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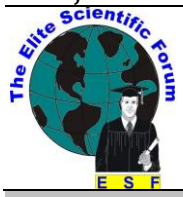

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RESEARCH ARTICLE

The Effect of Honey-Antioxidants on Blood Glycaemia in Normal Healthy Human Subjects

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ABSTRACT

This study was conducted to evaluate the correlation of antioxidants and Glycemic Indices (GI) of honey types in normal human subjects. In this study, antioxidative activity (AA), total phenolic contents (TPC) and total flavonoid contents (TFC) of 10 honey samples comprised of two natural honeys (locally harvested wild types) and eight commercial brands of honey were measured by three different analytical approaches i.e. 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$)) radical, Folin-Cioucalteau reagent and aluminum chloride, respectively. These antioxidative characteristics were correlated with the GI values calculated from postprandial blood glucose levels in human subjects, glucometerically. Ten normal healthy human adults were recruited for postprandial blood glucose measurement. Age of the participants ranged from 20-25 years. Comparatively, natural honeys had higher AA than commercial brands of honey (55.87±9.1 *v* 34.96±6.3 mg ascorbic acid/g). Similarly, TPC and TFC were also found higher in natural honey types than commercially available honey i.e. 8.01±0.63 *v* 4.52±0.64mg gallic acid/g and 53.15±2.47 *v* 29.23±4.32 mg rutin /g, respectively. Percent (%) increase of AA, TPC and TFC were found to be 55.24, 7.45 and 52.6, respectively in natural honeys as compared with the commercial blends. Statistical analyses revealed strong positive correlation ($P<0.05$) of AA with both TPC and TFC, whereas GI were inversely correlated ($r=-0.8539$, $p=0.0017$; $r=-0.9841$, $P<0.0001$ and $r=-0.7949$, $P<0.006$) with AA, TPC and TFC, respectively. Furthermore, natural honey types and commercial brands were found to have moderate to high GI values. The findings of this study support the concept that different honey types behave differently for their antiglycemic responses which might be due to differences in antioxidative properties.

INTRODUCTION

Diabetes is a heterogeneous metabolic disorder that is characterized by an abnormal increase in blood glucose level over the range of 126 mg/dl in fasting condition (Khan and Awan, 2012). Prolonged stay of glucose in blood leads to hyperglycemia, which is an established index of diabetes development. Hyperglycemia predisposes to different types of complications and is a key factor in the pathogenesis of diabetic complications by increasing protein glycation and gradual buildup of advanced glycated end products (AGEs) in body tissues (Creager et al., 2006). The AGEs receptors are found on different cells. Interaction of AGEs with their receptors is responsible to change gene expression, intercellular signaling pathways and an increased level of free radicals/reactive oxygen species (ROS) which contributes to the pathobiology of diabetic complications (Creager et al., 2006; Evans et al., 2003; Schalkwijk and Miyata, 2012). Among several diabetes treatment approaches, the bioactive compounds/ antioxidants obtained from different plants are becoming more attractive due to their multiple target sites in body (Mahomoodally et al., 2012).

Additionally, these antioxidants may also counterbalance the activity of two digestive enzymes i.e. α-amylase and α-glucosidase, stimulate insulin production/secretion from pancreatic β cells and also enhance glucose channels opening in various body tissues (Hanhineva et al., 2010). One of the natural products is palatable honey which is a juicy excretion of honeybees and is derived from nectar of plant flowers (Gheldof and Engeseth, 2002).

Bioactive compounds/antioxidants in honey are derived from plant (floral) origins (Eteraf-Oskouei and Najafi, 2013). However, in comparison with the other plant based natural products, antioxidants in natural honey are readily available for consumption and is delicious, semi-liquid form due to sugar contents (Alzahrani et al., 2012). Consequently, the consumption of floral/plant based antioxidants through honey suppress oxidative stress to prevent vital body organs from free radicals/ROS attacks (Gheldof and Engeseth, 2002). It also decreases starch digestibility in gastrointestinal tract, reducing blood glycemic response (Parada et al., 2016).Therefore, due to high antioxidative potential honey has been found as a highly efficacious and effective supplement for many of the pathologies/ ailments (Eteraf-Oskouei and Najafi, 2013; Noor et al., 2014; Pereira and Bartolo, 2016). But diabetic patients are reluctant to use this natural honeybee produce because of high sugar content (Alzahrani et al., 2012). However, previously published studies reported that blood glycemic response of various honey is not same because of variations in antioxidant activities and levels (Al-Hariri, 2011; Atayoglu et al., 2016; Gheldof and Engeseth, 2002). In addition to this, antioxidant capacity and physicochemical properties of honey are also affected by seasonal and climatic conditions (Al-Mamary et al., 2002). Differences in antioxidants may influence the glycemic index of honeys which is between 32 to 87 GI values (Atkinson et al., 2008; Foster-Powell et al., 2002; Ischayek and Kern, 2006).

Henceforth, on the basis of above descriptive facts, present study is designed to evaluate the impact of antioxidants on blood glycemic response, using domestically produced natural honey by honeybee keepers and commercially available honey brands, purchased from local market of Muzaffarabad, Pakistan.

MATERIALS AND METHODS

Honey samples

A total of 10 honey samples were included in this study, out of which 2 were domestically produced by beekeepers and 8 were commercially available samples including Young's honey, Langenese honey, Marhabah honey, Raaz honey, Organic Bee honey, Sue Bee honey, Al-Shifah honey and Life style honey. The samples collected from beekeepers were from two different localities of Muzaffarabad, which were as *Adhatoda vasica* (bhekkar) and *Rubus fruticosus* (rusberry) based on their floral origins/plant resources. All the collected samples were stored in plastic jars at room temperature for further processing.

Preparation of honey samples for antioxidant analyses

Prior to assess the antioxidant activity/level in above mentioned honey samples, extraction was carried out by dissolving 10g of each honey sample in 50ml of aqueous-methanolic (1:1v/v) solution, separately. Then, stirred at 25 ^{o}C for 24 hours and filtered. Filtrate was allowed to dry at room temperature for 2-4 days as adopted by (Islam et al., 2017), with modifications. Subsequently, semi-li°quid residual pellet was used to evaluate antioxidants in the said study honey samples, using UV/VIS-double beam spectrophotometer (UV-1700, Shimadzu, Japan).

The oxidants scavenging capacity of honey samples with ABTS·+ free radical was determined by using the methodology described by Lianda et al. (2012). Antioxidant activity was measured in honey samples with the help of calibration curve generated by serial dilutions of vitamin C instead of Trolox as reference at 734 nm. Activity was anticipated in milligram (mg) ascorbic acid/g honey by extrapolating line against given sample optical density (OD). Total phenolic contents (TPC) in honey samples were determined by using Folin-Cioucalteau reagent through standard calibration curve at 798 nm. Results were expressed as mg gallic acid/g of honey (Pontis et al., 2014), total flavonoid contents (TFC) were estimated in honey samples by aluminum chloride at 437 nm (Pontis et al., 2014). In TFC analysis rutin hydrate was used as standard in place of quercetin to determine calibration curve and results were expressed in mg rutin /g honey.

Evaluation of glycemic index

Present study was approved by Departmental Research Committee and Directorate of Advanced Studies and Research of the University and the current study was conducted with the prior consent of each participant. A total of 10 normal healthy human adults (5 males and 5 females) were considered in the present study to evaluate their postprandial blood glucose (PBG) levels. Study subjects were having age ranged from 20-25 year. For PBG estimation participants were intimated earlier to reach Obesity & Diabetes Research Laboratory, Department of Chemistry, University of Azad Jammu and Kashmir, Muzaffarabad in morning at 8:00-10:00 AM after 12 hours of fasting.

Glycemic index of each honey sample was assessed at zero time point in fasting condition, glucometerically (Infopia Easy Glucometer). Subsequently, blood was measured for other time points i.e. 15, 30, 45, 60, 75, 90, 105 and 120 minutes following the intake of 10g of

each honey sample comprising glucose as reference at different days. These blood glucose values were used to calculate **i**ncremental area under the curve (IAUC) of reference food and test honey samples (Table 1 & Figure 1). IAUC was further used to evaluate the glycemic index (Table 2) of each honey sample to rank/classify them as high, moderate and low glycemic indexed honey. Furthermore, nobody was met with any kind of disorder.

Statistical analyses

The correlations of glycemic index (GI) with antioxidant activity (AA), total phenolic contents (TPC) and total flavonoid contents (TFC) were computed using Pearson correlation coefficients (r) and univariate regression analysis, using Excel Software 2010 and Graph Pad prism (version 5.0). P value of <0.05 was considered statistically significant and the values were presented as mean±SD.

RESULTS

The glycemic index (GI) values of wild type/domestically collected bhekkar and rusberry honey samples were 65.86 and 68.18, respectively, which were found to be medium. Whereas GI values of the commercial honey brands i.e. Young's honey (71.80), Langenese honey (72.68), Marhabah honey (73.25), Raaz honey (74.17), Organic Bee honey (75.55), Sue Bee honey (73.94), Al-Shifah honey (73.80) and Life style honey (75.89) were found to be high and none of these were included in low GI group. Past findings revealed variable glycemic index values for different honey brands i.e. from 32 to 87; this variation counts real differences between honeys (Atkinson et al., 2008). Thus, honey with low/moderate GI value might be useful to incorporate in diabetic diet as a substitute of table sugar. Findings of this study are in line with those described previously (Atkinson et al., 2008; Brand-Miller et al., 2006). Interestingly, in this present study wild type natural honey samples had moderate GI values, whereas commercial honey brands showed high indexed values. Previously published studies reported that a plethora of antioxidants such as phenolic and flavonoid molecules might be effective to keep blood glucose level with in permissible range (Hanhineva et al., 2010; Parker et al., 2009). Thus, antioxidants such as antioxidant activity, total phenolic contents (TPC) and total flavonoid content (TFC) were also evaluated in honey samples.

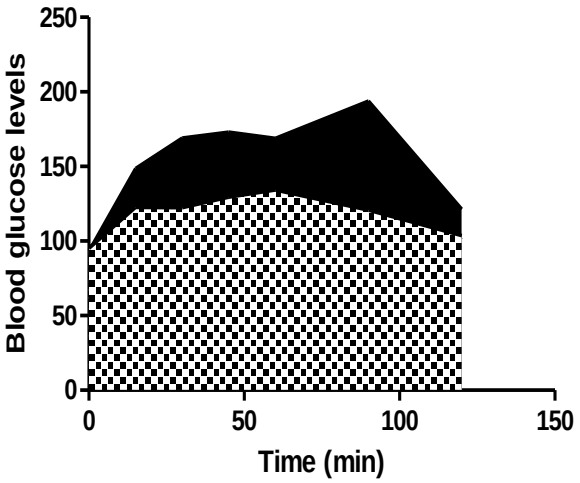

Fig. 1: Graphs of incremental area under the curve (IAUC) of reference and various honey test samples.

Table 1: Incremental area under the curve (IAUC) of the standard and test samples

Sr. No.	Standard and test samples	IAUC
1	Glucose	14410
2	*Adhatodavassica* (bhekkar)honey	9491
3	*Rubusfruticosus* (rusberry)	9825
4	Young's honey	10347
5	Langnese honey	10474
6	Marhabah honey	10556
7	Raaz honey	10688
8	Organic Bee honey	10888
9	Sue Bee honey	10655
10	Alshifa honey	10635
11	Life Style honey	10936

Table 2: GI, AA, TPC and TFC of the reference and honey samples

Sr. No.	Reference and test samples	GI	AA	TPC	TFC
1	Glucose	100			
2	*Adhatoda vasica* (bhekkar)honey	65.86	65	8.46	51.4
3	*Rubus fruticosus* (rusberry)	68.18	46.75	7.56	54.9
4	Young's honey	71.80	27	5.56	48.1
5	Langenese honey	72.68	31.5	5.26	39.1
6	Marhabah honey	73.25	42	4.78	26.6
7	Raaz honey	74.17	55.5	4.00	16.7
8	Organic Bee honey	75.55	46.5	4.14	36.8
9	Sue Bee honey	73.94	42.5	4.63	30.3
10	Alshifa honey	73.80	11.5	4.07	24
11	Life Style honey	75.89	23.25	3.75	10.7

Abbreviations: GI=glycemic index, AA=antioxidative activity, TPC=total phenolic contents, TFC=total flavonoid contents.

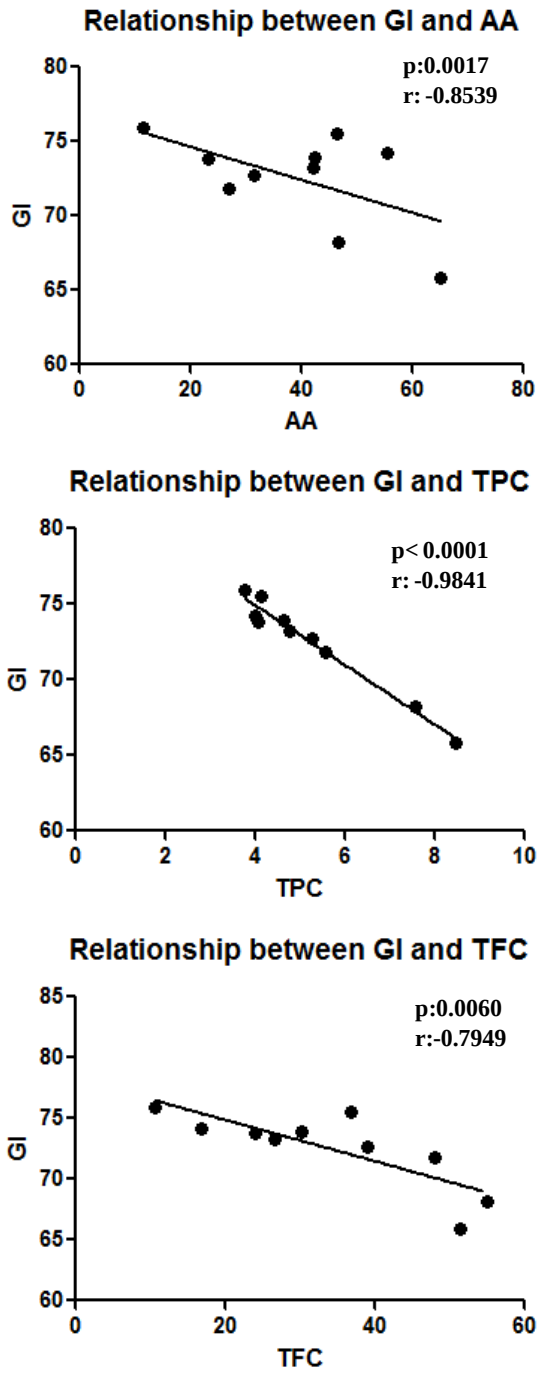

Fig. 2: The mutual relationships between GI with AA, TPC and TFC.

Abbreviations: GI=glycemic index, AA=antioxidative activity, TPC=total phenolic contents, TFC=total flavonoid contents

Antioxidant activity values of raw honey, Berry Raw honey, Young's honey, Langnese honey, Marhabah honey, Raaz honey, Organic bee honey, Sue bee honey, Alshifa brand honey and Life style honey were 65, 46.75, 27, 31.5, 42,55.5, 46.5, 42.5, 23.25 and 11.5, respectively. Total phenolic contents of same honey samples were 8.46, 7.56, 5.56, 5.26, 4.78, 4, 4.14, 4.63, 4.07 and 3.75, whereas total flavonoid contents were 51.4, 54.9, 48.1, 39.1, 26.6, 16.7, 36.8, 30.3, 24 and 10.7, respectively (Table 2). Furthermore, statistical analyses revealed that GI was significantly and inversely correlated to three variables; AA (r=-0.8539, p: 0.0017), TPC (r=-0.9841, P< 0.0001 and TFC (r=-0.7949, P< 0.006) (Figure 2).

DISCUSSION

Various kinds of drugs are used along with enhanced physical activity and particular meal pattern to regulate blood glucose level with in desired range. Despite of this disease pathogenesis is not reversed with available therapies (Chaudhury et al., 2017). Therefore, diabetic patients pay attention to alternative medicines originated from natural sources and believe that natural products might be more useful therapeutics as compared with the synthetic antidiabetic drugs (Kooti et al., 2016). One of the potent natural products is honey that is widely used against different aliments/pathologies in humans (Samarghandian et al., 2017).

Results of this study indicated that glycemic indices of honey are antioxidant dependent. Levels and activity of these antioxidants are inversely associated with GI values. Antioxidants found in honey are readily consumed by people, who are reluctant to use other plant based extracts/products (Ajibola et al., 2012). The climatic variations alongwith floral diversity of nectar from different regions influence the antioxidative activity/potential of honey. Phenolic and flavonoid compounds isolated from honey revealed beneficial activity against several disease conditions including diabetes (Alvarez-Suarez et al., 2010; Bertoncelj et al., 2007; Ferreira et al., 2009). However, diabetic patients show some sort of hesitation to use honey because of its sweet sugary taste of sucrose (Gheldof and Engeseth, 2002; Majid et al., 2013; Rehman, 2016). Therefore, further large scale comprehensive studies are needed to make honey as a vital therapeutics for diabetes management (Erejuwa, 2014; Nazir et al., 2014).

Based upon results, it was assumed that consumption of natural honey might lead to moderate decrease in blood glucose level in diabetic patients. Similar findings had also been reported previously (Azimi et al., 2014; Erejuwa et al., 2012; Khan et al., 2003; Alvarez-Suarez et al., 2013). To our knowledge, no any substantive research work was conducted before to assess antioxidants and/or glycemic index concomitantly, in honeys derived from local floral origins. Therefore, it is likely that results of this study will become baseline reference in future studies to identify low glycemic index honey varieties that will be helpful to improve the honey quality for diabetic patients. Furthermore,

antioxidative properties and glycemic index of honey type(s) could be used for marketing purposes to exploit the profitability of honey.

Authors' contribution

ARK designed and revised manuscript. WA did her M.Phil thesis research in Khan's lab. She generated data regarding antioxidative properties and glycemic indices. She also wrote initial draft of this manuscript. This manuscript was produced from her M.Phil thesis. While KAY, ZI, HF, SR, RAWK and GA participated in data analysis, interpretation and revision.

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