

In vitro hypoglycemic effects of selected dietary fiber sources

Faiyaz Ahmed · Sudha Sairam · Asna Urooj

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Abstract The physiological functions of dietary fiber and its role in health promotion and risk reduction of some chronic diseases has been well documented. In the present investigation, the effect of three dietary fiber sources, oats (OA), barley (BA) and psyllium husk (PH) on glucose adsorption, diffusion and starch hydrolysis were studied using *in vitro* techniques by simulating gastrointestinal conditions and compared with the commercial dietary fiber sources wheat bran (WB), acarbose (ACB) and guar gum (GG). The glucose binding capacity of all the samples was higher than WB and ACB at 5 mM concentration. In all the samples, the diffusion of glucose was directly proportional to the time and diffusion rate was significantly lower ($p \leq 0.01$) in the system containing various samples compared to control. Glucose dialysis retardation index (GDRI) was 100 for OA, BA and PH at 60 min, at 120 min the maximal GDRI was in PH. Whereas; WB and ACB exhibited maximal GDRI at 180 and 240 min. All of these mechanisms might create a concerted function in lowering the rate of glucose absorption and as a result, decrease the postprandial hyperglycemia.

Keywords Hypoglycemic effect · Glucose adsorption · Glucose diffusion · Amylolysis kinetics · GDRI

Introduction

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with

disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO 1999). Non-starch polysaccharides and resistant oligosaccharides, lignin, substances associated with NSP and lignin complex in plants, other analogous carbohydrates, such as resistant starch and dextrans, and synthesized carbohydrate compounds, like polydextrose are categorized as dietary fibre. They are mostly concentrated in cereals, pulses, fruits and vegetables. It has been proclaimed that daily dietary fibre intake helps in prevention of many nutritional disorders like gut related problems, cardiovascular diseases, type 2 diabetes, certain types of cancer and obesity (Verma and Banerjee 2010). Many other researchers have documented the relationship between the increase in fiber consumption and decrease in glycemic response in diabetics (Nishimune et al. 1991). However, the effectiveness of dietary fibers in controlling hyperglycemia is generally affected by their composition, source, and preparation. Plant fibers can moderate postprandial glucose and insulin concentrations in non-insulin dependent diabetics if administered with meals (Brigenti et al. 1995). In particular, water-soluble fibers including guar, soy, psyllium, and pectin are reported to be more effective than insoluble fibers such as wheat bran (Pastors et al. 1991).

Oats and Barley are rich in fiber, particularly the soluble fibers β -glucans and pectin. β -glucan is a viscous polysaccharide made up of units of the sugar D-glucose. Clinical studies with diets containing foods enriched in oat and barley β -glucans revealed a reduction of glycemic index (GI) and insulinemic response (GII). It is likely that the mechanism by which β -glucans decrease the postprandial glucose response is the result of not only high viscosity in the gastrointestinal track, but also the reduction of starch digestion by α -amylase (Biliaderis and Marta 2007). Psyllium is a source of water-soluble fiber, similar to the

F. Ahmed · S. Sairam · A. Urooj (✉)
Department of Studies in Food Science and Nutrition,
Manasagangotri, University of Mysore,
Mysore 570006, India
e-mail: asnaurooj@foodsci.uni-mysore.ac.in

fiber found in grains such as oats and barley. However, psyllium is known to contain higher amounts of soluble dietary fiber than oats and barley.

With the above background, the present study was undertaken to evaluate the hypoglycemic potential of dietary fiber sources of three different types (oats, barley and psyllium) in comparison with commercial dietary fiber sources *in vitro*.

Materials and methods

Oats (OA), barley (BA) and psyllium husk (PH) were purchased from local a store. Glucose oxidase peroxidase reagent kit was purchased from the Agappe Diagnostics, India. All the chemicals and the reagents used in the study were of extra pure analytical grade.

Determination of glucose adsorption capacity Glucose adsorption capacity of the samples was determined according to the method of Ou et al. (2001). Briefly, samples (1%) were added to 25 mL of glucose solution of increasing concentration (5, 10, 20, 50 & 100 mM), the mixture was stirred well, incubated in a shaker water bath at 37 °C for 6 h, centrifuged at 4,000×g for 20 min and the glucose content in the supernatant was determined. Glucose bound was calculated using the following formula.

$$\text{Glucose bound} = \frac{G1 - G6}{\text{Weight of the sample}} \times \text{Volume of solution}$$

G1 glucose concentration of original solution

G6 glucose concentration after 6 h

In case of fiber, acarbose (0.2%) was added to 25 mL of glucose solution (20 mM). The solution mixtures were dialyzed against 200 ml of distilled water at 37 °C. The glucose content in the dialysate was determined after 6 h. Glucose bound was calculated as follows:

$$\text{Glucose bound} = \frac{(G1 \times V1) - [G2 \times (V1 + V2)]}{\text{Weight of the sample}}$$

G1 glucose concentration in retentate before start of diffusion

G2 glucose concentration in dialysate after 6 h

V1 volume of retentate

V2 volume of dialysate

Effect of the selected samples on *in vitro* glucose diffusion The glucose-dietary fiber system comprising of 25 mL of glucose solution (20 mM) and the samples (1%) and

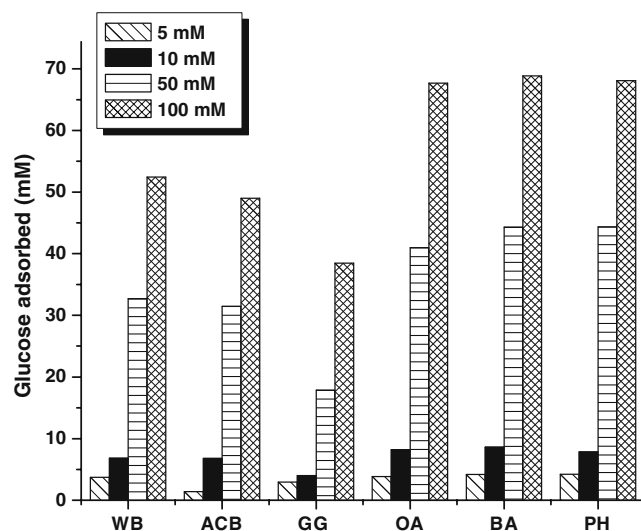
acarbose (0.2%) were dialyzed in dialysis bags against 200 mL of distilled water at 37 °C in a shaker water bath. The glucose content in the dialysate was determined at 60, 120, 180 and 240 min using glucose oxidase peroxidase diagnostic kit Ou et al. (2001). A control test was carried out without sample. The GDRI was calculated with the following formula,

$$\text{GDRI} = 100 - \frac{\text{Glucose content with the addition of fiber}}{\text{Glucose content of the control}} \times 100$$

Effect of the samples on *in vitro* amylolysis kinetics Forty grams of potato starch was added to ≈900 ml of 0.05 M phosphate buffer (pH 6.5). The solution after stirring at 65 °C for 30 min was made up to a final volume of 1,000 ml to give a 4% (w/v) starch solution. The starch-α-amylase-dietary fiber system comprising the above starch solution (25 mL), α-amylase (0.4%), and test sample (1%) were dialyzed in a dialysis bags against 200 mL of distilled water at 37 °C (pH 7.0) in a shaker water bath. The glucose content in the dialysate was determined at 60, 120, 180 and 240 min. A control test was carried out without sample Ou et al. (2001).

Results and discussion

Glucose adsorption capacity Glucose adsorption capacity of the selected samples is presented in Fig. 1. The



*WB-wheat bran ; ACB- acarbose ; GG- Guar gum ; OA-oats; BA-barley ; PH-psyllium husk

Fig. 1 Glucose binding capacity of the samples at different concentrations of glucose

Table 1 Effect of selected samples on glucose diffusion

Sample	Glucose content in the dialysate (mM)			
	30 min	60 min	120 min	180 min
Control	0.95 ^a ±0.02	1.3 ^c ±0.03	1.8 ^d ±0.03	2.0 ^d ±0.03
WB	0.75 ^a ±0.03	1.2 ^b ±0.03	1.6 ^c ±0.03	1.8 ^c ±0.05
ACB	0.63 ^a ±0.03	1.1 ^a ±0.02	1.5 ^b ±0.05	1.7 ^{bc} ±0.05
OA	0.67 ^a ±0.03	1.1 ^a ±0.03	1.5 ^b ±0.05	1.6 ^{ab} ±0.03
BA	0.77 ^a ±0.02	1.2 ^b ±0.01	1.5 ^b ±0.02	1.5 ^a ±0.07
PH	0.59 ^a ±0.03	1.0 ^a ±0.02	1.2 ^a ±0.06	1.6 ^a ±0.05

WB wheat bran; ACB acarbose; OA oats; BA barley; PH psyllium husk

Mean values with different superscript letters in columns differ significantly from each other ($p \leq 0.05$)

adsorption capacities of the samples were directly proportional to the molar concentration of glucose and higher amounts of glucose was bound with increased glucose concentration. No significant ($p \leq 0.05$) differences were observed between the adsorption capacities of OA, BA and PH. However, adsorption capacities of OA, BA and PH were significantly higher ($p \leq 0.05$) compared to that of WB, ACB and GG. The higher adsorption capacity of the samples maybe attributed to their dietary fiber content, as both insoluble and soluble fibers from different sources are reported to adsorb glucose (Adiotomre et al. 1990, Chau et al. 2003, Ou et al. 1999). Reports also indicate that, resistant starch and insoluble fibers derived from wheat bran could also adsorb glucose in the glucose solution of different concentration (Ou et al. 2001). *In vivo* and *in vitro* studies of glucose absorption have shown that the delay in glucose adsorption in the gastrointestinal tract is determined mainly by the viscosity of soluble polysaccharides (Jenkins et al. 1978, Adiotomre et al. 1990). The results also indicated that the samples could bind glucose even at lower concentrations of glucose (5 mM) thereby reducing the

Table 2 Glucose dialysis retardation index (GDRI) in glucose diffusion

Sample	GDRI			
	30 min	60 min	120 min	180 min
WB	34.8 ^c ±2.98	13.2 ^b ±3.36	2.0 ^a ±1.71	10.5 ^a ±1.55
ACB	45.2 ^c ±4.21	21.0 ^c ±3.57	3.3 ^a ±2.38	21.4 ^{cd} ±2.64
OA	29.7 ^b ±4.37	12.4 ^b ±1.17	17.1 ^b ±2.86	17.7 ^b ±1.04
BA	19.3 ^a ±3.84	2.2 ^a ±2.86	15.2 ^b ±2.3	23.1 ^d ±2.79
PH	38.3 ^d ±3.82	20.6 ^c ±4.94	30.8 ^c ±3.48	19.2 ^{bc} ±2.69

WB wheat bran; ACB acarbose; OA oats; BA barley; PH psyllium husk

Mean values with different superscript letters in columns differ significantly from each other ($p \leq 0.05$)

Table 3 Effect of selected samples on starch digestibility

Sample	Glucose content in the dialysate (mM)			
	60 min	120 min	180 min	240 min
Control	0.08 ^c ±0.02	0.14 ^c ±0.01	0.27 ^c ±0.02	0.38 ^c ±0.02
WB	0.09 ^c ±0.01	0.15 ^c ±0.05	0.20 ^b ±0.02	0.24 ^b ±0.02
GG	0.01 ^b ±0.03	0.10 ^b ±0.01	0.20 ^b ±0.01	0.25 ^b ±0.08
OA	0.0 ^a	0.08 ^b ±0.01	0.34 ^d ±0.02	0.16 ^a ±0.02
BA	0.0 ^a	0.11 ^b ±0.01	0.24 ^b ±0.01	0.15 ^a ±0.03
PH	0.0 ^a	0.04 ^a ±0.01	0.14 ^a ±0.01	0.28 ^b ±0.01

WB wheat bran; GG guar gum; OA oats; BA barley; PH psyllium husk

Mean values with different superscript letters in columns differ significantly from each other ($p \leq 0.05$)

amount of glucose available for transport across the intestinal lumen, consequently blunting the postprandial hyperglycemia. Similar observations are reported for insoluble fiber-rich fractions isolated from *Averrhoa carambola* (Chau et al. 2004).

Effect on in vitro glucose diffusion The effect of selected samples on glucose diffusion is presented in Table 1. In the present study, the movement of glucose across the dialysis membrane was monitored once in 30 min till 180 min and it was found that, all samples demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control. Glucose dialysis retardation index (GDRI) is a useful *in vitro* index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract (Lopez et al. 1996). In the present study, the GDRI values decreased over the time except in BA, wherein highest GDRI was found at 180 min (Table 2). Similar observations are reported for some fiber samples, wherein the GDRI values diminished over the time (Nishimune et al. 1991, Chau et

Table 4 Effect of samples on glucose dialysis retardation index (GDRI) in starch digestibility

Sample	GDRI			
	60 min	120 min	180 min	240 min
WB	25.0 ^a ±2.36	46.4 ^b ±1.17	60.0 ^d ±4.36	57.3 ^b ±1.01
GG	91.6 ^b ±6.87	65.1 ^c ±1.77	60.0 ^d ±2.78	66.7 ^d ±3.71
OA	100 ^c	43.8 ^b ±1.23	23.2 ^b ±2.47	57.8 ^b ±6.40
BA	100 ^c	21.6 ^a ±6.36	13.4 ^a ±6.09	60.5 ^c ±9.34
PH	100 ^c	69.6 ^c ±4.39	49.1 ^c ±6.10	25.7 ^a ±4.49

WB wheat bran; GG guar gum; OA oats; BA barley; PH psyllium husk

Mean values with different superscript letters in columns differ significantly from each other ($p \leq 0.05$)

al. 2003). ACB showed significantly higher ($p \leq 0.05$) GDRI at 30 min followed $PH < WB < OA < BA$. Similar trend was seen at 60 min, but at 120 min Ph exhibited maximum GDRI and at 180 min GDRI of barley was highest. The retardation in glucose diffusion might also be attributed to the physical obstacle presented by fiber particles towards the glucose molecules and the entrapment of glucose within the network formed by fibers (Lopez et al. 1996, Jenkins et al. 1978). The effect of insoluble dietary fiber in the inhibition of glucose diffusion in the small intestine is suggested to be due to the adsorption or inclusion of the smaller sugar molecules within the structure of the fiber particles (Lopez et al. 1996). The mechanism of action in polysaccharides for glucose reduction in diabetic patient is similar to that of other soluble fibers, because psyllium forms a viscous gel in aqueous solution, it may slow the access of glucose to the small intestine's absorptive epithelium, thereby blunting postprandial glucose peaks. Soluble fibers may delay gastric emptying, slowing carbohydrate uptake. A third mechanism that may contribute to the postprandial effect is the sequestration of carbohydrates ingested with the meal, retarding carbohydrate access to digestive enzymes. Reports indicate that psyllium can exert these effects hours after its administration and can produce a significant reduction in glucose after a second meal (Layce et al. 1991).

Effect on amylosis kinetics The diffusion rate of glucose and GDRI as affected by the addition of the samples in the starch- α -amylase-fiber system is shown in Tables 3 and 4. Compared to positive control, the diffusion rate of glucose in the systems containing samples, were significantly ($p \leq 0.05$) low at each interval of time. The glucose diffusion rate was nil at 60 min in the systems with samples. The GDRI values were 100 for OA, BA and PH at 60 min. The maximal GDRI was exhibited by GG, OA at 180 and 240 min followed by WB, PH, BA and OA. From the results obtained it was observed that the GDRI values of all the samples generally diminished as the time increased in the systems where glucose-samples/fiber were used. It is also been reported in an earlier study that GDRI values of the fiber samples diminished over the time (Chau et al. 2003). The effect of insoluble dietary fiber in the inhibition of glucose diffusion in the small intestine is suggested to be due to the adsorption or inclusion of the smaller sugar molecules within the structure of the fiber particles (Nishimune et al. 1991, Jenkins et al. 1978). The retardation of glucose diffusion is also due to the inhibition of α -amylase, thereby limiting the release of glucose from the starch. The inhibition of α -amylase activity by medicinal plants might be attributed to several possible factors such as fiber concentration, the presence of inhibitors on fibers, encapsulation of starch and enzyme

by the fibers present in the sample, thereby reducing accessibility of starch to the enzyme, and direct adsorption of the enzyme on fibers, leading to decreased amylase activity (Ou et al. 2001). Inhibitors of carbohydrate hydrolyzing enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise (Bailey 2003). These observations emphasize that inhibition of α -amylase is one of the probable mechanisms through which the samples exerts its hypoglycemic effect.

Conclusion

The dietary fiber sources selected are being used as functional ingredients in many food products. This investigation was undertaken to study and explore their potential hypoglycemic effect by employing suitable 'in vitro' techniques. The results on amylolysis kinetics and glucose diffusion suggested that the diffusion rate of glucose were affected by the selected samples. GDRI increased in the first 60 min for amylolysis kinetics and similar results were achieved at 30 min thereafter the GDRI decreased significantly. It was interesting to note that the ability of the samples to promote adsorption of glucose was concentration dependent. The adsorption of glucose increased with increase in glucose concentration. This study serves as a preliminary investigation to study and evaluate the hypoglycemic potential by using selected *in vitro* techniques. The hypoglycemic potential was further consolidated by the results on glucose adsorption, glucose diffusion, and amylolysis kinetics. However, further *in vivo* studies are needed to substantiate this observation.

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