In Vitro Inhibitory Potential Of Honey Against α - Glucosidase Activity

Nisha V. Warade
Department of Zoology, Shankarlal Khandelwal Arts,
Science and Commerce College, Akola, India.

Abstract

The health promoting characteristics of honey are mainly due to the presence of multiple metabolites including vitamins and essential minerals besides enzymes and co-enzymes. Honey is a valuable supplement for a healthy population as its benefits include "antioxidant", "anti-inflammatory" and "antimicrobial" activities. The nutritional value of honey depends on the floral source of nectar, types of bees and geographical and climatic conditions of the environment. In the present investigation, three unifloral honeys from three different floral sources *i.e. Helianthus* (Sunflower), *Citrus sinensis* (Orange), and *Punica granatum* (Pomegranate) were investigated to ascertained for the potential inhibitory effect *in vitro* on key enzyme linked to type-2 diabetes (α - glucosidase) in order to provide some possible mechanism by which it can be used in the management of the disease. The IC_{50} values of all the honeys analysed were in range from 308.780 to 767 (µg/ml). From the obtained IC_{50} values for different honeys it was clear that *Citrus sinensis* honey had the least IC_{50} value of 308.780 (µg/ml) followed by *Punica* granatum honey (485.714 µg/ml). The highest IC_{50} value was noticed in *Helianthus* honey of 767 (µg/ml).

Keywords: ***Alpha-glucosidase, Diabetes mellitus, Antihyperglycemia, Honey, Hypoglycemia***

Introduction:

Honey is a sweet viscous natural substance made by honey bees using the nectar part of the flowers. Honey contains amino acids, vitamin B, Vitamin B6, Vitamin C, niacin, folic acid, minerals, iron, zinc and antioxidants [1]. The health promoting characteristics of honey are mainly due to the presence of multiple metabolites including vitamins and essential minerals besides enzymes and co-enzymes. In principle, honey is a valuable supplement for a healthy population [2]. Among honey benefits are its anti-inflammatory [3], anti-oxidant [4] and anti-microbial effects [5]. Further-more, several studies have shown that honey produced an attenuated postprandial glycemic response when compared with sucrose in both patients with diabetes and normal subjects [6]. Honey administration was found to increase serum levels of insulin while it reduced serum concentrations of glucose and fructosamine in diabetic rats [7]. Honey, might be beneficial as an adjunct to anti-diabetic drugs in the treatment of diabetic patients [8].

Diabetes mellitus remains an incurable disorder which is associated with poor quality of life, cardiovascular complications and increased mortality [9]. Diabetes mellitus is characterized by abnormal elevated postprandial blood glucose levels. In treatment of type 2 diabetes, suppression of postprandial hyperglycemia can decrease the risk of various complications. Hence, control of postprandial hyperglycemia should be a primary goal in the prevention and management of type 2 diabetes. A sudden rise in blood glucose levels, causing hyperglycemia in type 2 diabetes patients happens due to hydrolysis of starch by pancreatic α-amylase and uptake of glucose by intestinal α-glucosidases [10]. Alpha-glucosidases are a series of enzymes, including sucrase and maltase, located in the brush-border surface of intestinal cell, which catalyze the final step in the digestive process of carbohydrates to release absorbable monosaccharides resulting in increased blood glucose levels [11]. If *α*-glucosidases are inhibited, the liberation of D-glucose from dietary complex carbohydrates can be retarded. Thus, *α*-glucosidase inhibitors delay the digestion and absorption of carbohydrates and hold back postprandial hyperglycemia. At present, some *α*-glucosidase inhibitors, such as

acarbose, miglitol, and voglibose, have been approved for clinical use in the management of type 2 diabetes. However, some synthetic *α*-glucosidase inhibitors have side-effects, such as flatulence, diarrhea, and abdominal cramping, all of which are associated with incomplete carbohydrate absorption [12]. As a result, many researchers have focused on novel *α*-glucosidase inhibitors from natural materials, which are used to develop functional foods or lead compounds for antidiabetic treatment including *Syagrus romanzoffiana*, *Adhatoda vasica Nees,* and *Syzygium cumini* (Linn.) seed kernel [13]. There has been an enormous interest in the development of alternative plant and animal based medicines for type 2 diabetes. The alternative approach to diabetes therapy includes the use of herbal preparations, dietary components or supplements and other natural products such as honey [14]. In the last few years, there has been an increased interest in the therapeutic uses of honey.

Hence, the goal of the present study is to investigate the potential inhibitory effect of *honey in vitro* on key enzyme linked to type-2 diabetes (α - glucosidase) in order to provide some possible mechanism by which it can be used in the management of type-2 diabetes.

Materials and Method:

Sample collection

A total of three unifloral honey samples from three different floral sources *i.e. Helianthus* (Sunflower), *Citrus sinensis* (Orange), and *Punica granatum* (Pomegranate) were collected from the market. All other chemicals used in this study were obtained commercially and were of analytical grade.

***In Vitro* Alpha (α) Glucosidase inhibition assay**

The α - glucosidase inhibitory activity can be measured *in vitro* by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by alpha – glucosidase enzyme. Alpha – glucosidase inhibitory activity was evaluated according to the chromogenic method, with some modification. This process was carried out by quantifying the reducing sugar (glucose equivalent) liberated under the assay condition.

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentration of honey for 5 min at 37ºC. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1 U/ml) to it, followed by incubation for 10 min at 37ºC. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method. The mixture was then incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water. Absorbance of the honey samples was measured at 540 nm while the reaction system without honey samples was used as control. The system without alpha-glucosidase was used as blank. Each experiment was conducted in triplicate.

Calculation of 50% inhibitory concentration (IC_{50})

The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The concentration of the honey required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations. Then, the α-amylase inhibitory activity was calculated as percentage inhibition. Inhibitory activity was expressed by subtracting relative:

$$\% \text{ Inhibition} = [(\text{Abs}_{(\text{Control})} - \text{Abs}_{(\text{Honey samples})}) / \text{Abs}_{(\text{Control})}] \times 100$$

Result and Discussion

Evaluation of *in vitro* α-glucosidase inhibitory activity using honey

All the honey for the assay were taken at concentrations ranging from 100-1000 µg/ml and the results of which reveal that at all the concentrations, honey from *Citrus sinesis* achieved highest alpha glucosidase inhibition. All the other honey exhibited different rate of inhibition at different concentrations.

The ability of honey from different floral sources to inhibit α-glucosidase activity *in vitro* was investigated and the results are presented in Table 1. The honey from different floral sources revealed a significant inhibitory action on α-glucosidase enzyme. The percentage inhibition at 100-1000 µg/ml concentrations of different honey showed a concentration dependant increase in percentage inhibition. The percentage inhibition varied from 36.78 to 81.67 for lowest concentration of 100 µg/ml to the highest concentration of 1000 µg/ml in *Citrus sinensis*, 22.52 to 62.93 in *Helianthus* and 28.86 to 73.28 in *Punica granatum*. The concentrations required for 50% inhibition (IC_{50}) was found to be 308.780 µg/ml in *Citrus sinensis*, 767 µg/ml in *Helianthus* and 485.714 µg/ml in *Punica granatum* whereas the α-glucosidase inhibitory activity of control acarbose produced percentage of 38.35 for 100 µg/ml and 91.76 for 1000 µg/ml. The IC_{50} value of standard drug acarbose against α-glucosidase was found to be 216.530 µg/ml (Table 1).

The anti-hyperglycemic activity of honeys under the study was compared with a standard antidiabetic drug acarbose and it could be noted that the alpha glucosidase inhibition capacity of acarbose ranged from 38.35 % to 91.76 %. Even though the honeys under the study had moderate inhibition capacity, the *Citrus sinensis* honey & *Punica granatum* honey proved to have comparatively good anti-hyperglycemic effect.

The efficacy of the honeys against alpha glucosidase was studied by fitting the regression equations and obtaining the IC_{50} value which denotes the concentration of honey to obtain 50% of inhibition over alpha glucosidase enzyme. The IC_{50} values of all the honeys analysed were in range from 308.780 to 767 (µg/ml). From the obtained IC_{50} values for different honeys it was clear that *Citrus sinensis* honey had the least IC_{50} value of 308.780 (µg/ml) followed by *Punica* granatum honey (485.714 µg/ml). The highest IC_{50} value was noticed in *Helianthus* honey of 767 (µg/ml).

Table 1: Alpha-glucosidase inhibition by honey from three different floral sources

Sample	Concentration (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Citrus sinensis (Orange plant)	100 200 400 800 1000	36.78 49.36 58.12 65.07 81.67	308.780
Helianthus (Sunflower plant)	100 200 400 800 1000	22.52 29.73 35.26 48.42 62.93	767
Punica granatum (Pomegranate plant)	100 200 400 800 1000	28.86 42.56 49.12 59.65 73.28	485.714

Standard (Acarbose)	100 200 400 800 1000	38.35 52.64 66.21 72.56 91.76	216.530

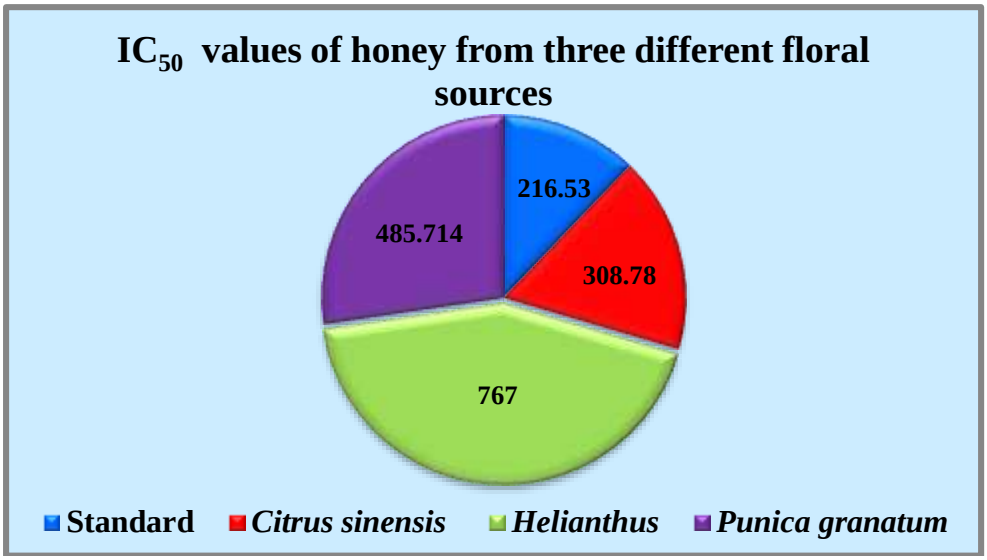

Fig 1: IC_{50} (µg/ml) values of honey from three different floral sources

Conclusion and Recommendations:

The findings of animal studies may not be justly generalized to human condition, before endorsing honey as a preferred sugar substitute, therapeutic or dietary supplement in diabetic patients. There is very less data and literature implying the possible impact of honey in diabetes mellitus. There are few research implications and issues raised from the studies which show the beneficial effects of honey in the treatment of diabetes mellitus. There is no doubt that much more well-designed, rigorously conducted randomized controlled studies are necessary to further validate these findings. The dietary or therapeutic use of honey in diabetic patients has obstacles and challenges and needs more large sample sized, multi-center clinical controlled studies to reach better conclusions. Further studies are required to investigate the effects of long term consumption of honey in these patients. This opens up scope for its utilization by diabetic patients who have a sweet tooth.

References:

1. B. Fatimah, G. Abubakar, S. Aliyu. (2013) Analysis of biochemical composition of honey samples from north-east Nigeria Biochem. Anal. Biochem. 2 (3) : 1000139
2. B. Denisow, M. Denisow-Pietrzyk. (2016) Biological and therapeutic properties of bee pollen. A review J. Sci. Food Agric. 10:1002
3. Al Waili, N. & Boni, N. (2003) Natural honey lowers plasma prostaglandin concentrations in normal individuals. J Med Food 6 (2) :129–133
4. Gheldof, N. & Engeseth, N. (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J Agric Food Chem. 50 (10) : 3050–3055
5. Theunissen, F.; Grobler, S. & Gedalia, I. (2001) The antifungal action of three South African honeys on Candida albicans. Apidologie. 32 (4) : 371–379

6. Shambaugh, P.; Worthington, V. & Herbert, J. (1990) Differential effects of honey, sucrose, and fructose on blood sugar levels. J Manip Physiol Ther. 13 : 322–325
7. Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KN, Salleh MS, Gurtu S. (2011) Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. Int J Biol Sci. 7: 244–252.
8. Das SK, Vijayakumar PA, Senthil R, Bhatt JK, Gupta S. (2012) Antioxidant effect of vitamin c on type 2 diabetes mellitus patients along with two different oral hypoglycemic agents for smooth glycemic control. WJPPS. 1:1113–1122.
9. American Diabetes Association (2011) Diagnosis and classification of diabetes mellitus. Diabetes Care. 34 (Suppl 1) : S62–S69.
10. Kwon YI, Apostolidis E, Kim YC, Shetty K. (2007) Health benefits of traditional corn, beans and pumpkin: In vitro studies for hyperglycemia and hypertension management. J Med Food. 10 : 266–275.
11. Shai L. J., Masoko P., Mokgotho M. P. (2010) Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African Journal of Botany*. 76 (3): 465 – 470. doi: 10.1016/j.sajb.2010.03.002.
12. Ghadyale V., Takalikar S., Haldavnekar V., Arvindekar A. (2012) Effective control of postprandial glucose level through inhibition of intestinal alpha glucosidase by *Cymbopogon martinii* (Roxb.) *Evidence-Based Complementary and Alternative Medicine*. 2012:6. doi: 10.1155/2012/372909.372909
13. Shinde J., Taldone T., Barletta M. (2008) *α*-Glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats. *Carbohydrate Research*. ; 343 (7) : 1278–1281. doi: 10.1016/j. carres.2008 . 03.003.
14. Chawla R, Thakur P, Chowdhry A, Jaiswal S, Sharma A, Goel R, Sharma J, Priyadarshi SS, Kumar V, Sharma RK. (2013) Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a dreadful lifestyle disorder of 21st century. J Diabetes Metab Disord. 12 : 35. doi: 10.1186/2251-6581-12-35