The potential of *Trigona* honey in reducing blood glucose levels: Evidence from an experimental Type 2 diabetes mellitus in *Rattus norvegicus*

Junaidin[1,2]*, Abdurachman[1] and I Ketut Sudiana[1]

[1]Faculty of Medicine, Airlangga University, 60132 Surabaya, Indonesia.
[2]Department of Nursing, Amanah School of Health Sciences, 90125 Makassar, Indonesia
**Email:junaidinlaode09@gmail.com ORCID: https://orcid.org/0000-0002-2060-3250*

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ABSTRACT

Prolonged administration of synthetic antihyperglycemic drugs, including metformin, sulfonylureas, and insulin, has been linked to several side effects such as gastrointestinal disturbances, liver toxicity, hypoglycemic events, and reduced therapeutic responsiveness. The present research investigated how stingless bee (Trigona) honey influences blood glucose regulation, considering different doses and treatment durations, in an alloxan-induced type 2 diabetic rat model. This study applied a true experimental method with a post-test-only control group design, involving 30 male Wistar rats that were randomly assigned into four categories: a normal group receiving distilled water, a diabetic group induced with alloxan at a dose of 150 mg/kg BW, and two experimental groups administered Trigona honey at 1.35 ml/200 g BW and 2.70 ml/200 g BW, respectively. Blood glucose concentrations were assessed using a glucometer device. The collected data were subjected to analysis through ANOVA, Kruskal–Wallis, and paired t-tests. Findings indicated a marked decline in glucose concentration on the seventh day in both treatment groups, recording 133 ± 95.6 mg/dL ($p = 0.006$) for the lower dosage and 236.3 ± 197.2 mg/dL ($p = 0.012$) for the higher dosage. Moreover, statistically meaningful differences in glucose levels among the groups were noted on day five ($p = 0.002$) and day seven ($p = 0.012$). The higher dose group approached normoglycemic levels, indicating a dose- and time-dependent effect when compared with the diabetic control group. In conclusion, Trigona honey demonstrated significant antihyperglycemic effects compared with diabetic controls, likely mediated through antioxidant and insulin-sensitizing bioactive compounds such as flavonoids and polyphenols, particularly at higher doses with prolonged administration.

Keywords: Antihyperglycemic, blood glucose, dose-dependent, rat model, Trigona honey, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) represents a major public health issue on a global scale, with more than 537 million individuals affected in 2021 and projections suggesting that this number could rise to approximately 783 million by 2045 (IDF Diabetes Atlas, 2021). Indonesia occupies the fifth position worldwide, showing a prevalence of 10.6% T2DM among adults. This metabolic disorder markedly increases the likelihood of developing complications such as kidney failure, visual impairment, nerve damage, and coronary heart disease, which collectively account for over half of the mortality among diabetic patients(WHO, 2016). From a pathophysiological standpoint, T2DM involves impaired insulin sensitivity together with pancreatic β-cell malfunction, resulting in persistent hyperglycemia and elevated oxidative stress due to excessive generation of reactive

oxygen species (ROS), thereby accelerating cellular and tissue injury(Galicia-Garcia *et al.*, 2020; Antar *et al.*, 2023). Hence, therapeutic strategies for T2DM should emphasize not only glycemic regulation but also the mitigation of oxidative stress.Conventional therapies such as metformin, sulfonylureas, and insulin injections remain the first-line treatment for T2DM. However, long-term use of these medications may result in various adverse effects, including gastrointestinal disturbances, hepatotoxicity, hematological disorders, and severe hypoglycemia (Maruthur *et al.*, 2016;Padhi *et al.*, 2020; ADA, 2023). This has prompted the search for safer, more accessible therapeutic alternatives that offer effective hypoglycemic effects without significant side effects.

In recent years, the use of naturally derived therapeutic approaches has gained growing scientific attention. Among these, honey stands out as a potential bioactive substance, especially Trigona honey, which is produced by stingless bees (*Trigona* spp.) and commonly referred to as kelulut or klanceng honey. This type of honey contains abundant bioactive constituents, including flavonoids, polyphenols, ascorbic acid (vitamin C), β-carotene, and vital minerals such as calcium, magnesium, and zinc components recognized for their strong antioxidant capacity (Northwestern *et al*., 2018; Riendriasari ans Krisnawati, 2017; Ranneh *etal.*, 2017). Additionally, this honey has a low pH (3.05–4.55), high moisture content (30–35%), and contains simple sugars that are absorbed more slowly than table sugar, making it safer for individuals with diabetes (Lustig *et al*., 2016).Given its high phenolic and flavonoid content, Trigona honey may counteract oxidative stress a key factor contributing to β-cell dysfunction in T2DM.

However, until now, only a limited number of investigations have comprehensively examined how Trigona honey administered at two distinct concentrations influences blood glucose regulation in experimental models of type 2 diabetes mellitus. Comparative data regarding the efficacy of 1.35 ml per 200 g body weight and 2.70 ml per 200 g body weight, as well as variations in glucose responses measured under fasting, two-hour postprandial, and treatment conditions, are still scarce(Dewi, 2024; Syamsul *et al.*, 2020; Junaidin *et al*., 2024). The majority of previous research employed a single dosage and therefore failed to fully illustrate the temporal pattern of glucose reduction.The present research seeks to address this knowledge gap by assessing how Trigona honey, given at two distinct dosage levels, influences blood glucose concentrations in alloxan-induced *Rattus norvegicus* serving as a type 2 diabetes model. Furthermore, it investigates the effects of varying doses and treatment durations on fasting and two-hour postprandial glucose responses topics that have seldom been independently analyzed in earlier investigations.

MATERIALS AND METHODS

Experimental animals and protocol design

This laboratory-based true experimental research utilized a post-test-only control group framework. A total of 32 healthy male *Rattus norvegicus* rats, approximately 10 weeks old and averaging 200 grams in body mass, were included in the study. The sample size (n = 8 per group) was determined based on previous experimental studies capable of detecting at least a 20% difference in blood glucose reduction with 80% statistical power ($\alpha = 0.05$) (Charan and Kantharia, 2013). Prior to treatment, all animals were acclimated for seven days under standardized housing conditions, maintaining a temperature range of 22–25°C, relative humidity of 50–60%, and a 12-hour alternating light–dark schedule. The normal control group (K0) was provided with distilled water, whereas the diabetic control group (K1) received an intraperitoneal injection of alloxan at 150 mg per kg of body weight. The two treatment groups (K2 and K3) were likewise induced with alloxan at the same dosage and then given oral doses of Trigona honey at 1.35 ml per 200 g body

weight and 2.70 ml per 200 g body weight, respectively. The honey treatment was administered once daily by oral gavage over a continuous 7-day period.

Blood glucose concentrations were determined using a validated digital glucose meter (Accu-Chek® Instant, Roche Diagnostics). Samples of blood were obtained from the lateral tail vein following a fasting period of 8–10 hours. Assessments were conducted on Days 1, 5, and 7, both under fasting conditions and two hours after glucose administration, to evaluate postprandial glucose fluctuations. All procedures took place between 08:00 and 10:00 a.m. to reduce variations associated with circadian rhythms. The schematic overview of the experimental protocol is presented in Figure 1.

The Trigona honey utilized in this research originated from Kolaka, Southeast Sulawesi, Indonesia, and was harvested from stingless bees (Trigona sp.) maintained by local apiculturists. The honey's physicochemical characteristics, including pH (3.2–4.5), moisture content (30–35%), and sugar composition, were analyzed to verify batch consistency and ensure standardization of the sample used. Verification of the honey's authenticity was carried out by experts from the Entomology Division, under the Faculty of Science and Technology, Airlangga University. The samples were kept at ambient temperature in clean, sealed containers until further application.

All experimental procedures involving animals received ethical clearance from the Ethics Committee for Animal Care and Use, Faculty of Medicine, Airlangga University, Surabaya, Indonesia (Ref. No.: 23777/B/UN3.FKH/PL/PK.03.08/2024), and were conducted following the internationally recognized standards for animal research as outlined in the OECD and ARRIVE guidelines.

Induction of type 2 diabetes mellitus

To establish a model of type 2 diabetes, the rats underwent a 12-hour fasting period prior to induction. They were then injected intraperitoneally with alloxan monohydrate at a dose of 150 mg per kilogram of body weight, prepared in sterile physiological saline. Because alloxan can cause a transient drop in blood glucose due to sudden insulin secretion, a 5% glucose solution was administered orally for the next 24 hours to avoid severe hypoglycemia. Seventy-two hours following induction, fasting blood glucose concentrations were assessed, and animals showing glucose levels of 200 mg/dl or higher were classified as diabetic and included for random distribution into treatment groups.

Statistical analysis

The data were expressed as the mean ± standard deviation (SD). The normality of distribution was examined through the Shapiro–Wilk test, while the equality of variances was verified using Levene's test. When the data fulfilled both normality and homogeneity assumptions, a one-way ANOVA was applied, followed by the Bonferroni post hoc analysis. In cases where homogeneity was not met, the Games–Howell procedure was employed. For data sets that deviated from normal distribution, the Kruskal–Wallis test was utilized, and pairwise comparisons were analyzed with the paired *t*-test. A significance level of $p < 0.05$ was adopted for all analyses. Statistical processing was carried out using IBM SPSS Statistics software version 26.0 (IBM Corporation, Armonk, New York, USA).

RESULTS AND DISCUSSIONS

Effect of Trigona honey administration 1.35 ml/200g BW (K2)

During the early phase of the intervention (day 1 to day 5), administration of Trigona honey at 1.35 ml/200g BW did not result in statistically significant effects on blood glucose levels. The average glucose level before the intervention was 384.88 ± 95.49 mg/dL, which remained nearly unchanged on Day 5 (385.00 ± 267.50 mg/dL). The calculated mean difference was 0.125 ± 223.88 mg/dL, with a p-value of

0.999, indicating no significant therapeutic effect during the first five days. This suggests that the lower dose may require a longer treatment period to produce a measurable effect.

However, when the administration period was extended to 7 days, a significant decrease in blood glucose levels was observed. The average glucose level decreased from 384.88 ± 95.49 mg/dL to 251.88 ± 49.35 mg/dL, with a mean difference of 133.00 ± 95.65 mg/dL ($p = 0.006$).A detailed comparison of blood glucose levels among all groups is presented in Table 1. These findings indicate that the lower dose of Trigona honey requires a longer duration to achieve hypoglycemic effects. The bioactive composition of Trigona honey, including its high content of reducing sugars and antioxidants, is believed to contribute to its hypoglycemic effects. These mechanistic aspects are illustrated in Figure 2.

Effect of Trigona honey administration 2.70 ml/200g BW (K3)

The higher dose of Trigona honey produced a faster and more pronounced glycemic response. On Day 5, glucose levels in the K3 group decreased from 363.38 ± 189.82 mg/dL to 143.75 ± 30.29 mg/dL, with a mean difference of 219.63 ± 185.88 mg/dL ($p = 0.012$), indicating a statistically significant effect. By Day 7, glucose levels further declined to 127.13 ± 111.57 mg/dL, corresponding to a mean reduction of 236.25 ± 197.24 mg/dL ($p = 0.012$). These findings demonstrate that the higher dose elicited a faster and more pronounced glucose-lowering effect. The accelerated glycemic response observed in the higher-dose group may reflect enhanced insulin secretion or improved peripheral glucose utilization, consistent with the insulin-mimetic activity reported for flavonoid-rich natural products. Overall, these results indicate that the hypoglycemic effects of Trigona honey are both dose- and time-dependent, with the higher dose achieving significant metabolic improvement within a shorter duration.

A comparative evaluation of glucose concentrations among the control and treatment groups on Days 1, 5, and 7 revealed significant differences between groups. Statistical analyses using ANOVA (for fasting and 2-hour postprandial data on Day 5) and the Kruskal–Wallis test (for Day 7) confirmed significant intergroup variations ($p < 0.05$). Detailed post hoc comparisons are presented in Table 2, while the overall trend in glucose changes throughout the study period is illustrated in Figure 3.

This line graph shows the changes in blood glucose levels across four rat groups over a seven-day period. The K0 group (Normal Control) maintained stable glucose levels throughout the study. The K1 group (Diabetes without intervention) exhibited consistently high glucose levels with minimal variation. In contrast, both intervention groups, K2 (received 1.35 ml honey) and K3 (received 2.70 ml honey), experienced significant reductions in blood glucose levels, with K3 showing the most pronounced decrease. These findings indicate the potential glucose-lowering effects of Trigona honey in diabetic rats. These results are consistent with previous studies demonstrating that Trigona honey inhibits α-amylase and α-glucosidase activity (Purnamasari, Marliyati and Damayanthi, 2022), thereby slowing carbohydrate digestion and reducing postprandial glucose excursions. This enzymatic inhibition, together with its antioxidant and insulin-sensitizing properties, contributes synergistically to improved glycemic control.

Post hoc Games–Howell analysis revealed statistically significant differences in fasting blood glucose levels between the K1 and K2 groups, as well as between the K2 and K3 groups ($p < 0.05$), confirming a dose-dependent hypoglycemic effect of Trigona honey.

This Table 3 displays the findings of the Games–Howell post hoc comparison used to evaluate differences in fasting

glucose concentrations among the treatment groups. Statistically and clinically meaningful variations were observed between group K1 (diabetic rats without treatment) and group K2 (administered 1.35 ml of Trigona honey) with a *p*-value of 0.037, as well as between group K2 and group K3 (administered 2.70 ml of Trigona honey) with a *p*-value of 0.047. These outcomes indicate that supplementation with Trigona honey particularly at specific dosages has a measurable impact on lowering fasting blood glucose.A comparative assessment of glucose concentrations before and after Trigona honey administration was performed through a paired *t*-test. The analysis revealed that group K2 (1.35 ml/200 g body weight) exhibited a decrease in glucose levels on Day 5 that was not statistically significant (p = 0.999). However, by Day 7, the reduction became significant ($\Delta = 133.0 \pm 95.6$ mg/dL; 95% CI: 53.0–213.0; $p = 0.006$).

Conversely, group K3 (2.70 ml/200 g body weight) demonstrated a marked reduction in glucose concentration on Day 5 ($\Delta = 219.6 \pm 185.9$ mg/dL; 95% CI: 64.2–375.0; $p = 0.012$), which continued to decline by Day 7 ($\Delta = 236.3 \pm 197.2$ mg/dL; 95% CI: 71.4–401.6; $p = 0.012$). A detailed summary of pre- and post-intervention glucose measurements is presented in Table 3.The paired *t*-test analysis comparing glucose values before and after Trigona honey treatment revealed that the K2 group (1.35 ml) exhibited a statistically significant decrease on Day 7, while the K3 group (2.70 ml) showed an earlier and sustained reduction beginning on Day 5 and persisting through Day 7. Specifically, this study shows that the high dose of Trigona honey significantly reduced blood glucose levels within 5 days, and its effect continued to increase on Day 7. This rapid response suggests that the higher dose may more effectively stimulate insulin-like activity or enhance insulin secretion in diabetic rats (Bobiş *et al.*, 2018). Furthermore, fasting glucose and 2-hour postprandial glucose profiles on Day 7 indicated that the 2.70 ml dose could normalize glycemic fluctuations, which is a critical consideration in managing T2DM (Ahmed *et al*., 2018). Previous investigations have shown that stingless bee products possess greater levels of antioxidant and antimicrobial constituents than conventional *Apis* honey, potentially offering enhanced metabolic advantages (Yanti and Kustiawan, 2023; Ranneh *et al.*, 2017).

In contrast, the K2 group (lower dose) showed poor postprandial glycemic control, characterized by a significant glucose spike after meals. This highlights the importance of selecting an adequate dose if honey is to be used as an adjunctive therapy for T2DM (Spoială *et al.*, 2022; Cichosz *et al.*, 2012). These outcomes indicate that the glucose-lowering potential of Trigona honey is influenced by both dosage and duration of administration.

Moreover, the absence of a glucose reduction in the diabetic control group (K1) throughout the observation period reinforces that the improvements observed in the treatment groups were indeed due to the administration of Trigona honey (Ode *et al*., 2024). The stability of glucose levels in the normal control group (K0) also confirms that the animal model used is valid and reliable (Nakahara *et al.*, 2014; Soni, 2019).

Fasting and postprandial glucose profiles

In addition to routine daily monitoring, the study also examined the immediate glycemic response following feeding on Day 7. Measurements of fasting glucose and two-hour postprandial (2PP) concentrations were performed to evaluate metabolic regulation. The K2 group (1.35 ml) demonstrated the greatest rise in post-meal glucose, suggesting suboptimal glycemic control. Conversely, the K3 group (2.70 ml) displayed fasting and 2PP glucose values that were nearly comparable to those of the K0 (normal control) group, indicating an almost normal metabolic state (Figure 4).The K0 group maintained stable glucose levels both in fasting and postprandial states. The K1 group showed high 2PP glucose levels despite normal fasting glucose, reflecting a

postprandial regulatory disturbance. The K2 group exhibited a high postprandial glucose spike, highlighting severe glycemic dysfunction. In contrast, the K3 group displayed a glucose profile nearly identical to the normal control.This research confirms that Trigona honey exerts a notable glucose-lowering activity in the *Rattus norvegicus* model of type 2 diabetes, with its effectiveness influenced by both dosage and treatment duration. Administration of Trigona honey at 2.70 ml per 200 g of body weight produced a more rapid and substantial decrease in blood glucose concentration than the lower dosage (1.35 ml per 200 g), which required a longer exposure period to elicit a significant improvement (Purnamasari *et al*., 2022; Aryaeian *et a*l., 2017).The combined antioxidant and enzyme-inhibitory mechanisms likely account for these improvements, as polyphenols and flavonoids in Trigona honey enhance insulin sensitivity while simultaneously limiting intestinal carbohydrate breakdown.Recent findings also confirm that plant-derived antioxidants can exert significant hypoglycemic effects through similar mechanisms of glucose metabolism modulation (Karim *et al*., 2025)

Although the results of this study suggest the potential of Trigona honey in lowering blood glucose levels, this study has limitations. The molecular mechanisms underlying these effects have not been explored, and important parameters such as insulin levels, oxidative stress markers, or pancreatic histology were not analyzed.

CONCLUSION

Trigona honey has a significant antihyperglycemic effect in the type 2 diabetes rat model. This glucose-lowering activity is shown to be dependent on both the dose and the duration of administration. The high dose significantly improves postprandial glucose regulation, approaching the glycemic levels found in the non-diabetic group. Therefore, Trigona honey, particularly at an optimal dose, has the potential to be used as a natural adjunctive therapy in the management of type 2 diabetes mellitus.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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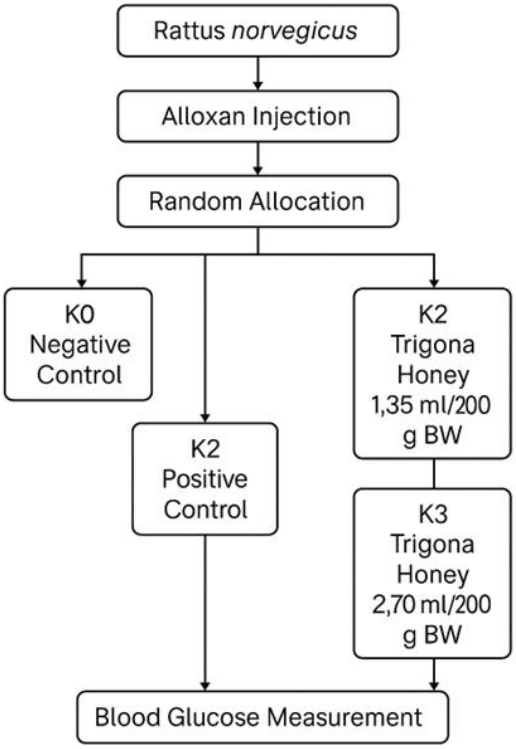

Figure 1: Flowchart of experimental design.

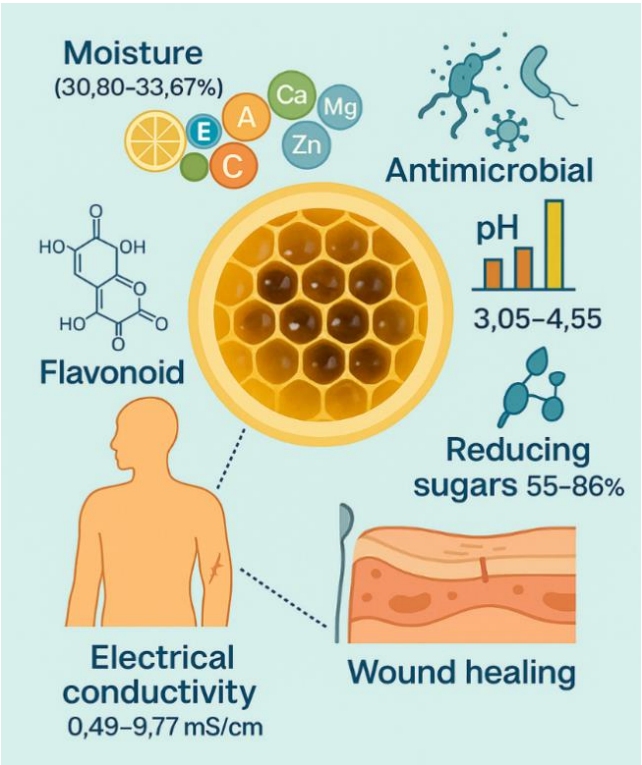

Figure 2: Key bioactive components and biological activities of Trigona honey

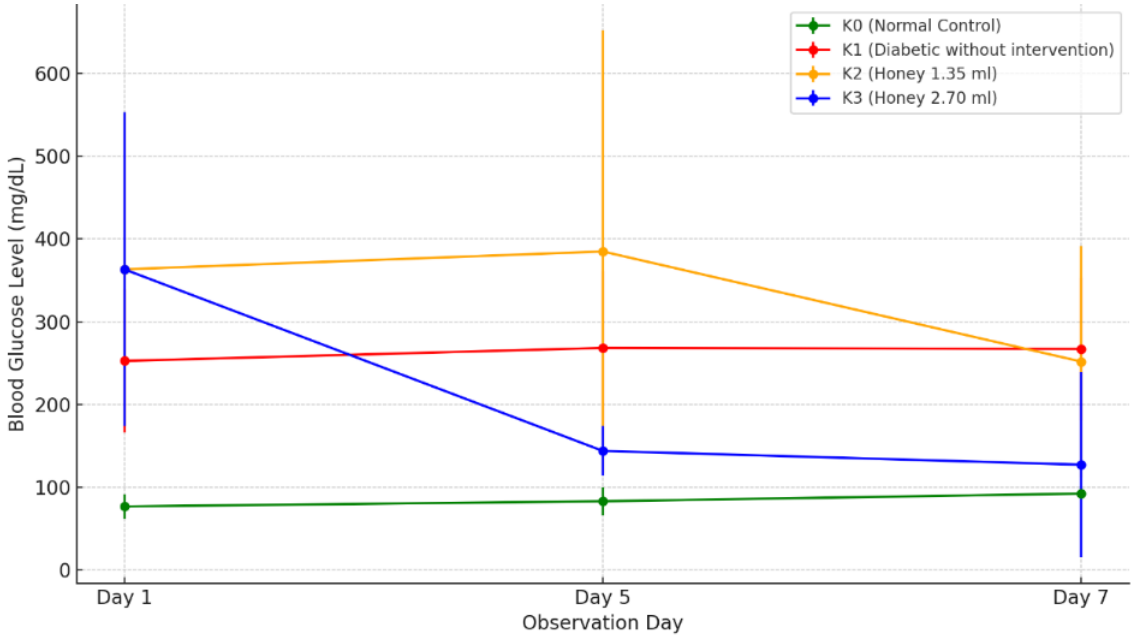

Figure 3: Trend of blood glucose levels in diabetic rats over 7 Days

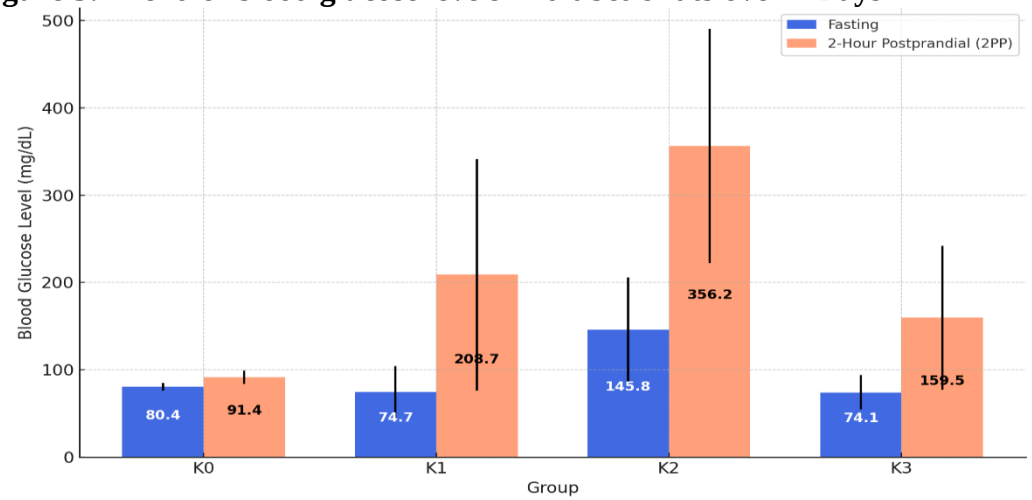

Figure 4: Comparison of fasting blood glucose and 2-hour postprandial glucose (2PP) between Groups

Table 1:Comparison of blood glucose levels between the control group and the intervention groups

Group	Day 1 (pre-intervention) (mg/dl)		Day 5 (mg/dl)		*p*	Day 7 (mg/dl)		*p*	Fasting blood glucose (mg/dl)		*p*	2-hour postprandial (mg/dl)		*p*
	Mean ± SD	Min - Max	Mean ± SD	Min - Max		Mean ± SD	Min - Max		Mean ± SD	Min - Max		Mean ± SD	Min - Max	
K0	76.57 (14.96)	58-95	82.85 (17.1)	69-100	.002*	92.1 (7.03)	85-105	.012*	80.43 ± 4.31	74–86	.001*	91.43 ± 7.93	78–99	.0001*
K1	252.57 (86.3)	118-376	268.3 (92.7)	128-361		267.1 (85.9)	131-364		74.71 ± 29.79	44–131		208.71 ± 132.59	54–407	
K2	363.38 (189.8)	139-755	385 (267.5)	61-755		251.8 (139.6)	73-438		145.75 ± 60.15	86–236		356.25 ± 133.74	94–526	
K3	363.38 (189.8)	139-755	143.75 (30.3)	87-175		127.1 (111.6)	65-400		74.12 ± 19.76	54–114		159.50 ± 82.42	81–331	

Table 2:Post Hoc analysis of fasting blood glucose comparisons between groups

Group	Difference in Mean	CI 95%		*p*
		Min	Max	
K0 vs K1	5.71	-33.2	44.6	.956
K0 vs K2	-65.32	-135.7	5.06	.068
K0 vs K3	6.30	-16.8	29.4	.816
K1 vs K2	71.03	1.94	144	**.037***
K1 vs K3	.58	0.58	13.3	1.000
K2 vs K3	71.63	0.89	142.4	**.047***

*Post Hoc games-howell test, CI: confidence interval. n K0,K1 = 7; n K2,K3= 8.

Table 3:Comparison of blood glucose reduction before and after administration of Trigona honey at 1.35 ml and 2.70 ml doses

Condition					Mean (SD)			
1.35 ml dose (K2)		Difference (SD)	CI 95%	p	Mean (SD)	Difference (SD)	CI 95%	p
Blood glucose level before Trigona honey administration								
Blood glucose level after Trigona honey administration	385 (265.5)	0.125 (223.9)	187-187.3	0.99	384.9	133 (95.6)	53-213	.006*
2.70 ml dose (K3)					251.9			
Blood glucose level before Trigona honey administration								
Blood glucose level after Trigona honey administration	143.8 (30.3)	219.6 (185.9)	64.2-375	.012*	363.4 (189.8)	236.3 (197.2)	71.4-401.6	.012*
Condition	Mean (SD)				127.1 (111.6)			

* Paired T Test, Values are statistically significant at $p < 0.05$. SD = standard deviation. n K0,K1 = 7; n K2,K3= 8.