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# 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 20$^{th}$ 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

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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 aestivam* 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 8$^{th}$ day, concentrated to 1/3 volume under reduced pressure in rotary evaporator (50±1ºC) 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, $ABTS^{\cdot+}$ cation, and $H_2O_2$, prevention of ABTS oxidation as reported methods (Tiwari *et al*., 2011). Reducing activity for $FeCl_3$ 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=

$[(A_t-A_c)/A_t x100]$ 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% ($RC_{50}$) of extract. Suitable regression analysis was applied to obtain $SC_{50}$ and $RC_{50}$ 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 for five minutes and centrifuged at 15000 rpm for 10 min at $4^0$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-mercaptoethanol 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$^0$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 ($AUC_{0\text{-}120minutes}$) 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 1.** Antioxidant components in methanolic extract of dietary formulations

| Formulation | Yield of extract | TPC | TF | TA |
|---|---|---|---|---|
| F1 | 7.6 | 29.8±0.6 | 16±2 | 0.61±0.19 |
| F2 | 7.4 | 32.8±0.4 | 35±2 | 0.25±0.001 |
| F3 | 6.4 | 37.8±0.1 | 26±4 | ND |

The % yield denotes yield %w/w; gm/100gm. Total polyphenols content (TPC) represents mg GAE/gm, total flavonoids (TF) as mg RE/gm and total anthocyanins (TA) as %w/w. GAE; gallic acid equivalent, RE; rutin equivalent, ND; not detected. Values are mean ± SD, n=3.

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 formula 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

**Table 2.** Protein content in food grains and formulations

| Barley | Brown Rice | Bengal gram | Wheat | White Rice | Pigeon Pea | F 1 | F 2 | F 3 |
|---|---|---|---|---|---|---|---|---|
| 20.9±0.2 | 18.4±1.7 | 44.1±4.7 | 17.9±0.5 | 17.8±4.7 | 24.2±3.8 | 22.1±0.3 | 24.6±0.65 | 16.6±0.16 |

Values (mean±SD) represent mg/gm of grain or formulations powder.

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), $ABTS^{\cdot+}$ cation (Figure 1b), $H_2O_2$ (Figure 1f), and reduction of $FeCl_3$ (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 $IC_{50}$ 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, $ABTS^{\cdot+}$, reducing $FeCl_3$ and NBT. However, methanolic extract of formulation F3 displayed more than twice ABTS oxidation prevention and $FeCl_3$ reducing activity than F1 extract. Formula F1 was more potent than formula 3 in scavenging DPPH radical however, $ABTS^{\cdot+}$ 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.

**Table 3.** $IC_{50}$ for methanolic extract of dietary formulations

| Formulation | DPPH (µg/mL) | $ABTS^{\cdot+}$ (µg/mL) | ABTS-ox (µg/mL) | NBT (µg/mL) | $FeCl_3$ (µg/mL) |
|---|---|---|---|---|---|
| F1 | 134.2 | 31.5 | 19.0 | 41.1 | 226.2 |
| F2 | 278.4 | 49.3 | 11.0 | 89.0 | 606.9 |
| F3 | 211.7 | 31.8 | 7.9 | 48.2 | 126.9 |

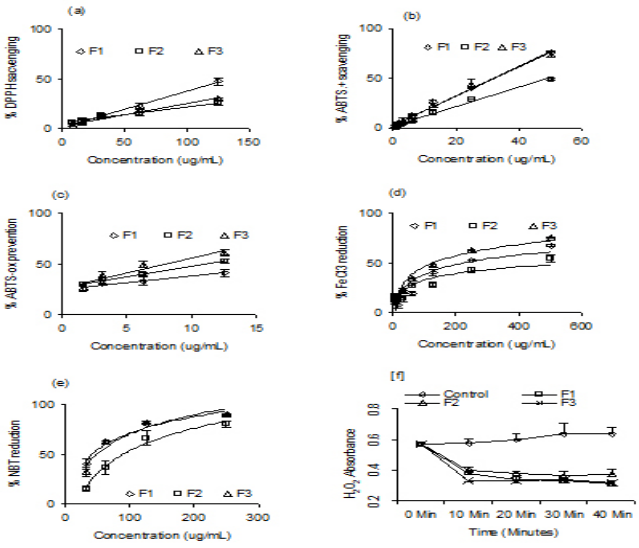


Figure 1. Concentration dependent antioxidant activities in methanolic extract of dietary formulations. [a] DPPH scavenging activity. [b] $ABTS^{\cdot+}$ scavenging activity. [c] Prevention of ABTS oxidation ($ABTS_{ox}$). Figures [d] and [e] represent $FeCl_3$ and NBT reducing activity respectively. [f] Kinetics of hydrogen peroxide ($H_2O_2$) scavenging at 100µg/ml concentration of samples. Data represent mean ± SD, n=3.

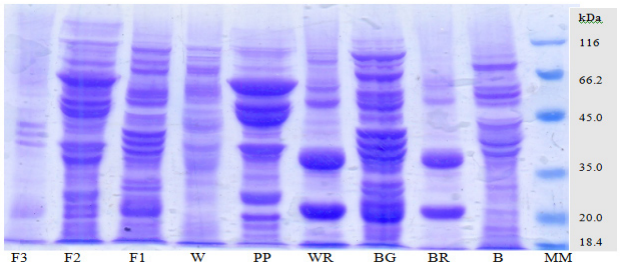


**Figure 2.** SDS-PAGE protein fingerprint of individual food grains and Ayurvedic dietary formulations. Formulation 3 (F3), formulation 2 (F2), formulation 1 (F1), wheat (W), pigeon pea (PP), white rice (WR), Bengal gram (BG), brown rice (BR), barley (B), Molecular weight marker (MM). Differences in protein band patterns of different food grains are clearly visible. It is clear therefore that each food grain has its unique protein band pattern. Formulation F1 was prepared by mixing barley, brown rice and Bengal gram. It is evident from the band pattern of F1 that it has a different fingerprint than the individual food grains despite the fact that some of the proteins of brown rice, Bengal gram and barley appear in its fingerprint. Similarly, F2 contains mixture of wheat, white rice and pigeon pea. Major proteins of pigeon pea are evident in F2 protein band pattern, altogether however, the protein fingerprint of F2 displays different protein profiles than individual food grains. It is interesting to note that the protein fingerprint of formula F3 is absolutely different than its parent mixture F1. Only few protein bands between 35-45 kDa ranges could be visualized in this formulation. This difference may be due to the reason that F3 was prepared from the parched grains. In the process of parching many of the proteins of individuals food grains might have been lost. The unique electrophoretic protein fingerprint of dietary formulations F1, F2, and F3 from their compositional food grains therefore, may serve as quality control tool and standardization of individual formulation.

In a biological system, multiple sources of free radical generation, oxidants and antioxidants have

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 (Sugiama *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 30$^{th}$ 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 30$^{th}$ 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.

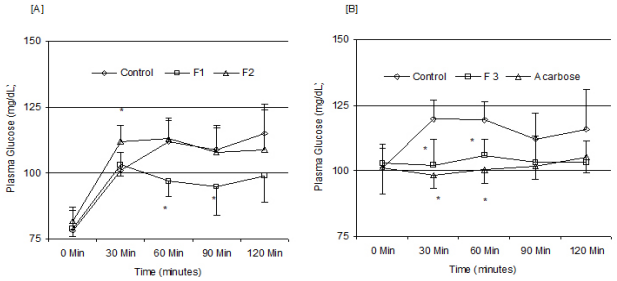


**Figure 3.** The glycemic response curve in rats following feeding of F1, F2, F3 and soluble potato starch (Control). [A] Glucose levels at different time points with dietary formulations F1 and F2. Control group received soluble potato starch. [B] Glucose levels with dietary formulation F3. Control group was treated sham as in [A]. Dosage was 2-gm/kg body weights in each group of animals. One group in the experiment [B] with standard antihyperglycemic drug acarbose was also kept wherein acarbose (10 mg/kg body weight) was orally administered 15 minutes before soluble potato starch feeding. ANOVA followed by Dunnett's multiple comparison tests was applied to compare the differences between the means. Degree of significance was chosen $^{*}p < 0.05$ when compared with control. Values represent mean ± SD, $n = 6$.

A verse (Ch.Chi. 6.21) from Ayurvedic text *Charaka Samhita* (Shastri *et al*., 1962) advocates that the diet of diabetic patients should consist predominantly of barley, wherein various food items may be prepared along with wild variety of rice and barley (Tiwari, 2008). This formed the basis for preparation of dietary formula F1. It was observed that the increase in blood glucose level in animals fed on this formulation was significantly less at 60$^{th}$ and 90$^{th}$ minute (Fig 1A). Although the increase in blood glucose level at 30$^{th}$ and 120$^{th}$ minute was also less in this group, it could not reach degree of significance when compared with either control group or the F2 diet fed group of animals.

Similarly, another verse (Ch. Chi. 6.48) from the same text (Shastri *et al*., 1962) mentions that use of parched barley flour prevents development of diabetes (Tiwari, 2008). In olden time's parched barley, parched brown rice and parched Bengal gram grains and their flour were used as munching snacks and drinks in India, particularly in Northern part of the country. This practice is still breathing in remote parts of Northern India. These facts laid down the basis for preparation of formula F3. It can be seen in figure 3(B) that by feeding formula F3, the rise in blood glucose level in rats was significantly less ($p<0.05$) when compared with starch fed control group of animals at 30$^{th}$ and 60$^{th}$ minute and remained lesser than starch fed group up to 120 minute.

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

glucose load in terms of area under the curve (AUC $_{0\text{-}120\ minutes}$) than Mediterranean-like diet.

It appears therefore that even though the Stone-Age Paleolithic diet may have better control over PPHG, it may not become diet of preference by the people in modern world. However, a diet or dietary formulation based on whole grains can easily become popular if helps prevent excessive postprandial glycemic excursion. Figure 4 presents postprandial incremental plasma glucose $AUC_{0\text{-}120\ min}$ 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).

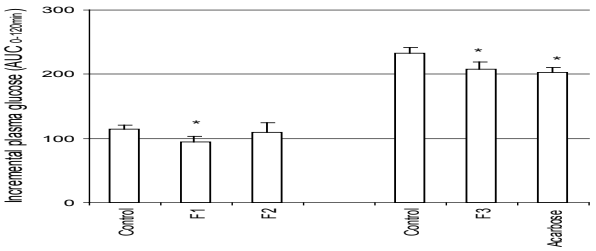


**Figure 4.** Postprandial incremental plasma glucose $AUC_{0\text{-}120\ min}$ with different dietary formulations and soluble potato starch feeding in rats. Area under the curve (AUC) was calculated following trapezoidal rules applying formula: $AUC_{0\text{-}120\ min} = (BG_0 + BG_{30}) \times 0.5/2 + (BG_{30} + BG_{60}) \times 0.5/2 + (BG_{120} + BG_{60}) \times 1/2$, 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 (control) or dietary formulations (F1, F2 and F3) feeding. ANOVA followed by Dunnett's multiple comparison tests was applied to find difference between the groups. *$p$<0.05 when compared with control. Values represent mean ± SD, $n$ = 6.

Consideration of hyperglycemia rates among domesticated mammals suggests that ethnic and national heterogeneity in diabetes prevalence is rooted in more recent events (Gerstein and Waltman, 2006). Therefore, the rising rates of diabetes in traditional living population of developing countries may be due to the recent adoption of western culture and dietary habits (to which they were least exposed) leaving aside their traditional dietary practices to which they were raised for long time. Infact, for at least 300 years Europeans have enjoyed a relatively stable food supply and increasingly available labour-saving devices (Diamond, 2003). The populations of developing countries are newly exposed to such an environment therefore, may have little resistance to its diabetogenic effect (Gerstein and Waltman, 2006). The Mediterranean diet was first described in the 1960s by Ancel Keys based on his observation of food habits of some populations in the Mediterranean region (Keys *et al*., 1986). So far, Mediterranean diet is one of the best-known and studied diets for its beneficial effects on human health that may act beneficially against development of T2DM, including reduced oxidative stress and insulin resistance (Kastorini and Panagiotakos, 2010). However, Indian Ayurvedic dietary formulations being claimed as one of the ancient medical dietary prescriptions for the people suffering from diabetes could not receive global attention. These dietary compositions (Shastri *et al*., 1962; Shastri, 1949) do not differ from that of the Mediterranean diet. The prevalence of T2DM in rural as well as urban populations of developing countries of the South/East Asia 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.

## Conclusion

The human genome and genetically determined biology of human was evolved and adapted to natural diet, environment, and hunter-gatherer life style before the dawn of Neolithic period. However, with the beginning of agricultural practices and domestication of animals during Neolithic period to the modern industrialized and globalized world, it got exposed to extreme changes in the lifestyle conditions and dietary patterns to which it is less adopted. Resultantly, 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 to which these disease symptoms were known, had developed several dietary and natural therapeutic formulations for prevention of the development and treatment of these diseases. Ayurveda, one of the oldest medical systems in the world, described barley-based dietary formulations beneficial for the people suffering from diabetes mellitus (*Prameha* and *Madhumeha*). The low-postprandial glycemic load as well as potent antioxidant activities present in Ayurvedic dietary formulations (F1 and F3) observed in this study when compared with modern

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

Alvarez-Parrilla, E., Rosa, L. A. L., Legarreta, P., Saenz, L., Rodrigo-Garcia, J. and Gonzalez-Agular, G.A. 2010. Daily consumption of apple, pear, and orange juice differently affects plasma lipids and antioxidant capacity of non-smoking adults. International Journal of Food Science and Nutrition 61: 369-380.

Arumugam, P., Murugan, R., Subathra, M. and Ramesh, A. 2010. Superoxide radical scavenging and antibacterial activities of different fractions of ethanol extract of *Mentha spicata* (L.). Medicinal Chemistry Research 19: 664-673.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry 72: 248-254.

Brownlee, M. and Hirsch, I. B. 2006. Glycemic Variability: A Hemoglobin $A_{1c}$–Independent Risk Factor for Diabetic Complications. Journal of American Medical Association 295: 1707-1708.

Brownlee, M. 2001. Biochemistry and molecular cell biology of diabetic complications. Nature 414: 813-820.

Carroll, M. F., Gutierrez, A., Castro, M., Tsewang, D. and Schade, D. S. 2003. Targeting Postprandial hyperglycemia: A comparative study of insulinotropic agents in type 2 diabetes. Journal of Clinical Endocrinology and Metabolism 88: 5248-5254.

Ceriello, A. 2000. The Post-Prandial state and cardiovascular disease: relevance to diabetes mellitus. Diabetes Metabolism Research Review 16: 125-132.

Chan, J. C ., Chan, K. W., Ho, L. L, Fuh, M. M., Horn, L. C, Sheaves, R., Panelo, A. A., Kim, D. K. and Embong. M. 1998. An Asian multicenter clinical trial to assess the efficacy and tolerability of acarbose compared with placebo in type 2 diabetic patients previously treated with diet. Asian Acarbose Study Group. Diabetes Care 21: 1058-1061.

Concklin, P. L., Saracco, S. A., Norris, S. R. and Last, R. L. 2000. Identification of Ascorbic acid-deficient *Arabidopsis thaliana* Mutants. Genetics 154: 847-856.

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., O'Keefe, J. H. and Brand-Miller, J. 2005. Origins and evolution of the western diet: health implications for the 21st century. American Journal of Clinical Nutrition 81: 341-354.

Crawford, G. W. 1992. The transitions to agriculture in Japan. In Gebauer, A. B. and Price, T.D. (Eds). Transitions to Agriculture in Prehistory. p. 117-132. Madison: Prehistory Press.

Diamond, J. 2003. The double puzzle of diabetes. Nature 423: 599-602.

Diano, S. 2009. Regulation of free radicals by the brain could play a role in weight control and appetite. Forefront (The American Diabetes Association's Research Magazine) 6: 26.

El-Hady, E. A. A. A., Haiba, A. A. A., El-Hamid, N. R. A. and Rizkalla, A. A. 2010. Phylogenetic diversity and relationships of some tomato varieties by electrophoretic protein and RAPD analysis. Journal of American Science 6: 434-441.

Emre, I., Turgut-Balik, D. and Sahin. A. 2007. Electrophoretic analysis of total protein profiles of some *lathyrus* L. (sect.cicerula) grown in turkey. Pakistan Journal of Biological Species 10: 2890-2894.

Gerstein, H. C. and Waltman, L. 2006. Why don't pigs get diabetes? Explanation for variations in diabetes susceptibility in human populations living in a diabetogenic environment. Canadian Medical Association Journal 174: 25-26.

Giugliano, D., Ceriello, A. and Paolisso, G. 1996. Oxidative stress and diabetic vascular complications. Diabetes Care 19: 257-267.

Haines, H., Hackel, D. B. and Schmidt-Nielsen, K.1965. Experimental diabetes mellitus induced by diet in the sand rat. American Journal of Physiology 208: 297-300.

Henry, C. J. K., Lightowler, H. J., Newens, K., Sudha, V., Radhika, G., Sathya R. M. and Mohan.V. 2008. Glycaemic index of common foods tested in the UK and India. British Journal of Nutrition 99: 840–845.

Hsieh, C. Y. and Chang, S. T. 2010. Antioxidant activities and Xanthine Oxidase inhibitory effects of Phenolic phytochemicals from *Acacia confusa* twigs and branches. Journal of Agricultural and Food Chemistry 58: 1578-1583.

Imai, S. and Kajiyama, S. 2010. Eating order diet reduced the postprandial Glucose and glycated hemoglobin levels in Japanese patients with type 2 diabetes. Journal of Rehabilitation and Health Sciences 8: 1-7.

Imamura, K. 1996. Jomon and Yayoi: The transition to agriculture in Japanese prehistory. In Harris, D. R. (Eds). The Origins and spread of Agriculture and Pastoralism in Eurasia. P.442-464. London: UCL Press.

Ishige, K., Schubert, D. and Sagara, Y. 2001. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radical Biology and Medicine 30: 433-446.

Jonsson, T., Granfeldt, Y., Erlanson-Albertsson, C., Ahren, B. and Lindeberg, S. 2010. A Paleolithic diet is more satiating per calorie than a Mediterranean- like diet in individuals with ischemic heart disease. Nutrition and Metabolism 7: 85.

Kastorini, Christina-Maria and Panagiotakos, D. B. 2010. Mediterranean diet and diabetes prevention: Myth or fact? World Journal of Diabetes 1: 65-67.

Keys, A., Menotti, A., Karvonen, M. J., Aravanis, C., Blackburn, H., Buzina, R., Djordjevic B. S., Dontas, A. S., Fidanza, F., Keys, M. H., Kromhout, D., Nedeljkovic, S., Punsar, S., Seccareccia, F. and Toshima, H. 1986. The diet and 15-year death rate in the seven countries study. American Journal of Epidemiology 124: 903-915.

Klonoff, D. C. 2009. The Beneficial Effects of a Paleolithic Diet on Type 2 Diabetes and Other Risk Factors for Cardiovascular Disease. Journal of Diabetes Science and Technology 3: 1229-1232.

Lindeberg, S. 2005. Palaeolithic diet ("stone age" diet). Scandinavian Journal of Nutrition 49: 75-77.

Lindeberg, S., Jonsson, T., Granfeldt, Y., Borgstrand, E., Soffman, J., Sjostrom, K. and Ahren, B. 2007. A Palaeolithic diet improves glucose tolerance more than a Mediterranean-like diet in individuals with ischaemic heart disease. Diabetologia 50: 1795-1807.

Milicevic**,** Z., Raz, I., Beattie, S. D., Campaigne, B. N., Sarwat, S., Gromniak, E., Kowalska, I., Galic, E., Tang, M. and Hanefeld, M.2008. Natural history of cardiovascular disease in patients with diabetes: role of hyperglycemia. Diabetes Care 31: 155-160.

Misra, A. and Khurana, L. 2011. Obesity-related non-communicable diseases: South Asians vs. White Caucasians. International Journal of Obesity 35: 167-187.

Monnier, L., Dunseath, G. J., Colette, C. and Owens, D. R. 2007. The loss of Postprandial Glycemic Control Precedes Stepwise Deterioration of Fasting with Worsening Diabetes. Diabetes Care 30: 263-269.

O'Keefe, J. H., Gheewala, N. M. and O'Keefe, J. O. 2008. Dietary strategies for improving post-prandial Glucose, Lipids, Inflammation, and cardiovascular health. Journal of American College of Cardiology 51: 249-255.

Prior, R. L., Wu, X. and Schaich, K. 2005. Standardized methods for the determination of Antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53: 4290-4302.

Raju, B. C., Tiwari, A. K., Kumar, J. A., Ali, A. Z., Agawane, S. B., Saidachary, G. and Madhusudhana, K. 2010. α-Glucosidase inhibitory antihyperglycemic activity of substituted chromenone derivatives. Bioorganic and Medicinal chemistry 18: 358-365.

Richards, M. P. 2002. A brief review of the archaeological evidence for Palaeolithic and Neolithic subsistence. European Journal of Clinical Nutrition 56: 1270-1278.

Sadia, M., Malik, S. A., Rabbani, M. A. and Pearce, S. R. 2009. Electrophoretic characterization and the relationship between some Brassica Species. Electronic Journal of Biology 5: 1-4.

Scheen, A. J. 2009. Voglibose for prevention of type 2 diabetes mellitus. Lancet 373: 1579-1580.

Shastri, D. 1949. Sharangadhara Samhita, p.337, Babu Baijnath Prasad Bookseller, Banaras, India.

Shastri, R. 1962. Charaka Samhita of Agnivesha. Vidyabhavan Ayurveda Granthamal 32 part II, p 236-242, Chaukhambha Vidya Bhawan, Varanasi. India.

Sivasankaran, S. 2010. The cardio-protective diet. Indian Journal of Medical Research 132: 608-616.

Sugiyama, M., Tang, A. C., Wakaki, Y. and Koyama, W. 2003. Glycemic Index of single and mixed meal foods among common Japanese foods with white rice as a reference food . European Journal of Clinical Nutrition 57: 743-752.

Tiwari, A. K., Swapna, M., Ayesha, S. B., Zehra, A., Agawane, S. B. and Madhusudana, K. 2011. Identification of proglycemic and antihyperglycemic activity in antioxidant rich fraction of some common food grains. International Food Research Journal 18(3): 915-923.

Tiwari, A. K., Viswanadh, V., Gowri, P. M., Ali, A. Z., Radhakrishnan, S. V. S., Agawane, S. B., Madhusudana, K. and Rao, J. M. 2010. Oleanolic acid-an α-Glucosidase inhibitory and antihyperglycemic active compound from the fruits of *Sonneratia caseolaris*. Open Access Journal of Medicinal and Aromatic Plants 1: 19-23.

Tiwari, A. K. 2005. Wisdom of Ayurveda in perceiving diabetes: Enigma of therapeutic recognition. Current Science 88: 1043-1051.

Tiwari, A.K. 2008. Invigorated barely in diabetes. Current Science 95: 25-29.

Tiwari, A.K. 2006. Ayurveda will survive till Bharat breathes. Current Science 90: 1589-1590.

Whitfield, J. 2003. Fat pigs ape obese humans. Nature Science Update.

Wootton-Beard, P. C., Moran, A. and Ryan, L. 2011. Stability of the total antioxidant and total Polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP DPPH, ABTS and Folin- Ciocalteu methods. Food Research International 44: 217-214.

Zimmet, P. 2000. Globalization, coca-colonization and the chronic disease epidemic: can the doomsday scenario be averted? Journal of Internal Medicine 247: 301-310.

Zimmet, P. Z. and Alberti, K. G. M. M. 2006. Introduction: Globalization and the Non-communicable disease epidemic. Obesity 14: 1-3