Ayurvedic dietary formulations and postprandial glycemia in rats

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Abstract: Two dietary formulations were prepared according to the prescriptions made for diabetic people in Ayurvedic classics. One formulation was prepared with barley, brown rice and Bengal gram and parched grains of these food grains were used to prepare second formulation. These formulations were evaluated for their postprandial glycemic effect in rats and were compared with a dietary formula containing mixture of commonly used modern dietary food grains (wheat, polished white rice and pigeon pea). Methanolic extract of these formulations were evaluated for antioxidant activities applying multiple in vitro test methods. It was observed that time dependent increase in the postprandial blood glucose levels of rats up to two hours as well as over all postprandial glycemic loads induced by Ayurvedic dietary formulations were significantly less than the mixture of modern dietary food grains and starch. Total polyphenolic content in the extract of Ayurvedic dietary mixtures and protein content in constituent food grains were higher than the comparative modern dietary food grains. Free radicals scavenging activities, reducing powers as well as antioxidant activity in two Ayurvedic dietary formulations were superior to modern dietary mixture’s extract. A SDS-PAGE based protein fingerprint of these formulations was also prepared to identify genuine food grains and standardize dietary preparations. This is the first report of its kind that evaluated and compared postprandial glycemic effect, antioxidant activities of Ayurvedic dietary formulations with mixture of modern dietary food grains and provides protein fingerprint as a quality control tool for identification of genuine food ingredients and standardize the finished product.

Keywords: Ayurvedic diet, postprandial glycemia, antioxidant activities, diabetes

Introduction

Modern epidemic of lifestyle related diseases like type-2 diabetes mellitus (T2DM), cardiovascular disorders (CVD) and obesity are thought to be the product of industrialization, progressive modernization and globalization (Zimmet and Alberti, 2006) that has made calorie rich, processed, cheap, and convenient marketed foods the main menu for common man (Sivasankaran, 2010). Researchers argue that human genome which evolved and adjusted to Palaeolithic diet and hunter-gatherer lifestyle some 2.5 million years ago, is finding difficult to adjust to these advancements made during and after Neolithic period (Richards, 2002). The transition and transformation in human dietary patterns and lifestyle conditions started with the dawn of agriculture and animal husbandry practices in Neolithic period merely 10,000 years ago on evolutionary scale. Apart from cultivation of plants and domestication of animals, the phenomenon like increase in population, sedentism and eventually urbanization started with “Neolithic revolution” itself (Richards, 2002). Industrialization, progressive modernization and late 20th century phenomenon of globalization added fire to these practices. Therefore, the inharmonious link between our ancient and genetically determined biology with the progress made in nutritional, cultural and activity pattern during the course of modernization, may be held responsible for emergence of many of the so-called diseases of civilization (Cordain et al., 2005).

Improvements in public facilities, lifestyle conditions and availability of ample nutrition, paradoxically led to a remarkable increase in the prevalence of risk factors for diseases like T2DM, CVD, obesity, hypertension and Strokes (Zimmet and Alberti, 2006; Zimmet, 2000). These explanations find support with the observations made during animal studies. The Israeli sand rat, which is adapted to a desert environment with frequent scarcities of food, develops obesity and diabetes when maintained in laboratory on a ‘westernized rat diet’ with abundant food (Haines et al., 1965). OSSabaw Island pigs habituated to live in an environment of uncertain food supply and high physical activity develop obesity and hyperglycemia when raised with food-producing pigs consuming high-calorie diets and living in low activity environment (Whitfield, 2003). Diabetes epidemic among captive population of many primate species in Los Angeles Zoo has also been observed whose zoo lifestyle approximates the high-calorie diet and low-exercise lifestyle of urban humans (Diamond, 2003).

It is important to mention here that the prevalence...
of diabetes mellitus has not been a new incidence in India. Ayurvedic texts that originated in India some 4000-2000 year BC (Tiwari, 2006) provide detail descriptions of diabetes mellitus (Madhumeha). Furthermore, the descriptions about the cause and course of diabetes mellitus development made in these texts do not differ with the understanding of modern medical sciences (Tiwari, 2005). Current researches highlight beneficial effects of Paleolithic (Lindeberg et al., 2007; Klonoff, 2009) and Mediterranean diets (Kastorini and Panagiotakos, 2010) on T2DM and other risk factors of CVD. Paleolithic diet has become now a popular diet in Sweden (Lindeberg, 2005). Interestingly, several dietary formulations in the traditional Indian medical texts of Ayurveda have been advocated beneficial for diabetic persons (Shastri, 1949; Shastri et al., 1962). These original texts were scripted in ancient language Sanskrit. In the course of cultural and linguistic transformations in the country, the then known Bharatvarsha through Hindustan to the present day India, the apathy towards this language could not bring this knowledge on the international scientific platform. Therefore, little attention could be paid to evaluate and authenticate the scientific basis of Ayurvedic dietary prescriptions based on modern scientific understandings.

Ayurvedic classics prescribe barley based dietary food preparations and snacks for diabetic persons (Tiwari, 2008). In this research, we compared postprandial glycemic effect of two dietary formulations prepared according to Ayurvedic prescriptions with a dietary mixture containing food grains commonly used in Indian diet today, and soluble potato starch on Wistar rats. Furthermore, this report also evaluated antioxidant properties of these formulations applying various in vitro methods and provides an electrophoretic protein-fingerprint of the formulations and individual food grains for standardization and quality control of the mixtures.

Material and Methods

Dietary materials

Barley (Hordeum vulgare Linn.), brown rice (dehusked brown grains of Oryza sativa Linn.), Bengal gram (Cicer arietinum Linn.), wheat (Triticum aestivum Linn.), polished rice (dehusked polished-white grains of Oryza sativa Linn.), and pigeon pea (Cajanus indicus Spreng.), was procured from local food grain stores of Hyderabad city (India). Soluble potato starch was purchased from LOBA CHEMIE, Bombay (India).

Preparation of dietary mixtures

Whole grains of barley, brown rice and Bengal gram were taken in the ratio of 50:25:25 (w/w) and coded as formula 1 (F1). Similarly, whole grains of wheat, polished rice and pigeon pea were taken and coded as formula 2 (F2). Whole grains of barley, brown rice and Bengal gram were parched separately and taken in the same ratio as above and coded as formula 3 (F3). Individual formulations were grinded in flourmill to obtain fine quality raw flour and kept in airtight containers.

Extraction of formulations for determination of antioxidant activities

Extraction of formulations was carried out with slight modification in the method described earlier (Tiwari et al., 2011). In 85% methanol (acidified with 1.0N HCl) each formulation in the ratio of 1:5 (w/v) was soaked for 7 days at room temperature. The formulations in solvent were shaken occasional daily. Supernatant was vacuum filtered on 8th day, concentrated to 1/3 volume under reduced pressure in rotary evaporator (50±1°C) and lyophilized to dryness. Extracts were stored refrigerated until analysis.

Determination of chemical components in methanol extract

Percentage yield, total polyphenols content (TPC), and total anthocyanins content (TA) were determined as described earlier (Tiwari et al., 2011). The total flavonoids content (TF) in the extracts was determined following method described by Hsieh and Chang (2010).

Evaluation of antioxidant activities

The extracts were evaluated for scavenging of free radicals DPPH, ABTS+ cation, and H2O2, prevention of ABTS oxidation as reported methods (Tiwari et al., 2011). Reducing activity for FeCl3 was assayed as described by Arumugam et al. (2010). Nitroblue tetrazolium (NBT) reducing activity as a measure of presence of ascorbic acid (Concklin et al., 2000) in formulations was determined as follows. In a 96-well plate containing 100 µL phosphate buffer (50 mM, pH10) and equal volume of NBT (1mM), prepared in same buffer), 50 µL (5mg/ml DMSO solution) samples were mixed and incubated for 15 minutes. A blank with each extract in the absence of NBT was run to correct background absorbance. Reduction of NBT was measured at 560 nm using BioTek Synergy 4 multimode microplate reader (BioTek Instruments Inc, USA). The percentage of NBT reduction was calculated applying following formula % reduction=...
[(A_t-A_c)/Atx100] where, (A_t) represents absorbance of reduced NBT by extracts and (A_c) the absorbance of NBT solution in reaction mixture in the absence of extract. At least four to five dilutions in triplicate of each extract was run in every experiment to find out either free radical scavenging concentration 50% or the reducing power 50% (RC₅₀) of extract. Suitable regression analysis was applied to obtain SC₅₀ and RC₅₀ values.

SDS-PAGE protein fingerprinting

100 mg powder of each formulation and individual food grains was taken into test tubes containing 4 ml of ice-cold protein extraction buffer [62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% glycerol, 3% β-mercaptoethanol, 5M Urea] according to the method of Sadia et al. (2009) with slight modifications. Mixture was vortexed intermittently to the method of Sadia et al. (2009) with slight modifications. Mixture was vortexed intermittently for five minutes and centrifuged at 15000 rpm for 10 min at 4°C. Supernatant was collected and used for protein estimation (Bradford, 1976) and SDS-PAGE protein separation.

20 µL equal concentration protein samples were mixed with appropriate volume of 2X SDS loading buffer containing 0.5M Tris-HCl (pH 6.8), 10% SDS, Glycerol, 2-mercaptopethanol and bromophenol blue and heated for 5 minutes in boiling water. 20 µl of samples were separated on 12% SDS-PAGE (Bio-Rad mini Protein gel apparatus) along with molecular weight marker. The gel was allowed to run in 1X Tris-Glycine buffer (10X buffer- 250mM Tris base, 1.92M Glycine, 1% SDS), at constant voltage of 100V. The gel was stained with 0.5% Coomassie brilliant blue (250 R) solution for an hour and de-stained several time with fresh methanol: acetic acid: water (50:10:40) solution until appropriate staining of the gel reached. Destained gels were photographed using BioDoc-It™ Imaging System, UV Transilluminator UVP.

Postprandial glycemic test

Postprandial glycemic activity of dietary formulations was determined by feeding individual formulation to group of rats. Male Wistar rats (176±11 gram body weight) were obtained from National Institute of Nutrition (CPCSEA Reg. No.154, Government of India), Hyderabad. Rats were housed in standard polyvinyl cages in the institute’s (IICT) animal house. Room temperature was maintained at 22±1°C with an alternate 12 h light dark cycle. Food and water were provided ad libitum. Approval of experimental protocol was obtained from Institutional Animal Ethical Committee (IAEC-IICT). All the animals were kept for overnight fasting. Next day forenoon, blood was collected from the retro orbital plexus in EDTA containing tubes. Basal plasma glucose level (‘0’ h) was estimated by glucose oxidase method with auto blood analyzer (Bayer EXPRESS PLUS). Rats were divided into various groups. Each group contained six rats. Animals were grouped such that the mean basal glucose levels do not differ significantly among the groups. Light slurry of formulations F1, F2 and F3 was prepared in normal saline and administered orally (2-gm/kg body weight) to respective group of animals. Control groups of animals were given soluble potato starch in the same dose. A positive control group with standard drug acarbose was also taken. Acarbose (10 mg/kg body weight) was given to animals fifteen minutes before starch feeding. Blood was collected at the intervals of 30, 60, 90 and 120th min post-starch or the formulations feeding. Plasma glucose levels were measured as described above. Time dependent increase in plasma glucose level and two-hour postprandial glycemic load as a measure of area under the curve (AUC₀₋₁₂₀minutes) was calculated following trapezoidal rules (Raju et al., 2010).

Statistical analysis

One-way ANOVA followed by Dunnett’s multiple comparison tests was applied to compare difference between animal study groups. To determine degree of significance between the groups p< 0.05 was considered.

Results and Discussion

Chemical components

Table 1 presents percentage yield and different antioxidant compositions in methanolic extract of three formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Yield of extract</th>
<th>TPC (mg GAE/g)</th>
<th>TF (mg RE/g)</th>
<th>TA (mg %w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>7.6</td>
<td>29.8±0.6</td>
<td>16±2</td>
<td>0.61±0.19</td>
</tr>
<tr>
<td>F2</td>
<td>7.4</td>
<td>32.8±0.4</td>
<td>35±2</td>
<td>0.25±0.001</td>
</tr>
<tr>
<td>F3</td>
<td>6.4</td>
<td>37.8±0.1</td>
<td>26±4</td>
<td>ND</td>
</tr>
</tbody>
</table>

The percentage methanolic yield did not differ among the formulations. Total polyphenols content (TPC) in formula F3 was 21% more than in F1. In formulation F2, total flavonoids content (TF) was found double than F1 and 25% more than F3. Total anthocyanins (TA) could not be detected in formula F3. However, in formula F1 it was 69% more than F2. It requires mention here that with increasing time of extraction percentage yield of the extract increases as in our previous study (Tiwari et al., 2011) with only
24-hour extraction time fewer yields was obtained.

In barley, protein content was 14% higher than wheat (Table 2). These two food grains are used mainly to prepare chapatti and breads. Pulse grain Bengal gram contained more protein (45%) than pigeon pea (Table2). Equal protein content in white rice and brown rice was observed. Similarly, there was no difference in protein content of formula F1 and F2. However, protein content in formula F3 was 29% less than formula F1 and 47% less than formula F2.

**Antioxidant activities**

Highly processed, calorie-dense, nutrient depleted diet leads to exaggerated postprandial spikes in blood glucose and lipids that induces immediate oxidative stress. Induction of oxidative stress has been observed to increase in direct proportion to the increase in postprandial blood glucose level (O’Keefe et al., 2008). Hyperglycemic spikes even in non-diabetic individuals have been shown to markedly increase free radicals generation (Brownlee and Hirsch 2006). Furthermore, oxidative stress has been recognized recently as a major physiological link between CVD and diabetes (Giugliano et al., 1996) and also in the development of diabetic complications (Brownlee, 2001). Therefore, presence of free radicals scavenging antioxidant properties in a diet becomes an important consideration.

Figure 1 presents concentration dependent multiple free radical scavenging properties of the methanol extract of formulations. It was observed that scavenging activities for free radicals like DPPH (Figure 1a), ABTS·+ cation (Figure 1b), H2O2 (Figure 1f), and reduction of FeCl3 (Figure 1d) and NBT (Figure 1e), or ABTS oxidation (Figure 1c) prevention activities, all the three extract displayed varying degrees of activity potentials. With respect to the IC50 values (Table 3), it was found that extract from the formulation F1 displayed far better antioxidant activity than extract from formulation F2 in scavenging free radicals DPPH, ABTS·+, reducing FeCl3, and NBT. However, methanolic extract of formulation F3 displayed more than twice ABTS oxidation prevention and FeCl3 reducing activity than F1 extract. Formula F1 was more potent than formula 3 in scavenging DPPH radical however, ABTS·+ scavenging potency of these two formulas remain same. The antioxidant potency of formula F2 was close to double than formula F1. These results show that whether free radicals scavenging, reducing power or the antioxidant activities, extract of Ayurvedic formulations displayed far better activity than extract of formulation F2.
been identified that possess different chemical and physical characteristics (Prior et al., 2005). Therefore, to balance the multiplicity of oxidants, multiple characteristics in antioxidants are required. It has been observed that in some cases, antioxidants present multiple mechanism of action in a single system (Ishige et al., 2001), however, may display different mechanism of action depending on the reaction system and can respond in a different manner to different radical or oxidant sources (Prior et al., 2005). Because of these multiplicities involved in the characteristics as well as mechanism of antioxidants action, no single assay can optimally reflect true characteristics of antioxidants in a mixture (Wootton-Beard et al., 2011). Variations in the activity level and response to different free radicals in this study presented by dietary Ayurvedic formulations may be due to the presence of mixtures of different type of antioxidants, their synergistic mode of actions, and differences in their physical and chemical characteristics.

Despite the fact that antioxidants present in human diet play important beneficial role in reducing oxidative stress and preventing free radicals induced biomolecules damage, it has recently been observed that the timing (Diano, 2009) and order of antioxidants consumption with meal (Imai and Kajiyama, 2010) affect other metabolic parameters differently. It has been observed that antioxidant when taken on an empty stomach affects appetite and when consumed along with diet affects satiety (Diano, 2009). Furthermore, some antioxidant rich fruits (Alvarez-Parrilla et al., 2010) and antioxidant rich fractions from food grains (Tiwari et al., 2011) have been alleged to induce hyperlipidemia and hyperglycemia respectively. Similarly, antihyperglycemic compounds isolated from fruits have also been reported to increase postprandial blood glucose level when carbohydrate diet is fortified with such compounds (Tiwari et al., 2010). Therefore, selection of antioxidant rich food items meant for diabetic patients that do not adversely affect blood glucose level requires special attention.

**SDS-PAGE protein fingerprint**

One of the major problems associated with natural food products is the availability of appropriate standardization and quality control tools. Lack of such tools increases the malpractice of adulterations and also erroneous identification of the natural material. Based on molecular characteristics of electrophoretic protein fingerprints, it has become possible to gather information about genetic variations, taxonomic relationship, phylogenetic diversity and even identification of sub-species of a plant material around the world (Emri et al., 2007; El-Hady et al., 2010). The unique band patterns of protein electrophoregram of food grains or the mixture therefore, could serve as an important supplemental tool that can provide passport data for its identification and standardization. Figure 2 presents distinct spectrum of electrophoretic protein band patterns of individual food grains and the formulations prepared by mixing these food grains. Distinct protein band patterns for each food grains were observed. However, protein fingerprint of the mixture of dietary formulations present a clear distinction and differences in protein band pattern than the respective food grains used to prepare formulations (Figure 2). The presence or the absence of a protein band of constituent food grain protein in the respective formula may help identify genuine product. This tool may serve the purpose of correct identification of food grain as well as proper standardization of Ayurvedic dietary formulations.

**Postprandial glycemia**

Postprandial hyperglycemia (PPHG) has emerged as a prominent and early defect in ensuing T2DM (Carroll et al., 2003). The deterioration of glucose homeostasis in individuals with T2DM progresses from postprandial to fasting hyperglycemia in several steps (Monnier et al., 2007). PPHG has also been identified as an independent predictor of the development of future cardiovascular events even in non-diabetic individuals (O’Keefe et al., 2008). The postprandial state is characterized by several metabolic alterations that may play role in the pathogenesis of CVD. PPHG has been reported as an important independent risk factor contributing to the development of atherosclerosis and amplify generation of oxidative stress (Ceriello, 2000). Indian population has been observed to present increased prevalence of PPHG than other ethnic groups around the world on a daily seven-point scale (Milicevic et al., 2008). Higher intake of carbohydrate in the diet of Asians and South Asians in particular, has been observed in comparison to White Caucasians (Misra and Khurana, 2011). Evolutionarily, this may be due to the reason that adoption of agriculture in Neolithic period occurred independently in different parts of the world that influenced selection of dietary materials and hence dietary patterns. The starch rich food plants such as rice became main domesticated plant in Asia (Imamura, 1996; Crawford, 1992). Now, for most Asian population, white rice has become staple food. Rice is readily available, considered more palatable (Sugiami et al., 2003) and constitute major portion in the menu of this region. The high level of carbohydrate content in the diet of Asian population
along with use of white rice as a staple food may be the reason of increased incidence of postprandial hyperglycemia and increasing prevalence of T2DM in this part of the world. Therefore, a diet that can control precipitous rise in postprandial glycemia may become helpful in bringing down the risk factors associated with PPHG.

Figure 3 presents time dependent increase in postprandial blood glucose level in animals fed on different dietary formulations. It was observed that rats fed on F2 diet (which includes mixture of present day common dietary food grains like white rice, wheat flour made breads and Chapatti, and pulse made of pigeon pea), there was a sharp and significant rise in blood glucose level even at the first 30th minute after oral feeding (Fig. 3A) and later blood glucose level paralleled close to the blood glucose level of animal group who received control starch diet. The F2 diet induced increase in blood glucose level was significantly (p<0.05) higher at this time point (first 30th minute), than that induce even by pure starch diet (Control group) fed animals. This may explain the reason Asian population display higher PPHG levels that consume this type of food today. It requires mention here that the development and adoption of this type of fast-glucose releasing diet might have been the requirement of fast paced lifestyle of modernizing society, which looked-for quick energy giving diet.

Intestinal α-glucosidase inhibitor drugs like acarbose and voglibose slow down the digestion of carbohydrate rich diet. These drugs have been observed better therapeutic in controlling PPHG in Asian people (Scheen, 2009) presumably because of their specific food habits (Chan et al., 1998). Such drugs are now getting special prescription in the population particularly Indian whose diet is starch rich and demonstrate higher glycemic response to all foods (Henry et al., 2008) and present increased prevalence of PPHG (Milicevic et al., 2008) than other ethnic groups around the world. It was interesting to note that postprandial glucose level of formulation F3 treated group of animals was comparable to the postprandial glucose level of acarbose treated group of starch fed animals (Figure 3B). It is possible therefore that if these dietary formulations (F1 and F3) become substitute as dietary therapies, the use of drugs like acarbose can be avoided.

The Paleolithic diet (based on lean meat, fish, fruits, vegetables, root vegetables, eggs and nuts) has been reported significantly potent in improving glucose tolerance test in ischemic heart disease (IHD) patients with impaired glucose tolerance or T2DM than the Mediterranean-like diet that is based on whole grains, low-fat dairy products, fish, fruit and vegetables (Lindeberg et al., 2007.) In another study, Paleolithic diet was found more satiating per calorie than a Mediterranean-like diet in individuals with IHD (Jonsson et al., 2010). However, Paleolithic diet was less liked by the study participants and the drop out rate was observed more in this study group (Lindeberg et al., 2007) despite the fact that it significantly improved over all two hour postprandial...
Applying formula: \( \text{AUC}_{0-120} = \frac{(BG_0 + BG_30) \times 0.5}{2} + \frac{(BG_30 + BG_60) \times 1}{2} + (BG_120 + BG_60) \times \frac{1}{2} \), where \( BG_0, BG_30, BG_60, \) and \( BG_{120} \) represent plasma glucose level at 0, 30, 60 and 120 min after soluble potato starch feeding in rats. Area under the curve (AUC) was calculated following trapezoidal rules with different dietary formulations. It is evident from figure 4 that substitution of Ayurveda based dietary formulations like F1 and F3 if prescribed for impaired glucose tolerant people and diabetic patients, may have significant potential in improving glucose intolerance, and also prevalence and incidence of PPHG caused by modern diet in general population. These formulations may become a suitable dietary substitute to control modern epidemic of PPHG because restoring to drug therapy for an epidemic caused by maladaptive hyperglycemic diet (which represent conventional diet of today like F2) is less rational than simply restoring to olden day’s time tested diets (F1 and F3) and realigning dietary habits with the physiological needs (O’Keefe et al., 2008).

**Conclusion**

The human genome and genetically determined biology of human was evolved and adapted to natural diet, environment, and hunter-gatherer lifestyle before the dawn of Neolithic period. However, with the beginning of agricultural practices and domestication of animals during Neolithic period, human population around the world is facing epidemic of several metabolic disorders like T2DM, CVD, obesity, stress and cancer etc. Nevertheless, ancient civilizations in the world is taking shape of epidemic. These countries share many of the common features in their dietary practices and pattern. Therefore, scientific exploration, design and development of dietary formulations mentioned in Ayurvedic texts for the prevention of development of T2DM epidemic in these regions may offer better therapeutic dietary substitute than the dietary prescriptions explored, studied, designed and developed to suite white Caucasian population.
high-postprandial glycemic value diets, approves their preventive as well as therapeutic effectiveness. Therefore, development and adoption of such diet may help prevent the prevalence of PPHG and epidemic of T2DM in the people of South/East Asian origin in particular where such diet was in traditional practice. To the best of our knowledge this is the first report of its kind that explored effect of Ayurvedic diet on postprandial glycemia and evaluated antioxidant properties present in such dietary formulations.

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References


