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RESEARCH ARTICLE

ANTIHYPERGLYCEMIC AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF HONEY IN ALLOXAN-INDUCED DIABETIC MALE ALBINO RATS

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Abstract

Background and objectives: Natural products are investigated as interventions for diabetes-related alterations. This study assessed the antihyperglycemic and *in vitro* antioxidant potential of honey.
Methods: The glucose-lowering effect of honey was investigated in alloxan-induced diabetic male albino rats treated with 0.2, 0.5, and 0.8 mL/kg honey for 21 days, with glibenclamide as the standard drug. Fasting blood glucose (FBG) was monitored before induction and on Days 3, 7, 14, and 21. Antioxidant activity was assessed using DPPH and ABTS radical-scavenging assays, ferric reducing antioxidant power (FRAP), total phenolic content (TPC), and total flavonoid content (TFC).
Results: Alloxan administration increased FBG from approximately 91–92 mg/dL to above 318 mg/dL. Untreated diabetic rats showed progressive hyperglycemia, reaching 358.23±19.74 mg/dL on Day 21. Honey significantly ($p<0.05$) reduced FBG in a dose-dependent manner. The 0.8 mL/kg dose reduced FBG to 116.42±8.51 mg/dL, approaching the glibenclamide value of 108.56±8.13 mg/dL. Honey showed substantial antioxidant activity, with DPPH and ABTS scavenging capacities of 78.42±2.16% and 81.57±1.94%, respectively, FRAP of 623.45±18.62 µmol Fe^{2+}/g, TPC of 68.84±3.25 mg GAE/g, and TFC of 24.37±1.41 mg QE/g.
Conclusion: Honey demonstrated significant dose-dependent antihyperglycemic activity and antioxidant capacity, indicating potential as an adjunct for diabetes management.
Keywords: alloxan, antioxidant activity, diabetes mellitus, honey, fasting blood glucose.

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial endocrine-metabolic disorder marked by impaired glucose homeostasis arising from insufficient insulin secretion, diminished insulin action, or a combination of both. Beyond disturbances in glucose metabolism, diabetes also disrupts normal lipid and protein homeostasis, contributing to its widespread health burden and making it a major global public health challenge. Prolonged elevation of blood glucose levels initiates a cascade of biochemical alterations, including elevated ROS production, which contributes to oxidative stress, compromises pancreatic β-cell integrity, and promotes the development of diabetes-associated complications such as kidney injury, liver dysfunction, nerve damage, and cardiovascular abnormalities[1,2].

Honey is a biologically active natural food containing diverse bioactive constituents, such as phenolic acids, flavonoids, enzymes, vitamins, amino acids, and organic acids. These compounds contribute to its anti-oxidant, anti-inflammatory, antimicrobial, and metabolic modulating effects[3]. Growing experi-mental evidence indicates that honey possesses anti-hyperglycemic activity by improving glucose homeo-stasis, reducing oxidative stress, and preserving pancreatic β-cell integrity[4,5]. These properties have made honey an attractive complementary therapeutic agent for the management of diabetes mellitus.

Previous investigations by Alaebo *et al.*[6], showed that evidence from experimental studies suggests that

honey may mitigate oxidative stress and preserve renal function in diabetic animals by strengthening endogenous antioxidant defenses and reducing biochemical indicators of kidney damage. Alaebo *et al.*[7], similarly demonstrated that honey administration improved lipid homeostasis and attenuated hepatic dysfunction in alloxan-induced diabetic rats. These protective effects may be associated with the phenolic and flavonoid compounds present in honey, which can enhance cellular antioxidant mechanisms and scavenge reactive oxygen species[8]. The antioxidant capacity of natural products can be characterized using complementary analytical methods, including DPPH and ABTS radical-scavenging assays, ferric-reducing antioxidant power (FRAP), and quantification of total phenolic and flavonoid contents[9,10].

The present investigation was undertaken to assess the glucose-lowering potential of honey in male albino rats with alloxan-induced diabetes and to characterize its antioxidant capacity under *in vitro* conditions.

MATERIALS AND METHODS

Experimental design

A completely randomized design was adopted to investigate the antihyperglycemic potential and *in vitro* antioxidant properties of honey in male albino rats with alloxan-induced diabetes. The animal experiment was conducted over a period of 21 days, whereas the antioxidant properties of the honey were determined *in vitro* using established analytical methods.

Experimental animals

Thirty-six healthy male albino rats, weighing 150–180 g, were sourced from the Animal House of the Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike. The animals were maintained in well-ventilated, clean steel cages under controlled environmental conditions of 25±2°C temperature, 50–60% relative humidity, and a 12-hour light/12-hour dark photoperiod. Before the commencement of the experiment, the rats were allowed to acclimatize for two weeks. Throughout the acclimatization and experimental periods, they had unrestricted access to commercially formulated growers' mash and potable drinking water.

Honey collection and preparation

Raw natural honey was procured from a certified beekeeper located in Isuochi, Umunneoche Local Government Area, Abia State, Nigeria. The collected honey was carefully filtered to eliminate visible particulate matter and other extraneous materials. Following filtration, the sample was transferred into sterile, airtight, amber-colored containers and maintained at room temperature until required for the experimental procedures.

Chemicals and reagents

Alloxan monohydrate and glibenclamide were procured from established pharmaceutical suppliers. The reagents employed for the antioxidant analyses included 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin–Ciocalteu reagent, gallic acid, quercetin, potassium ferricyanide, ferric chloride, and trichloroacetic acid. All other chemicals and reagents used in the study were of analytical grade and obtained from Sigma-Aldrich (USA) or other accredited suppliers.

Induction of experimental diabetes

Diabetes was experimentally induced following an overnight fast by administering a single intraperitoneal injection of alloxan monohydrate at 120 mg/kg body weight. Immediately following alloxan administration, the animals were provided with a 5% glucose solution for 24 hours to prevent possible hypoglycaemic shock associated with the initial insulin-releasing effect of alloxan. After 72 hours, fasting blood glucose (FBG) concentrations were determined from tail-vein blood using a digital glucometer. Animals exhibiting FBG levels of ≥200 mg/dL were classified as diabetic and selected for subsequent experimentation[11].

Experimental grouping

Following confirmation of diabetes, the animals were randomly distributed into six experimental groups, with six rats assigned to each group (n=6), as follows:

Group 1: Normal control rats receiving distilled water only.

Group 2: Diabetic control rats induced with alloxan but left untreated.

Group 3: Diabetic rats administered glibenclamide at 5 mg/kg body weight.

Group 4: Diabetic rats administered honey at 0.2 mL/kg body weight.

Group 5: Diabetic rats administered honey at 0.5 mL/kg body weight.

Group 6: Diabetic rats administered honey at 0.8 mL/kg body weight.

All treatments were delivered orally once daily for 21 consecutive days. The animals in the normal and diabetic control groups received the corresponding vehicle according to the experimental protocol.

Determination of fasting blood glucose

Fasting blood glucose (FBG) concentrations were measured at baseline before diabetes induction (Day 0), 72 h following alloxan administration (Day 3), and subsequently on Days 7, 14, and 21. Before each measurement, the rats were fasted overnight, after which blood was collected from the tail vein. Glucose concentrations were determined using an Accu-Chek® glucometer (Roche Diagnostics, Germany) according to the manufacturer's instructions.

In vitro antioxidant assays

DPPH free-radical scavenging test

The radical-scavenging capacity of the honey sample was assessed using the method of Brand-Williams *et al.*[12], with appropriate modifications where necessary. Briefly, a measured volume of the prepared honey solution was combined with freshly prepared DPPH solution and maintained in the dark for 30 min. The absorbance of the reaction mixture was subsequently determined at 517 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the reference antioxidant. The DPPH scavenging activity was calculated and reported as percentage inhibition.

Assessment of ABTS radical-scavenging activity

The ABTS radical-scavenging capacity of honey was determined following the procedure reported by

Re *et al*.[10]. The prepared ABTS radical cation solution was mixed with appropriately diluted honey samples and allowed to react for the specified incubation period. Absorbance was then measured at 734 nm, and the antioxidant activity was expressed as the percentage inhibition of the ABTS radical.

Determination of ferric-reducing antioxidant potential

The reducing capacity of honey was determined using the FRAP procedure described by Benzie and Strain[9]. The freshly prepared FRAP reagent was combined with the honey sample and incubated at 37°C for 30 min. Absorbance was measured at 593 nm, and the reducing antioxidant capacity was calculated as µmol Fe^{2+} equivalents per gram of honey.

Determination of Total Phenolic Content

The total phenolic content (TPC) of honey was estimated using the Folin–Ciocalteu colorimetric procedure described by Singleton *et al*.[13]. Gallic acid was used as the reference standard for constructing the calibration curve. The phenolic concentration of the honey sample was expressed as milligrams of gallic acid equivalents per gram (mg GAE/g).

Determination of total flavonoid concentration

Total flavonoid content (TFC) was quantified using the aluminium chloride colorimetric method of Chang *et al*.[14]. Quercetin was employed as the standard for calibration, and the flavonoid concentration was expressed as milligrams of quercetin equivalents per gram (mg QE/g).

Statistical analysis

Results were presented as mean±standard deviation (SD). Statistical analyses were conducted using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA). Differences between experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test for post hoc comparisons.

RESULTS AND DISCUSSION

Values are expressed as mean±standard deviation (SD) for six animals per group (n=6). Fasting blood glucose (FBG) levels were determined with a glucometer at baseline (Day 0), 72 h following alloxan administration (Day 3), and subsequently on Days 7, 14, and 21. Diabetes was induced through a single intraperitoneal administration of alloxan monohydrate at 120 mg/kg body weight. Honey was administered orally once daily at 0.2, 0.5, and 0.8 mL/kg body weight for 21 days, while glibenclamide (5 mg/kg body weight) was used as the reference antidiabetic treatment. Within each column, mean values carrying different superscript letters differ significantly at $p<0.05$, based on one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test. Values are expressed as mean±standard deviation (SD) from three independent determinations (n=3). DPPH denotes 2,2-diphenyl-1-picrylhydrazyl; ABTS represents 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); FR AP refers to ferric-reducing antioxidant power; GAE indicates gallic acid equivalents; and QE denotes quercetin equivalents. Greater DPPH and ABTS radical-scavenging percentages and higher FRAP values are indicative of stronger antioxidant capacity. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test, with significance established at $p<0.05$. Honey exhibited appreciable antioxidant capacity under *in vitro* conditions, as evidenced by its ability to scavenge DPPH and ABTS radicals and reduce ferric ions. The substantial levels of phenolic and flavonoid compounds detected in the sample may account, at least in part, for its antioxidant activity. These phyto-chemical constituents could contribute to the biological effects of honey observed in the diabetic animal model, particularly its glucose-lowering and tissue-protective actions. Overall, the findings indicate that honey possesses both antihyperglycemic and antioxidant properties in alloxan-induced diabetic male albino rats. The improvement in fasting blood glucose, together with the marked antioxidant capacity demonstrated *in vitro*, suggests that honey may exert beneficial effects through mechanisms involving regulation of glucose homeostasis and reduction of oxidative stress. These observations further support investigations into honey and other bee-derived products as potential complementary interventions in diabetes management.

Table 1: Effect of honey on Fasting Blood Glucose (FBG) levels (mg/dL) in alloxan-induced diabetic male wistar rats.

Groups	Day 0 (Pre-induction)	Day 3 (Post-induction)	Day 7	Day 14	Day 21
Group 1: Normal Control	91.25±4.18^{a}	92.16±3.95^{a}	90.84±4.27^{a}	91.36±3.82^{a}	90.92±4.11^{a}
Group 2: Diabetic Control	92.43±4.65^{a}	322.84±18.74^{c}	335.12±21.63^{c}	347.41±20.15^{c}	358.23±19.74^{c}
Group 3: Glibenclamide (5 mg/kg)	90.78±3.87^{a}	318.75±16.52^{c}	245.36±14.27^{d}	168.47±11.28^{c}	108.56± 8.13^{b}
Group 4: Honey (0.2 mL/kg)	91.84±4.26^{a}	320.63±17.36^{c}	281.52±15.83^{d}	226.41±13.65^{c}	172.38±11.42^{c}
Group 5: Honey (0.5 mL/kg)	90.95±4.01^{a}	321.46±18.14^{c}	266.18±14.69^{d}	195.74±12.83^{c}	136.84± 9.76^{b}
Group 6: Honey (0.8 mL/kg)	91.16±3.95^{a}	319.92±17.88^{c}	248.36±13.92^{d}	172.65±10.74^{c}	116.42± 8.51^{b}

Table 2: Evaluation of *in vitro* antioxidant activity and bioactive phytochemical contents of honey.

Parameter	Honey	Ascorbic Acid (Standard)
DPPH Radical Scavenging Activity (%)	78.42±2.16	91.63±1.04
ABTS Radical Scavenging Activity (%)	81.57±1.94	94.28±0.87
Ferric Reducing Antioxidant Power (FRAP, µmol Fe^{2+}/g)	623.45±18.62	782.51±15.37
Total Phenolic Content (mg GAE/g)	68.84±3.25	—
Total Flavonoid Content (mg QE/g)	24.37±1.41	—

A major observation was the significant ($p<0.05$) decline in fasting blood glucose following honey administration. At baseline, FBG values across the experimental groups were relatively similar, ranging from approximately 90 to 92 mg/dL, indicating comparable glycaemic status before diabetes induction. Administration of alloxan monohydrate at 120 mg/kg body weight resulted in a pronounced elevation of FBG, with concentrations exceeding 318 mg/dL among the diabetic groups. This marked increase confirmed the successful establishment of experimental diabetes. In contrast, the untreated diabetic group demonstrated persistent hyperglycaemia throughout the study, with FBG reaching 358.23±19.74 mg/dL on Day 21. The sustained hyperglycaemia is consistent with alloxan-mediated pancreatic β-cell damage and consequent impairment of insulin secretion[11].

Honey administration produced a progressive decline in FBG, with the magnitude of the response increasing with dose. The 0.8 mL/kg treatment produced the greatest reduction, followed by the 0.5 and 0.2 mL/kg doses. By Day 21, FBG in rats receiving 0.8 mL/kg honey had declined to 116.42±8.51 mg/dL, which was relatively close to the 108.56±8.13 mg/dL recorded for the glibenclamide-treated group. The greater response observed at the highest honey dose suggests a dose-related glucose-lowering effect. This finding indicates that increasing honey administration within the tested range may enhance its ability to restore glycaemic balance in experimentally diabetic animals.

The antihyperglycemic effect observed following honey administration may be related to the diverse bioactive constituents present in honey. In addition to glucose and fructose, honey contains amino acids, vitamins, minerals, phenolic acids, flavonoids, enzymes such as catalase and glucose oxidase, and other biologically active compounds that may influence glucose metabolism and cellular redox balance[3,8]. Although honey contains naturally occurring sugars, its biological effects extend beyond its carbohydrate content. Previous studies have suggested that honey may improve insulin sensitivity, facilitate peripheral glucose utilization, modulate hepatic glucose production, and attenuate oxidative stress[4,5,15]. These properties provide a plausible biochemical basis for the reduction in fasting blood glucose observed in the present study.

The progressive decline in fasting blood glucose following honey administration is consistent with previous experimental evidence. Alaebo *et al.*[7], reported that honey administration improved glycaemic regulation and attenuated diabetes-associated dyslipidaemia without producing evidence of hepatic toxicity. Similarly, Erejuwa *et al.*[4], demonstrated that honey supplementation reduced hyperglycaemia, oxidative stress, and inflammatory responses in experimental diabetes. Samarghandian *et al.*[5], also reported beneficial effects of honey on glucose regulation and oxidative status, suggesting that its anti-diabetic activity may involve preservation of pancreatic β-cell function and improvement of cellular antioxidant defenses. In human subjects, Abdulrhman *et al.*[16], reported improved glycaemic parameters following honey supplementation alongside conventional diabetic therapy. The agreement between these findings and the present results suggests that the glucose-lowering effect of honey warrants further investigation in both experimental and clinical settings.

The biochemical basis of the observed reduction in blood glucose may involve the ability of honey-derived polyphenols and flavonoids to modulate pathways associated with glucose homeostasis. Phenolic compounds can act as free-radical scavengers and may protect pancreatic β-cells from oxidative injury. This is particularly relevant in diabetes, where persistent hyperglycaemia promotes excessive reactive oxygen species (ROS) generation. Oxidative stress can impair β-cell function, interfere with insulin signaling, and contribute to progressive metabolic dysfunction[2]. By reducing oxidative burden, antioxidant constituents of honey may help preserve residual pancreatic β-cell activity and support glucose utilization.

The untreated diabetic rats in the present study showed progressive hyperglycaemia throughout the experimental period, whereas honey-treated animals demonstrated a dose-related reduction in fasting blood glucose. This difference is physiologically important because sustained hyperglycaemia is a major contributor to the development of diabetic complications. Chronic elevation of glucose activates several interconnected biochemical pathways, including the polyol pathway, formation of advanced glycation end products (AGEs), protein kinase C activation, and mitochondrial ROS generation. These processes promote oxidative stress, inflammation, endothelial dysfunction, and tissue injury[17-19]. Therefore, the reduction in blood glucose observed following honey treatment may have implications beyond glycaemic regulation by potentially reducing the biochemical burden associated with diabetes-related complications.

The dose-dependent response observed in this study is particularly noteworthy. Honey administration at 0.2, 0.5, and 0.8 mL/kg produced progressively greater reductions in fasting blood glucose, with the 0.8 mL/kg treatment producing the most pronounced response. This pattern suggests that the biological activity of honey may be influenced by the quantity of its active constituents delivered to the animals. Increasing the administered amount may provide greater exposure to polyphenols, flavonoids, organic acids, peptides, enzymes, and other antioxidant constituents capable of influencing glucose metabolism and oxidative balance. Similar dose-related responses have been documented with other polyphenol rich natural products, in which increasing phytochemical exposure was associated with improved metabolic and antioxidant outcomes[20,21].

By Day 21, the highest honey dose reduced fasting blood glucose to 116.42±8.51 mg/dL, compared with 108.56±8.13 mg/dL in the glibenclamide-treated group. Although the response to the highest honey dose approached that observed with glibenclamide, the two treatments should not be interpreted as therapeutically equivalent without direct statistical comparison between the groups and confirmation in additional experimental and clinical studies. Nevertheless, the finding demonstrates the considerable glucose-

lowering potential of honey under the conditions of the present experiment. Glibenclamide acts primarily by stimulating pancreatic insulin secretion, whereas honey is likely to exert a broader biological effect involving antioxidant, anti-inflammatory, metabolic, and possibly insulin-sensitizing mechanisms[4,24].

The antioxidant findings provide additional insight into the possible mechanism underlying the anti-hyperglycemic response. Honey demonstrated DPPH radical-scavenging activity of 78.42±2.16%, ABTS radical-scavenging activity of 81.57±1.94%, and FRAP of 623.45±18.62 µmol Fe^{2+}/g. The sample also contained 68.84±3.25 mg GAE/g total phenolics and 24.37±1.41 mg QE/g total flavonoids. Collectively, these findings demonstrate that the honey sample possesses appreciable free-radical-scavenging and ferric-reducing capacities. The use of several complementary antioxidant assays is important because each method reflects a different aspect of antioxidant behavior. DPPH and ABTS assays primarily evaluate radical-scavenging capacity, whereas FRAP reflects the ability of antioxidant compounds to reduce ferric ions.

The relatively high DPPH and ABTS scavenging activities observed in the present study indicate that honey contains constituents capable of donating electrons or hydrogen atoms to reactive radicals. Such activity is relevant to diabetes because excessive ROS generation contributes to oxidative damage of lipids, proteins, nucleic acids, and cellular membranes. Brand-Williams *et al.*[12], described DPPH reduction as an indicator of the radical-scavenging ability of anti-oxidant compounds, while Re *et al.*[10], established ABTS radical decolourization as a useful measure of antioxidant capacity. The substantial responses obtained with both assays therefore suggest that the antioxidant constituents of the honey operate through more than one radical-scavenging mechanism.

The FRAP result further supports the antioxidant potential of the honey sample. The ferric-reducing capacity of 623.45±18.62 µmol Fe^{2+}/g indicates the presence of compounds capable of donating electrons and reducing oxidized iron. Although FRAP does not directly measure all antioxidant mechanisms occurring in biological systems, its result complements the radical-scavenging findings obtained from DPPH and ABTS assays. The combined results therefore provide stronger evidence of the overall antioxidant capacity of the honey than reliance on a single assay would provide.

The phytochemical findings may explain, at least partly, the antioxidant activity observed. The honey contained 68.84±3.25 mg GAE/g total phenolics and 24.37±1.41 mg QE/g total flavonoids. Phenolic compounds possess hydroxyl groups capable of donating hydrogen atoms or electrons to reactive species, while some phenolics can chelate transition metals involved in oxidative reactions[23]. Flavonoids similarly possess structural features that enable them to neutralize free radicals and modulate cellular antioxidant defenses. Consequently, the phenolic and flavonoid constituents detected in the honey may have contributed substantially to its DPPH, ABTS, and FRAP activities.

The present findings are consistent with the observations of Cianciosi *et al.*[3], who emphasized the contribution of phenolic compounds to the antioxidant properties of honey. Pasupuleti *et al.*[8], likewise reported that the antioxidant characteristics of honey are strongly influenced by its phenolic composition and botanical origin. Variations in phenolic and flavonoid concentrations among honey samples may arise from differences in floral source, geographical location, climatic conditions, processing, storage, and harvesting practices. Therefore, the antioxidant values obtained in this study should be interpreted in the context of the specific honey sample investigated rather than generalized to all varieties of honey. A potentially important relationship emerged between the antioxidant and antihyperglycemic findings. Diabetes is characterized not only by disturbed glucose metabolism but also by increased oxidative stress. Pancreatic β-cells are particularly vulnerable to oxidative injury because of their relatively limited antioxidant defense capacity. Excessive ROS production can impair insulin synthesis and secretion, damage cellular membranes, and exacerbate insulin resistance[2]. Consequently, an antioxidant-rich intervention capable of reducing oxidative stress may indirectly contribute to improved glycaemic regulation. The strong antioxidant activity demonstrated by the honey *in vitro* therefore provides a plausible mechanistic explanation for part of its glucose-lowering effect observed in the diabetic rats[24].

The progressive improvement in fasting blood glucose with increasing honey dose may further support this proposed relationship. The highest dose produced the greatest reduction in blood glucose and the honey sample demonstrated substantial antioxidant activity together with appreciable phenolic and flavonoid concentrations. It is therefore plausible that antioxidant mediated protection of pancreatic tissue, together with modulation of glucose metabolism, contributed to the observed response[25]. However, because antioxidant assays were conducted *in vitro*, the present study cannot establish a direct causal relationship between the measured antioxidant capacity and the *in vivo* anti-hyperglycemic effect. Additional studies involving pancreatic oxidative stress markers, insulin concentrations, β-cell histology, and molecular indicators of insulin signaling would be necessary to confirm these mechanisms[26].

An important strength of the present investigation is the combined assessment of honey using complementary *in vivo* and *in vitro* approaches. The animal experiment demonstrated its glucose-lowering potential in an alloxan-induced diabetic model, while DPPH, ABTS, FRAP, total phenolic, and total flavonoid assays characterized its antioxidant and phytochemical properties[27]. This integrated approach provides a broader understanding of the potential biological activity of honey than an assessment of blood glucose alone[28]. The observed dose-response relationship also provides useful preliminary information

regarding the influence of honey quantity on glycaemic outcomes.

Overall, the findings indicate that honey possesses considerable antihyperglycemic and antioxidant potential under the experimental conditions employed. The reduction in fasting blood glucose, particularly at the 0.8 mL/kg dose, together with the substantial DPPH, ABTS, and FRAP activities and measurable phenolic and flavonoid contents, supports the possibility that honey may influence diabetes through interconnected glucose-regulatory and antioxidant mechanisms. Nevertheless, these findings should be regarded as preliminary experimental evidence. Further investigations are required to determine the specific bioactive compounds responsible for the observed effects, establish dose-response relationships, assess long-term safety, standardize honey according to botanical and physicochemical characteristics, and determine whether the findings can be translated into clinically meaningful benefits in humans.

Limitations of the study

The interpretation of the findings is subject to certain limitations. First, the investigation was conducted using an experimental animal model; therefore, the observed effects may not necessarily reflect the physiological and therapeutic responses that would occur in humans. In addition, the individual bioactive constituents of the honey sample were not isolated or chemically characterized, making it difficult to attribute the observed biological effects to specific compounds. The antioxidant assessment was also performed using *in vitro* assays, which may not completely reproduce the complex biological environment *in vivo*. Further studies involving detailed phytochemical characterization, mechanistic investigations, longer-term toxicity assessment, and well-controlled clinical trials are recommended to establish the therapeutic relevance of honey in diabetes management.

CONCLUSIONS

The findings of this study demonstrate that honey possesses appreciable antihyperglycemic and antioxidant properties in alloxan-induced diabetic male albino rats. Honey administration produced a dose-related reduction in fasting blood glucose, with the highest tested dose producing the most pronounced glucose-lowering response. The honey sample also demonstrated substantial DPPH and ABTS radical-scavenging capacities, ferric-reducing activity, and appreciable levels of total phenolic and flavonoid compounds. These findings suggest that the biological activity of honey may involve complementary glucose-regulatory and antioxidant mechanisms, including attenuation of oxidative stress. Although the results provide promising experimental evidence for the use of honey as a potential complementary nutritional intervention in diabetes management, further investigations are necessary to establish its active constituents, optimal dosage, safety profile, and clinical efficacy.

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AUTHORS' CONTRIBUTIONS

Ogochukwu AP: conceived and designed the study, supervision, data interpretation, writing original draft. **Ndubuisi AP:** experimental procedures, laboratory analyses, data collection. **Amarachi CNP:** experimental procedures, laboratory analyses, data collection. **Okwudiri OA:** sample analysis, data interpretation, literature review. **Somtochukwu MB:** data organization, critical review. **Esther CEC:** sample analysis, data interpretation. **Cemaluk EAC:** sample analysis, interpretation of results, literature review. **Ebere UP:** data organization, critical review. All authors reviewed and approved the final version of the manuscript.

DATA AVAILABILITY

The datasets generated and/or analyzed during the present study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this study.

REFERENCES

1. American Diabetes Association. Standards of care in diabetes-2024. Diabetes Care 2024; 47(1): 1-350. *https://doi.org/10.2337/dc24-SDIS*
2. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev 2013; 93(1): 137-188. *https://doi.org/10.1152/physrev.00045.2011*
3. Cianciosi D, Forbes-Hernández TY, Afrin S, *et al*. Phenolic compounds in honey and their associated health benefits: A review. Molecules 2018; 23(9): 2322. *https://doi.org/10.3390/molecules23092322*
4. Erejuwa OO, Sulaiman SA, Wahab MSA. Honey: A novel antioxidant. Molecules 2012; 17(4): 4400-4423. *https://doi.org/10.3390/molecules17044400*
5. Samarghandian S, Farkhondeh T, Samini F. Honey and health: A review of recent clinical research. Pharmacogn Res 2017; 9(2): 121-127.
6. Alaebo PO, Onyeabo C, Oriaku CE, *et al*. Hepato-protective effect and lipid profile of honey on alloxan-induced diabetic rats. Asian J Res Biochem 2022; 10(1): 16-24. *https://doi.org/10.9734/AJRB/2022/v10i130212*
7. Alaebo PO, Umeh VA, Njoku GC, *et al*. Antioxidant and nephroprotective effects of honey in alloxan-induced diabetic rats. J Appl Life Sci Int 2022; 25(2): 26-32. *https://doi.org/10.9734/JALSI/2022/v25i230285*
8. Pasupuleti VR, Sammugam L, Ramesh N, *et al*. Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. Oxid Med Cell Longev 2020; 2020: 1864168.

9. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power. Anal Biochem 1996; 239(1): 70-76. *https://doi.org/10.1006/abio.1996.0292*
10. Re R, Pellegrini N, Proteggente A, *et al*. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26(9-10): 1231-1237. *https://doi.org/10.1016/S0891-5849(98)00315-3*
11. Balde GH, Gautam PK, Bhoumik MK, *et al*. Investigation of a polyherbal formulation's anti-diabetic effectiveness in rats. Biochem Cell Arch 2026; 26(1): 629-634. *https://doi.org/10.51470/bca.2026.26.1.629*
12. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 1995; 28(1): 25-30. *https://doi.org/10.1016/S0023-6438(95)80008-5*
13. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299: 152-178. *https://doi.org/10.1016/S0076-6879(99)99017-1*
14. Chang CC, Yang MH, Wen HM, *et al*. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10(3): 178-182. *https://doi.org/10.38212/2224-6614.2748*
15. Bogdanov S, Jurendic T, Sieber R, *et al*. Honey for nutrition and health: A review. J Am Coll Nutr 2008; 27(6): 677-689. *https://doi.org/10.1080/07315724.2008.10719745*
16. Al-Waili N, Salom K, Al-Ghamdi A, *et al*. Antibiotic, pesticide, and microbial contaminants of honey: Human health hazards. Sci World J 2011; 12: 766-787. *https://doi.org/10.1100/tsw.2011.78*
17. Abdulrhman MA, El-Hefnawy MH, Ali RA, *et al*. Effects of honey, sucrose, and glucose on blood glucose levels in diabetic children: A randomized clinical trial. Acta Diabetol 2013; 50(6): 851-856.
18. Brownlee M. The pathobiology of diabetic complications: A unifying mechanism. Diabetes 2005; 54(6): 1615-1625. *https://doi.org/10.2337/diabetes.54.6.1615*
19. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010; 107(9): 1058-1070. *https://doi.org/10.1161/CIRCRESAHA.110.223545*
20. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol 2018; 14(2): 88-98. *https://doi.org/10.1038/nrendo.2017.151*
21. Ríos JL, Francini F, Schinella GR. Natural products for the treatment of type 2 diabetes mellitus. Planta Med 2018; 84(5): 257-267.
22. Kondreddy VK, Saini D, Bhoumik MK, *et al*. Analytical method development and validation of antihypertensive drug telmisartan and hydrochlorothiazide by using RP-HPLC. Biochem Cell Arch 2025; 25(2):1987-1993. *https://doi.org/10.51470/bca.2025.25.2.1987*
23. Kim Y, Keogh JB, Clifton PM. Polyphenols and glycemic control. Nutrients 2016; 8(1): 17. *https://doi.org/10.3390/nu8010017*
24. Al-Ishaq RK, Abotaleb M, Kubatka P, *et al*. Flavonoids and their anti-diabetic effects: Cellular mechanisms and therapeutic potential. Nutrients 2019; 11(6): 1247. *https://doi.org/10.3390/biom9090430*
25. Molan PC. The evidence supporting the use of honey as a wound dressing. Int J Low Extrem Wounds 2015; 14(2): 110-116.
26. Ahmed S, Othman NH. Review of the medicinal effects of tualang honey and a comparison with manuka honey. Malays J Med Sci 2013; 20(3): 6-13.
27. Sies H. Oxidative stress: Concept and some practical aspects. Antioxidants 2020; 9(9): 852. *https://doi.org/10.3390/antiox9090852*
28. Alvarez-Suarez JM, Gasparrini M, Forbes-Hernández TY, *et al*. The composition and biological activity of honey: A focus on its antioxidant, antimicrobial and anti-inflammatory properties. Nutrition 2013; 30(10): 1182-1189.