In vitro Study of the Effects of Viscous Soluble Dietary Fibers of *Abelmoschus esculentus* L in Lowering Intestinal Glucose Absorption

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Abstract

The edible green fruits or pods of *Abelmoschus esculentus* L (vendi/lady's finger) are popular all over the world for possessing valuable nutrients including fibers, vitamins, minerals, proteins and carbohydrates, nearly half of which is soluble fiber in the form of gums and pectins. Traditionally, they have been used in lowering serum cholesterol, relieving constipation and healing ulcers.The objective of the present study was to investigate the effects of Na-Carboxymethylcellulose (Na-CMC) and viscous soluble dietary fibers (VSDF) of the fruits of *Abelmoschus esculentus* L on intestinal glucose absorption using *in-vitro* model. Different diffusion systems were prepared using different concentrations of viscous water soluble portion of the fruits of *Abelmoschus esculentus* L and Na-CMC solution with fixed concentration of glucose solution (0.1g/ml) and the diffusion of glucose from these systems into outer solution through the ultra-fine membrane was measured. Substantial reductions of diffusion of glucose from water soluble portion of the pods of *Abelmoschus esculentus* L and Na-CMC diffusion systems were observed compared to control in a concentration-dependant manner ($P<0.05$) which implicates a possible potential role of VSDF of fruits of *Abelmoschus esculentus* L in lowering postprandial serum glucose.

Keywords: Viscous Soluble Dietary Fiber (VSDF), *Abelmoschus esculentus* L, Glucose diffusion, Diabetes.

Introduction

Consumption of a diet high in soluble fiber has been suggested as a strategy to reduce the risk factors for development of obesity through the regulation of satiety and energy intake (Pereira *et al*., 2001). In addition, beneficial long-term effects of soluble viscous dietary fibers on blood glucose and lipids have been observed in diabetic subjects (Aro *et al.,* 1981 and Ray *et al.,* 1983). Glucose tolerance and insulin sensitivity are the key issues involved in the development of non-insulin dependent diabetes mellitus (NIDDM) in humans and soluble dietary fibers (SDF) have been shown to improve glucose tolerance and insulin sensitivity (Smith *et al*., 1997 and Ellis *et al*., 1996). Several studies suggest that these fibers exert such an effect by reducing the glucose absorption rate (Vaugelade *et al*., 2000). Moreover, viscous water-soluble dietary fibers have the effects of hampering the diffusion of glucose and postponing the absorption and digestion of carbohydrates, thus resulting in lower postprandial blood glucose (Yokoyama *et al*., 1997).

The green edible pod of *Abelmoschus esculentus* L. (Family: Malvaceae) is extensively used globally as a vegetable for its nutritional and health benefits (Begum *et al*., 2005). In Bangladesh, it is called vendi and one of the most popular vegetables because of its nutritional benefits, availability, and low cost. It constitutes minerals, vitamins, proteins, carbohydrates, enzymes and very high quantities of mucilage which are rich in soluble dietary fiber as pectin,

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guar gum, carboxymethylcellulose etc. (Alam *et al*., 2007). It is believed that it is useful in the treatment of inflammatory disorders, constipation, retention of urine, lowering blood cholesterol and blood glucose level.

The previous studies reported that vendi possess hypo-lipidemic effect (Trinh *et al*., 2009). However, the effect of vendi on long term glycemic control is less clear. This study reveals the effects of viscous water-soluble portion of the pods of *Abelmoschus esculentus* L (vendi) and Na-CMC on intestinal glucose absorption using *in vitro* model.

Materials and Methods

Preparation of viscous water soluble portion of *Abelmoschus esculentus* L

500gm of fresh pods or fruits of *Abelmoschus esculentus* L were collected in the month of May, 2009 from a local market of Rajshahi, Bangladesh. Then, the pods were thoroughly washed with distilled water, cut into small slices (Figure 1) by a sharp knife and 15, 30, 45, 60 and 75gm of the sliced pods were immersed into 150ml distilled water in different beakers.

Figure 1: Slices of ***Abelmoschus esculentus***

The mixture was then stirred gently for 10 to 15 minutes with a glass rod. After 24 hours the contents were filtered using a thin layer of cotton to remove the insoluble matters. The viscous water soluble portions (VWSP) that contain the soluble dietary fibers were passed through the filter and filtrate was collected. The amount of filtrates were measured and numbered as solution A to E from low to high concentration (Table 1). In 50ml beakers 1ml of each solution was taken and dried in an oven at 85°C for 12 hours and weighed to measure the amount of dried constituents (Table 2).

Table 1: Amount of VWSP of *Abelmoschus esculentus* L obtained after filtration

Amount of sliced *Abelmoschus esculentus* L	Amount of distilled water	Amount of water soluble portion obtained after filtration
15 gm	150 ml	135 ml (Solution A)
30 gm	150 ml	130 ml (Solution B)
45 gm	150 ml	110 ml (Solution C)
60 gm	150 ml	100 ml (Solution D)
75 gm	150 ml	90 ml (Solution E)

Table 2: Amount of dried constituents in 1 milliliter of filtrate

Solution	Amount of solution	Amount of dried constituents obtained
Solution A	1 ml	0.1 gm
Solution B	1 ml	0.2 gm
Solution C	1 ml	0.34 gm
Solution D	1 ml	0.52 gm
Solution E	1 ml	0.57 gm

1% w/v Na-CMC solution was prepared by warming the solution at 30°C in a water bath for 5 minutes and then vortexing at 4000rpm for 5 minutes. The Na-CMC used in this study was obtained from Fluka, Switzerland. It had a dynamic viscosity of 1000mPa.s (Pa.s= Pascal second) in 2% w/v water solution. The pH was 6.5-8.0.

Preparation of diffusion systems of glucose with Na-CMC and VWSP of the fruits of *Abelmoschus esculentus* L

Different diffusion systems were prepared according to the proportion mentioned in Table 3.

Table 3: Formulation of different systems (Control Na-CMC solution: System G to L)

No. of Test Tube	Glucose Solution (0.1gm/ml)	Na-CMC solution (0.01gm/ml)	Water soluble portion of ladies finger	Distilled water	Designation of systems
No. (1)	10 ml	0 ml	0	10 ml	System G
No. (2)	10 ml	2 ml	0	8 ml	System H
No. (3)	10 ml	4 ml	0	6 ml	System I
No. (4)	10 ml	6 ml	0	4 ml	System J
No. (5)	10 ml	8 ml	0	2 ml	System K
No. (6)	10 ml	10 ml	0	0 ml	System L
No. (7)	10 ml	0	10 ml of Solution A	0	System M
No. (8)	10 ml	0	10 ml of Solution B	0	System N
No. (9)	10 ml	0	10 ml of Solution C	0	System O
No. (10)	10 ml	0	10 ml of Solution D	0	System P
No. (11)	10 ml	0	10 ml of Solution E	0	System Q

Determination of diffusion of glucose from the different systems

To observe the effects of dietary fibers on intestinal glucose and drug absorption, *in vitro* model using dialysis method has been reported (Yokoyama *et al*., 1997). But due to limited facilities in our laboratory we have fabricated a process to simulate the effect of dialysis using Whatman ultra-fine membrane filter (retention down to 0.7µm). The test tubes containing different systems were covered with the filters and fastened well (Figure 2).

Figure 2: Fastened test tube with ultra fine filter paper

Then each test tube was inverted and soaked in the distilled water (50ml) containing in glass beakers (Figure 3). Then the beakers were stirred at 3.5rpm in a thermostat shaker at 37.5^{0}C (Figure 4). After

Figure 3: Inverted test tube in a beaker

Fig 4: Shaking of systems in a thermostat shaker

20, 40, 60, 80, 100 and 120 minutes 1ml of solution from each beaker was taken out and 1ml of Fehling solution was added, heated at 85°C for

30 minutes, cooled. Then, 1ml of arsenic molybdate solution and 7ml of distilled H_2O were added and the absorbance of each solution was measured in UV spectrophotometer at 520nm. The diffusion of glucose into the solution was determined using standard glucose curve (Figure 5).

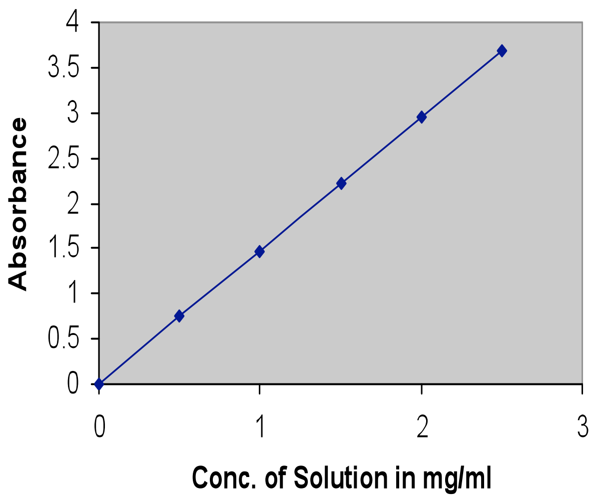

Figure 5: Standard Glucose Curve (5 concentrations of glucose solution were used: 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml)

Statistical Analysis:

Significance of differences between the mean values were determined by analysis of variance (ANOVA), followed by Dunnett's test. Results were considered significant when the p values were less than 0.05. Statistical calculations and the graphs were prepared using GraphPad Prism version 4.00 for Windows.

Results and Discussion

Dialysis tubing technique is a simple model to evaluate the potential of soluble dietary fibers to retard the diffusion and movement of glucose in the intestinal tract (Adiotomre, *et al*., 1990). Our fabricated technique to test the diffusion of glucose perfectly simulates this model. In our fabricated technique, substantial reduction of diffusion of glucose from Na-CMC-glucose systems was found in a concentration-dependant manner (Table 4). Increasing the concentration of Na-CMC decreased the diffusion of glucose from the systems. Diffused glucose from systems H to L (Na-CMC and glucose diffusion systems) was 0.30-0.35 mg/ml at 20 min and 75-85.07% of control, respectively. In the highest concentration of Na-CMC (10mg/ml) significant reduction of glucose diffusion ($P<0.05$) was found at 100 and 120 minutes (Figure 6). From the system L maximum reduction of diffusion was observed at 60 minutes (70% of control).

Table 4: Effect of Na-CMC-glucose diffusion systems on diffusion of glucose

	Diffusion of glucose (mg/ml) in outer solution from systems					
	20 min	40 min	60 min	80 min	100 min	120 min
Solution G	0.40±0.2[a]	0.80±0.5	1.20±0.3	1.60±0.7	2.10±0.8	2.45±0.8
System H	0.35±0.6	0.70±0.8	1.10±0.1	1.45±0.5	1.85±0.2	2.20±0.1
System I	0.35±0.1	0.68±0.9	1.05±0.1	1.44±0.2	1.75±0.3	2.10±0.8
System J	0.34±0.6	0.65±0.2	0.95±0.1	1.28±0.2	1.64±0.5	1.95±0.1
System K	0.32±0.6	0.60±0.2	0.94±0.4	1.25±0.3	1.60±0.8	1.85±0.2
System L	0.30±0.3	0.58±0.3	0.90±0.4	1.22±0.8	1.55±0.2	1.84±0.5

[a]Mean values ± standard deviation

VWSP of vendi exerted the same effect as Na-CMC. From the system G to Q, reduction of the diffusion of glucose was observed in a concentration-dependant manner ($P<0.05$) (Figure 7, Table 5). The system Q contains maximum amount of mucilaginous constituents and the diffusion of glucose from this system was minimum. At 20 minute, the diffusion of glucose from the system M, N, O, P and Q were 0.34, 0.25, 0.20, 0.15 and 0.12mg/ml, respectively. The diffusion of glucose from system Q was 30% of the control where there was no dietary fiber component. Thus, it is clear that glucose entrapping capacity of VWSP of vendi was significantly higher than that of Na-CMC.

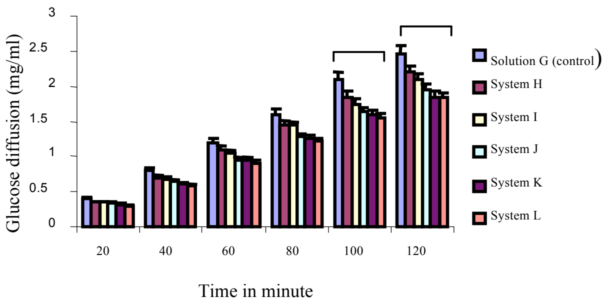

Figure 6: Diffusion of glucose (mg/ml) from Na-CMC-glucose diffusion system where different systems contain different conc. of Na-CMC (system H, 2mg/ml; system I, 4mg/ml; system J, 6mg/ml; system K, 8mg/ml; system L, 10mg/ml) with fixed conc. of glucose solution (0.1g/ml). Data were presented as mean ± SEM; n=3, *p<0.05 compared to control (ANOVA followed by Dunnett's test)

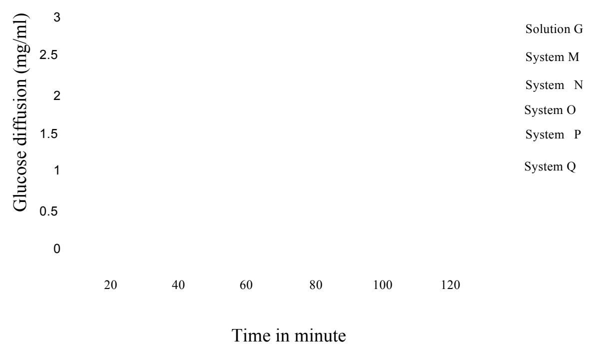

Figure 7: Diffusion of glucose (mg/ml) from VWSP of *Abelmoschus esculentus* L.-glucose diffusion system where different systems contain different conc. of VWSP of *Abelmoschus esculentus* L (system M, 0.1g/ml; system N, 0.2g/ml; system O, 0.34g/ml; system P, 0.52g/ml; system Q, 0.57g/ml) with fixed conc. of glucose solution (0.1g/ml). Data were presented as mean ± SEM; n=3, *p<0.05 compared to control (ANOVA followed by Dunnett's test)

Table 5: Effect of VWSP of *Abelmoschus esculentus* L. on diffusion of glucose

	Diffusion of glucose (mg/ml) in outer solution from systems					
	20 min	40 min	60 min	80 min	100 min	120 min
Solution G (control)	0.40±0.4 [a]	0.80±0.3	1.20±0.6	1.60±0.8	2.10±0.8	2.45±0.5
System M	0.34±0.6	0.65±0.8	0.80±0.2	0.90±0.5	1.01±0.2	1.75±0.4
System N	0.25±0.6	0.62±0.7	0.70±0.9	0.80±0.2	0.90±0.6	1.10±0.8
System O	0.20±0.5	0.54±0.2	0.68±0.4	0.72±0.2	0.854±0.4	1.02±0.5
System P	0.15±0.6	0.48±0.3	0.55±0.4	0.65±0.8	0.82±0.8	0.98±0.2
System Q	0.12±0.3	0.36±0.9	0.50±0.4	0.60±0.8	0.75±0.2	0.90±0.5

[a]Data are expressed as Mean values ± SEM

Studies reported that consumption of viscous water-soluble dietary fibers reduced postprandial blood glucose by reducing the diffusion of glucose and postponing the absorption and digestion of carbohydrates (Yokoyama *et al*., 1997). It has also been reported that different types of dietary fibers (especially soluble) reduced the diffusion of glucose *in vitro* (Ou *et al*., 2001) and also *in vivo* (Lafrance *et al*., 1998 and Torsdottir *et al*., 1991). Our data are consistent with the earlier studies. The decreased extent of diffusion of glucose by Na-CMC and VWSP of the pods of *Abelmoschus esculentus* L across the ultra fine membrane was almost similar to that reported by Ou, S. *et al.,* 2001. Although the situation for absorption in the small intestine could not be completely judged by the results but from results, we can assume the possible reason. It has been assumed by Ou *et al.,* that the possible mechanism may be the increase of the viscosity of the systems, retardation of the diffusion of glucose and/or adsorption of glucose on the dietary fibers which prevent its diffusion. Further studies are required to evaluate the exact mechanism by which intestinal glucose absorption was reduced by soluble dietary fiber.

Conclusion

In this study, we observed that viscous water-soluble portion of the fruits of *Abelmoschus esculentus* L has significant capacity to reduce the glucose diffusion form the dietary fiber- glucose systems. As the *Abelmoschus esculentus* L is widely distributed and the fruits commonly used as an edible item, it therefore represents a potentially dietary adjunct in diabetic people to control their blood glucose.

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