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***In vitro* Postprandial Glucose Lowering Effects of Dietary Fibers Isolated from *Tamarindus indica* and *Cassia fistula* Seeds**

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ABSTRACT

The present study investigates the postprandial serum glucose lowering mechanism of soluble and insoluble dietary fibers isolated from *Cassia fistula* and *Tamarindus indica* seed comprised with natural Guar Gum (GG) and Tragacanth (TRAG) *in vitro* (glucose dialysate and α -amylase activity). The study was performed by two methods that's were effect on diffusion of glucose in glucose-dietary fiber system and effect of dietary fiber on the activity of α -amylase. The results have shown that these isolated dietary fibers diminish the postprandial glucose level more than 50% as compared to control. *Tamarindus indica* soluble fiber (TSF) has more inhibitory action against glucose dialysate in comparison to *Cassia fistula* soluble fiber (CSF), GG and TRAG. *Tamarindus indica* insoluble fiber (TIF) and *Cassia fistula* insoluble fiber (CIF) also had shown significant glucose lowering result against the control and standards. All the tested fibers had also shown the significant hindered α -amylase activity on starch as compared to control. The results of this research suggested that isolated dietary fiber could be potentially effective in the treatment of hyperglycemia in human as per other classical sources of dietary fibers.

Key words: Soluble dietary fiber, insoluble dietary fiber, guar gum, tragacanth, glucose dialysate, α -amylase

INTRODUCTION

Diabetes Mellitus (DM) is a major chronic disease caused by an improper balance of glucose homeostasis. At least 171 million people worldwide have diabetes; this figure is likely to be more than double by 2030 and around 3.2 million deaths every year are attributable to complications of diabetes; six deaths every minute. Two types of DM are currently known, one being insulin-dependent diabetes mellitus, IDDM and the other being type 2 non-insulin-dependent diabetes mellitus, NIDDM (Kwon *et al.*, 2008).

Postprandial hyperglycemia is one of the earliest abnormalities of glucose homeostasis associated with type-2 diabetes and is markedly exaggerated in diabetic patients with fasting hyperglycemia. Postprandial hyperglycemia is independent of risk factors in the development of macro-vascular complications of diabetes mellitus (Brennan *et al.*, 1996). Soluble dietary fibers (SDF) have the effects of hampering diffusion of glucose and postponing the absorption and digestion of carbohydrates, that results in lowering of postprandial blood glucose (Gin and Rigalleau, 2000). In general, SDF such as GG and pectin have been shown to lower blood glucose level and insulin, when given to human simultaneously with a carbohydrate-containing meal

(Ebihara *et al.*, 1981). Dietary fibers lowers the glycemic level in serum by (1) increasing the growth and function of the upper gastrointestinal tract as well as the plasma levels of the intestinotrophic factor, glucagons-like peptide 2, (2) lowering the insulin response and (3) slowing glucose absorption through an effect on gastric emptying and/or entrapment of materials in the viscous digesta. Soluble dietary fibers have the effects of hampering the diffusion of glucose and postponing the absorption and digestion of carbohydrates (Ou *et al.*, 2001; Galisteo *et al.*, 2008).

α -Amylase and α -glucosidase are key enzymes involved in starch breakdown and intestinal absorption, respectively. It is now believed that inhibition of these enzymes involved in the digestion and uptake of carbohydrates can significantly decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet and therefore can be an important strategy in the management of hyperglycemia linked to type 2 diabetes (Kwon *et al.*, 2008).

Thus, DF can have an impact on food by reducing the rate of glucose breakdown and absorption, hence avoiding an excess of glucose in the body and facilitating the steady breakdown of carbohydrate and release of glucose (Ou *et al.*, 2001; Lyly *et al.*, 2004). Dietary fiber has become more and more important since the work of Burkitt *et al.* (1972), Burkitt (1973) and Trowell (1973), who hypothesized a direct relationship between fiber deficient diets and acceleration in the development of certain chronic and degenerative diseases which were prevailing in industrialized countries (Chau *et al.*, 2004).

The objective of this study was to investigate the effect of postprandial glucose lowering effect of isolated dietary fibers in comparison with classical used dietary fiber sources such as Guar and Tragacanth Gum.

MATERIALS AND METHODS

This study was conducted from July, 2007 to August, 2008 at the Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra-Ranchi, Jharkhand, India.

Materials: Isolated DFs, guar gum, tragacanth gum, α -amylase, glucose, God Pod sugar estimation kit (Autospan, Span Diagnostics Ltd., India) and diffusion cell (Franz diffusion cell). All chemicals used in the study were reagent grade.

Methods

Collection of seed: *Cassia fistula* L. and *Tamarindus indica* L. seed were collected in the month of December, 2006 from adjoining areas of Birla Institute of Technology, Mesra, Ranchi. It was identified by Dr. S. Jha, Associate Professor, Department of Pharmaceutical Sciences B.I.T, Mesra, Ranchi. The seeds and plant herbariums were deposited in the departmental museum. Powder of seeds was prepared by hammer mill and sieved by 60 and 100 mesh size sieves.

Dietary fiber extraction: The dietary fibers were extracted from *T. indica* and *C. fistula* defatted seeds as per the procedure describe by Jha *et al.* (2008). Defatted seeds powder was added in 0.2 M phosphate buffer (pH 6.0) followed by the addition of the heat stable α -amylases. The mixture was incubated in a boiling water bath (95°C) for 30 min. NaOH (0.2 N) was dispensed into the mixture and pH of the mixture was adjusted to 7.5-7.9 at 60°C by using 0.2 N HCl or 0.2 N NaOH. Immediately before use, solution of protease in phosphate buffer, was added to the mixture and was incubated at 60°C for 30 min with continuous agitation. Subsequently, HCl (0.2 N) was dispensed into the mixture and pH of the mixture was adjusted to 4.0-4.7 at 60°C by using 0.2 N

NaOH or 0.2 N HCl. Then fungal amyloglucosidase was added into the mixture and incubated at 60°C for further 30 min with continuous shaking. The heat stable α -amylase enzyme used to gelatinize and depolymerize the starch into monosaccharide and dextrin, while the protease and amyloglucosidase were used to hydrolyze protein and to further break down dextrin's into glucose.

The enzyme treated mixture containing buffer solution and nondigestible material was separated by using centrifugation (5000 rpm for 20 min at 25°C) and filtered with the vacuum filtration unit. The residue recovered (IDF) was washed twice with preheated water (70°C), 78% ethanol, 90% ethanol and acetone sequentially and dried in vacuum air oven at 40°C overnight. The filtrate was precipitated with four volumes of 90% ethanol overnight. Then the ethanol insoluble residue (SDF) recovered from centrifugation (5000 rpm, 10 min, 25°C) was washed with the solvent 78% ethanol, 90% ethanol and acetone sequentially and dried in the same manner as in the IDF.

Effect of fiber on diffusion of glucose in glucose-dietary fiber system: The glucose DF system comprised 100 mg L⁻¹ of glucose and one of the following DFs: CSF, CIF, TSF, TIF, guar gum (GG) and tragacanth gum (TRAG). The concentrations of the DFs used in the system were 1% (w/v) for SDFs and 2% (w/v) for IDFs (3 replicates of DFs and a blank.); 5 mL of each were dialyzed in diffusion cell by using dialysis membrane with cutoff molecular weight of 12000 (High media) against 20 mL of double distilled water at pH 7.0 and 37°C (Ou *et al.*, 2001). The glucose content in 1 mL of the dialysate was determined after 10, 20, 30, 60, 90, 120, 150, 180 and 300 min according to the manual provided by God Pod kit.

Effect of dietary fiber on the activity of α -amylase: One gram of the DF was mixed with 100 mL of the 4% potato starch solution in a 200 mL beaker. One gram of α -amylase was then added to this mixture and stirred vigorously at 37°C. After 30 min, 0.1 mol L⁻¹ sodium hydroxide was added to terminate the α -amylase activity. The glucose content of the solution was determined as per manual provided by God Pod kit (Ou *et al.*, 2001).

Statistical analysis: The results were expressed as Mean \pm SEM. Statistical significance between groups was assessed by One-way analysis of variance followed by Dunnett multiple tests for the determination of level of significance.

RESULTS AND DISCUSSION

The results of diffusion rate of glucose were clearly showed dietary fibers affected diffusion of glucose (Table 1), diffused glucose was hindered by water-soluble treatment 45 to 55% as compared to control at 3 h. As compared to water-soluble fibers, diffused glucose had less effect in water insoluble fibers with 28 to 36% of the control, respectively. The maximum diffusion decreased in the order Trag > TIF > CIF > CSF > GG > TSF. Table 2 proved that the activity of α -amylase was directly influenced by dietary fibers. According to the view of Ou *et al.* (2001), dietary fibers can be absorbed starch and thus hinder hydrolysis of starch by α -amylase. In this experiment, 0.1 g of insoluble dietary fibers or 1 mL of 1% soluble fibers was mixed with 1 ml of α -amylase solution for 30 min and then the mixture was put into 1000 mL of starch-phosphate buffer; the results was shown that the activity of α -amylase (CSF 534.46 \pm 17.45**; TSF 537.31 \pm 14.62**; CIF 640.37 \pm 26.84**; TIF 817.31 \pm 24.62 and Control 857.17 \pm 15.26) was influenced significantly (Table 2).

Table 1: Effect of dietary fiber on diffusion of glucose

Time (min)	Glucose in dialysate (µg)						
	Control	GG	TRAG	CSF	TSF	CIF	TIF
10	426.16±6.67	403.85±17.63	423.85±6.7	407.69±17.63	406.93±6.7	459.62±11.55	437.3±17.63
20	518.31±6.67	450.77±13.28*	511.92±0.89	453.04±12.44*	437.44±16.79**	484.62±6.7	463.84±23.79
30	557.31±19.09	506.54±11.54	547.92±24.02	519.62±26.65	488.46±17.63	559.37±6.66	575.77±23.08
60	740.77±17.61	554.62±23.08**	738.85±6.66	540.38±34.62**	541.15±43.95**	670.00±6.67	677.69±17.62
90	892.31±29	580.38±24.02**	888.85±6.66	592.07±37.09**	624.61±48.04**	739.23±13.31**	752.31±17.63*
120	921.31±35.25	697.69±47.87**	923.08±23.08	633.46±33.31**	675.31±56.92**	758.46±50.29	807.69±23.07
150	945.38±52.03	768.38±29.07*	1049.23±17.61	717.69±37.09**	718.46±29.04**	816.92±43.68	836.76±23.08
300	1553.46±35.25	868.46±35.25**	1253.08±23.08**	912.69±34.61**	740.77±27.08**	1000.23±24.56**	1123.45±23.08**

CSF: *C. fistula* soluble fiber; CIF: *C. fistula* insoluble fiber; TSF: *T. indica* soluble fiber; TIF: *T. indica* insoluble fiber; TRAG: Tragacanth powder; GG: Guar gum. The values represent the Means±SEM for three per treatment sample. The level of significance *p<0.05, **p<0.01 was found in all group in compared of control.

Table 2: Effect of dietary fiber on the activity of α-amylase

Time (min)	Control	GG	TRAG	CSF	TSF	CIF	TIF
Glucose produced (µg/h/g)	857.17±15.26	523.85±18.78**	587.69±26.49**	534.46±17.45**	537.31±14.62**	640.37±26.84**	817.31±24.62

CSF: *C. fistula* soluble fiber; CIF: *C. fistula* insoluble fiber; TSF: *T. indica* soluble fiber; TIF: *T. indica* insoluble fiber; TRAG: Tragacanth powder; GG: Guar gum. The values represent the Means±SEM for three per treatment sample.*p<0.05, **p<0.01

The nature of the starch was found to be an important determinant of the blood glucose and insulin responses to food, giving rise to the concept of glycemic index. The fiber content of the food may also play a role as cooking modifies the composition of starch. It has also been shown that when different foods are consumed together the mean index of the meal is preserved. So, the nature of the indigested food can influence the excursion in blood glucose level. This present study dealt with fibers from *Tamarindus indica* and *Cassia fistula* on glucose lowering effect in *in vitro* system. Both showed significant glucose lowering effects.

Diffusion rate of glucose would decrease when time increases. Glucose diffusion of dietary fiber treatment did not slow until 60 min. Especially for insoluble dietary fiber, the diffusion rate of glucose was decreased even if they contributed little to the viscosity of the solution. There was a difference in diffused glucose between dialysate from dietary fibers and control when dialysis reached equilibrium (300 min, the results at 12 h proved 300 min was enough for equilibrium) (Ou *et al.*, 2001). The phenomena can be explained by adsorption of insoluble dietary fibers for glucose. At the beginning of dialysis, diffusion of glucose was affected by adsorption and viscosity of dietary fibers; thus, the diffusion rate of glucose was slow, although, the concentration in the dialysis bag was high. As the adsorption saturated, the diffusion of glucose was affected only by the viscosity of dietary fibers and the diffusion rate was not significantly decreased even when the concentration of glucose in the dialysis bag decreased. As dialysis reached equilibrium, the difference of glucose in the dialysate between control and treatment can be regarded as the amount of glucose adsorbed. Chau *et al.* (2004) studied three insoluble fiber-rich fractions, that's could effectively adsorb glucose, retard glucose diffusion, postpone the release of glucose from starch and inhibit the activity of α-amylase to different extents. All of these mechanisms results showed decrease the postprandial serum glucose concentration and this study suggested that they could be incorporated as low-calorie bulk ingredients in high-fiber foods to reduce calorie level and help control blood glucose concentration.

SDF have the effects of hampering the diffusion of glucose and postponing the absorption and digestion of carbohydrates, thus resulting in lowered postprandial blood glucose level. However, the viscosity increase by dietary fiber does not seem to give an adequate explanation of the extent of the decrease in blood glucose level (Weickert and Pfeiffer, 2008). The high-fiber cereal reduced glucose responses to the same extent in normal and hyperinsulinemic men, but reduced insulin responses only in hyperinsulinemic subjects (Wolever *et al.*, 2004). The results also showed that *C. fistula* with high viscosity had comparatively less glucose inhibition than *T. indica*. Liljeberg *et al.* (1996) reported that the blood glucose was decreased by more than 50% in healthy men with high dietary fiber bread intake within 30-70 min as compared to those who ingested barley or oat porridge. Schweizer *et al.* (1990) compared serum glucose response of a study group with low enzyme-resistant starch (RS) intake and a group with high RS intake and found that the glucose index of the former was 100% higher than that of the latter, although the former only took 16% more starch than the latter. RS is a water-insoluble dietary fiber, which would not greatly increase the viscosity of the small intestine's digesta. *In vitro* starch degradation of these products had shown that the addition of DF had an effect in reducing the amount of glucose produced, following digestion with amylase (Tudorica *et al.*, 2002).

Results of the above studies showed that SDF of *C. fistula* had more viscosity than the *T. indica* but it possesses comparatively less effect on postprandial glucose level as compared to TSF fiber. All data showed that the viscosity was not a matter to inhibition of glucose. Future studies shall be taken to check the role of viscosity and nature of DF in postprandial glucose absorptions.

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