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IN VITRO ANTIDIABETIC ACTIVITY AND *IN VIVO* POST PRANDIAL GLYCEMIC RESPONSE OF ALOE GEL ENRICHED CURD

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ABSTRACT

In vitro anti diabetic activity of *dahi* enriched with Aloe gel powder (AG) at various concentrations (0, 0.1%, 0.15%, 0.2% and 0.25%) was carried out in terms of amylase and α glucosidase inhibition. With increasing concentration of Aloe gel, the inhibitory activity against the tested enzymes increased steadily. *Dahi* with 0.15% AG (0.15 AG) was selected for the *in vivo* study to evaluate the post prandial glycemic response. A group of healthy and diabetic volunteers received at breakfast equal carbohydrate portions of control meal (plain curd/PC with whole wheat flour chapati/WFC), test meal (Aloe gel curd/AGC with WFC), and glucose (as reference). Blood glucose measurements were carried out at periodic intervals. The incremental area under curve (IAUC) and relative glycemic effect (RGE) of the meals were assessed. In both normal and diabetic subjects, the IAUC was significantly lower by 31% and 21%, respectively, compared to control. The RGE of the test meal was also significantly lower in normal (42) and diabetic subjects (46), compared to the respective control meals (54 and 57). The present investigation provides clinical evidence for the improved glucose lowering efficacy of a dairy product containing Aloe gel. This indicates the potential of using Aloe gel for commercial formulation of low glycemic dairy products.

Keywords: *Aloe vera* gel, Curd, Chapati, Diabetes, Relative glycemic effect.

INTRODUCTION

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). Reduction of post-prandial glycemia is therefore a desirable goal. Intervention using low glycemic foods and diets is an important strategy to attain this goal.

Therapeutic role of low glycemic diets in the treatment of diseases related to insulin resistance syndrome/ metabolic syndrome has been also suggested. The preventive potential of low glycemic foods against development of Type II 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.

Major sources of blood glucose are dietary carbohydrates, such as starch, hydrolyzed by α glucosidase and pancreatic α amylase to monosaccharides for absorption by the small intestine (Barret and Udani, 2011). An effective strategy for type-2 diabetes management is inhibition of α glucosidase and pancreatic α amylase. Although there are several synthetic α glucosidase and pancreatic α amylase inhibitors available commercially for medical use, their effectiveness is limited by deleterious side-effects such as abdominal distention and flatulence (Maruhama *et al*., 1980). In this regard, a number of natural ingredients from plant sources are being explored as attractive strategies for the effective management of diabetes, and one of them is *Aloe vera* gel.

Aloe barbadensis Miller, popularly known as *Aloe vera*, has been used since time immemorial for the treatment of several disorders and ailments, owing to its curative and therapeutic properties. In recent years, it is being promoted as a valuable ingredient in the food, pharmaceutical and cosmetic industries (Eshun and He, 2004). The *Aloe vera* gel has been said to be responsible

for a wide array of biological activities exhibited by the *Aloe vera* plant, one of them being its glucose lowering potential (Reynolds and Dweck, 1999).

Various products containing Aloe gel as a functional ingredient are being marketed by the food industry (Rodr'iguez *et al*., 2010). But, scientific information on the therapeutic effects of such products is lacking. Fermented milk products such as yoghurt and curd enjoy wide popularity owing to their taste, nutritional and therapeutic properties. The purpose of the present investigation was to develop a dairy product similar to yoghurt i.e. *dahi*/curd with different concentrations of Aloe gel and to evaluate its blood glucose lowering efficacy. The *in vitro* anti diabetic activity of the formulated products was studied against the key enzymes related to pathogenesis of type 2 diabetes i.e. pancreatic α amylase and α glucosidase. The formulation found to be the most acceptable was selected for *in vivo* glycemic response study.

Curd is a low carbohydrate (approximately 5g/100g) fermented dairy food and is usually consumed as an adjunct in meals in various parts of Asia. Hence, its glycemic response was studied along with a meal comprising of whole wheat flour chapati (unleavened flat bread) and tomato chutney, in normal as well as in diabetic subjects.

MATERIAL AND METHODS

IN VITRO STUDY

PREPARATION OF DAHI

The milk used in the present study was purchased from the local supermarket (Goodlife brand, fat 3.6%, protein 3.2% and carbohydrate 4.6%). For the preparation of *dahi*, the homogenized milk was heated at 85-90^{0}C for 5 minutes and cooled to 45^{0}C. Skimmed milk powder was added at 2.5% level and then inoculated with 10% standard *dahi* starter (Hatsun brand, fat 3.1%, protein 3.3% and carbohydrate 4.4%). Amount of starter was selected based on preliminary experiments. The inoculated milk was incubated at 45^{0}C till the *dahi* was set.

For the preparation of Aloe gel enriched *dahi*, skimmed milk powder was replaced with spray dried Aloe gel powder (AG, 200 X powder, procured from Excel Industries, Hyderabad, A.P., India). It was added at concentrations of 0%, 0.1%, 0.15%, 0.2% and 0.25% levels and the developed products were coded as C, 0.1 AG, 0.15 AG, 0.2 AG and 0.25 AG, respectively.

PREPARATION OF SAMPLE EXTRACTS

The formulated *dahi* samples were homogenized with a sterile spatula and were subjected to centrifugation twice at 5000 rpm. The supernatants were collected and stored at 4^0 C and the experiments were performed within two days.

α- AMYLASE INHIBITION

Amylase activity was assayed by the spectrophotometric method using 3, 5-dinitrosalicylic acid (Pinto *et al*., 2010). Sample extracts (100-500 µl) were taken in test tubes. To that, 70 µL of 50% methanol, 80 µL of enzyme solution and 1mL of starch solution was added and incubated at 37°C for 5 minutes. DNSA reagent (2ml) was added and the tubes were heated in boiling water bath for 5 minutes followed by cooling to room temperature. The absorbance of the colour developed was read at 540 nm. Blank and control tubes were also set up simultaneously without enzyme and sample respectively.

$$\% \text{ inhibition of } \alpha \text{ amylase} = \frac{O.D.control - O.D.sample}{O.D.control} X100$$

α-GLUCOSIDASE INHIBITION ASSAY

The assay was performed according to the method given by Mc Cue *et al* (2005). The assay mixture consisting of different concentrations of sample extracts (100-500µg/ml) and 1000µl of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 U/ml) was incubated at 25^0 C for 10 min. After pre-incubation, 500 µl of 5mM para-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added and incubated at 25^{0}C for 5 min. Before and after incubation, absorbance was recorded at 405 nm using UV spectrophotometer and compared to a control containing 500 µl buffer solutions instead of the extract. The α-glucosidase inhibitory activity (%) was calculated as following:

$$\% \text{ inhibition of } \alpha \text{ glucosidase} = \frac{O.D.control - O.D.sample}{O.D.control} X100$$

IN VIVO GLYCEMIC RESPONSE STUDY

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 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 (m^2). The details of the subject characteristics are given in table 1.

STUDY DESIGN

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 programme 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.

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.

EXPERIMENTAL MEALS

The glycemic effect elicited by test meal consisting of Aloe gel enriched curd (AGC) and whole wheat flour chapati (WFC) and was compared to that of a control meal consisting of plain curd (PC) and WFC. Tomato chutney (TC) was served as an accompanying dish in both the meals.

For the preparation of WFC, commercial whole wheat flour (Aashirwad brand) was purchased from the local supermarket. The dough (prepared from 60g flour) was rolled to approximately 15 cm in diameter, cooked well on both sides on a hot girdle by applying 1-2 ml oil. Plain curd and Aloe gel enriched curd was prepared as described in section 2.1.1. The curd sample chosen for the study was 0.15 AG wherein Aloe gel powder was added at 0.15% level. This level of Aloe gel powder was decided based upon an initial study conducted at our laboratory (Pushkala and Srividya, 2011), wherein 0.15% level of addition 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 AGC was used which would equate to administering 225 mg Aloe gel powder.

In the experimental 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).

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.

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.

CALCULATION OF RELATIVE GLYCEMIC EFFECT

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 (Wolever *et al.*, 1991). 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). For the above reasons, RGE approach was used in the present study to evaluate the glycemic response of the experimental 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.

RGE of test/control meal (%) = (Blood glucose IAUC value for test or control meal)/ (Mean IAUC value for reference food) ×100

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 $P<0.05$.

RESULTS AND DISCUSSION

INHIBITION OF A AMYLASE AND A GLUCOSIDASE BY DAHI EXTRACTS

It was observed that the sample extracts demonstrated higher α glucosidase inhibitory effect than α amylase, since inhibition of α glucosidase could be achieved at lower concentrations. α amylase inhibitory effect of the control and Aloe gel enriched *dahi* extracts (doses 100, 200, 300, 400 and 500µl)is demonstrated in Fig. 1. Figure 2 demonstrates the α glucosidase inhibitory effects of the *dahi* extracts (doses of 10, 20, 30, 40 and 50µl).

Comparison of the α amylase percentage inhibition of the Aloe gel enriched dahi extracts over control revealed that 0.15AG, 0.2AG and 0.25AG exhibited 16.2%, 30.2% and 44.5% higher α amylase inhibition compared to control, respectively, at the highest dose level studied. Similarly in case of α glucosidase, at the highest concentration studied, 0.15AG, 0.2AG and 0.25AG samples showed 30.7%, 40.1% and 43.6% greater inhibition compared to control.

IN VIVO GLYCEMIC RESPONSE

HEALTHY SUBJECTS

The *in vivo* study was conducted on ten healthy and ten diabetic individuals with body mass index ranging between 19 and 29. The characteristics of the subjects who participated in the study are given in table 1. Table 2 presents the macronutrient composition of the experimental meals. The control meal comprising of plain curd and whole wheat flour chapati, and the test meal wherein the plain curd was replaced by Aloe gel enriched curd were similar in terms of macronutrient composition and provided portion sizes with equal carbohydrate.

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.1. Fig.2 represents the mean blood glucose concentrations (mmol/L) for diabetic subjects at 0, 30, 60, 90, 120, 150 and 180 minutes.

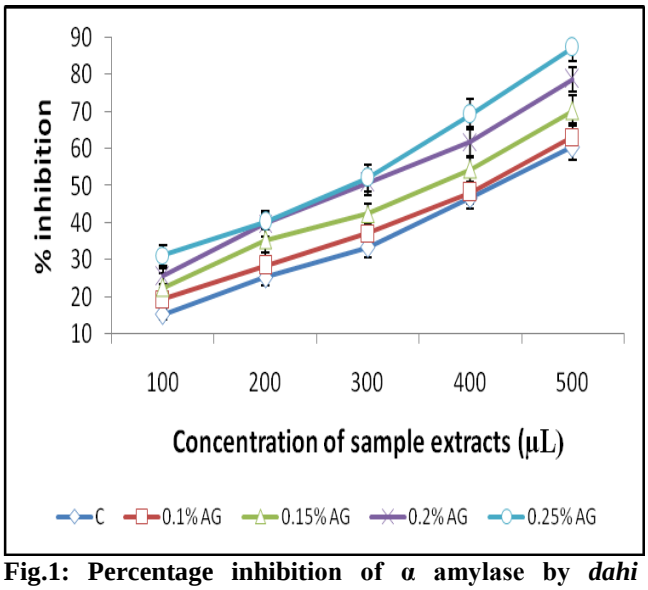


Fig.1: Percentage inhibition of α amylase by *dahi* aqueous extracts

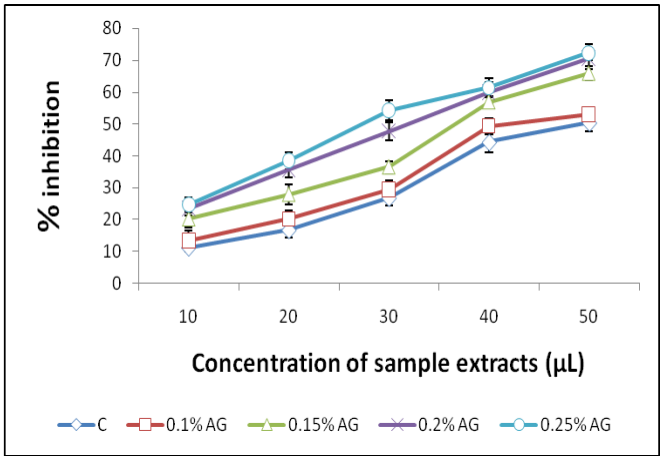


Fig.2: Percentage inhibition of α glucosidase by *dahi* aqueous extracts

The mean fasting blood glucose level for the healthy subjects was similar before the ingestion of glucose (4.75 ± 0.35 mmol/L, mean ± SEM), control (4.84 ± 0.30 mmol/L) and test meal (4.84 ± 0.39 mmol/L). The peak rise in the blood glucose concentration was observed at 30 minutes after ingestion of glucose (6.73 ± 0.69 mmol/L), whereas, in case of control and test meals the peak rise was observed at 45 minutes. The blood glucose response was found to be significantly lower ($P<0.05$) for the test meal at 30 ($P=0.018$), 60 ($P=0.006$) and 90 ($P=0.002$), compared to the control meal. The incremental area under the curve (IAUC) and the relative glycemic effect elicited by the control and test meals in case of normal subjects are shown in table 3. The IAUC of the test meal (71 ± 27.2 mmol/L, mean ± SEM) was found to be significantly lower ($P=0.01$) than that of control meal (102 ± 38.8 mmol/L). The relative glycemic effect elicited by control and test meals followed a similar trend, with the RGE of test meal being 42, significantly lower ($P=0.04$) than that of control meal (54).

Table 1 Subject characteristics (*n* 10) (Mean ± SEM)

Subjects	Age	Height (m)	Weight (Kg)	BMI (Kg/m^2)	Fasting blood glucose (mmol/L)
Normal	19.3 ± 1.07	1.52 ± 0.05	53.6 ± 11.4	23.0 ± 3.31	4.61 ± 0.23
Diabetic	46.0 ± 5.83	1.65 ± 0.08	69.0 ± 12.3	25.3 ± 2.35	8.50 ± 1.01

Table 2 Inclusion/Exclusion criteria followed for selection of normal and diabetic subjects

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

Table 3 Composition of experimental meals providing 50g of total carbohydrates

Product	Quantity	Energy(KJ)	Protein(g)	Fat(g)	Total dietary fibre (g)
WFC§	2 in no.(91g)	911.3	7.75	0.39	10.9
PC†/AGC‡	Curd - 150ml	392.8	3.34	5.02	--
Total		1304.2	11.09	5.41	10.9

§ Whole wheat flour chapati (WFC); † Plain curd (PC); ‡ Aloe gel enriched curd (AGC)
Experimental meal 1 (Control) – WFC and PC; Experimental meal 2 (Test) – WFC and AGC

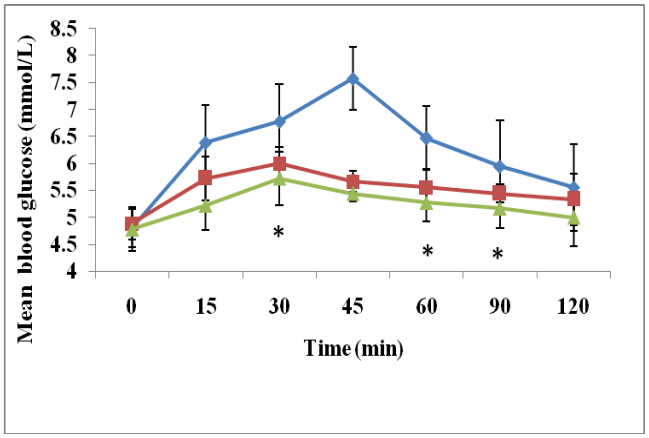
DIABETIC SUBJECTS

In case of diabetic subjects also, mean fasting blood glucose levels were similar after ingestion of glucose (7.5 ± 0.59 mmol/L, mean ± SEM), control (7.33 ± 0.46 mmol/L) and test meal (7.44 ± 0.35 mmol/L). The peak rise in blood glucose response was observed at 60 minutes (15.2 ± 0.85 mmol/L) for glucose, whereas for control and test meal, the peak rise was observed at 90 minutes. The blood glucose response was found to be significantly lower for the test meal at 30 ($P=0.002$), 60 ($P=0.01$), 90 ($P=0.02$), 120 ($P=0.01$), 150 ($P=0.02$) and 180 ($P=0.02$) minutes, compared to the control meal. Table 4 depicts the incremental area under the curve (IAUC) and the relative glycemic effect elicited by the control and test meals. The IAUC of the test meal (374 ± 38.3 mmol/L, mean ± SEM) was found to be significantly lower ($P=0.02$) than that of control meal (469 ± 40.8 mmol/L). The relative glycemic effect elicited by control and test meals followed a similar trend, with the RGE of test meal being 46, significantly lower ($P=0.03$) than that of control meal.

Table 4 Mean IAUC and RGE of control and test meals in normal and diabetic subjects

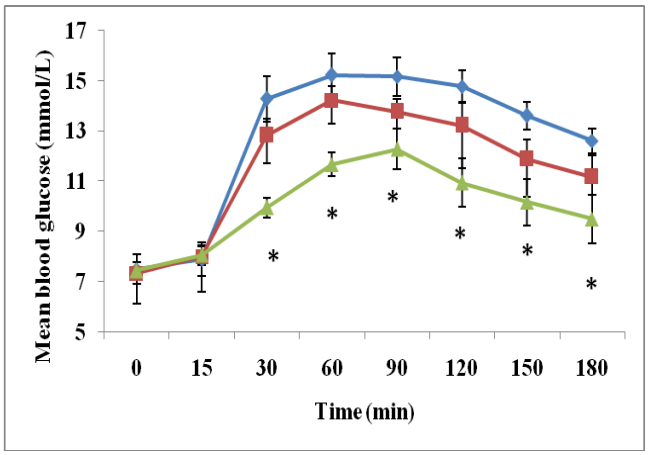
	Normal subjects		Diabetic subjects	
Meal type	IAUC (mmol/L)	RGE	IAUC (mmol/L)	RGE
Control meal	102 ± 38.8	54 ± 20.8	469 ± 40.8	57 ± 14.2
Test meal	71* ± 27.2	42* ± 9.2	389* ± 29.5	48* ± 10.2

* Values significantly different at P value (<0·05) between control and test meal.



* Statistically significant difference between meals

Fig.3. Mean blood glucose concentrations for glucose, control and *Aloe* test meal in normal subjects



* Statistically significant difference between meals

Fig.4. Mean blood glucose concentrations for glucose, control and *Aloe* test meal in diabetic subjects

DISCUSSION

This observation is in consonance with a study which reported natural α amylase and α glucosidase inhibitors from plants to have lower inhibitory effect against α amylase activity and a stronger inhibitory activity against α glucosidase (Kwon *et al.*, 2006).

In an *in vitro* study (Sudha *et al*., 2011), the cold water extract and cyclohexane extract of *Aloe vera* was reported to inhibit porcine pancreatic α amylase activity. To our knowledge, there are no reports available on the *in vitro* anti diabetic activity of food products incorporating Aloe gel, in particular *dahi*. An early study by Korean investigators (Shin *et al*., 1995) and a recent study from our laboratory (Pushkala and Srividya, 2011) examined the effect of Aloe gel incorporation into yoghurt and *dahi*, respectively, and reported its quality characteristics. The present could therefore be the first of its kind to demonstrate the anti diabetic effect of a food product enriched with Aloe gel.

The second part of the study was undertaken to evaluate the glycemic response of curd enriched with Aloe gel as part of whole wheat flour based meal. Curd was chosen for value-addition with Aloe gel mainly for two reasons, the first one being its popularity among large masses of population. Secondly, the minimal processing operation to which the curd is subjected could help in retaining the biological activity of the sensitive Aloe gel constituents, thereby providing maximal functional benefits.

Earlier studies evaluating the glycemic response of whole wheat based foods/meals have reported the impact as glycemic index. This includes, however, few studies which are based on total carbohydrate content. Also, there is a paucity of reports on glycemic response of dahi independently or in combination with other foods. The present study could be considered as one of the first scientific reports evaluating the glycemic response of dahi in combination with chapati, a common meal pattern followed among the Asian population.

There seems to be a paucity of studies on the short term glycemic effects of curd alone or as a part of a meal. However, few long term feeding studies on the antidiabetic effects of curd and yoghurt has been reported. A study conducted on diabetic rats reported that supplementation of probiotic dahi in the diet significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress (Yadav *et al*., 2007). In a recent long term study on human diabetic subjects, consumption of probiotic yogurt was reported to significantly improve fasting blood glucose and antioxidant status, thereby suggesting it as a promising agent in the management of diabetes (Ejtahed *et al*., 2012). To our knowledge, the present study seems to be the first to report the glycemic response of Aloe gel enriched curd.

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

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 containing *Aloe vera* gel along with the 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 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.

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

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, 2006) 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).

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). 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.

CONCLUSION

In conclusion, the present study demonstrates Aloe gel supplementation in the form of curd to be helpful in lowering the glycemic response to wheat based meal. It further indicates the potential of using Aloe gel as a hypoglycemic ingredient in dairy products. Low glycemic foods incorporating Aloe gel nutraceuticals could also result in additional functional benefits.

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