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Antioxidative properties of spices and their impact on postprandial blood glucose in humans

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Abstract

Background: Diabetes mellitus represents disrupted orderly processes of carbohydrate metabolism, in which body cells become unable to utilize glucose. Consequently, glucose molecules pile up in blood and exceed upper normal limit. Spices are vital source of bioactive molecules, which could be useful to treat different diseases including diabetes. Present study was conducted to determine the effect of turmeric, cloves, green cardamom and cinnamon intake on postprandial blood glucose (PBG) levels in normal healthy human subjects.

Method: A total of 10 participants including both genders were considered to assess blood glycemic response of said four culinary spices. Participants' ages were 20-25 years. Incremental area under the curve (IAUC) method was employed for glycemic index (GI) determination. In addition to this, antioxidative properties were estimated by 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$)) radical, Folin-Cioucalteau reagent and aluminum chloride.

Results: GI values of turmeric, cloves, green cardamom, cinnamon and their combined blend were 83.06, 87.48, 82.27, 73.59 and 69.48, respectively. Antioxidative activity (AA) of spices was 2.63, 1.55, 2.55 2.8 and 3.33, respectively. Regarding antioxidant levels cinnamon contained the highest amount (32.78 mg/g) of total phenolic compounds (TPC) than turmeric (28.7 mg/g), cloves (29.6 mg/g) and green cardamom (15.04 mg/g). Similarly, total flavonoid contents (TFC) were found maximum (6.17 mg/g) in cinnamon relative to the other three spices i.e. 2.66, 4.6 and 1.6 mg/g, respectively. Furthermore, GI was inversely related to antioxidative properties i.e. AA (r=-0.88), TPC (r=-0.5625) TFC (r=-0.7716).

Conclusion: The results obtained from this present study indicate that spices' antioxidants interfere with gastrointestinal digestion, lowering starch conversion into blood glucose, effectively. An appropriate intake of spices may be wanted to keep blood glucose level within an optimum limit.

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Introduction

Several previously published studies reported that elevated blood glucose level in postprandial state is a major risk factor/clinical sign for the development of various metabolic disorders, not only in diabetic victims but also in normal healthy obese human subjects [1]. It is also speculated that decrease in postprandial blood glucose levels might be a key target for the management and treatment of diabetes mellitus [2]. Spontaneously, elevated blood glucose causes proteins' glycation in form of advanced glycated end products (AGEs) [3]. Receptors of AGEs (RAGEs) situated on various cells interact with glycated proteins in order to alter gene expression, intercellular signaling pathways and antioxidants-free radicals/reactive oxygen species (ROS) balance. These derangements during the course of disease pathobiology predispose to diabetic macro- and micro-vascular complications [4-6].

Generally, various kinds of drugs are suggested in order to keep blood glucose level in optimum limit [7]. But disease phenotype is not reversed by these available drugs [8]. Therefore, attention is paying to explore alternative medicines developed from plant sources [9-11]. Among natural products, culinary spices are supposed to be effective against several ailments [12, 13]. Additionally, past researches indicated that bioactive compounds/antioxidants in spices are promising to reduce dietary carbohydrates conversion into blood glucose and successive prevention of oxidative stress and glycation processes in diabetic patients [14].

Spices are commonly used in human diets and most of them have been proved effective to reduce blood glucose elevation [15-17]. Glycemic index (GI) is a parameter which is used to rank foods for their conversion into blood glucose [18]. Among numerous spices turmeric, cloves, green cardamom and cinnamon are commonly used to prepare different kinds of foods/dishes [19, 20]. Past studies demonstrated the significant effects of spices on postprandial blood glucose (PBG) levels [17, 21]. A measurable impact of a culinary intake on diabetes markers was also manifested [22]. In another previous study it is reported that cinnamon reduces PBG remarkably [23]. It is reported that bioactive compounds/antioxidants in spices are playing role to decrease blood glucose, oxidative stress and glycation processes in diabetic patients [24, 25]. Therefore, investigating commonly consumed spices i.e. turmeric, cloves, green cardamom and cinnamon in terms of antioxidative properties and GI values may become useful to understand the medicinal value of culinary spices in perspective of blood glucose regulation for diabetes patients.

Methods

Sampling and processing

Initially, four culinary spices i.e. turmeric, cloves, green cardamom and cinnamon were purchased from local market Muzaffarabad, Azad Kashmir. Subsequently, spices were air dried, crushed and ground to form fine powders. A powdered blend was also prepared by mixing equal quantity (20g) of each said spice, using mincer and mortar. Each powdered sample was divided into two parts to determine antidiabetic activity and antioxidative properties i.e. antioxidant activity, total phenolic and flavonoid contents.

Antidiabetic activity

In all, ten (10) normal healthy human subjects including males (n=5) and females (n=5) were considered for blood glycemic response of four commonly used culinary spices such as turmeric, cloves, green cardamom, cinnamon and their blend. For glycemic response, 30g of each said sample was ingested with water. Successively, with uniform interval of time i.e. 15 minutes, postprandial blood glucose (PPB) concentrations were measured by glucometer (Infopia Easy Glucometer). Before conducting PPB, blood glucose level of each participant was also measured in fasting state at zero time point. Average of value of PPB for each time point was used to calculate incremental area under the curve (iAUC). Then, iAUC values were used to compute glycemic index (GI) of turmeric, cloves, green cardamom and cinnamon. Participants involved for PPB evaluation were informed one day earlier to come at specified place with an overnight fast. Their age and other demographic and health related information were also written in predesigned proforma. Consent was also received from each volunteer. This study was approved by Board of Advanced Studies and Research (BASR) of the University.

Antioxidant properties

Antioxidants investigation of turmeric, cloves, green cardamom and cinnamon was carried out by following [26], with few changes. The antioxidants extraction of said fine powder was carried out in methanol. For this, 2g of each sample was extracted with 50ml of 80% methanol at room temperature by placing at stirring for 24 hours using water bath. Successively, the sample was filtered using Whatman filter paper and filtrate was evaporated on heating at room temperature. Resulted residue was divided into three portions and used for antioxidative activity, total phenolic and flavonoid contents.

Determination of antioxidant activity

Re et al. method was applied to assess oxidants scavenging capacity of spices' extracts with 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$))

free radical, as adopted earlier with some modifications [26]. Initially, 5mg of residue was dissolved in 1 ml of methanol with successive aqueous (4ml) dilution. Vitamin C was used as standard instead of Trolox. Standard curve was made with serial dilutions of vitamin C. Finally, antioxidant activity was measured in milligram (mg) ascorbic acid/g extract against optical density (OD) at 734 nm.

Estimation of total phenolic contents

Total phenolic contents were estimated with Folin–Ciocalteau colorimetric method as described previously [26], with few alterations. First of all, 10mg residue part was dissolved in 1ml of water and out of them 0.5ml residual solution was mixed with 0.5ml of 10% Folin-Ciocalteau reagent and 2ml of 7.5% sodium carbonate. This mixture was kept 60 minutes at room temperature and to get maximum absorbance at 765nm wavelength, spectrophotometrically. Gallic acid was used as standard and results were declared as mg of gallic acid equivalent/g extract. The obtained results were expressed in mg rutin /g spice extract.

Estimation of total flavonoid contents

According to an analytical method which was adopted by [27], total flavonoid contents (TFC) were measured with changes. A total of 10 mg of sample residue was dissolved in 1ml of water and 0.5ml of this was further diluted with 2ml of water, then mixed with 0.15ml of 5% $NaNO_2$ and kept the solution for 6 minutes. After this 1.5ml of 10% AlCl3 added and allowed to incubate at room temperature for 10 minutes, then added 2ml of 4% NaOH in it. Absorbance was measured spectrophotometrically (UV-1700 of Shimadzu) at 510nm. Flavonoids were quantified in said samples by plotting their obtained absorbance against the standard calibration graph of rutin hydrate. The obtained results were expressed in mg rutin /g spice extract.

Statistical analyses

Pearson correlation coefficients (r) and univariate regression were applied for GI, AA, TPC and TFC mutual correlation analyses, using Graph Pad prism 5 and Excel Software 2010. P value of <0.05 was considered statistically significant.

Results

The incremental area under the curve (IAUC), antioxidative properties and glycemic index (GI) values are given in Figure 1 and Table 1. GI values of turmeric, cloves, green cardamom and cinnamon were 83.06, 87.48, 82.27 and 73.59 respectively. Spices combined blend showed 69.48 GI value which was the lowest as compared with the other individual culinary spice GI value. Furthermore, 16.9, 12.51, 17.73, 26.41 and 30.52% postprandial blood glucose levels were decreased by the consumption of each culinary relative to the reference GI value i.e. 100).

Antioxidant activity of turmeric, cloves, green cardamom, cinnamon and mixed blend was 2.63, 1.55, 2.55, 2.8 and 3.33mg of ascorbic acid equivalent/g, respectively. Total phenolic contents obtained from spices samples were 28.7, 29.6, 15.04, 32.7 and 36.89mg of gallic acid equivalence/g, respectively. Total flavonoid contents obtained were 2.66, 4.6, 1.3, 6.17 and 8.8mg of Rutin hydrate equivalence/g, respectively, also given in the Table 1. This work has also reported, for the first time, an inverse relationship between GI and three antioxidative parameters i.e. AA (r=-0.8539), TPC (r=-0.9841) and TFC (r=-0.7949) in four culinary spices.

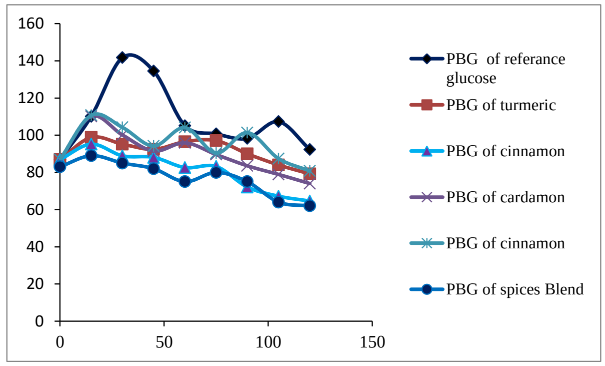

Figure 1: Graphs show IAUC of spices.
Abbreviations: PBG=Postprandial blood glucose, IAUC=incremental area under the curve.

Standard and spice samples	GI (%)	AA	TPC	TFC
Glucose	100			
Turmeric	83.06	2.63	28.7	2.66
Cloves	87.48	1.55	29.6	4.6
Cardamom	82.27	2.55	15.04	1.3
Cinnamon	73.59	2.8	32.78	6.17
Mixed blend	69.48	3.33	36.89	8.8

Table 1: GI, AA, TPC and TFC of the standard and spice samples
Abbreviations: AA=antioxidative activity, GI=glycemic index, TFC=total flavonoid contents, TPC=total phenolic contents.

Discussion

Comparatively, cinnamon intake showed the lowest GI value which was comparable with the previously published results [28]. It is also studied that chronic blood glucose elevation causes enhanced reactive oxygen species (ROS) production, which ultimately leads to oxidative stress development and vital organs damage [29, 30]. Several studies reported that plants including different spices are rich source of phytochemicals such as phenolic and flavonoid compounds [22, 26, 31, 32]. Synergistically, these antioxidants counter two digestive enzymes' activity including α-glucosidase and α-amylase, induce insulin production and increase glucose channels (GLUTs) opening in body tissues [17]. It is also reported that antioxidants-amylose adduct constitution may trigger starch digestion [33, 34]. These lead to a new avenue to regulate hyperglycemia in diabetic victims by reducing the associated morbidities and mortalities [35].

Inverse relationship of GI with oxidative properties obtained from this study is supported by other studies. As a past study reported the oxidative attenuating capability of eight Thai culinary plants [36]. Recently, a study conducted in US manifested that a spice blend enhances some plasma values of antioxidative properties in humans [22]. Similarly, a study screened out 26 spices originated from different countries as effective free-radical scavengers and vital natural antioxidants source [26]. For exploring vital antioxidants, several efforts have been made on various plants, culinary herbs and spices. Still, researchers are paying profound attentions to explore alternative medicines for metabolic disorders, including diabetes mellitus [37, 38]. Diets enriched with plethora of antioxidants have been supposed as potential candidates to manage diabetes mellitus [39, 40]. A few recent studies indicated that the inclusion of culinary spices in diets of diabetic patients might be helpful to reduce the adverse effects of diabetes and its associated complications [39]. For examples; a past study manifested euglycaemic and enhanced antioxidative activities of spices, concomitantly [22]. It has been manifested that consumption of about 6 g of cinnamon/day decrease blood plasma of glucose, and lipids in diabetic patients [23]. In another study, it has been studied that a polyphenol from cinnamon behave like insulin regarding blood glucose regulation [41]. Past studies reported the antioxidants and enzymes mutual binding to explain their antidiabetic capacity [17, 42]. Restriction of glycation processes might lead to improve insulin sensitivity in diabetic patients [43, 44].

On the basis of findings obtained from this study, it could be concluded that blood glucose lowering effects of culinary spices intakes was antioxidants dependent. Since GI was inversely related to antioxidative properties which provide new insight of bioactive components interaction with blood glucose. An expected synergism of the bioactive compounds from spices blend on postprandial blood glycemic response in humans was also obtained. Thus, this study suggests that incorporation of appropriate amount of spices in diet might be beneficial to normalize postprandial glucose and enhance antioxidative defenses, reducing risk factors associated with increased blood glucose level/hyperglycemia. Furthermore, correlation between GI and antioxidative properties obtained from this study is needed to validate in large prospective cohort studies in order to define adapted recommendations for healthy persons and diabetic patients.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' Contribution

ARK & NM designed and conducted the study. HF supported in additional lab work while ZI superivised the research.

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