COMPARATIVE STUDY OF *IN VITRO* ANTIDIABETIC & ANTIOXIDANT ACTIVITIES OF HONEYS OBTAINED FROM APIARIES

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Abstract

The source of honey heavily influences its antidiabetic and antioxidant properties. In the present study, on comparing the IC_{50} values (for antidiabetic and antioxidant activity) of three honeys i.e. honey from *Citrus sinensis*, *Punica* granatum and *Helianthus*, honey from *Citrus sinensis* showed lowest IC_{50} value, while the honey from *Helianthus* showed the highest IC_{50} value for both activities. Hence, it can be concluded that though all the honeys showed significant antidiabetic and antioxidant activity, honey from *Citrus sinensis* demonstrated significantly higher antidiabetic as well as antioxidant activity simultaneously amongst all the different types of honeys. Further, it can be concluded that honey from *Citrus sinensis* would be more potent and a healthier natural diet supplement, in not only managing diabetes and but also in reducing the oxidative stress caused by it, simultaneously and effectively.

Keywords: *Honey, Antioxidant, pharmaceutical, Apiculture by-products.*

Introduction

Diabetes (hyperglycemia) remains an incurable disorder throughout the world and is associated with poor quality of life, cardiovascular complications and increased mortality [1]. 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 [2]. Along with other complications, recent evidence suggests that diabetes is closely associated with oxidative stress [3, 4]. Hyperglycemia promotes auto-oxidation of glucose to form free radicals. This results in oxidative stress due to an imbalance between radical- generating and radical-scavenging (antioxidant defence)systems.The balance between the rate of free radical generation and elimination is important. The generation of free radicals beyond the scavenging abilities of endogenous antioxidant defenses results in macro- and microvascular dysfunction [5].Several studies have shown that diabetes mellitus (types 1 and 2) is accompanied by increased formation of free radicals and decreased antioxidant capacity, leading to oxidative damage of cell components. Oxidative stress may play a role in the pathophysiology of T_2DM and cardiovascular disease by increasing insulin resistance or impairing insulin secretion [6].

The inhibition of intracellular free radical formation would provide a therapeutic strategy to prevent oxidative stress and thus, related diabetic complications. Even though, different well-established artificial antioxidants (N-acetylcysteine, vitamin C (ascorbic acid), E (tocopherol), carotenoids and α-lipoic acid) effective in reducing diabetic complications have been studied intensively [5,7] but then also, now-a-days searching for more natural antioxidants, having no side-effects, are proving essential tools in the investigation of oxidative stress-related diabetic pathologies [8].

At the moment, the management of this disorder entails increased physical activity, healthy eating or diet and administration of anti-diabetic drugs and/or insulin (for the inhibition of α-amylase and α-glucosidase activity), along with other antioxidant drugs. There has been an enormous interest in the development of alternative plant and animal based medicines for the management of 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. In the last few years, there has been an increased interest in the therapeutic uses of honey [9].

The goal of the present study is to compare the antidiabetic and antioxidant properties *in vitro* of three different honeys and to find a single better source of honey, which is more potent and a healthier natural diet supplement, in not only managing diabetes and but also will also reduce the oxidative stress caused by it, simultaneously and effectively.

Materials and Method:

i) 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 apiaries directly. All other chemicals used in this study were obtained commercially and were of analytical grade.

ii) In Vitro alpha amylase inhibition assay

Alpha (α) - amylase inhibitory activity was determined according to the method of Bernfield (1951) with some modification. It can be measured *in vitro* by hydrolysis of starch in presence of alpha – amylase enzyme. This process was carried out by quantifying the reducing sugar (glucose equivalent) liberated under the assay condition. The enzyme inhibitory activity was expressed as decrease in units of glucose liberated [5]. A non-proteinaceous alpha amylase inhibitor (acarbose) was utilized as a standard or reference amylase inhibitor to compare the activity of the honeys against this enzyme.

Appropriate dilutions of the honey (500 µl) and 500 µl of 0.02 mol/l sodium phosphate buffer (pH 6.9 with 0.006 mol/l NaCl) containing α-amylase (0.5 mg/ml) were incubated at 25 °C for 10 minutes. Then, 500 µl of 1% starch solution in 0.02 mol/l sodium phosphate buffer (pH 6.9 with 0.006 mol / l NaCl) was added to the reacting mixture. Thereafter, the reaction mixture was incubated at 25 °C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid (DNSA) colour reagent. 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-amylase was used as blank. Each experiment was conducted in triplicate.

iii) Study of Quantitative antioxidant activity of honey by DPPH assay

The antioxidant activity (total free radical-scavenging capacity) of different types of honey was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay *in vitro* as previously reported [10,11] with some modifications. The honey samples were prepared to various concentrations by dissolving in methanol. A volume of 0.5 ml of the honey was mixed with 1.5 ml of the DPPH reagent and incubated in the dark at room temperature for 20 minutes. The absorbance of the reaction was measured at the wavelength 517 nm using UV visible spectrophotometer. The DPPH without honey was used as the control and methanol was used as the blank solution. The DPPH radical scavenging activity (antioxidant activity) and IC_{50} value was then calculated.

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

The IC_{50} value is defined as the concentration of inhibitor (honey) to inhibit 50% of the activity under the assayed conditions. The antioxidant and antidiabetic efficacy of the honey was studied by fitting the regression equation and obtaining the IC_{50} values. The IC_{50} *i.e.* concentration of the honey required to scavenge 50% of the free radicals and to inhibit α- amylase enzyme was calculated by using the percentage scavenging/ inhibiting activities at different concentrations. The inhibitory activity was calculated as percentage inhibition.

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

Result and Discussion

i) Evaluation of *in vitro* α-amylase inhibitory activity using honey

The ability of honey from different floral sources to inhibit α-amylase or antidiabetic activity *in vitro* was investigated and the IC_{50} values are presented in Table 1. The honey from different floral sources revealed a significant inhibitory action on α-amylase enzyme. The IC_{50} values of all the honeys analysed were in range from 281.590 to 751.628 (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 281.590 µg/ml followed by *Punica* granatum honey (428.292 µg/ml). The highest IC_{50} value of 751.628 µg/ml was noticed in *Helianthus* honey. Even though the honeys under the study had moderate to significant inhibition capacity, the *Citrus sinensis* honey proved to have comparatively good antidiabetic effect with highest α-amylase inhibition.

ii) Study of Quantitative antioxidant activity of honey by DPPH assay

The quantitative antioxidant activities of honey from different floral sources were evaluated using DPPH assays. The IC_{50} value for free radical scavenging ability of all the honeys analysed were in range from 88 to 114 mg/ml (Table 1). From the obtained IC_{50} values for different honeys, it was clear that *Citrus*

sinensis honey had the least IC_{50} value (88 mg/ml) for free radical scavenging ability followed by *Punica granatum* honey (110 mg/ml). The highest IC_{50} value of 114 mg/ml was noticed in *Helianthus* honey.

Table 1: IC_{50} (mg/ml) value of different honeys for free radical scavenging ability and inhibition of Alpha - amylase enzyme

Source of Honey	IC_{50} value for inhibition of Alpha-amylase enzyme (Antidiabetic activity)	IC_{50} value for free radical scavenging ability (Antioxidant activity)
Citrus sinensis (Orange) honey	281.590 µg/ml	88 mg/ml
Punica granatum (Pomegranate) honey	428.292 µg/ml	110 mg/ml
Helianthus (Sunflower) honey	751.628 µg/ml	114 mg/ml

The assays showed that honey from *Citrus sinensis* had the highest antioxidant activity *i.e.*DPPH scavenging ability, compared to other types of honey, whereas the honeys from *Helianthus* (Sunflower) had the lowest activity. As honey from *Citrus sinensis* demonstrated better activity than honey from *Helianthus* (Sunflower) and *Punica granatum* (Pomegranate), it suggests that *Citrus sinensis* honey iscomparatively a good antioxidant.

Conclusion

The source of honey heavily influence its antidiabetic and antioxidant activities. Comparative antidiabetic and antioxidant study in this article showed that even though the honeys under study had moderate inhibitory capacity, the honey from *Citrus sinensis* showed significantly higher antidiabetic as well as antioxidant activity simultaneously amongst all the different types of honeys.On comparing the IC_{50} values of three honeys i.e. honey from *Citrus sinensis*, *Punica* granatum and *Helianthus*, the results demonstrated that the greatest antidiabetic and antioxidant activity was found in the honey collected from the nectar of *Citrus sinensis*, while the honey obtained from the *Helianthus* flower demonstrated the lowest DPPH radical scavenging activity and antidiabetic property. This study revealed that the honey could provide novel compounds useful for the treatment of various diseases and that these honeys could be a good sources of nutrition as an antidiabetic as well as antioxidant molecules and may be used as potential components in medicines and cosmetic products as well.

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