore, reducing caffeine content in coffee extract enhances the ability of chlorogenic acid to lower blood glucose levels.

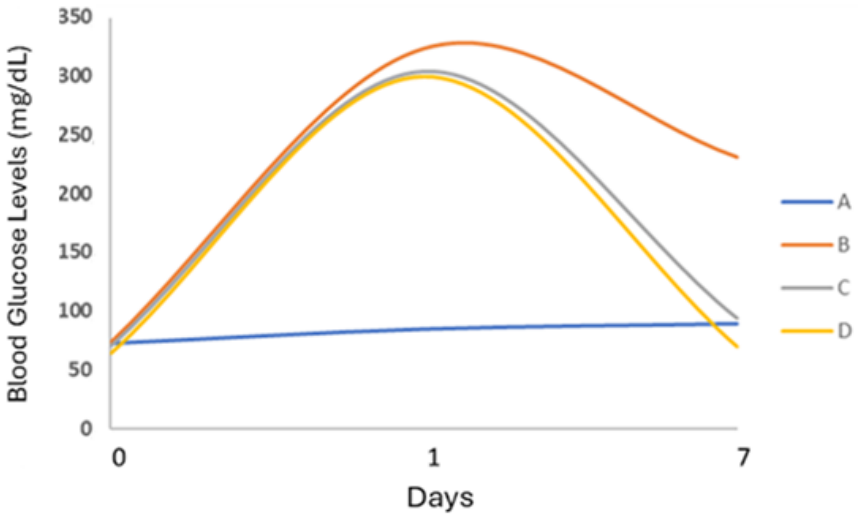
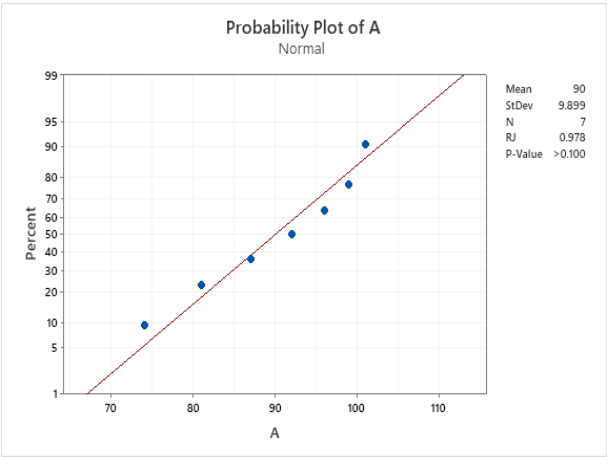


Figure 3. Blood glucose reduction test results: (A) normal control rats; (B) diabetic control rats without treatment; (C) diabetic rats treated with green coffee extract without caffeine adsorption; (D) diabetic rats treated with green coffee extract with caffeine adsorption.

3.3 Statistical Analysis

3.3.1 Normality

The normality test was performed to determine whether the data followed a normal distribution. A normal distribution is represented by a bell-shaped curve that is symmetrical, tapers at the edges, and peaks in the middle [33,34]. The Shapiro–Wilk test was used with Minitab 18 software because the sample size was fewer than 50 [35,36]. Based on the curves in Figure 4, the data distribution was linear, and the *P*-values (>0.1) were greater than the significance level. These results indicate that all data were normally distributed.



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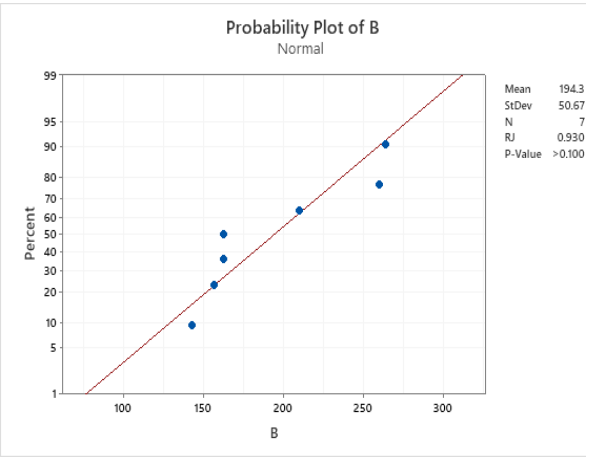

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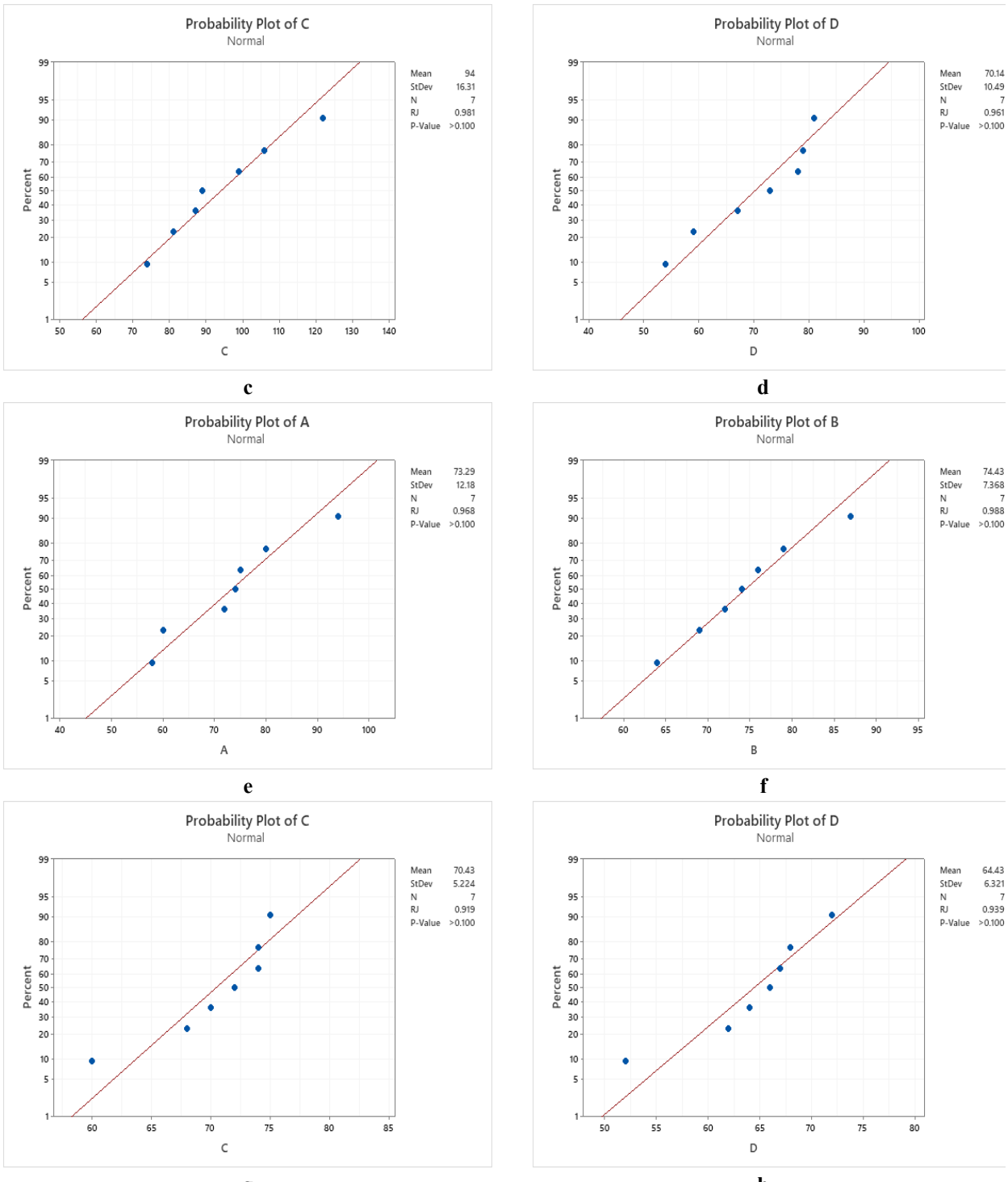

Figure 4. Normality test results: (a) Group A on day 7; (b) Group B on day 7; (c) Group C on day 7; (d) Group D on day 7; (e) Group A on day 0; (f) Group B on day 0; (g) Group C on day 0; (h) Group D on day 0.

3.3.2 Homogenity

A homogeneity test was performed to assess whether the obtained data were derived from the same population (i.e., homogeneous) [37,38]. As shown in Table 2, the P-value obtained from Levene's test was 0.439, which exceeded the predetermined significance level of 0.05. These results indicate that the dataset is homogeneous, suggesting that all observations originate from the same population.

Table 2. Homogeneity test results for Groups C and D on day 7

Groups	N	Significance (α)	Lavene test (P-value)
C on day 7	7	0.05	0.439
D on day 7	7		

3.3.1 T-test

Independent-samples t-tests were performed using Minitab 18 after verifying assumptions of normality and variance homogeneity [39–41]. There is a statistically significant difference between the normal control and Group C (Table 3), indicating Group C did not return to normal levels by Day 7. Meanwhile, no significant difference was observed between Group A and Group D (Table 4), indicating Group D reached values comparable to normal rats by Day 7. Thus, caffeine adsorption in the coffee solution appears to lower blood glucose more effectively.

Comparisons between Group B (diabetic, untreated control) and each of the experimental groups (Groups C and D) revealed significant differences in mean blood glucose in both cases (Tables 5 and 6; $p < 0.05$). The findings show that both coffee solution treatments reduced blood glucose levels, with the adsorption-treated group (Group D) achieving near-normal glucose values similar to those observed in Group A (non-induced rats).

Table 3. T-test between Group A and C on Day 7

Groups	N	Significance (α)	P-value
A	7	0.05	0.021
C on day 7	7		

Table 4. T-test between Group A and D on Day 7

Groups	N	Significance (α)	P-value
A	7	0.05	0.615
D on day 7	7		

Table 5. T-test between Group B and C on Day 7

Groups	N	Significance (α)	P-value
B	7	0.05	0.002
C on day 7	7		

Table 6. T-test between Group B and D on Day 7

Groups	N	Significance (α)	P-value
B	7	0.05	0.001
D on day 7	7		

Table 7 shows a p-value below the significance level ($\alpha = 0.05$), indicating a statistically significant difference in mean blood glucose between Day 0 and Day 7. This finding suggests that the reduction in blood glucose in Group C by Day 7 was not sufficient to restore levels to baseline (Pre-alloxan induction). Table 8 indicates a p-value above the significance level ($\alpha = 0.05$), showing no significant difference in mean blood glucose between Day 0 and Day 7. This suggests that Group D's blood glucose levels by Day 7 had returned to baseline values. The results show that Group D (caffeine treatment with activated carbon) brought blood glucose back to levels seen in Group C day 0, while Group C (without adsorption) did not. This suggests that the adsorption step was more effective.

Table 7. T-test of Group C on day 0 dan 7

Groups	N	Significance (α)	P-value
C on day 0	7	0.05	0.004
C on day 7	7		

Table 8. T-test of Group D on day 0 dan 7

Groups	N	Significance (α)	P-value
D on day 0	7	0.05	0.219
D on day 7	7		

The statistical analysis supports that caffeine adsorption using activated carbon improves the hypoglycemic efficacy of green coffee extract: treatments (both non-decaffeinated and decaffeinated) significantly reduced blood glucose compared to untreated diabetic controls ($p = 0.002$ and $p = 0.001$, respectively). Importantly, only the decaffeinated extract produced blood glucose values on Day 7 that were not significantly different from the normal control ($p = 0.615$), whereas the non-decaffeinated treatment remained significantly different from normal ($p = 0.021$). Within-group comparisons confirm that Group D returned to baseline ($p = 0.219$), while Group C did not ($p = 0.004$). These corrected interpretations indicate that selective caffeine removal enhanced the extract's ability to restore near-normal glycemia.

3.4 Comparison Study

The results of this study demonstrated that adsorption using activated carbon effectively reduced caffeine levels while maintaining a significant proportion of chlorogenic acid in the green coffee extract. This finding aligns with the results of Shiono et al. (2017) and Muchtadiri et al. (2021), who reported that adsorption-based decaffeination can selectively remove caffeine depending on the adsorbent's pore size and surface chemistry. The use of activated carbon with high surface area and polar functional groups facilitated the adsorption of caffeine molecules through π–π interactions and hydrogen bonding [21,42].

Compared to solvent extraction and supercritical CO_2 methods, the adsorption approach used in this study offers several advantages: lower operational cost, minimal solvent residue, and better preservation of thermolabile compounds such as chlorogenic acid. This supports findings by Bouhzam et al. (2023), who observed reduced chlorogenic acid degradation when milder extraction conditions were employed [43].

In terms of biological activity, the decaffeinated extract showed a greater reduction in blood glucose levels compared with the non-decaffeinated extract, confirming that caffeine can interfere with the hypoglycemic effects of chlorogenic acid. This observation is consistent with Alshawi (2020), who reported that caffeine acutely decreases insulin sensitivity and glucose tolerance in both healthy and diabetic models [9].

Interestingly, the influence of caffeine on insulin sensitivity may vary depending on the type of coffee and processing method. For instance, Arabica coffee generally contains lower caffeine but higher chlorogenic acid levels than Robusta, which could explain why different coffee species exhibit distinct metabolic effects [44]. Furthermore, roasting can degrade chlorogenic acid and increase other bioactive compounds such as melanoidins, which may alter the glycemic response. Thus, the present findings emphasize that optimizing decaffeination processes and coffee bean selection are critical to maximizing coffee's hypoglycemic potential.

Overall, this study provides strong evidence that selective caffeine removal through activated carbon adsorption can enhance the antidiabetic efficacy of green coffee extract. The results bridge the existing gap between chemical composition modification and biological activity, highlighting the potential of adsorptive decaffeination as a scalable and environmentally friendly approach for functional food development.

3.5 Mechanistic Link between Caffeine Removal and Glucose Regulation

The improved hypoglycemic activity observed in the decaffeinated extract group suggests that removal of caffeine plays a crucial role in enhancing the glucose-lowering effect of green coffee extract. Caffeine is known to transiently increase plasma glucose by stimulating catecholamine release and enhancing hepatic glycogenolysis and gluconeogenesis, leading to a short-term rise in blood glucose and insulin resistance [9]. Therefore, the presence of caffeine in the non-decaffeinated extract may counteract the beneficial action of chlorogenic acid, a compound that enhances insulin sensitivity and glucose uptake through modulation of glucose-6-phosphatase and AMP-activated protein kinase (AMPK) activity [45].

By selectively removing caffeine through activated carbon adsorption, the antagonistic metabolic effect between caffeine and chlorogenic acid is minimized. This allows chlorogenic acid and related polyphenols to exert their full hypoglycemic potential without interference. Furthermore, caffeine adsorption was achieved under mild thermal and pH conditions, preventing degradation of chlorogenic acid, as confirmed by the minimal change (0.70%) in its concentration after adsorption. The optimized decaffeination process therefore not only improved

extract purity but also enhanced its functional efficacy in vivo, as evidenced by blood glucose levels in the decaffeinated group that were statistically comparable to the normal control ($p = 0.615$).

These findings demonstrate a synergistic relationship between selective chemical processing and biological response: by reducing caffeine content while preserving chlorogenic acid, the extract's pharmacological consistency improves. Consequently, the decaffeination step using activated carbon can be considered a critical optimization stage in developing functional green coffee formulations for diabetes management.

4. CONCLUSION

The present study demonstrates that selective caffeine removal from green coffee extract using activated carbon effectively enhances its hypoglycemic potential. Activated carbon proved to be an effective adsorbent for removing caffeine from coffee solutions, achieving a reduction of 23.71%. FTIR analysis confirmed the formation of hydrogen bonds between the –OH groups of activated carbon and the C=O or N functional groups in caffeine, qualitatively validating successful adsorption.By reducing caffeine content while maintaining chlorogenic acid levels, the decaffeinated extract showed greater efficacy in lowering blood glucose in diabetic rats. This improvement is attributed to the removal of caffeine's antagonistic effect on glucose metabolism—caffeine stimulates catecholamine release and transiently increases blood glucose—thereby allowing chlorogenic acid to exert its full biological activity in enhancing insulin sensitivity and glucose uptake.

These findings highlight that optimizing the decaffeination process can improve the functional properties of green coffee extract without compromising its bioactive components. The study provides important insights for developing functional food formulations or complementary therapies aimed at diabetes management. Further investigations focusing on dose optimization and long-term metabolic effects are recommended to strengthen the practical application of this decaffeinated extract.

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