JPPIPA 12(6) (2026)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

The Effect of Arabica Coffee Extract (*Coffea arabica* L.) on the Expression of Inflammatory Biomarkers and Histopathological Features of the Liver-Pancreas in Alloxan-Induced Diabetic Mice

Suyarta Efrida Pakpahan[1*], Putri Miftahur Rizki[2], Putri Najwa Az-Zahra[2], Rendi Ramdhani[2]

[1] Faculty of Health, Rajawali Health Institute, Bandung, 40184, West Java, Indonesia
[2] Diploma III Medical Laboratory, Rajawali Health Institute Bandung, 40184, West Java, Indonesia

Received: March 25, 2026
Revised: June 06, 2026
Accepted: June 29, 2026
Published: June 30, 2026

Corresponding Author:
Suyarta Efrida Pakpahan
suyartapakpahan@gmail.com

DOI: 10.29303/jppipa.v12i6.15586

Abstract: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, oxidative stress, inflammation, and organ damage, particularly in the liver and pancreas. This study aimed to evaluate the antidiabetic and hepatoprotective effects of Arabica coffee extract (Coffea arabica L.) in alloxan-induced diabetic mice. Thirty male mice were divided into five groups consisting of a negative control, a positive control treated with glibenclamide, and three treatment groups receiving Arabica coffee extract at concentrations of 50%, 75%, and 100% for 21 days. Chlorogenic acid content was analyzed using High-Performance Liquid Chromatography (HPLC), while blood glucose levels and liver histopathology were assessed after treatment. The results showed that chlorogenic acid content reached 20.8% in the tubruk extract and 16.9% in the macerated extract. Administration of Arabica coffee extract significantly reduced blood glucose levels, with the highest reduction observed in the 100% extract group (65% on day 14 and 62% on day 21). Histopathological examination revealed improvement in liver tissue architecture, characterized by reduced parenchymatous degeneration, hydropic degeneration, and necrosis compared with the negative control group. These findings suggest that Arabica coffee extract possesses antihyperglycemic and hepatoprotective activities and may serve as a promising natural therapeutic agent for diabetes mellitus management.

Keywords: Alloxan-induced mice; Arabica coffee extract; Chlorogenic acid; Diabetes mellitus; Liver histopathology

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease that is a global health problem, with its prevalence increasing annually. This disease is characterized by hyperglycemia due to impaired insulin secretion, insulin action, or a combination of both. According to the International Diabetes Federation (IDF), the number of diabetes sufferers worldwide is estimated to exceed 500 million and continues to increase, including in Indonesia, which has one of the highest prevalence rates in Southeast Asia. Uncontrolled chronic hyperglycemia can lead to various microvascular and macrovascular complications, which impact quality of life and increase morbidity and mortality (Genitsaridi et al., 2025).

Prolonged hyperglycemia not only disrupts glucose metabolism but also triggers increased production of reactive oxygen species (ROS) and oxidative stress (Volpe et al., 2018). ROS accumulation plays a role in the activation of inflammatory pathways, characterized by increased expression of various inflammatory biomarkers such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), nuclear factor-kappa

How to Cite:
Pakpahan, S. E., Rizki, P. M., Az-Zahra, P. N., & Ramdhani, R. (2026). The Effect of Arabica Coffee Extract (Coffea arabica L.) on the Expression of Inflammatory Biomarkers and Histopathological Features of the Liver-Pancreas in Alloxan-Induced Diabetic Mice. *Jurnal Penelitian Pendidikan IPA*, *12*(6), 544–553. https://doi.org/10.29303/jppipa.v12i6.15586

B (NF-κB), and C-reactive protein (CRP) (Burgos-mor et al., 2019; Yaribeygi et al., 2020). The activation of these inflammatory mediators contributes to tissue damage and organ dysfunction, particularly in the liver and pancreas, which are crucial for regulating glucose metabolism (Fadillah et al., 2024; Wijayanti et al., 2026).

The pancreas is the primary organ responsible for insulin synthesis and secretion through β cells in the islets of Langerhans (Rorsman & Ashcroft, 2018). In diabetes, oxidative stress and chronic inflammation damage pancreatic β cells, resulting in decreased insulin production and worsening hyperglycemia (Donath & Shoelson, 2011; Yaribeygi et al., 2020). In addition to the pancreas, the liver is also a primary target for damage caused by diabetes. The liver plays a crucial role in carbohydrate, lipid, and protein metabolism (Petersen et al., 2018). Prolonged exposure to hyperglycemia can cause histopathological changes in the liver, including parenchymatous degeneration, hydropic degeneration, inflammatory cell infiltration, and even hepatocyte necrosis (Mohamed et al., 2016; Yaribeygi et al., 2020). Damage to both organs is often closely related to the increase in inflammatory mediators and oxidative stress that occurs during the development of diabetes (Yaribeygi et al., 2020).

A common animal model of diabetes used in research is the alloxan-induced mouse (*Mus musculus*). Alloxan is a diabetogenic compound that selectively damages pancreatic β-cells through the formation of free radicals, resulting in a hyperglycemic state that mimics diabetes in humans (Ighodaro et al., 2018; Lenzen, 2008). In addition to causing pancreatic damage, alloxan induction is also known to cause histopathological changes in the liver and an increased systemic inflammatory response (Etuk, 2010; Rohilla & Ali, 1943). Therefore, this model is widely used to evaluate the effectiveness of antidiabetic compounds and protective agents against diabetes-induced organ damage (Forbes & Cooper, 2013).

Currently available pharmacological therapies for diabetes, such as sulfonylureas and biguanides, have been shown to be effective in controlling blood glucose levels. However, long-term use is often associated with various side effects, prompting the search for alternative, safer, natural-based therapies with broad biological activity (Inzucchi et al., 2015; Kalra et al., 2015). One plant with potential as an antidiabetic agent is Arabica coffee (*Coffea arabica* L.), which contains various bioactive compounds with antioxidant, anti-inflammatory, and glucose-lowering properties (Nguyen et al., 2024; Reis et al., 2018). Arabica coffee contains various bioactive compounds, particularly chlorogenic acid, flavonoids, polyphenols, trigonelline, and caffeine, which have antioxidant and anti-inflammatory activities (Peng et al., 2025; Socała et al., 2021).

Chlorogenic acid is the main phenolic component of Arabica coffee and has been reported to lower blood glucose levels by inhibiting hepatic gluconeogenesis, increasing insulin sensitivity, and protecting pancreatic β-cells (Nguyen et al., 2024). Furthermore, the antioxidant activity of chlorogenic acid plays a role in suppressing reactive oxygen species (ROS) formation, thereby reducing the activation of inflammatory pathways and preventing tissue damage (Huang et al., 2023; Nguyen et al., 2024). Several studies have shown that consuming Arabica coffee or coffee extract can improve metabolic parameters, including insulin resistance, glucose metabolism, and hepatic steatosis, in animal models of diabetes (Shokouh et al., 2019). Although several studies have investigated the metabolic and anti-inflammatory effects of coffee in diabetic animal models, comprehensive studies integrating inflammatory biomarkers with liver and pancreatic histopathological assessments are still scarce (Shokouh et al., 2019; Subagiantara et al., 2024).

This study was conducted to evaluate the effect of Arabica coffee (*Coffea arabica* L.) extract on the expression of inflammatory biomarkers and histopathological features of the liver and pancreas in alloxan-induced diabetic mice. The results are expected to provide scientific information on the potential of Arabica coffee extract as an antidiabetic, anti-inflammatory, and protective agent against organ damage caused by diabetes mellitus, thus providing a basis for the development of natural-based complementary therapies in the future.

Method

Research Sample

The research sample used in this study was 30 male mice, divided into five test groups, each consisting of six male mice. The samples in this study were male mice that met the following requirements:

1. Male mice
2. Body weight 20-30 grams
3. Active/healthy movements
4. Age 1-3 months
5. No diarrhea

Research Tools and Materials

The tools and materials for this research consist of:

Alcohol 70%, 80%,90%, 100%. Mice. Alloxan 0.9% physiological NaCl. HE staining with distilled waterCoffee beans, alcohol swab. Eosin, Xylol Glibenclamide 5 mg. Stirring Rod. 100 ml beaker glass. Blood Gluces Stick. Funnel. Cover Glass. Disposable syringe 1 ml. Erlenmeyer. Evaporator. Safety pin.

Cotton. Watch glass. Measuring cup 10, 100 ml. Glucometer. Hot plate. Experimental animal cage. Filter paper. 100 ml measuring flask. Micropipette. Microscope. Glass object. Oven. Paraffin. Tweezers. Filter. Gloves. Razor blade. Syringe 1cc and 3cc. Electric scale. Blue and yellow tips. Water bath.

Coffee Extraction Using the Tubruk Method

Weigh 10 grams of Arabica coffee powder. The weighed coffee powder is placed in a beaker containing 150 ml of RO water. The solution is transferred to an Erlenmeyer flask and heated for 1 hour using a hot plate. Let it stand for 4 minutes, then filter the extraction results.

Coffee Extraction Using the Maceration Method

306 grams of coffee grounds were macerated with 3 liters of 96% ethanol on a shaker for 9 hours, then allowed to stand for 15 hours. The first filtrate was filtered and macerated twice using 1.5 liters of solvent. The liquid ethanol extract was evaporated using a rotary evaporator at 55°C to produce a thick extract.

Determination of Chlorogenic Acid Content Using the HPLC Method

Weigh 20 grams of coffee extract. Add 75 ml of methanol and 75 ml of distilled water. Heat the solution at boiling point for 1 hour and cool to room temperature. The sample is filtered through a 0.2µm filter cartridge and injected into the HPLC apparatus.

Alloxan Production in Test Animals

The dose of Alloxan used was 130 mg/kg of mouse body weight with 3.6 mg of Alloxan weighed. Put the weighed Alloxan powder into a glass beaker and add 10 ml of 0.9% NaCl then homogenize until dissolved.

Preparation of Glibenclamide Solution

Glibenclamide was weighed at 1.3 mg. The weighed glibenclamide powder was placed into a glass beaker containing 20 ml of distilled water, left for 15 minutes until a mucilage formed, then homogenized using a hot plate magnetic stirrer mixer. After dissolving, transfer it to a 100 ml volumetric flask, then add distilled water little by little until the mark is reached and homogenized.

Conditioning of Diabetic Melittus Mice and Intraperitoneal Injection

Male mice weighing 20-30 grams were purchased and adapted for 1 week. After 1 week of adaptation and 16 hours of fasting, blood glucose levels were measured with a glucometer before Alloxan injection. Alloxan was injected at a dose of 0.1 ml intraperitoneally, then the mice were observed for 7 days. After 3 days of Alloxan injection, their blood glucose levels were rechecked. On the 7th day after Alloxan induction, the mice's blood glucose levels were measured again. Mice are said to have DM if their fasting blood glucose levels exceed 126 mg/dl. Mice are grouped into V sample groups, namely group I (Negative Control, Alloxan induced and given distilled water), group II (Positive Control, Alloxan induced and given 5mg Gliblenclamide), group III, group IV (Alloxan induced and given Arabica coffee bean extract at a dose of 50%), group V (Alloxan induced and given Arabica coffee bean extract at a dose of 75%), group V (Alloxan induced and given Arabica coffee bean extract at a dose of 100%). Treatment was given to mice orally for 21 days. On the 14th and 21st days after treatment, the blood glucose levels of the mice were measured again and 3 mice from each group were euthanized for observation of the histology of the mice's liver.

Liver Histology Examination
Network Block Creation

The liver tissue was then fixed in 10% formalin for 10 hours. The fixed tissue was placed in a stainless steel block and the surface was smoothed. Liquid paraffin was poured into the 1/2-filled stainless steel block mold, then a plastic filter was placed to adhere the tissue to the plastic filter. Liquid paraffin was poured back up to the full height of the stainless steel block and allowed to solidify, allowing the paraffin to easily be removed.

Tissue cutting

The paraffin block containing the tissue is trimmed on a microtome to remove the wax layer covering the slide. Once the slide is clearly visible, the tissue section cut with the microtome is placed in a water bath. The section is then taken with a glass slide. The slide containing the tissue section is briefly placed on a hot plate to remove any remaining paraffin.

Hematoxylin-Eosin Staining

Tissue slides still containing paraffin were deparaffinized by immersing them in xylene for 1 hour. Rehydration was performed by immersing the slides in graded alcohol solutions ranging from high to low concentrations: 90%, 80%, 70%, 60%, 50%, 40%, and 30%, for 5 minutes at each alcohol concentration. After immersion in 30% alcohol, the slides were immersed in distilled water for 10 minutes. The slides were then immersed in hematoxylin for 5 minutes. The slides, which had been immersed in hematoxylin, were rinsed under running water for 5 minutes, then dehydrated by immersing them in alcohol solutions ranging from low to high concentrations: 30%, 40%, 50%, 60%, 70%, 80%, and 90%. The slides were immersed in Orange-G solution for 3 minutes, then dipped in 96% alcohol 8

times. The slides were immersed in eosin-alcohol solution for 3 minutes, then dipped again in 96% alcohol 8 times.

Clearing Process

The slides were dipped into a 3:1 alcohol:xylol solution four times, a 1:1 alcohol:xylol solution four times, and a 1:3 alcohol:xylol solution four times. Xylol 1 was dipped for 5 minutes, Xylol 2 for 5 minutes, and Xylol 3 for 5 minutes. The remaining xylol solution was then covered with a cover glass using Entelan.

Preparation Observation

Liver tissue preparations were observed under a microscope at 40x magnification.

Result and Discussion

Arabica Coffee Extract

Arabica coffee is extracted using two methods: the tubruk method and the maceration method. The results of this Arabica coffee extraction are as follows table 1. Based on Table 1, the results of the coffee extraction using the tuburuk method produced 136 ml of liquid extract, while the maceration method produced 26 g of thick extract.

Table 1. Results of Arabica Coffee Extract

Method	Sample Weight (g)	Solvent	Extract Results
Crash	10	RO water	Liquid Extract
Maceration	306	Ethanol	Condensed Extract

Analysis of Chlorogenic Acid Content Using High Performance Liquid Chromatography (HPLC)

Coffee contains biochemical compounds including sucrose, trigonelline, caffeine, and chlorogenic acid. Chlorogenic acid in coffee acts as an antihyperglycemic agent by increasing insulin secretion in pancreatic beta cells. Chlorogenic acid levels can be identified using HPLC. Chlorogenic acid levels were analyzed using High Performance Liquid Chromatography (HPLC). Coffee grounds extracted using the maceration method yielded a chlorogenic acid content of 16.99%. In a study (Khairunnisa et al., 2022), chlorogenic acid levels in Arabica coffee beans were found to be 4.0-8.4%. In this study, the chlorogenic acid content using the maceration method was higher than that in the study (Khairunnisa et al., 2022) because the coffee beans used were unroasted.

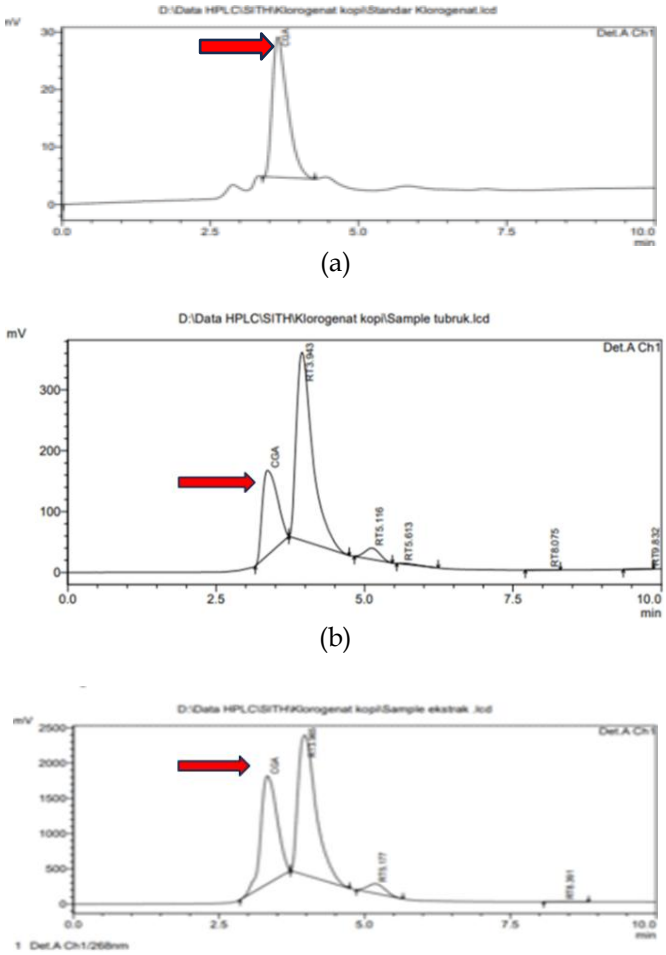

Figure 1. Chlorogenic Acid Chromatogram

Information:

a. Standard Chromatogram of Chlorogenic Acid
b. Chromatogram of Chlorogenic Acid
c. Macerated Chlorogenic Acid Chromatogram

Perrone et al., (2012) reported that chlorogenic acids are degraded during coffee roasting and become incorporated into melanoidins, whereas (Coelho et al., 2014) demonstrated that roasting progressively decreases free chlorogenic acid content through degradation and transformation reactions. In addition to using the maceration method, researchers also used the tubruk method, which was analyzed using HPLC, resulting in a higher chlorogenic acid content of 20.8%. The tubruk method is simpler in making extracts, the costs incurred for making the extract are very economical and the time used to make the extract is faster. This is the reason researchers used the tubruk method in making Arabica coffee extract, which will then be given to mice orally as a treatment to reduce blood sugar levels.

Table 2. Results of Chlorogenic Acid Chromatogram

Extraction	Name	Ret Time	Area	Height
Standard	CGA	3.635	438356	24430
Contact	CGA	3.362	24311551	139641
Maceration	CGA	3.325	29799993	1534568

The area produced in the standard chromatogram of chlorogenic acid shows that chlorogenic acid is at 3,635 minutes, this chromatogram is a benchmark for analyzing chlorogenic acid qualitatively and quantitatively. Based on the retention time obtained for Arabica coffee tubruk chromatogram of 3,362 minutes and the retention time obtained for maceration chromatogram of 3,325 minutes, it can be concluded that the compound is chlorogenic acid because its retention time is the same as the chlorogenic acid standard. In the analysis of chlorogenic acid levels, it can be calculated using one standard concentration with the following Formula 1.

$$\frac{Sample\ area}{Standard\ area\ size} x\ Sample\ concentration \quad (1)$$

This equation shows that the chlorogenic acid content in the Arabica coffee sample is 20.8% while the macerated sample is 16.9%.

Measurement of Blood Sugar Levels Before and After Treatment

The results of this study demonstrated that Arabica coffee extract was able to reduce blood glucose levels in alloxan-induced diabetic mice. The greatest reduction was observed in the group treated with 100% Arabica coffee extract, which showed a decrease of 65% on day 14 and 62% on day 21. Meanwhile, the positive control group treated with glibenclamide exhibited a decrease of 43% on day 14 and 49% on day 21. These findings indicate that Arabica coffee extract possesses antihyperglycemic activity and that the effect tends to increase with higher extract concentrations.

Table 3. Data on Blood Sugar Levels on Day 14

Group	Mice	Before Alloxan Injection	After Alloxan Injection		After Extract Treatment	Percentage of
			H-3	H-7	H-14	Decreased
	1	124	115	209	256	18%
KN	2	119	147	201	146	27%
	3	116	241	144	272	47%
Average		120	168	184	224	27%
	1	96	131	234	78	66%
KP	2	111	142	160	101	37%
	3	111	129	163	122	25%
Average		106	134	185	100	43%
	1	100	149	219	99	54%
50%	2	128	143	156	93	40%
	3	96	129	211	96	50 %
Average		108	140	195	96	50%
	1	128	135	235	95	60%
75%	2	102	127	339	147	56%
	3	96	236	250	112	52%
Average		108	166	274	121	56%
	1	112	105	274	74	73%
100%	2	114	262	433	183	58%
	3	103	124	342	128	62,5%
Average		109	163	349	128	65%

Table 4. Blood Sugar Level Data for Day 21

Group	Mencit	Before *Alloxan* Injection	After *Alloxan* Injection		After Extract	Percentage of
			H+3	H+7	Treatment H+21	Decreased
	1	128	125	209	276	24%
KN	2	125	152	208	167	19%
	3	118	241	253	301	15%
Rata-rata		124	173	223	248	19%
	1	98	133	237	71	70%
KP	2	115	144	163	96	41%
	3	120	138	188	102	37%
Rata-rata		111	138	188	90	49%
	1	116	113	182	73	55%
50%	2	122	216	275	104	40%

Group	Mencit	Before *Alloxan* Injection	After *Alloxan* Injection		After Extract Treatment H+21	Percentage of Decreased
			H+3	H+7		
	3	105	163	219	115	55%
	Rata-rata	114	164	225	97	50%
	1	132	137	233	98	60%
75%	2	104	136	313	136	56%
	3	100	216	250	101	52%
	Rata-rata	112	163	262	112	56%
	1	116	113	281	73	74%
100%	2	122	275	334	174	47%
	3	105	163	342	115	66%
	Rata-rata	114	163	319	120	62%

The findings of the present study are consistent with those reported by (Campos-florián et al., 2013), who found that aqueous extracts of *Coffea arabica* significantly reduced fasting blood glucose levels in alloxan-induced diabetic rats after 8 and 15 days of treatment compared with untreated diabetic controls. The authors suggested that bioactive compounds in coffee, including chlorogenic acid and polyphenols, may contribute to the antihyperglycemic effect by improving glucose metabolism and reducing oxidative stress.

Similarly, a study by (Martina et al., 2019) reported that administration of Arabica Gayo coffee bean extract resulted in a significant reduction in blood glucose levels compared with the control group. Although their study was conducted in healthy mice and used different dosages, the results support the notion that Arabica coffee contains bioactive compounds capable of modulating glucose homeostasis.

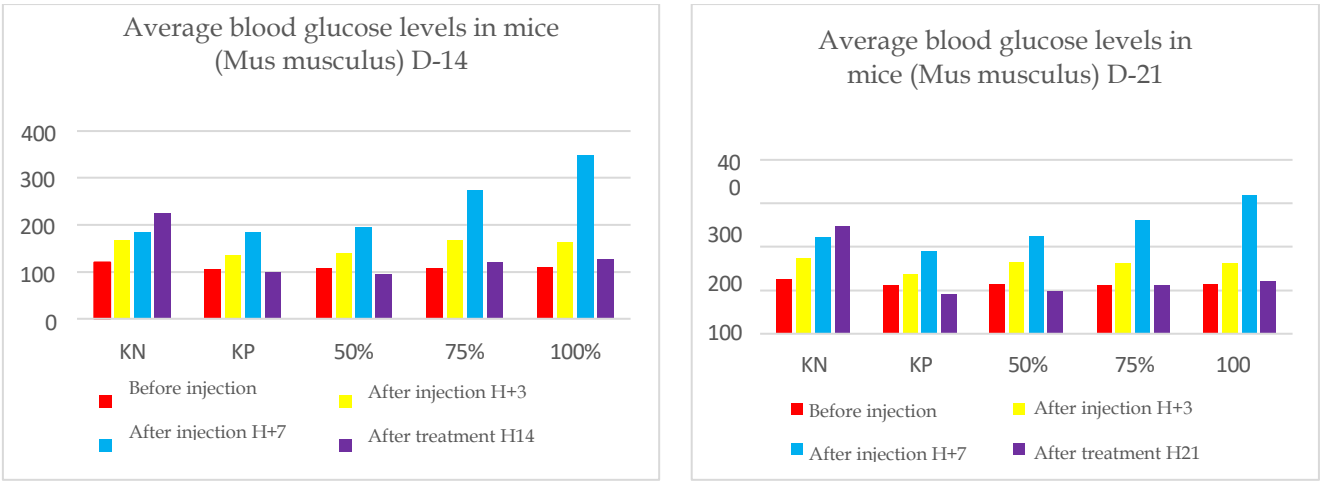

Figure 2. Average Blood Glucose Graph of Mice (*Mus muculus*) D-14 and D-21

The present findings are also in agreement with the systematic review conducted by (Reis et al., 2018), which concluded that coffee consumption may improve glucose metabolism and insulin sensitivity in both healthy and diabetic individuals. According to the review, chlorogenic acid can suppress hepatic glucose production, delay intestinal glucose absorption, and enhance insulin-mediated glucose uptake. These mechanisms may explain the reduction in blood glucose levels observed in the coffee extract-treated groups in the current study. Furthermore, the results are supported by the study of (Mitiku et al., 2022), who reported that *Coffea arabica* extracts inhibited intestinal α-glucosidase activity and significantly reduced postprandial hyperglycemia in experimental animals. This finding suggests that the glucose-lowering effect of Arabica coffee may involve both enhanced insulin action and reduced carbohydrate digestion and absorption.

Although the present study and the study by (Reis et al., 2018) both demonstrated beneficial effects of coffee on glucose homeostasis, there are important methodological differences. Reis et al., (2018) reviewed clinical trials involving human participants, whereas the present study evaluated the effect of Arabica coffee extract in an alloxan-induced diabetic mouse model. In addition, Reis et al. focused primarily on metabolic outcomes such as blood glucose regulation and insulin sensitivity, while the present study provides experimental evidence of the antihyperglycemic potential of Arabica coffee extract under controlled

laboratory conditions. Nevertheless, both studies support the potential use of Arabica coffee as a natural therapeutic agent for diabetes management.

Histological Preparation Observation Results

This study used male mice (*Mus musculus*) as test animals because male mice do not have estrogen hormones, even if they do, they are only in relatively small amounts and the hormonal conditions in males are more stable when compared to female mice, because female mice experience changes in hormonal conditions at certain times such as during the estrus cycle, pregnancy and breastfeeding which can affect the psychological condition of the test animals (Becker et al., 2015; Prendergast et al., 2014). The test animals used were 1-3 months old with a body weight of 20-30 grams, and the body condition of the mice was healthy and active (Guo et al., 2014). The mice used in the study consisted of 30 mice divided into 5 groups, each group consisting of 6 mice.

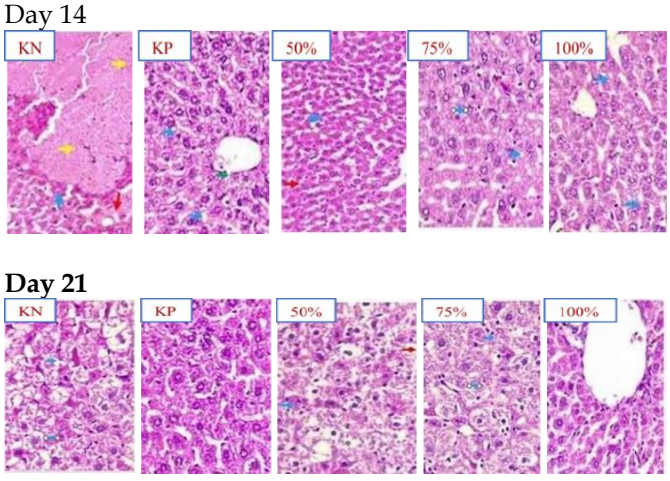

Figure 3. Histological image of the liver of mice induced by Alloxan, glibenclamide, Aquades, and given Arabica coffee extract, then stained using (Hematoxylin Eosin) at 400x magnification.

Group I (negative control) namely mice that were injected with Alloxan intraperitoneally and without treatment were only given distilled water, group II (positive control) namely mice that were injected with Alloxan and given glibenclamide treatment, group III mice were injected with Alloxan and given Arabica coffee extract treatment with a concentration of 50%, group IV were injected with Alloxan and given Arabica coffee extract treatment with a concentration of 75% and group V were injected with Alloxan and given Arabica coffee extract treatment with a concentration of 100% orally.

The results of the study can be seen in Tables 3 and 4. Before injection, all blood sugar levels were within normal limits. After Alloxan injection, blood sugar levels in all treatment groups had reached diabetes, exceeding 126 mg/dl. Alloxan was chosen for this study because it can damage pancreatic beta cells, which then causes hyperglycemia in mice (*Mus musculus*). After all treatment groups had reached diabetes, the KN group (shown in Tables 3 and 4) was not given treatment after Alloxan injection, resulting in no decrease in blood sugar levels. However, in the KN group, mice 2 experienced a decrease 14 days after treatment. This may have been due to the mice being stressed due to the uncomfortable procedure. Furthermore, Alloxan is a highly unstable chemical compound with a hydrophilic, glucose-like molecule.

In the positive control group with Glibenclamide administration, there was a significant increase in the percentage of blood glucose levels, which was better on day 14 (43%) and on day 21 (49%). Glibenclamide is the most commonly used oral antidiabetic drug for the treatment of diabetes mellitus, which has a mechanism of action as an antidiabetic that stimulates pancreatic β cells to release stored insulin, thereby increasing insulin secretion as a result of glucose stimulation (DeFronzo et al., 2013). Glibenclamide was chosen as a comparative control in this study because the price of Glibenclamide is more economical and easily available at the nearest pharmacy.

On day 14 and day 21 of the 50% group, the percentage decrease in blood glucose levels on day 14 was 50% and on day 21 was 50%, then in the 75% group there was a comparable percentage decrease in blood glucose levels, namely on day 14, 56% and day 21, 56% and 100% the percentage decrease in blood glucose levels on day 14, 65% and day 21, 62%. All experienced a decrease in blood glucose, this means that in accordance with cohort studies in the United States, Asia, and Europe have shown that consuming coffee can reduce the risk of type 2 diabetes mellitus (Fuente et al., 2020; Poole et al., 2017). The compound contained in Arabica coffee that plays a major role in reducing blood glucose levels in hyperglycemic mice is chlorogenic acid. The chlorogenic acid content in coffee can stimulate the production of glucagon-like peptide, which is a gastrointestinal hormone and has an effect on the function of pancreatic beta cells, which strengthens glucose-dependent insulin secretion.

The results of histological observations of the liver organs of mice (*Mus musculus*) on days 14 and 21 based on Figure 4.3, the negative control group injected with Alloxan without treatment showed severe damage marked by parenchymal degeneration, hydropic degeneration to the most severe, necrosis or cell death. Parenchymal degeneration is the mildest degree of damage, parenchymal degeneration has a larger cell size than normal cells due to cells experiencing swelling and

a more cloudy and granular cytoplasmic structure after parenchymal degeneration, increasing the degree of damage to the 2nd is hydropic degeneration (swollen cells with a typical picture in the form of small to large vacuoles) the 3rd damage, is necrosis or death of necrotic tissue such as pyknotic (purple to black nuclei with a smaller size due to compaction of the cell nucleus), or karyolysis which has a picture of empty cells without a cell nucleus (Yana & Budijastuti, 2022).

The damage that occurred in the negative control group was due to the administration of Alloxan which can cause increased blood glucose levels, Alloxan works by inhibiting insulin secretion so that pancreatic cells become damaged and form Reactice Oxygen Species (ROS) which can cause liver cells to experience necrosis. The positive control, namely the group induced by Alloxan and given glibenclamide, showed mild liver damage on day 14 and on day 21 showed improvement in liver damage and from mild to normal, this proves that Glibenclamide is able to repair liver cell damage composed of parenchymal degeneration and hydropic degeneration (Altan et al., 1994).

The 50% Arabica coffee extract group showed severe liver damage on day 14 and mild liver damage improvement on day 21, characterized by parenchymal degeneration and hydropic degeneration. The 75% Arabica coffee extract group showed mild liver damage on day 14 and normal liver damage improvement on day 21, demonstrating that coffee extract administration was comparable to glibenclamide. The 100% coffee extract group showed no liver damage on days 14 and 21, characterized by the presence of normal hepatocytes, a perfectly shaped central vein, regular nuclei, and no degeneration.

Conclusion

Based on the research conducted, the following conclusions were drawn:The chlorogenic acid content using the tubruk method was 20.8%, and the maceration method was 16.9%. Blood glucose levels decreased by 50% on day 14 in Group III and 50% on day 21. Group IV experienced a 56% decrease on day 14 and 56% on day 21. Group V experienced a 100% decrease in blood glucose, with a 65% decrease on day 14 and 62% on day 21. On days 14 and 21, the histological features of the livers in Group II (the positive control) and Groups III, IV, and V showed improvement compared to the negative control group. Liver improvement was characterized by only parenchymal degeneration and hydropic lesions.

Acknowledgments

The author would like to thank the Faculty of Health, Rajawali Health Institute, for facilitating the author in completing this article.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

References

Altan, N., Onguna, C., Hasano, E., Engin, A., & Tuncerb, C. (1994). Effect of the sulfonylurea glyburide on superoxide dismutase activity in alloxan-induced diabetic rat hepatocytes. *Diabetes Research and Clinical Practice*, *22*(3), 95–98. https://doi.org/https://doi.org/10.1016/0168-8227(94)90041-8

Becker, J. B., Arnold, A. P., Berkley, K. J., Blaustein, J. D., Eckel, L. A., Hampson, E., Herman, J. P., Marts, S., Sadee, W., Steiner, M., & Taylor, J. (2015). Strategies and Methods for Research on Sex Differences in Brain and Behavior. *Endocrinology*, *146*(4), 1650–1673. https://doi.org/10.1210/en.2004-1142

Burgos-mor, E., Abad-jim, Z., Mart, A., Marañ, D., Iannantuoni, F., Escribano-l, I., Sandra, L., Salom, C., Jover, A., Mora, V., Roldan, I., Sol, E., Rocha, M., & V, M. (2019). Relationship between oxidative stress, ER stress, and inflammation in type 2 diabetes: The battle continues. *Journal of Clinical Medicine*, *8*(9), 1–22. https://doi.org/https://doi.org/10.3390/jcm8091385

Campos-florián, J., Bardales-valdivia, J., Caruajulca-guevara, L., & Cueva-llanos, D. (2013). Anti-diabetic effect of Coffea arabica in alloxan-induced diabetic rats. *Emirates Journal of Food and Agriculture*, *25*(10), 772–777. https://doi.org/10.9755/ejfa.v25i10.16409

Coelho, C., Ribeiro, M., Cruz, A. S. C., M, M. R., Coimbra, M. A., Bunzel, M., & Nunes, F. M. (2014). Nature of Phenolic Compounds in Coffee Melanoidins Nature of Phenolic Compounds in Coffee Melanoidins. *Journal of Agricultural and Food Chemistry*, *62*(31), 7843–7853. https://doi.org/10.1021/jf501510d

DeFronzo, R. A., Goodman, A. M., & Abdul-Ghani, M. (2013). Pathophysiologic approach to therapy in patients with newly diagnosed type 2 diabetes. *Diabetes Care*, *37*(1), S95–S102. https://doi.org/10.2337/dcS13-2011

Donath, M. Y., & Shoelson, S. E. (2011). Type 2 diabetes

as an inflammatory disease. *Nature Publishing Group*, *11*(2), 98–107. https://doi.org/10.1038/nri2925

Etuk, E. U. (2010). Animals models for studying diabetes mellitus. *Agriculture and Biology Journal of North America*, *1*(2), 130–134. https://doi.org/https://doi.org/10.5251/abjna.2010.1.2.130.134

Fadillah, M. F., Rezaldi, F., Fadila, R., Andry, M., Pamungkas, B. T., Mubarok, S., & Susiyanti, S. (2024). Studi Bioteknologi Komputasi (Bioinformatika) Senyawa Vitexin Pada Kombucha Bunga Telang Vitexin Sebagai Antioksidan dan Antikanker. *Jurnal Gizi Kerja Dan Produktivitas*, *5*(1), 60–67. https://doi.org/https://dx.doi.org/10.62870/jgkp.v5i1.24993

Forbes, J. M., & Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiological Reviews*, *104*(1), 1–56. https://doi.org/10.1152/physrev.00045.2011

Fuente, F. P., Nocetti, D., Sacristán, C., Ruiz, P., Guerrero, J., Jorquera, G., Uribe, E., Bucarey, J. L., Espinosa, A., & Puente, L. (2020). Physalis peruviana L . Pulp Prevents Liver Inflammation and Insulin Resistance in Skeletal Muscles of Diet-Induced Obese Mice. *Nutrients*, *12*(1), 1–11. https://doi.org/10.3390/nu12030700

Guo, Z. V, Hires, S. A., Li, N., Connor, D. H. O., Komiyama, T., Ophir, E., Cox, J., & Svoboda, K. (2014). Procedures for Behavioral Experiments in Head-Fixed Mice. *PloS One*, *9*(2), 1–16. https://doi.org/10.1371/journal.pone.0088678

Huang, J., Xie, M., He, L., & Song, X. (2023). Chlorogenic acid: A review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems. *Frontiers in Pharmacology*, *14*(2), 1–10. https://doi.org/10.3389/fphar.2023.1218015

Ighodaro, O. M., Adeosun, A. M., & Akinloye, O. A. (2018). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, *53*(6), 1–10. https://doi.org/10.1016/j.medici.2018.02.001

Inzucchi, S. E., Bergenstal, R. M., Buse, J. B., Diamant, M., Ferrannini, E., Nauck, M., Peters, A. L., & Tsapas, A. (2015). Management of hyperglycemia in type 2 diabetes, 2015: A patient-centered approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*, *38*(1), 140–149. https://doi.org/10.2337/dc14-2441

Kalra, S., Madhu, S. V, & Bajaj, S. (2015). Sulfonylureas: Assets in the past, present and future. *Indian Journal of Endocrinology and Metabolism*, *19*(3), 314–316. https://doi.org/10.4103/2230-8210.149925

Khairunnisa, F., Alhmahdy, A., & Armenia, A. (2022). Pengaruh Ekstrak Biji Kopi Hijau Ro- busta dan Liberika Terhadap Histopatologi Pankreas Pada Mencit Diabe- tes. *Medical Sains: Jurnal Ilmiah Kefarmasian*, *7*(3), 513–522. https://doi.org/https://doi.org/10.37874/ms.v7i3.391

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*(2), 216–226. https://doi.org/10.1007/s00125-007-0886-7

Martina, S. J., Govindan, P. A. P., & Wahyuni, A. S. (2019). The difference in effect of Arabica Coffee Gayo beans and leaf (Coffea arabica Gayo) extract on decreasing blood sugar levels in healthy mice. *Open Access Macedonian Journal of Medical Sciences*, *7*(20), 3363–3365. https://doi.org/https://doi.org/10.3889/oamjms.2019.423

Mitiku, H., Kim, T. Y., Kang, H., Apostolidis, E., Lee, J. Y., & Kwon, Y. I. (2022). Selected coffee (Coffea arabica L .) extracts inhibit intestinal α - glucosidases activities in - vitro and postprandial hyperglycemia in SD Rats. *BMC Complementary Medicine and Therapies*, *22*(2), 1–10. https://doi.org/10.1186/s12906-022-03726-7

Mohamed, J., Nazratun Nafizah, A. H., Zariyantey, A. H., & Budin, S. B. (2016). Mechanisms of diabetes-induced liver damage: The role of oxidative stress and inflammation. *BMC Complementary Medicine and Therapies*, *16*(2), 132–141. https://doi.org/10.18295/squmj.2016.16.02.002

Nguyen, V., Taine, E. G., Meng, D., Cui, T., & Tan, W. (2024). Chlorogenic acid: A systematic review on the biological functions, mechanistic actions, and therapeutic potentials. *Nutrients*, *16*(7), 1–36. https://doi.org/https://doi.org/10.3390/nu16070924

Peng, R., Lan, M., Zhang, Y., & Zhang, S. (2025). Transforming coffee from an empirical beverage to a targeted nutritional intervention : health effects of coffee ' s core functional components on chronic diseases. *Frontiers in Nutrition*, *12*(1), 1–13. https://doi.org/10.3389/fnut.2025.1690881

Perrone, D., Farah, A., & Donangelo, C. M. (2012). Influence of coffee roasting on the incorporation of phenolic compounds into melanoidins and their relationship with antioxidant activity of the brew. *Journal of Agricultural and Food Chemistry*, *60*(17), 4265–4275.

Petersen, M. C., Vatner, D. F., & Shulman, G. I. (2018). Regulation of hepatic glucose metabolism in health and disease. *Nature Reviews Endocrinology*, *13*(10), 572–587. https://doi.org/10.1038/nrendo.2017.80.Regulatio

n

Poole, R., Kennedy, O. J., Roderick, P., Fallowfield, J. A., Hayes, P. C., & Parkes, J. (2017). Coffee consumption and health : umbrella review of meta-analyses of multiple health outcomes. *BMJ*, *1*(1), 1–18. https://doi.org/10.1136/bmj.j5024

Prendergast, B. J., Onishi, K. G., & Zucker, I. (2014). Neuroscience and Biobehavioral Reviews Female mice liberated for inclusion in neuroscience and biomedical research. *Neuroscience and Biobehavioral Reviews*, *40*(1), 1–5. https://doi.org/10.1016/j.neubiorev.2014.01.001

Reis, C. E. G., Dórea, J. G., & da Costa, T. H. M. (2018). Journal of Traditional and Complementary Medicine Effects of coffee consumption on glucose metabolism : A systematic review of clinical trials. *Journal of Traditional and Complementary Medicine*, *9*(3), 184–191. https://doi.org/10.1016/j.jtcme.2018.01.001

Rohilla, A., & Ali, S. (1943). Alloxan induced diabetes: Mechanisms and effects. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *3*(2), 819–823.

Shokouh, P., Jeppesen, P. B., Christiansen, C. B., Mellbye, F. B., Hermansen, K., & Gregersen, S. (2019). Efficacy of Arabica versus Robusta coffee in improving weight, insulin resistance, and liver steatosis in a rat model of type-2 diabetes. *Nutrients*, *11*(9), 1–15. https://doi.org/https://doi.org/10.3390/nu11092 07

Socała, K., Szopa, A., Serefko, A., Poleszak, E., & Wlaź, P. (2021). Neuroprotective Effects of Coffee Bioactive Compounds: A Review. *International Journal of Molecular Sciences*, *22*(1), 1–64. https://doi.org/https://doi.org/10.3390/ijms220 10107

Subagiantara, P. A. D. K., Widianti, I. G. A., Widhiartini, I. A. A., Griadhi, I. P. A., Iswari, I. S., & Darwinata, A. E. (2024). Administration of arabica coffee green bean extract (Coffea arabica L .) reduces tumor necrosis factor-α levels and increases superoxide dismutase levels in excessive physical exercise male Wistar rats (Rattus norvegicus). *Intisari Sains Medis*, *15*(2), 825–830. https://doi.org/10.15562/ism.v15i2.2104

Volpe, C. M. O., Villar-Delfino, P. H., Dos Anjos, P. M. F., & Nogueira-Machado, J. A. (2018). Cellular death , reactive oxygen species (ROS) and diabetic complications. *Cell Death & Disease*, *9*(2), 1–19. https://doi.org/10.1038/s41419-017-0135-z

Wijayanti, W., Noviyanti, R. D., Luthfianto, D., & Haryanti, R. S. (2026). Peran Kader Kesehatan dan Inovasi Olahan Bekatul serta Kombucha Bunga Telang dalam Penanganan Diabetes Mellitus di Desa Dagen , Karanganyar. *Jurnal Medika*, *5*(1), 1010–1018. https://doi.org/https://doi.org/10.31004/pcjpm w74

Yana, E. F., & Budijastuti, W. (2022). Gambaran Histopatologi Toksisitas Hepar Tikus Jantan (Rattus norvegicus) Pasca-Pemberian Sirup Umbi Yakon (Smallanthus sonchifolius) Histopathological Overview of Liver Toxicity of Male Rats (Rattus norvegicus) After Administration of Yakon Tuber Sy. *LenteraBio: Berkala Ilmiah Biologi*, *11*(1), 202–207. https://doi.org/https://doi.org/10.26740/lentera bio.v11n1.p202- 207

Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020). Molecular mechanisms by which oxidative stress and inflammation contribute to diabetes and its complications. *Oxidative Medicine and Cellular Longevity*, *1*(1), 1–13. https://doi.org/https://doi.org/10.1155/2020/86 09213