Inhibitory effects of Nigerian Sweet and Bitter Honey on Pancreatic Alpha Amylase Activity

***Adeoye Bayo Olufunso[1], Iyanda Abolape Ayobola[1], Oyerinde Ayodeji Michael[2], Oyeleke Ibukunoluwa Oyebimpe[3], Fadeyi Blessing Olayinka[4]**

[1]*Department of Chemical Pathology, Faculty of Basic Medical sciences, College of Health Sciences, Ladoke Akintola University of Technology (LAUTECH), P.M.B 4000, Ogbomoso, Oyo State, Nigeria.*

[2]*Department of Forestry and Wood Technology, Federal University of Technology Akure (FUTA), Ondo State, Nigeria.*

[3]*Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University (O.A.U), Ile – Ife, Osun State, Nigeria.*

[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University (O.A.U), Ile – Ife, Osun State, Nigeria.*

***Corresponding author**: adeoye.ilemobayo@gmail.com*

ABSTRACT

***Background:** Nigeria has the highest prevalence of diabetes in Sub - Saharan Africa. The relative contraindications associated with conventional oral hypoglycemic drugs demand the search for nutraceuticals as ideal alternatives.*

***Objective**: In this study, we aimed at elucidating possible antiglycemic properties of Nigerian sweet and bitter honey from Apis Mellifera Andasonii through the pathway of alpha amylase inhibition.*

***Method**: The pH of the honey samples was determined using a digital pH meter (pHs-2F, Harris, England). Standard assay methods using 3, 5 - dinitrosalicylic acid (DNSA) was used to analyse alpha amylase activity invitro. The honey concentration which inhibited 50% alpha amylase activity (IC_{50}) was determined using the dose response curve. Data were analysed using student t-test on graph pad prism 6.1.*

***Result:** The sweet and bitter honey samples exhibited low pH values of 3.30 and 3.38 respectively. The dose dependent inhibition of alpha amylase activity was significantly ($p<0.05$) higher in sweet honey (94.73% at 1 mg/mL) than in bitter honey (92.06% at 1 mg/mL) with IC_{50} values of 0.157 ± 0.023 mg/mL and 0.255 ± 0.049 mg/mL respectively.*

***Conclusion**: Both sweet and bitter honey varieties used for this study are potential new sources of alpha amylase inhibitor. They can be appraised as novel indigenous functional foods for regulating postprandial hyperglycemia.*

Keywords: Diabetes, Alpha Amylase, Hyperglycemia, Honey.

INTRODUCTION

The raging epidemics of diabetes mellitus (DM) is a global public health concern. The disease which was earlier thought to be peculiar to affluent countries is now very rampant among developing nations. Nigeria has the highest occurrence of diabetes in sub – Saharan Africa with a prevalence of 5.7% (4.7 million people)(1). The baseline symptom of diabetes is hyperglycaemia which occurs as a result of delayed clearance of glucose from blood(2). Long term

hyperglycaemia takes in its toll a plethora of damaging effect on vital organs of the body(3). Ameliorating hyperglycaemia alone will be a landmark breakthrough for improving treatment outcomes of DM.

Alpha Amylase (1, 4 – α – D – glucan - glucanohydrolase, EC 3.2.1.1) is a digestive enzyme which initiates the catalytic breakdown of carbohydrates to specific glucose containing monomers(4). The pathway of its inhibition is one of the therapeutic targets for controlling hyperglycaemia in the diabetic state. The optimum pH for α – amylase is between 6.7 and 7.1(5). A lowered pH condition in its microenvironment may likely be a major limiting factor to its catalytic efficiency.

Meanwhile in developing nations, poor compliance to conventional antidiabetic drugs due to affordability, availability and undesirable contraindications has been reported(6). Much so, the contraindications associated with oral hypoglycaemic agents make the search for ideal nutritional supplements inevitable.

The role of honey as a nutritional supplement has been widely documented. It is a natural medium which conserves essential plant nutrients which may be organic or inorganic. The role of honey as an ideal functional food is solely attributable to its bioactive components such as phytochemicals, enzymes, amino acids, fructose, vitamins and trace elements . Owing to the botanical source, the physicochemical properties and corresponding nutraceutical value of honey can be divergent (7). Sweet honey is commonly available worldwide, but the bitter variety is quite rare. Within the past decade, the scientific community is beginning to explore their antidiabetic propensities. There are conflicting reports concerning the roles of honey as an ideal component of medical nutrition therapy (MNT) particularly for the management of diabetes. While several studies have documented its therapeutic value in the diabetic state (8), its detrimental (9), (10), and non-significant effects (11), (12) have also been reported. The α – amylase inhibitory potentials of Nigerian honey varieties are scarcely explored. Although some of the indigenous plants which constitute the botanical sources of our sweet and bitter honey have been documented for their roles in inhibiting α – amylase activity, it is not known whether this health benefit can be replicated by supplementation with those honey samples. Till date, the effect of these sweet and bitter honey variety on pancreatic α – amylase activity has neither been reported or compared. The present study was therefore designed to do just.

MATERIALS

Honey

Each honey sample was collected at different botanical source in Southwestern Nigeria. Bitter honey was harvested at a branch of Community Lifestyle Improvement Project Farm (RC: 2930642) located at Modakeke (7° 27' 19.6704" N and 4° 32' 39.8112" E), Osun State, Nigeria. The sweet honey was harvested at Federal University of Technology Akure (FUTA) (7^0 15' 2.7756" N and 5^0 12' 36.9576" E), Ondo State Nigeria.

Reagents

Pancreatic α - amylase, 3, 5- dinitrosalicylic acid (DNSA) and starch soluble products were purchased from Sigma Aldrich Co., St Louis, USA. Other chemicals and reagents used were of analytical grade and purchased from reputable organizations.

METHOD

Determination of pH of Honey

The pH of the honey samples was determined according to standard method described by the Association of Official Analytical Chemists. The digital pH meter (pHs-2F, Harris, England) used was calibrated with a standard buffer solutions having a pH of 4.0 and 7.0. Afterwards, ten millilitres (10 mL) of each honey sample was transferred into a beaker and the pH was determined. The readings were taken after equilibration was ensured.

Alpha - Amylase Inhibition Assay

The analysis was done according to the procedure of (13). Each honey sample (1.25–10 mg/mL) was placed in a tube and 250 L of 0.02 M sodium phosphate buffer (pH 6.9) containing amylase solution (0.5 mg/mL) was added. The solution was initially incubated at 25^0 C for 10 min, after which 250 L of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at timed intervals and then further incubated at 25^0C for 10 min. The reaction was terminated by

adding 0.5 mL of dinitrosalicylic acid (DNSA). The tubes were then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture was diluted with 5 mL distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure by replacing the honey samples with distilled water. The amylase inhibitory activity was calculated as shown below.% Inhibition = [Absorbance of control - Absorbance of Honey] × 100

The honey concentration resulting in 50% inhibition of enzyme activity (IC_{50}) was determined from a dose response curve on graph pad prism (verion 6.1).

Statistical Analysis

The raw data were analyzed using Graph Pad Prism® software (version 6.1). Mean differences were determined by student t – test. Significance was set at $p < 0.05$. All data are presented as mean ± standard error of mean (SEM).

RESULT

Percentage Inhibition

As shown in table 1, the sweet and bitter honey samples at a concentration of 1mg/mL inhibited alpha amylase activity by 94.73% and 92.06% respectively.

pH and IC_{50} Values

As shown in table 2, the IC_{50} value of sweet honey (0.157 ± 0.023 mg/mL) is significantly lower than that of bitter honey (0.255 ± 0.049 mg/mL). Also, the pH value obtained for bitter honey is significantly higher than the pH value of sweet honey.

DISCUSSION

Both Nigerian sweet and bitter honey varieties used for this study showed a dose dependent inhibition of pancreatic α – amylase activity. However, inhibition by sweet honey was

Table 1: Percentage Inhbition of α - Amylase Activity by Sweet and Bitter Honey

Concentration (mg/mL)	Inhibition by Sweet Honey (%)	Inhibition by Bitter Honey (%)
1	94.73 ± 1.43	92.06 ± 1.03
0.5	81.29 ± 0.67	80.14 ± 1.01
0.25	56.84 ± 6.84	74.47 ± 0.20
0.125	48.66 ± 1.67	58.30 ± 5.31
0.0625	46.55 ± 1.63	30.50 ± 4.40
0.03125	33.33 ± 1.35	14.61 ± 0.51

Data are presented as mean ± SEM of triplicate values

Table 2: pH and IC_{50} Values of Sweet and Bitter Honey

Honey Type	pH	IC_{50} (mg/mL)
Sweet Honey	3.30 ± 0.000 *	0.157 ± 0.023 *
Bitter Honey	3.38 ± 0.003	0.255 ± 0.049

Data are presented as mean ± SEM of triplicate values.
Value is significant at (* $p < 0.05$)

significantly higher than bitter honey. The inhibition of α – amylase activity by our honey samples agrees with the report of previous investigators. Acarbose, a conventional oral hypoglycaemic agent has an α – amylase inhibitory activity of 91.97% (14). Bee propolis from Morroco have also been reported for remarkable inhibition of α – amylase activity (15). It is well known that the nutraceutical value of honey is predominantly a function of botanical source (16). This shows that the α – amylase inhibitory potency of the sweet and bitter honeys used for this study may be related to the indigenous plant source of its bioactive mechanisms.

Honey is a natural repository of plant secondary metabolites (17). Some of these phytochemicals are quite specific in there interactions with the active site of alpha amylase so as to retard its catalytic efficiency. Flavonoids and phenols have gained the best attention in this regard (18). (19) proposed that the 95.4% inhibition of α – amylase activity exerted by Syzygium cumini was a function of its flavonoid content. Using an insilico approach, the flavonoid kaempferol exhibited a relatively higher docking score against α – amylase activity than other compounds (20). In another insilico study, certain phenolic compounds competitively displaced acarbose from the active site of alpha amylase (21). The nutraceutical potency of honey is usually homologous to the botanical source of its phytochemicals (22). Thus, the significant variations in the alpha amylase inhibitory properties of both honey varieties. This suggests that the α – amylase inhibitory potentials of the honey samples used for this study may have contributed by their constituent phytochemicals.

In the present work, the pH value obtained for each honey sample is distinct from the report of previous investigators. Much lower pH values of 3.27 and 3.24 was reported for two Malaysian stingless bee honey (23) whereas Malaysian Manuka honey has a higher pH of 4.10 (24). Moreover, pH range of 3.73 to 4.50 was obtained for bitter honey samples native to five different communities in Ekiti State, South western Nigeria (25). A bitter honey sample native to Algeria was reported to have pH values of 4.59 (26) while pH values of 4.83 and 4.85 were obtained for two samples of bitter honey from different botanical sources in India (27).

Considering the optimum pH of α – amylase enzyme, the pH value obtained for either the sweet or bitter honey used for this study can as well contribute to the inhibition of α – amylase activity. This therapeutic approach does not effect a clearance of glucose from the blood unlike insulinomimetics or insulin analogues, but rather ensure a strict regulation of glucose available for absorption through the hepatic portal vein, thereby limiting postprandial glucose surge (28). The denaturation of α – amylase can be ensured under a pH condition below 4.0 (29). Therefore, a temporary alteration in the functional microenvironment of α – amylase without upsetting the biochemistry of the gastrointestinal tract is a promising therapeutic approach for curtailing hyperglycaemia in the diabetic state.

Therefore, recommending a daily intake value for any branded honey variety requires due consideration to its alpha amylase inhibitory propensity. This will spare the populace of the acute ailments associated with indiscriminate consumption of honey.

CONCLUSION

The Nigerian sweet and bitter honey samples used for this study possess remarkable α – amylase inhibitory mechanisms. Both honey varieties may therefore serve as ideal nutraceuticals which can potentially regulate post prandial glucose surge in the diabetic state.

Author's Contributions

Adeoye B.O conceived and designed the study. Iyanda A.A supervised the study. Adeoye B.O sourced for the bitter honey while Oyerinde A.M sourced for the sweet honey. Adeoye B.O, Oyeleke I.O and Fadeyi B.O conducted the laboratory analysis. Oyerinde A.M conducted the statistical analysis. Adeoye B.O wrote the first draft of the manuscript. All authors proof read and approved the final manuscript.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no competing interest of any kind which may have appeared to influence the work reported in this paper.

REFERENCES

1. Adeloye D., Ige JO, Aderemi AV, Adeleye N., Amoo E.O., Auta A., Oni G. (2017).

"Estimating the prevalence, hospitalisation and mortality from type 2 diabetes mellitus in Nigeria: a systematic review and meta-analysis," BMJ Open, 7:e015424

2. Sheriff M, Tukur M, Sera S. (2011). The effect of oral administration of honey and glucophage alone or their combination on the serum biochemical parameters of induced diabetic rats. Research in Pharmaceutical Biotechnology, 39: 118-122.

3. American Diabetes Association. (2021). Children and Adolescents: Standards of Medical Care in Diabetes. Diabetes Care, 44:S180–99.

4. Williams J.A. (2019). Amylase. Pancreapedia, doi: 10.3998/panc.2019.02

5. Worthington P, Hoang V, Perez-Pomares F, Blum P. (2003). Targeted Disruption of the α-Amylase Gene in the Hyperthermophilic Archaeon Sulfolobus solfataricus. Journal of Bacteriology, 185:482–488.

6. Chaudhary N, Tyagi N. (2018). Medicinal plants used for Diabetes mellitus: An Overview. International Journal of Research and Development in Pharmacy & Life Sciences,7:3022–30299.

7. Bogdanov S. (2011). Honey Composition. Bee Product Science. www.bee-hexagon.net

8. Asuquo AE, Obia O, Chuemere AN. (2018). Prolonged Effect of Niger Delta Honey on Blood Glucose and Haematological Parameters in Alloxan Induced Diabetic Rats. International Journal of Biochemistry Research & Review, 2018; 21:1–10.

9. Bahrami M, Ataie-Jafari A, Hosseini S, Foruzanfar M, Rahmani M, Pajouhi M. (2009). Effects of natural honey consumption in diabetic patients: An 8-week randomized clinical trial. International journal of food sciences and nutrition, 60:618–626.

10. Abdulrhman M. Honey as a Sole Treatment of Type 2 Diabetes Mellitus. (2016). Endocrinology & Metabolic Syndrome, 5:232.

11. Al Aamri ZM, Ali BH. (2017). Does honey have any salutary effect against streptozotocin- induced diabetes in rats? Journal of Diabetes and Metabolic Disorders https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5260029/.

12. Sahlan M, Rahmawati O, Pratami D, Raffiudin R, Mukti R, Hermasyah H. (2019). Effects of Stingless Bee (Tetragonula biroi) Honey on Streptozotocin-Induced Diabetes Mellitus in Rats. Saudi Journal of Biological Sciences, https://doi.org/10.1016/j.sjbs.2019.11.039

13. Okonji Raphael, Akinwunmi K, Madu J, Bamidele F, Funmilola A. (2015). In Vitro study on α-amylase inhibitory activities of Digitaria exilis, Pentadiplandra brazzeana (Baill) and Monodora myristica . International Journal of Biological and Chemical Sciences, 25: 2306-2313.

14. Vadasery K, Ukkuru P. (2017). In vitro antidiabetic activity and glycemic index of bee honeys. Indian journal of traditional knowledge, 16:134–140.

15. El-Guendouz S, Smail A, Lyoussi B, Antunes M, Faleiro ML, Miguel M. (2016). Anti-acetylcholinesterase, antidiabetic, anti-inflammatory, antityrosinase and antixanthine oxidase activities of Moroccan propolis. International Journal of Food Science & Technology, 51:1762–1773.

16. Codex. (2001). Standards | CODEX ALIMENTARIUS FAO-WHO

17. Ajibola A. (2015). Novel Insights into the Health Importance of Natural Honey. Malaysian Journal of Medical Science, 22:7–22.

18. Martínez-González A, Díaz-Sánchez Á, De la Rosa L, Bustos-Jaimes I, Alvarez-Parrilla E. (2018). Inhibition of α-amylase by flavonoids: Structure activity relationship (SAR). Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 5:437-447.

19. Prabakaran K, Shanmugavel G. (2017).

Antidiabetic Activity and Phytochemical Constituents of Syzygium cumini Seeds in Puducherry Region, South India. International Journal of Pharmacognosy and Phytochemical Research, 9: 985-989

20. Jackie B, Sagar S, Alamgir H, Razowanul Md F, Sumaiya F, Emon D, et al. (2018). In silico Molecular Docking and ADME/T Analysis of Some Selected Isolated Compounds of Phyllanthus emblica against Type 2 Diabetics. American Journal of Ethnomedicine. 5(2), doi: 10.21767/2348-9502.100009

22. Selvaraju K, Vikram P, Soon JM, Krishnan KT, Mohammed A. (2019). Melissopalynological, physicochemical and antioxidant properties of honey from West Coast of Malaysia. Journal of food science and technology, 56:2508–2521

23. Abu Bakar MF, Sanusi S, Abu Bakar F, Cong O, Mian Z. (2017). Physicochemical and Antioxidant Potential of Raw Unprocessed Honey From Malaysian Stingless Bees. Pakistan Journal of Nutrition, 16:888–94.

24. Moniruzzaman M, Khalil MI, Sulaiman SA, Gan SH. (2014). Physicochemical and antioxidant properties of Malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera. BMC Complementary and Alternative Medicine, http://dx.doi.org/10.1155/2014/359890

25. Oyeyemi SD, Kayode J, Owolabi MO. (2015). Comparative Nutritional Studies on Honey Samples in Ado Ekiti, Ekiti State. Donnish Journal of Medicinal Plant Research, 2: 016-020.

26. Otmani I, Abdennour C, Dridi A, Kahalerras L, Halima-Salem A. (2019). Characteristics of the bitter and sweet honey from Algeria Mediterranean coast. Vetenary World, 12: 551–557.

27. Joshna K, Gopal V, Kavitha B. (2019). Phytochemical Screening, Physicochemical Properties And Total Phenolic Content Of Bitter Honey Samples. International Research Journal of Pharmacy. 10:121–125.

28. Tundis R, Loizzo Mr, Menichini F. (2010). Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: Mini reviews in medicinal chemistry. https://pubmed.ncbi.nlm.nih.gov/20470247/.

29. Yadav JK, Prakash V. Stabilization of α-Amylase, the Key Enzyme in Carbohydrates Properties Alterations, at Low pH. International Journal of Food Properties, 14:1182–1196.