Original Article

Effect of Honey on Postprandial Hyperglycemia in Alloxan-Induced Diabetic Rats

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ABSTRACT

The role of postprandial hyperglycemia (PPH) has been identified as a contributing factor in the development of diabetes mellitus and its associated complications. The objective of this study was to investigate the effect of honey on high glucose-induced PPH in alloxan-induced diabetic rats. Diabetes mellitus was induced in overnight-fasted rats by administering alloxan [150 mg/kg body weight (BW)]. The diabetic rats were administered either drinking water (1 ml/kg BW) or honey (1, 2 or 3 g/kg BW) via oral gavage. Each group consisted of six rats. Prior to the administration of either the drinking water or the honey, the baseline fasting blood glucose (BG) was measured and recorded as BG0. Subsequently, BG levels (BG60, BG120 and BG180) were assessed at 60, 120 and 180 minutes, respectively. The estimation of the BG parameters concentration was performed, including the area under the curve (AUC), the peak BG (PBG), the percentage change in BG. The AUC and PBG did not differ between the diabetic groups (regardless of administered agents) and the diabetic control group. Compared with baseline fasting blood glucose (BG0), the BG60 significantly ($p < 0.05$) increased in diabetic rats that received drinking water or honey (2 or 3 g/kg BW) but not in diabetic rats that received 1 g/kg BW of honey. The diabetic rats that received 1 g/kg BW of honey exhibited significantly ($p < 0.05$) lower percentage change in BG compared with the diabetic control rats. The study demonstrated that the administration of honey (regardless of dosage) did not exacerbate high glucose-induced PPH in diabetic rats. The study also indicated that a dose of 1 g/kg BW of honey was the most effective dose in suppressing PPH.

Keywords: Diabetes Mellitus, Blood Glucose, Postprandial Hyperglycemia, Honey, Alloxan.

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1. Introduction

Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia, which is a manifestation of relative or absolute insulin deficiency (1). Recent statistics indicate that there were 529 million people with diabetes mellitus in 2021. This figure is predicted to increase to more than 1.31 billion people by 2050 (2). Diabetes mellitus represents a significant health burden globally and is associated with PPH. This reflects impairments in the body's glucose-regulatory pathways, which exert deleterious effects (3). The deleterious effect of PPH is further substantiated by evidence indicating that PPH is a more accurate predictor of cardiovascular risk than fasting hyperglycemia (4). It is therefore crucial to emphasize the importance of targeting PPH in the management of diabetes mellitus. In addition to the absence of a targeted therapeutic approach for PPH, there is a pressing need to investigate the potential of complementary and alternative medicines in the treatment of PPH. The consumption of beverages rich in polyphenols such as coffee and green tea has been linked to a reduction in PPH and an improvement in endothelial function (5). These alternative interventions offer a number of advantages, including a reduced incidence of side effects and cost-effectiveness. Honey is a natural product comprising a variety of bioactive substances. It is a cost-effective and widely accessible commodity. Similar to coffee, and green tea, honey is a rich source of flavonoids and phenolic compounds. Its antioxidant effect has been the subject of extensive research (6). As has been documented for a number of medicinal plants (7-9), a substantial body of evidence exists to support the assertion that honey has beneficial effects on fasting hyperglycemia and diabetic complications including diabetic wounds (10-12). However, with regard to the effect of honey on PPH, there are limited or no data. While a study by Erejuwa and colleagues demonstrated the role of honey in suppressing postprandial hyperlipidemia in rats (13), it remains unclear whether honey can exert a similar effect on PPH. Accordingly, the present study was carried out to investigate the effect of honey on PPH in alloxan-induced diabetic rats.

2. Materials and Methods

2.1 Materials

Alloxan was procured from Sigma-Aldrich, MO, USA. All other chemicals and reagents were of analytical grade.

2.2 Honey

The raw, untreated, and unadulterated honey was procured from Umuebe Bee Farm, Ezzamgbo, Abakaliki, Ebonyi State, Nigeria.

2.3 Animals

The study employed male and female Wistar rats weighing between 180 – 220 grams. An animal ethical approval (EBSU/DRIC/UREC/Vol.04/083) was obtained from the University Research Ethics Committee of Ebonyi State University. The animals were allowed to acclimate to their surroundings two weeks before the commencement of the research project. The animals were provided with commercial feeds and had access to drinking water *ad libitum*. The rats were maintained in a well-ventilated animal facility throughout course of the study.

2.4. Induction of Diabetes Mellitus

The induction of diabetes mellitus was conducted in overnight fasted Wistar rats. The rats were administered alloxan (150 mg/kg BW; intraperitoneally), which was dissolved in normal saline. A second group of fasted rats was injected with normal saline without alloxan. Seventy-two hours after the injection of alloxan, the fasting BG concentrations were measured using an Accu-Chek Active glucometer (Roche Diagnostics, Mannheim, Germany). Rats with fasting BG concentrations of $\geq$ 250 mg/dL were considered as diabetic and selected for the study.

2.5. Study Design

This study was conducted in accordance with the established institutional guidelines and NIH guidelines for the use of experimental animals. The rats were randomly assigned to one of four groups based on their diabetic status. Each group comprised six rats. Fasting BG (FBG) concentration at the initial time point was determined. The FBG concentration measured at 0 minute was utilized as the baseline BG (BG_0) value. The rats were administered drinking water or honey via intragastric administration, in accordance with the treatment groups outlined below:

Group 1: 1.0 ml/kg BW of drinking water was administered to fasted non-diabetic rats.

Group 2: 1.0 g/kg BW of honey was administered to fasted non-diabetic rats.

Group 3: 1.0 ml/kg BW of drinking water was administered to fasted diabetic rats.

Group 4: 1.0 g/kg BW of honey was administered to fasted diabetic rats.

Group 5: 2.0 g/kg BW of honey was administered to fasted diabetic rats.

Group 6: 3.0 g/kg BW of honey was administered to fasted diabetic rats.

2.6. Oral Glucose Tolerance Test (OGTT)

Thirty minutes following the administration of either drinking water or honey, the rats were given an oral gavage of 1g/kg BW of glucose solution. Following the administration of glucose, blood glucose (BG) concentrations (BG_{60}, BG_{120}, and BG_{180}) were measured at 60, 120, and 180 minutes, respectively. The recorded BG concentrations were utilized for the determination of the PBG, the percentage (%) variation in BG, the AUC, and the percentage (%) change in BG. The PBG concentration represents the maximum BG concentration. AUC was calculated using the following formula:

$$AUC = [0.25 \times (BG0)] + [0.5 \times (BG60)] + [0.75 \times (BG120)] + [0.5 \times (BG180)]$$

The percentage variation in BG was calculated using the following formula:

$$\% \text{ Variation in BG} = [(BG60 + BG120 + BG180 - BG0) / BG0] \times 100\%$$

The percentage change in BG was determined using the following formula:

% Change in BG = [(BG180 – BG0) / BG0] × 100%

where BG0, BG60, BG120, and BG180 represent the BG values at 0, 60, 120, and 180 minutes, respectively.

2.7. Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23. All data were subjected to normality test. The results are presented as mean ± standard error of the mean (SEM). The BG concentrations measured at 0, 60, 120, and 180 minutes were subjected to repeated measures analysis of variance (ANOVA). A OneWay ANOVA was utilized to compare the existence of differences among groups, with a Tukey *post-hoc* test subsequently utilized to detect discrepancies between two specific groups. A $p < 0.05$ was considered as the statistically significant.

3. Results

3.1. Effect of Honey on BG Levels at 0, 60, 120 and 180 Minutes

The data regarding the effect of honey on BG concentrations at 0, 60, 120 and 180 minutes following glucose administration are presented in Figure 1. The BG concentrations at 0, 60, 120 and 180 minutes post- glucose administration in diabetic groups were statistically significantly ($p < 0.001$) higher compared to the values of the non-diabetic groups. No statistically significant difference ($p > 0.05$) in BG levels was observed between the diabetic groups that received honey (1, 2 or 3 g/kg BW) and the diabetic control group. A statistically significant increase ($p < 0.05$) was observed in BG60 compared with BG0 in groups 3, 5 and 6. The BG60 was not statistically significantly different from BG0 in groups 4 ($p > 0.05$).

3.2 Effects of Honey on PBG, AUC Glucose, % Change in BG and % Variation in BG Following Glucose Ingestion in Diabetic Rats

The data on the effects of honey on PBG, AUC glucose, % change in BG and % variation in BG following glucose ingestion in diabetic rats are presented in Table 1. The data in the table demonstrate that PBG levels in the diabetic groups were statistically significantly ($p < 0.001$) higher compared to the non-diabetic groups. No statistically significant difference in PBG was observed between the diabetic rats that received honey (1, 2 or 3 g/kg BW) and the diabetic control group ($p > 0.05$). The AUC values for diabetic rats were found to be statistically significantly ($p < 0.001$) higher in comparison to the non-diabetic groups. No statistically significant difference was observed between the diabetic groups that received honey (1, 2 or 3 g/kg BW) and the diabetic control rats($p > 0.05$). The percentage change in BG of diabetic rats that received 1 g/kg BW of honey was statistically significantly ($p < 0.05$) lower compared to that of the diabetic control group. The percentage variation in BG in the diabetic rats that received 1 g/kg BW of honey was lower but not statistically significant ($p > 0.05$) than in the non-diabetic control rats.

4. Discussion

The levels of BG60, BG120 and BG180 were significantly higher in all the diabetic groups compared to the non-diabetic groups. This is a consequence of impaired postprandial glucose homeostasis between individuals with and without diabetes (14,15). In contrast, no significant change was observed in BG60, BG120 and BG180 between the non-diabetic control and honey-administered non-diabetic groups. This can be attributed to the presence of functional glucose-stimulated insulin mechanism in non-diabetic rats. The two highest doses (2 and 3 g/kg BW) demonstrated a lesser efficacy in suppressing PPH when compared to 1 g/kg BW of honey. This may not be surprising because the results corroborate existing data on dose increments of honey. A previous study demonstrated that diabetic rats administered 1 or 2 g/kg BW of honey exhibited significantly lower FBG concentrations and % change in FBG while no such effects were observed with 3.0 g/kg BW of honey (16). Similarly, the administration of 1 g/kg BW of honey resulted in a significant improvement in obesity-related anthropometric parameters in rats with diet-induced obesity. In contrast, the administration of 2 or 3 g/kg BW of honey did not result in the observed beneficial effects (17). The PBG and AUC values derived from the OGTT were significantly elevated in the diabetic groups compared to the non-diabetic groups. Higher PBG and AUC in diabetic rats is indicative of glucose intolerance in rats that have been injected with alloxan. This is a consequence of insulin deficiency resulting from alloxan-induced destruction of pancreatic beta cells (18). The findings reflect the occurrence of a dysfunctional glucose-stimulated insulin response in the diabetic rats. No significant difference was observed in PBG and AUC between the honey-administered groups (1, 2 or 3 kg/BW) and the diabetic control group. At present, no study has investigated the effect of honey on glucose- or meal-induced PPH in rats or humans. Consequently, it is difficult to correlate these findings with existing data. It is noteworthy that the diabetic control rats received distilled water and a glucose solution (1 g/kg BW), whereas the honey groups received varied amounts of honey (1, 2 or 3 g/kg BW) and glucose solution (1 g/kg BW). Honey is primarily composed of glucose and fructose. In other words, the diabetic groups that received honey consumed greater amounts of glucose than did the control diabetic rats. Therefore, the lack of significant difference in PBG and AUC among the three honey- consuming diabetic groups and the diabetic control group is interesting. This is because it is well-established that the quantity of carbohydrate or sugar consumed influences the severity of PPH (19). In this study, the second (2 g/kg BW) and third (3 g/kg BW) honey-administered diabetic groups consumed twice and thrice, respectively, as much

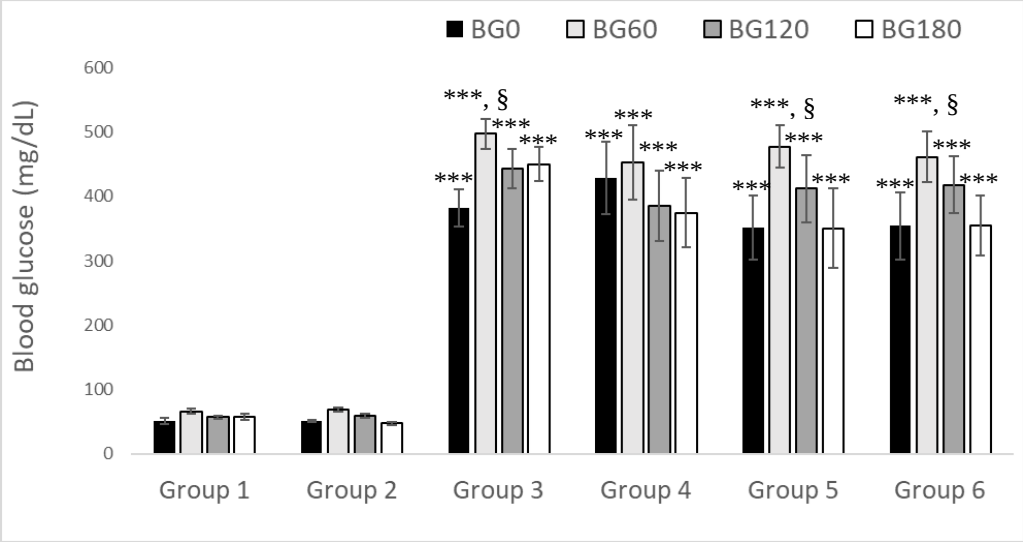

Figure 1. Effect of honey on blood glucose (BG) concentrations at 0, 60, 120 and 180 minutes post-glucose ingestion in diabetic rats

Group 1: Non-diabetic control rats + tap water (1 ml/kg BW).
Group 2: Non-diabetic rats + Honey (1 g/kg BW).
Group 3: Diabetic control rats+ tap water (1 ml/kg BW).
Group 4: Diabetic rats + Honey (1 g/kg BW).
Group 5: Diabetic rats + Honey (2 g/kg BW).
Group 6: Diabetic rats + Honey (3 g/kg BW)
BG0, BG60, BG120 and BG180 refer to BG at 0, 60, 120 and 180 minutes
*** $p < 0.001$: A significantly higher BG value compared with the corresponding BG value of group 1.
§ $p < 0.05$: A significant increase in BG value at 60 min compared with BG value at 0 min within the same group.

Table 1. Effect of honey on PBG, AUC glucose, % change in BG and % variation in BG following glucose ingestion in diabetic rats

GROUP				
	PBG Mean ± SEM	**AUC Mean ± SEM**	**% Change in BG Mean ± SEM**	**% Variation in BG Mean ± SEM**
Group 1	69.2 ± 3.2	117.2 ± 5.2	14.2 ± 7.6	264.6 ± 31.6
Group 2	68.7 ± 2.6	114.8 ± 4.0	-6.5 ± 7.0	240.5 ± 14.0
Group 3	507.5 ± 18.2 ***	901.8 ± 50.9 ***	18.9 ± 4.1	267.7 ± 11.2
Group 4	480.0 ± 55.8 ***	809.4 ± 104.5 ***	-13.8 ± 2.6 †	183.4 ± 13.6
Group 5	472.0 ± 27.9 ***	811.4 ± 97.2 ***	-1.1 ± 7.8	257.3 ± 17.6
Group 6	469.8 ± 44.0 ***	810.4 ± 80.3 ***	3.4 ± 8.3	264.0 ± 24.7

PBG: Peak blood glucose, AUC: Area under curve, BG: Blood glucose.
Group 1: Non-diabetic control rats + tap water (1 ml/kg BW).
Group 2: Non-diabetic rats + Honey (1 g/kg BW).
Group 3: Diabetic control rats+ tap water (1 ml/kg BW).
Group 4: Diabetic rats + Honey (1 g/kg BW).
Group 5: Diabetic rats + Honey (2 g/kg BW).
Group 6: Diabetic rats + Honey (3 g/kg BW).
*** $p < 0.001$: versus group 1; † $p < 0.05$: Group 4 versus Group 3

carbohydrate/glucose as the first honey (1 g/kg BW)-ingested diabetic group. Indeed, when considering the total amount of carbohydrate/glucose ingested, the order of the diabetic groups can be written as follows: DH3>DH2>DH1>DC. It was anticipated that the data on glucose parameters would exhibit a similar pattern to that observed in the aforementioned order. It was anticipated that the greatest significant differences would be observed in the BG60, BG120, BG180, PBG, AUC, and percentage variation in BG measurements in DH3 group, followed by DH2, DH1, and DC. However, this was not the case. These findings demonstrate that, despite the high sugar content of honey, honey did not elevate PPH in these diabetic rats compared to the diabetic control. This may be attributed to the low glycemic index profile of honey and the presence of several bioactive substances in honey. Therefore, honey can be considered as a potentially unique sugar substitute and beneficial agent in diabetes treatment (20-21). The diabetic rats that received 1 g/kg BW of honey exhibited a non-significantly lower percentage variation in BG and significantly lower percentage % change in BG compared to diabetic control rats. In contrast, the diabetic groups administered 2 or 3 g/kg BW of honey did not demonstrate these effects. The data suggest that the rate of decline of PPH to pre-meal glycemia was more rapid/greater with 1g/kg BW of honey than with other higher doses of honey. These findings corroborate previous research indicating that moderate doses of honey are essential for achieving a desired therapeutic goal (16,17,22). In conclusion, the results of this study indicate that a dose of 1g/kg BW of honey is the most effective for the treatment of PPH in diabetic rats. Furthermore, the findings suggest that honey may be a suitable alternative for the treatment of PPH in diabetes.

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Authors' Contribution
Study concept and design: N.N.E, S.I.G. and O.O.E.
Acquisition of data: N.N.E. and O.O.E.
Analysis and interpretation of data: N.N.E. and O.O.E.
Drafting of the manuscript: N.N.E.
Critical revision of the manuscript for important intellectual content: S.I.G. and O.O.E.
Statistical analysis: O.O.E.
Administrative, technical, and material support: N.N.E. and E.S.
Study supervision: S.I.G. and O.O.E.

Ethics
The study was approved by the Research Ethics Committee of Ebonyi State University, Abakaliki, Ebonyi State, Nigeria (EBSU/DRIC/UREC/Vol.04/083).

Conflict of Interest
The authors declare that they have no conflict of interest.

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Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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