Chapter 8

Evaluation of post prandial glycemic response of Aloe gel enriched dahi and chitosan enriched chapati in normal and diabetic subjects

8.1 Introduction

Type 2 diabetes is emerging as a global public health crisis, particularly among the Asian population, which accounts for 60% of the world’s diabetic population (Chan et al., 2009). Increased urbanization, sedentary lifestyle and higher intake of refined carbohydrates are some of the factors leading to its epidemic growth which in turn lead to losses in the nation’s economy (Hu, 2011).

Post prandial hyperglycemia has been identified as an important risk factor with mounting evidence indicating the undesirable consequences of high blood glucose concentration (Brand-Miller, 2007). It also creates oxidative stress, affecting cellular function, lipid oxidation, protein glycation, clotting tendency and inflammatory processes (Brand-Miller, 2007). Management of post prandial hyperglycemia is considered to be the keystone in the control of diabetes and its complications (Sudhir and Mohan, 2002). Intervention using low glycemic foods and diets is an important strategy to attain this goal.

The preventive potential of low glycemic foods against development of Type 2 diabetes and cardiovascular diseases (Riccardi et al., 2008), and the favorable effects on weight loss (Toeller et al., 2001) has been documented. The recognition of these facts has led to formulation of foods eliciting low glycemic response. Traditional, staple foods consumed on a daily basis by a majority of the population are suitable vehicles for this approach. Enrichment of these traditional foods with nutraceutical ingredients could lead to the development of low glycemic food products providing useful benefits beyond their traditional nutritive value.
*Aloe vera* gel, a recently explored nutraceutical, has been attributed with a wide array of biological activities, one of them being its glucose lowering potential (Reynolds and Dweck, 1999). Though studies conducted in animal models and human subjects demonstrate the blood glucose lowering efficacy of Aloe juice/gel (Jones, 2007), scientific reports on the antidiabetic effects of Aloe gel incorporated food products are lacking.

Adequate dietary fibre consumption, a food based approach, is also considered to be useful in controlling post prandial hyperglycemia. Chitosan, as a new source of dietary fibre (Tungland *et al*., 2002) could help to regulate post prandial glycemic response. Chitosan has also been successfully used as a shelf life enhancer in various foods namely bread, milk, eggs, pasta and other foods (No *et al*., 2007). The cholesterol lowering and weight loss potential of chitosan tablets and capsules has been well explored (Xia *et al*., 2012). Few investigations have studied the effect of chitosan on blood glucose levels in animal models (Miura *et al*., 1995). With regard to food products, hypolipidemic potential of chitosan incorporated bread has been reported (Ausar *et al*., 2003).

The present study attempted to study the post prandial glycemic response of a complete meal, in order to understand and quantify the anti-hyperglycemic efficacy of Aloe gel and chitosan biopolymers as part of a food matrix. Chapati is a traditional staple food consumed in many regions of Asia, and is often consumed with vegetables and curd/dahi as adjuncts. Hence, among the various functional foods developed (chapter 5 and 6), Aloe gel enriched dahi and chitosan enriched chapati were chosen for the *in vivo* glycemic response study in normal and diabetic subjects.

### 8.2 Review of literature

#### 8.2.1 Glycemic response of foods- influencing factors

‘Glycemic response’ is defined by Wolever (1991) as the incremental area under the blood glucose response curve. It describes the effects that carbohydrate containing foods have on blood glucose concentration during the time and course of digestion,
and can be accurately utilized to measure the impact of a consistent consumer-friendly portion of food on the blood sugar levels (Ebbeling et al., 2005). Several factors influence the variability in glycemic response, and some of them are discussed below

8.2.1.1 Macronutrient composition and content

- **Carbohydrates**

  Glycemic responses are reported to be influenced by both amount of carbohydrate consumed and its source. Ingestion of complex carbohydrates was found to result in lower glucose and insulin response (Crapo et al., 1977). Most flour based bread products have been reported to produce high responses of glucose and insulin whereas pumpernickel – type bread products or sour dough fermented bread (Liljeberg et al., 1992) gave rise to lower glucose response than white bread (Granfeldt et al., 1994).

- **Nature of starch**

  Among cereals, the amylase / amylopectin content may differ considerably among genotypes. Miller et al., (1992) reported that high amylose containing rice causes higher blood glucose and insulin responses than low amylose rice. Behall et al., (1988) reported reduced postprandial response of glucose and insulin in healthy subjects following ingestion of crackers made from high amylose corn starch compared to product made from low amylose starch. Schoch and Maywald (1968), reported that legumes whose starch granules contains 30-40% amylose, compared to other starchy foods containing 25-30% amylose, produce lower glycemic response.

- **Resistant starch**

  The fraction of starch that is not hydrolyzed in the small intestine but later fermented by the colonic microflora has been designated as resistant starch, in the sense that it resists hydrolysis by the amylolytic enzymes (Champ et al., 1999). Chemically modified resistant starch has been reported to increase the glycemic
response of foods without any impact on the insulin response (Zhou and Kaplan, 1997). In contrast, natural resistant starch from high amylose corn has been shown to reduce the glycemic response and insulin response of foods when used as a flour substitute (Jones et al., 2005).

- **Protein and fat**

Several studies have emphasized that addition of protein and fat was found to decrease the blood glucose responses and enhance insulin secretion when added to a carbohydrate test meal (Nuttall et al., 1984; Welch et al., 1987). Administration of 10, 30 or 50 g of protein through 50 g glucose loads to NIDDM subjects has been found to show increased insulin secretion (Nuttall et al., 1984). Addition of 12g protein and 25g fat to 50g carbohydrate in the form of bread did not alter the glycemic response in NIDDM subjects, when fat was added as cheese but did have a significant effect when added as peanut butter (Jenkins et al., 1984).

- **Fibre**

Many studies demonstrated that increasing dietary fibre may be useful means of lowering plasma glucose in some diabetic patients (Miranda and Horwitz, 1978). Early studies showed that the gel forming fibers were able to reduce the excursions of blood glucose and insulin after a meal (Jenkins et al., 1977). Studies on high fibre foods have led to the observation that cereal fiber as such has no effect on the glycemic response; (Jenkins et. al., 1981; 1983). Whereas foods containing viscous fiber, such as barley (Wolever et. al., 1988) and legumes (Jenkins et. al., 1981;1983) have been reported to have a low GI. Anderson (1987) reported that insoluble oat bran decreased fasting serum glucose levels, LDL and apolipoproteins significantly.

8.2.1.2 **Antinutrients**

A number of studies have demonstrated the ability phytic acid, tannins, lectins and hemagglutinins to inhibit alpha - amylase activity indicating that these have
potential in interfering with starch digestibility, thereby lowering the glycemic response.

Lectins inhibit the access of starch by either binding to the enzyme (amylase) or to starch. They also bind to the surface of the cells in the gut and impair the uptake of sugars (Donatucci et al., 1987). The effect of phytic acid on starch digestibility and blood glucose levels has been demonstrated (Yoon et al., 1983; Thompson et al., 1987) in humans where higher phytic acid intake has been reported to correlate negatively with glycemic index (GI). In vitro studies have shown that phytic acid can suppress the activity of amylase in sprouted wheat, maize, chickpea, barely, peanut, and bovine pancreas (Deshpande and Cheryan, 1984) thereby interfering with starch digestion and absorption.

The polyphenolic extracts from bean varieties were shown to inhibit glucose transport across rat ileal mucosa in vivo (Motilva et al., 1983). Thompson et al., (1984) demonstrated the role of polyphenols in reducing starch digestibility in vitro and their possible role in lowering glucose response.

8.2.1.3 Starch-nutrient interaction

The protein matrix in cereal (Jenkins et al., 1987) as well as in legume products (Tovar et al., 1990) has been reported to limit the accessibility of starch to amylase. In accordance with the observation of Jenkins et al., (1987), protein – starch interactions reduces the availability of starch in bread products and bread from gluten – free flour was found to elicit a higher glucose response than ordinary wheat. An amylase – lysolecithin complex has been reported to be hydrolysed by amylase at a reduced rate in vitro and to produce a reduced blood glucose and insulin response in vivo (Holm et al., 1983).

8.2.1.4 Effect of food processing / cooking

Cooking causes starch gelatinization; i.e. swelling of starch granules in the presence of heat and water. This increases its susceptibility to enzymic digestion (Snow and O’ Dea, 1981). A greater rise in blood glucose and insulin has been
reported after consumption of cooked as opposed to raw starch (Collings et al., 1981). Increased glycemic responses have been reported to be associated with increased cooking of rice (Wolever et al., 1986) and potato, but not carrot (Vaaler et al., 1984) or spaghetti (Wolever et al., 1986). It has been reported that modern methods of food processing are associated with increased glycemic responses. These methods include grinding (Jenkins et al., 1982), extracting (Bjorck et al., 1984), flaking and popping (Holm et al., 1985). Traditional methods of food processing that may be associated with reduced glycemic response include parboiling of wheat (Gannon et al., 1986) and rice (Wolever et al., 1986), the use of whole grain in rye breads (Jenkins et al., 1986), and cold extrusion as in the making of pasta (Wolever et al., 1986).

8.2.1.5 Other factors

Other factors influencing the glycemic response include variability of the method of assessment and presentation of result, variation due to test-meal related factors, variability from day-to-day within the same subject and variation between different subjects (Wolever, 1990).

Variability due to different methods of assessment and presentation of results has also been found. Factors that affect the glycemic response to the same food include the presence of diabetes, type and treatment of diabetes, weight, age, sex and race. It has also been reported that men have larger glycemic response than women and this difference could be explained by the fact that men consumed more carbohydrates than women in absolute terms (Wolever, 1991).

One of the earliest methods used to ascertain the glycemic response to foods is the Glycemic Index (GI). It is a ranking of foods based on the post meal rise in blood glucose they produce compared to the rise in glucose after ingestion of a reference food. Technically, GI is defined as the area under the glucose response curve after an individual consumes a portion of the test food that provides 50 grams of carbohydrate (Wolever et al., 1991).
Low – glycemic index carbohydrates are generally considered to be those with a GI below 40 (using white bread as reference); those that have a GI between 40 and 70 have a moderate glycemic index and those greater than 70 can be considered high – glycemic index carbohydrates (Bell and Sears, 2003).

The accuracy of certain aspects in the measurement of glycemic index (GI) is currently being questioned. In this approach, the calculation of 50g available carbohydrate portion size is usually carried out by subtracting dietary fibre from total carbohydrate and only includes those carbohydrate sources that are assumed to be fully digestible, absorbable and glycemic. It has been suggested that this technique may not truly reflect the in vivo available carbohydrate content and may lead to overestimation as in case of products containing indigestible carbohydrates that are not recovered as dietary fibre (Monro, 2003). Also, this approach does not actually indicate the blood glucose response elicited by a whole food or meal, which would in turn depend upon the amounts and types of fat, protein, fibre and other constituents present and their interactions (Monro and Shaw, 2008).

In this regard, a ‘food-based GI’ approach has been suggested as an alternative to GI. This approach recommends the calculation and application of GI on a whole food basis as a food effect, including all its constituents (Witwer, 2005). It takes into account the quantity of food consumed, the proportion of carbohydrate in it, as well as the glycemic potency of the carbohydrate. To describe this approach, certain glycemic expressions have been developed, one of them being relative glycemic effect (RGE).

RGE is based on total carbohydrate instead of available carbohydrate and is determined by measuring the glycemic response elicited by 50g total carbohydrate expressed relative to 50g glucose (Brouns et al., 2005). An advantage of this approach is that, unlike the GI approach, it is does not penalize for the inclusion of dietary fibre. The total carbohydrate approach is more feasible to compare identical portions of food and could be utilized to measure the impact of a consistent consumer friendly food portion on blood sugar (Witwer, 2005).
8.2.2 In vivo antidiabetic activity of Aloe gel

Animal studies and human clinical trials have described the action of Aloe vera as an antidiabetic agent in different forms, as discussed below:

In studies conducted by Rajasekaran et al., (2005, 2005a), oxidative stress in STZ induced diabetic rats was evaluated post administration of 300mg/kg Aloe vera ethanolic extract once a day for 21 days, in comparison of reference drug glibenclamide. The authors reported similar ameliorative effects on oxidative stress compared to diabetic control group, which includes significantly reduced levels of plasma glucose, lipid peroxides, TBARS and elevated insulin levels.

Abo – Youssef and Messiha (2013) studied the antidiabetic efficacy of Aloe vera whole leaf pulp extract in comparison with the standard drug glimperide, in STZ induced diabetic rats. Aloe extract (10ml/kg) and glimperide (10mg/kg) were reported to significantly reduce the serum glucose level, levels of the enzymes MDA, SOD and increased serum insulin levels and levels of GSH, with Aloe reported to show better results compared to the synthetic drug.

Kim et al., (2009) investigated the antidiabetic effects of processed Aloe vera gel in mice exhibiting diet-induced obesity (DIO), which is an animal model demonstrating metabolic abnormalities such as hyperglycemia, obesity, and insulin resistance, that closely resemble those found in human NIDDM subjects.

Relatively few human clinical trials report the anti diabetic activity of Aloe gel, but the results are consistent with those of animal studies. In an early long term human clinical trial, bread with Aloe vera gel (100g) and husk of isabgol (20g) administered to subjects was reported to bring about marked reduction in serum cholesterol, triglycerides, decrease in LDL and increase in HDL cholesterol. More than 90% of diabetic subjects were reported to achieve normalization in fasting and post prandial blood glucose levels after 2 months of the study (Agarwal, 1985).

In the first of two related clinical trials, one tablespoon of Aloe vera gel or placebo was administered for 6 weeks to 72 diabetic subjects, who were not taking
any diabetic medication. Significant reduction in blood glucose and serum triglyceride levels was observed with Aloe gel administration (Yongchaiyudha et al., 1996). The second trial investigated the effects of *Aloe vera* gel or placebo in combination with glibenclamide (a commonly prescribed antidiabetic medication), with significant reduction in plasma glucose and serum triglyceride levels (Bunyapraphatsara et al. 1996).

A pilot study conducted to evaluate the effect of two Aloe products UP780 (*Aloe vera* inner leaf gel powder standardized with 2% aloesin) and AC952 (*Aloe vera* inner leaf gel powder) in patients with pre diabetes and impaired glucose tolerance, reported AC952 to bring about significant reduction in total and LDL cholesterol, glucose, and fructosamine and UP780 to cause reduction in HbA1c, fructosamine, fasting glucose, insulin, and HOMA (Homeostasis Model Assessment, an indicator which estimates steady state beta cell function (%B) and insulin sensitivity (%S), as percentages of a normal reference population (Devaraj et al., 2013).

A study by Yagi et al., (2009) reported hypoglycemic effect and lowering of triglycerides in patients administered *Aloe vera* L. high molecular weight fractions after six weeks and four weeks of treatment, respectively.

### 8.2.3 In vivo antidiabetic activity of chitosan

Chitosan has been evaluated for its antidiabetic activity in many animal and human studies. These studies reveal chitosan to increase insulin sensitivity, glucose tolerance and lower the plasma lipoprotein concentrations.

Studies conducted by Lee et al. (2003) on the antidiabetic effect of chitosan on diabetic rats have shown that the glucose tolerance levels significantly increased in the diabetic rats treated with 0.3% chitosan for 4 weeks. Although the exact mechanism is unknown, one of the putative mechanisms is thought to be the induction of glucose-inducible secretions of insulin in the diabetic rats.

Yao et al. (2008) have conducted experiments on streptozotocin-induced diabetic rats. They reported that high molecular weight (HMW) chitosan significantly reduced
the activities of intestinal disaccharidases, suggesting that glucose absorption in the small intestine might be slowed down on feeding HMW chitosan. This has been postulated to increase the insulin sensitivity of the peripheral tissues, thereby reducing the plasma glucose (Doi, 1995; Choi et al., 1998).

The effect of chitosan on insulin sensitivity of obese subjects was studied by Hernández-Gonzalez, et al. (2010). They observed that chitosan improved insulin sensitivity significantly as measured by euglycemic – hyperinsulinemic clamp technique. Also, there was a decrease in weight, body mass index, waist circumference and triglycerides in the chitosan fed subjects. It was suggested that the increase in insulin sensitivity may be due to significant decrease in the weight and triglyceride levels, though exact mechanism is not known. Lowering of LDL cholesterol levels, another lipid parameter of significance has been also reported (Tai et al., 2000) in Type 2 diabetic subjects with hypercholesterolemia on chitosan administration.

8.3 Materials and methods

8.3.1 In vivo glycemic response study

8.3.1.1 Selection of subjects

For the stage 1 of the study, ten non-diabetic, healthy female volunteers aged between 18-23 years, were recruited to take part. For stage 2, ten non-insulin dependent diabetic subjects aged between 40-60 years were selected. The inclusion and exclusion criterion for selection of the subjects is given in table 8.1. Anthropometric measurements such as height and weight were taken in the fasting state. For measuring the height, standard measuring tape was used and the measurements carried out to the nearest cm by making the subjects stand erect against the wall without shoes.

Body weight was recorded using a standard weighing machine, with subjects wearing light clothing without shoes. Body mass index was calculated using the
standard formula of weight (kg)/ height (m²). The details of the subject characteristics are given in table 8.2.

8.3.1.2 Study design

The experimental design followed has been outlined in Fig 8.1. The study design followed was randomized, single-blind, controlled, crossover trial. The glycemic response was measured as per FAO/WHO protocol (1998), which recommends the tests to be repeated in six or more subjects. In the present study, ten normal and ten diabetic subjects were tested to provide a greater degree of precision.

In order to reduce the effect of day-to-day within-subject variation in glycemic response, control was exercised over the lifestyle confounding factors. Subjects were asked to standardize their exercise habits (Venter et al., 2003) by following the same exercise program especially the day before the tests were conducted. Subjects were advised not to participate in the tests during their menstrual period as hormonal fluctuations have been reported to affect the blood glucose response (Poirier-Solomon, 2001). Subjects were also asked not to take part in the test if they had any infection such as cold, toothache etc. which also has been reported to usually affect the blood glucose levels (Hanas, 1998). Care was taken to ensure that all the subjects consumed a similar standard meal the night before all the tests and were asked not to consume anything after 20:00 hours though water was allowed in moderation. The tests were carried out in the morning after 10-12 h of overnight fast. Diabetic subjects were asked to abstain from taking their daily diabetic medication on the day of the study and the day before under the supervision of a physician.
Table 8.1 Inclusion/Exclusion criteria followed for selection of normal and diabetic subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal subjects</th>
<th>Diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>18-23 yrs.</td>
<td>40-60 yrs.</td>
</tr>
<tr>
<td>BMI</td>
<td>18-30 kg/m²</td>
<td>18-30 kg/m²</td>
</tr>
<tr>
<td>Health condition</td>
<td>Subjects excluded in case of:</td>
<td>Subjects excluded in case of:</td>
</tr>
<tr>
<td></td>
<td>1. Any illness/infection</td>
<td>1. Any illness/infection</td>
</tr>
<tr>
<td></td>
<td>2. Food allergy</td>
<td>2. Food allergy</td>
</tr>
<tr>
<td></td>
<td>3. Medication known to modify glucose metabolism.</td>
<td>3. Presence of complications related to heart, liver, kidney and thyroid.</td>
</tr>
<tr>
<td>Fasting blood glucose level</td>
<td>&lt;100 mg/dl</td>
<td>&lt;150 mg/dl</td>
</tr>
</tbody>
</table>

Table 8.2 Subject characteristics (n= 10) (Mean ± SEM)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Height (m)</th>
<th>Weight (Kg)</th>
<th>BMI (Kg/m²)</th>
<th>Fasting blood glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19.3 ± 1.07</td>
<td>1.52 ± 0.05</td>
<td>53.6 ± 11.4</td>
<td>23.0 ± 3.31</td>
<td>4.61 ± 0.23</td>
</tr>
<tr>
<td>Diabetic</td>
<td>46.0 ± 5.83</td>
<td>1.65 ± 0.08</td>
<td>69.0 ± 12.3</td>
<td>25.3 ± 2.35</td>
<td>8.50 ± 1.01</td>
</tr>
</tbody>
</table>
Fig 8.1 Experimental design of *in vivo* glycemic response study

![Experimental Design Diagram]

- **Selection of healthy subjects** (n=10)
- **Administration of 50g glucose**
- **Administration of control and test meals in random order on different days**
  - **Control meal**
    - Whole wheat flour chapati (WFC)
    - Plain curd (PC)
  - **Test meals**
    - 1. Aloe test meal: WFC and Aloe curd (AC)
    - 2. Chitosan test meal: Chitosan chapati (CHC) and PC
- **Testing of blood glucose levels at 0, 15, 30, 45, 60, 90 and 120 minutes after administration of glucose and control/test meals, using finger prick capillary method**
- **Evaluation of glycemic response and calculation of Incremental area under curve (IAUC) and Glycemic Index (GI) (FAO/WHO guidelines, 1998)**
Ethical approval for the study was obtained from the institutional human ethical committee. Written informed consent was obtained from all the subjects prior to their participation in the study, after explanation of the study objectives in detail.

8.3.1.3 Experimental meals

Test meal 1 (Aloe test meal) consisted of Aloe gel enriched dahi (AGD) and whole wheat flour chapati (WFCh) and test meal 2 (chitosan test meal) consisted of chitosan enriched chapati (CHCh) with plain dahi (PD). The glycemic responses of the test meals were compared to that of a control meal consisting of PD and WFCh. Tomato chutney (TC) was served as an accompanying dish in both the control and test meals.

Preparation of plain/control dahi (PD) and AGD was carried out as per the method mentioned in chapter 5, section 5.3.1. Among the different concentrations of Aloe gel powder incorporation in dahi studied, 0.15% was chosen for the glycemic response study. This concentration was found to give optimum results in terms of physico-chemical, functional and sensory parameters. Also, an earlier clinical trial in humans reported blood glucose lowering potential of 80% Aloe gel juice when administered at 30ml per day (Yongchaiyudha et al., 1996). This would amount to around 200mg of Aloe gel powder. In the present study 150ml AGD was used for the in vivo study which would equate to administering 225 mg Aloe gel powder.

Whole wheat flour chapati and chitosan enriched chapati (WFCh and CHCh) were prepared as described in chapter 6, section 6.3. Chapati prepared from wheat flour incorporated with 5% chitosan was selected as it exhibited optimum sensory properties and better inhibition of enzymes related to glucose metabolism (α amylase and α glucosidase), compared to 1% and 3% chitosan incorporated wheat flour chapati (chapter 7).
8.3.1.4 Nutritional analysis of the experimental meals

In the meals, moisture, protein, fat and total dietary fibre were estimated as per AOAC method (1990). Total carbohydrate was estimated by difference method (FAO, 2003) and energy (KJ) was calculated using conversion factors (Codex Alimentarius, 1991).

8.3.1.5 Reference food

Glucose (50g Glucon-D glucose powder) dissolved in 200ml of water was used as reference food. The reference food was consumed in the first and last sessions, while the control and test meals were consumed in random order in between the reference food sessions. As per the FAO/WHO recommendations, (1998), a minimum of one day gap was maintained between the measurements to minimize the carry over effects.

The tests were carried out in the morning after a 10-12 h overnight fast. The subjects were asked to consume the reference food/control/test meal within 10-15 minutes at a comfortable pace. The reference food, the control and test meals were served with 200ml water. Subjects were asked to have minimum physical activity during the test.

8.3.1.6 Blood glucose measurement

After consumption of the reference/test and control meals, capillary finger-prick samples were obtained from the normal subjects at 0 minutes and after 15, 30, 45, 60, 90 and 120 minutes of ingestion of meals. For diabetic subjects, blood glucose measurements were carried out at 0, 30, 60, 90, 120, 150 and 180 minutes. Finger tip capillary blood has been reported to give the greatest sensitivity and has also been reported to remove the potential for variation in measurement due to fluctuations in factors such as ambient temperature (Brouns et al., 2005). Blood glucose measurements were carried out using a glucometer (One Touch Ultra, Lifescan healthcare products) which utilizes the glucose oxidase method to estimate glucose. It was calibrated with a control solution.
supplied by the manufacturer, on a daily basis and each time a new test strip box was used. An automatic lancet device provided along with the glucometer was used for blood sampling.

8.3.1.7 Calculation of incremental area under curve (iAUC) and relative glycemic effect (RGE) of control and test meals

The incremental area under the blood glucose response curve (iAUC) to reference, control and test meals were calculated geometrically using the trapezoid rule, as recommended by the FAO/WHO, (1998). The glycemic response for each of the experimental meals was calculated for each subject as a percent ratio between iAUC of test and control meals and the same subject’s iAUC for reference food (Wolever et al., 1991). The mean glycemic response was expressed as RGE.

\[
\text{RGE of test/control meal (\%) = } \frac{(\text{Blood glucose IAUC value for test or control meal})}{(\text{Mean iAUC value for reference food})} \times 100
\]

8.3.1.8 Statistical analysis

Statistical analysis was carried out using Microsoft Office Excel software. Data are shown as mean with standard deviations. Paired t-test was utilized to study the significant difference between the blood glucose response, iAUC and RGE of control and test meals. Statistical significance was set at P<0.05.

8.4 Results

8.4.1 Effect of Aloe gel and chitosan enrichment on the nutritional composition of the experimental meals

The equicarbohydrate (total CHO) control and test meals (Table 8.3) were found to be similar in terms of energy, protein, and fat. Available carbohydrate content of 42.9g and 41.6g was recorded by the Aloe test meal and chitosan test meal, respectively, significantly lower than that of control meal (44.2g). Chitosan test meal recorded
significantly higher total dietary fibre content of 8.91g, compared to control and Aloe test meal (6.51g). Resistant starch contents were similar between the meals.

8.4.2 In vivo postprandial glycemic response of Aloe test meal

8.4.2.1 Normal subjects

The mean blood glucose concentrations (mmol/L) for normal subjects at 0, 15, 30, 45, 60, 90 and 120 minutes after ingestion of glucose, control and test meals are depicted in Fig.8.2a.

The mean fasting blood glucose level for the healthy subjects was similar before the ingestion of glucose (4.79 mmol/L), control (4.88 mmol/L) and test meal (4.77 mmol/L). The peak rise in the blood glucose concentration observed at 45 minutes for glucose was found to be 7.57 mmol/L, whereas, for control and Aloe test meal it was found to be significantly lower (5.66 and 5.44 mmol/L, respectively). The blood glucose response was found to be significantly lower (P<0.05) for the test meal at 30, 60 and 90 minutes, compared to the control meal. The IAUC of the test meal (71 ± 27.2 mmol/L, mean ± SEM) was also found to be significantly lower than that of control meal (102 ± 38.8 mmol/L). The relative glycemic effect (RGE) elicited by control and test meals followed a similar trend, with the RGE of test meal being 42, significantly lower than that of control meal (54) (Table 8.4).

8.4.2.2 Diabetic subjects

The mean blood glucose concentrations (mmol/L) for diabetic subjects at 0, 15, 30, 45, 60, 90, 120, 150 and 180 minutes after ingestion of glucose, control and Aloe test meal are depicted in Fig.8.2b.

In case of diabetic subjects also, mean fasting blood glucose levels were similar after ingestion of glucose (7.44 mmol/L), control (7.54 mmol/L) and test meal (7.44 mmol/L). The peak rise in blood glucose response was observed at 60 minutes (15.2 mmol/L) for glucose, whereas for control and test meal, the peak rise was observed at 90 minutes.
Table 8.3 Nutritional composition of control, Aloe test meal and chitosan test meal

<table>
<thead>
<tr>
<th>Product</th>
<th>Components</th>
<th>Quantity</th>
<th>Energy (KCal)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Total CHO (g)</th>
<th>Available CHO (g)</th>
<th>TDF (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control meal</td>
<td>WFCh</td>
<td>2 in no. (86g) 150 ml</td>
<td>188</td>
<td>7.17</td>
<td>0.43</td>
<td>46.4</td>
<td>39.9</td>
<td>6.51</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td></td>
<td>81</td>
<td>5.14</td>
<td>5.02</td>
<td>4.74</td>
<td>4.33</td>
<td>--</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>269</td>
<td>12.3</td>
<td>5.45</td>
<td>51.1</td>
<td>44.2</td>
<td>6.51</td>
</tr>
<tr>
<td>Aloe test meal</td>
<td>WFCh</td>
<td>2 in no. (86g) 150 ml</td>
<td>188</td>
<td>7.17</td>
<td>0.43</td>
<td>46.4</td>
<td>39.9</td>
<td>6.51</td>
</tr>
<tr>
<td></td>
<td>AGD</td>
<td></td>
<td>77</td>
<td>5.42</td>
<td>5.02</td>
<td>5.36</td>
<td>2.97</td>
<td>--</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>265</td>
<td>12.6</td>
<td>5.45</td>
<td>51.8</td>
<td>42.9</td>
<td>6.51</td>
</tr>
<tr>
<td>Chitosan test meal</td>
<td>CHC</td>
<td>2 in no. (86g) 150 ml</td>
<td>184</td>
<td>8.35</td>
<td>0.48</td>
<td>46.2</td>
<td>37.3</td>
<td>8.91</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td></td>
<td>81</td>
<td>5.14</td>
<td>5.02</td>
<td>4.74</td>
<td>4.33</td>
<td>--</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>265</td>
<td>13.5</td>
<td>5.50</td>
<td>50.9</td>
<td>41.6</td>
<td>8.91</td>
</tr>
</tbody>
</table>
Table 8.4 iAUC and RGE of Aloe and chitosan enriched meals

<table>
<thead>
<tr>
<th>Meal type</th>
<th>Normal subjects</th>
<th></th>
<th>Diabetic subjects</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>iAUC (mmol/L)</td>
<td>RGE</td>
<td>iAUC (mmol/L)</td>
<td>RGE</td>
</tr>
<tr>
<td>Control meal</td>
<td>102 ± 38.8</td>
<td>54 ± 20.8</td>
<td>469 ± 40.8</td>
<td>57 ± 14.2</td>
</tr>
<tr>
<td>Aloe test meal</td>
<td>71* ± 27.2</td>
<td>42* ± 9.2</td>
<td>389* ± 29.5</td>
<td>48* ± 10.2</td>
</tr>
<tr>
<td>Chitosan test meal</td>
<td>63* ± 9.9</td>
<td>36* ± 7.7</td>
<td>374* ± 38.3</td>
<td>46* ± 12.1</td>
</tr>
</tbody>
</table>
Fig 8.2 Mean postprandial blood glucose response curves after consumption of glucose, control meal and Aloe test meal in a) Normal subjects and b) Diabetic subjects

* Indicates significant difference with respect to control meal
Significance calculated using a multisample t-test (*p < 0.05)
The blood glucose response for the test meal was found to be significantly lower at all measurement intervals up to 180 minutes, compared to the control meal. The IAUC of the Aloe test meal (389 mmol/L) was, therefore, found to be significantly lower than that of control meal (469 mmol/L). The relative glycemic effect elicited by control and test meals followed a similar trend, with the RGE of test meal being 48, significantly lower than that of control meal (57) (Table 8.4) in diabetic subjects.

8.4.2.3 Efficacy of Aloe gel enriched meal in attenuating blood glucose response

In the present study, the test meal supplemented with Aloe gel enriched curd was found to elicit a significantly lower glycemic response compared to the control meal in normal and diabetic subjects (22% and 19%, respectively).

The experimental meals studied had similar macronutrient composition. Hence, the greater blood glucose lowering effect observed in case of Aloe test meal could be attributed to the presence of Aloe gel, the only component varied between the two meals.

A study by Korean investigators (Shin et al., 1995) examined the effect of Aloe gel incorporation into yoghurt and studied only its quality characteristics. An early human clinical trial reports the administration of wheat bread given along with Aloe vera gel and husk of isabgol to 5000 patients having atheromatous heart disease, out of which more than 3000 subjects also had diabetes (Agarwal, 1985). The results of the study revealed that the combination product administered showed profound effects, mainly the normalization of blood glucose and lipid profile in more than 90% of the cases. Except for the above study, other clinical trials demonstrating the blood glucose lowering potential, have utilized Aloe gel directly as gel fractions or juice, without incorporating in a product. Such studies include those in animals (Ajabnoor, 1990; Rajasekharan et al., 2005) and in diabetic subjects (Beppu et al., 2006; Arora et al., 2009). In all the above studies long term supplemental effect of Aloe gel has been determined. The present study is probably also the first acute response study of Aloe gel supplemented meal.
Various components present in Aloe gel have been hypothesized to be responsible for its blood glucose lowering effect. In a recent investigation, a high molecular weight Aloe gel fraction containing the polysaccharide acemannan was found to exhibit hypoglycemic activity (Yagi et al., 2009). Saponins, a phytochemical, present in Aloe vera gel (Rajasekharan et al., 2005; Park and Lee, 2003) are reported to have blood glucose lowering effect (Lu et al., 2008).

Phytosterols isolated from Aloe vera gel have been also reported to markedly reduce blood glucose levels in a mouse model with Type II diabetes (Tanaka et al., 2006). A number of flavonoid compounds have been reported to be present in Aloe gel (Rajsekharan et al., 2005). These compounds suppress levels of plasma glucose, increase hepatic glucokinase activity thereby enhance insulin release from pancreatic islet cells (Vessa et al., 2003).

Qualitative and quantitative studies on Aloe gel indicate the presence of compounds like saponins, terpenoids, glycosides and ferulic acid (Nejatzadeh-Barandozi, 2013) which have been reported to stimulate the release of insulin in the blood stream and improve uptake of glucose (Ng et al., 1986; Nomura, 2003). Presence of saponins, alkaloids and glycosides were also observed in our present study as reported in chapter 2. The mechanisms of action by which Aloe vera modulates blood glucose are unknown, but it has been suggested that it may interact with insulin. It has been hypothesized that Aloe stimulates insulin synthesis or its release from pancreatic β cells (Ajabnoor 1990). Processed Aloe vera gel was found to suppress the expression of the adipogenic genes SREBP-1a, FAS, and GPAT, suggesting that the gel improves insulin resistance by the reducing toxic effects of lipids in the liver (Kim et al., 2009).

The anti-hyperglycemic activity exhibited by Aloe gel could be through prevention of death of β-cells, permitting recovery of partially destroyed β-cells and initiating cell proliferation (Noor et al., 2008). Further, administration of Aloe gel extract in mice (Perez et al., 2007) has been found to control insulin resistance, one of the underlying causes of diabetes.
Further, diabetic patients are known to have decreased antioxidant defenses with lower levels of antioxidants such as vitamins C and E and also reduced activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Studies conducted on animals have revealed that supplementation with Aloe gel stimulate the body’s own antioxidant defenses (Beppu et al., 2006). In human subjects, a study by Vinson et al., (2005) demonstrated marked enhancement in the bioavailability and half-life of the antioxidant vitamins, ascorbate (vitamin C) and tocopherol (vitamin E).

8.4.3 In vivo glycemic response of chitosan test meal

8.4.3.1 Normal subjects

Fig 8.3a illustrates the mean blood glucose concentrations (mmol/L) for normal subjects after ingestion of glucose, control and chitosan test meal.

The mean fasting blood glucose level for the healthy subjects was similar before the ingestion of glucose (4.79 mmol/L), control (4.88 ± 0.40 mmol/L) and test meal (4.77 mmol/L). As in the case of Aloe test meal, peak rise observed at 45 min after consumption of glucose was significantly higher than that of control and test meal. A significantly (P<0.05) lower blood glucose levels at 45, 60, 90 and 120 minutes was observed by the chitosan test meal, compared to the control meal. The test meal also helped in bringing glucose levels to baseline after 120 minutes. The chitosan enriched test meal exhibited 38% and 33% significantly lower iAUC and RGE, respectively, compared to control meal.

8.4.3.2 Diabetic subjects

The mean blood glucose concentration after ingestion of glucose, control and chitosan test meal for diabetic subjects is given in fig 8.3b. Similar initial blood glucose concentration ranging from 7.8-8 mmol/L was seen after consumption of reference and experimental meals. Peak rise of 15.2 mmol/L was seen after consumption of glucose at 60 min, significantly higher than that of control (14.2 mmol/L) and chitosan test meal (12.6 mmol/L).
Fig 8.3 Mean postprandial blood glucose response curves after consumption of glucose, control meal and chitosan test meal in a) Normal subjects and b) Diabetic subjects

* Indicates significant difference with respect to control meal. Significances calculated using a multisampler-test (*p < 0.05).
Test meal enriched with chitosan was found to significantly lower (P< 0.05) the glucose levels compared to control meal at 30, 60, 120 and 150 minutes. The iAUC and RGE of the control meal was reduced by 20% and 19%, respectively, on administration of chitosan test meal in diabetic subjects.

8.4.3.3 Efficacy of chitosan enriched meal in attenuating blood glucose response

The blood glucose lowering effect of chitosan test meal could be attributed to chitosan, the only component varied between the control and chitosan test meals.

Addition of chitosan brought about a significant increase in the total dietary fibre (TDF) content of the meal. As reported in our study (chapter 2), chitosan is a good source of dietary fibre. It is also known to be a viscous dietary fibre. Viscous fibres have been reported to have greater potential of reducing glycemic response and increasing insulin sensitivity (Jenkins et al., 1978). In a study (Rodríguez, 2008) using in vitro gastrointestinal model, chitosan was reported to reduce glucose bioavailability by entrapping glucose and reducing its rate of absorption. A similar mechanism could have led to the flattened glucose curve observed in subjects after intake of chitosan test meal.

An earlier study reported an increase in glucose tolerance and insulin secretion in neonatal streptozotocin (STZ)-induced noninsulin-dependent diabetes mellitus rats treated with 0.3% chitosan oligosaccharide (Lee et al., 2003). In a recent study, administration of 2.25g chitosan per day for three months has been reported to increase insulin sensitivity in obese patients (Hernández-González et al., 2010).
8.5 Conclusions

The present investigation is probably the first to report the glycemic response of Aloe gel enriched dahi and chitosan enriched chapati in normal and diabetic individuals. A significant observation in the present study was that both Aloe gel and chitosan, apart from lowering the overall increase in blood glucose level, also brought about a significant reduction in the peak rise of blood glucose concentrations. Also, additional benefit of the chitosan test meal was that it brought the blood glucose levels almost to baseline after 2h in normal subjects.

The study indicates potential of using Aloe gel as a hypoglycemic agent in dairy based products and chitosan in cereal based products. Such low glycemic products could be of immense benefit in the staple diets of populations highly susceptible to metabolic diseases.