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In vitro Effect of Aqueous Extract of Fresh Leaves of *Abroma augusta* L on the Diffusion of Glucose

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Abstract

There have been a number of reports concerning the role of dietary fiber in hampering the diffusion of glucose and lowering the postprandial serum glucose. The present study investigates the effect of viscous aqueous leaf extract of *Abroma augusta* L (Family: Sterculiaceae, Bengali name: Ulatkambal, English name: Devil's cotton, DC) on the diffusion of glucose *in vitro*. Different mixtures were prepared using varying concentrations of sodium carboxymethylcellulose (Na-CMC) and aqueous extract of *A. augusta* with a fixed concentration of glucose. The diffusion of glucose from these systems into the outer medium through the ultra-fine membrane was measured. The results showed that both Na-CMC and aqueous extract of ulatkambal significantly ($p<0.05$) reduced the diffusion of glucose compared to control in a concentration-dependent manner. The result of this study suggested that dietary fiber present in the aqueous leaf extract of *A. augusta* may be potentially effective in the management of type 2 diabetes mellitus by reducing post-prandial glucose absorption from the gastrointestinal tract.

Key words: Water-soluble fraction, *Abroma augusta*, glucose diffusion, diabetes mellitus.

Introduction

Diabetes mellitus is a major chronic disease caused by an improper balance of glucose homeostasis. Type 2 diabetes comprises 90% of people with diabetes around the world and is one of the major public health challenges of the 21st century (Bailey, 2002). In 2000, the number cases worldwide are estimated to be about 171 million (Wild *et al.,* 2004) and are projected to rise to 366 million in 2030. According to World Health Organization without urgent action, diabetes-related deaths will increase by more than 50% in the next 10 years (World Health Organization, November 2008).

Glucose tolerance and insulin sensitivity are the main reasons in the development of non-insulin dependent diabetes mellitus (NIDDM) in humans. Soluble dietary fibers have been shown to improve glucose tolerance and insulin sensitivity (Cameron-Smith *et al.,* 1997; Ellis *et al.,* 1996). Viscous water-soluble dietary fibers hamper the diffusion of glucose and postponing the absorption and digestion of carbohydrates (Galisteo *et al.,* 2008; Ou *et al.,* 2001) thus resulting in lowered postprandial blood glucose level (Gin *et al.,* 2000; Yokoyama *et al.,* 1997). Soluble dietary fiber such as guar gum and pectin have been shown to lower blood glucose level and insulin in human, when given simultaneously with a carbohydrate-containing meal (Ebihara *et al.,* 1981). Dietary fibers lower the glycemic level in serum by slowing glucose absorption through an effect on gastric emptying and/or entrapment of materials in the viscous digest (Baghurst *et al.,* 1996). Chandalia *et al.* (2000) reported that high intake of soluble dietary fibers, improve glycemic control, decreases hyperinsulinemia, and lowers plasma lipid concentrations in patients with type 2 diabetes. Dietary fibers extracted from white bean produced significant reduction of blood glucose (Al-Okbi *et al.,* 1989) but viscous fibers extracted from *Laminaria digitata* resulted in a dramatically reduced glucose absorption balance (Vaugelade *et al.,* 2000).

The anti-diabetic activity of aqueous extract of dried root bark of *A. augusta* has been observed in diabetic rats by Eshrat *et al.* (2002, 2003). They showed that the water extract of the roots of *A. augusta* resulted in significant fall in fasting blood glucose and improvement in glucose tolerance. Nahar *et al.* (2010) reported that the methanolic extract of leaves of *A. augusta* significantly reduced the blood glucose level in alloxan-induced diabetic rats when administered at a dose of 300 mg/kg/day. These findings established the rationality of ethnomedicinal use the roots

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and leaves of *A. augusta* in management of diabetes.

But, the mechanisms by which the plant extracts containing high soluble dietary fiber improve glucose tolerance in diabetic patients have been poorly investigated. In this *in vitro* study we examined the possible mechanism by which the highly viscous water soluble portion of the leaves of *A. augusta* is useful in managing type 2 DM.

Materials and methods

Materials: Na-CMC (pH = 6.5-8.0; 400-1000 mPa.s, Deg of sub: 0.6-0.75, medium viscosity, Fluka, Switzerland), Glucose (Powdered dextrose monohydrate 100%, Glaxo SmithKline), Ultra-fine filter paper (Whatman-42, Ash less Circles, 125mm), Fehling's solution (A and B), Arsenic molybdate were purchased from the local suppliers.

Collection of fresh leaves and preparation of viscous aqueous extract: Fresh leaves of *A. augusta* were collected in the month of September, 2010 from Botanical garden and garden of Madar Bux Hall, Rajshahi University, Rajshahi, Bangladesh. After collection, the fresh leaves were thoroughly washed with distilled water and sliced into small pieces by a sharp scissor. The sliced leaves were (15, 30, 45, 60 and 75 gm) immersed into 250 ml distilled water in different beakers and stirred gently for 10 to 15 minutes with a glass rod to facilitate the solubilization of the water-soluble fibers. After 24 hours, the mixture was filtered using a filter and the viscous WSF of ulatkambal was collected as a filtrate. The amount of filtrate was measured and numbered as solution A to E from low to high concentration (Table 1). In 50 ml beakers 1 milliliter 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). Finally, the viscosity was measured using Brookfield Dial Viscometer (LV series, Brookfield Engineering Laboratories, INC, USA) (Table 3).

Preparation of solutions and diffusion systems of glucose with Na-CMC and VWSF of leaves of A. augusta:

i. *Preparation of glucose solution:* Glucose solution (8 mg/ml) was prepared by dissolving 1.60 gm of glucose in 200 ml of distilled water.
ii. *Preparation of Na-CMC solution:* Na-CMC solution (1% w/v) was prepared by warming the mixture of water and Na-CMC at 30°C in a water bath for 5 minutes and vortexing at 4000 rpm for 5 minutes in 100 ml of distilled water.
iii. *Preparation of different mixture systems to test the diffusion of glucose:* The different mixture systems were prepared by mixing different amounts of Na-CMC solution and a fixed amount (10 ml) of each viscous WSF of ulatkambal (solution A, solution B, solution C, solution D and solution E) with a fixed amount of glucose solution (10 ml) as shown in Table 4.

Determination of diffusion of glucose from the dietary fiber-glucose system: The test tubes (25 × 200) containing different systems including the control solution of glucose were covered with ultra-fine filter paper with scotch tape and fastened well. Then, each test tube was inverted and soaked in the distilled water (50 ml) in glass beakers. The beakers were shaken well at 35 rpm in a thermostatic shaker (Advantec, Model: TS-20, Advantec Toyo Kaisha, Ltd).

After 10, 20, 30, 60, 90, 120, 150, 180 and 300 minutes 1ml of solution from each beaker was taken out. To each solution, 1ml of Fehling's solution was added, shaken (vortexed) for 1 min for uniform mixing, heated at 85°C for 30 minutes, cooled and then 1ml of arsenic molybdate solution was added, vortexed for 1min for proper reaction and finally 7 ml of distilled water was added and vortexed for 1min for proper dilution. Finally, the absorbance of each solution was measured using UV-spectrophotometer (UV- 1200, UV-VIS Spectrophotometers, Shimadzu, and Japan) at 520 nm to measure the diffusion of glucose into the solution. The diffusion of glucose into the solution was determined using standard glucose curve.

Statistical analysis: All results were expressed as mean ± S.D. of three experiments. All data were analyzed with two-way analysis of variance (ANOVA) followed by Dunnet's test using statistical software SPSS 15.0. The level of significance was set at $p<0.05$. The figures were prepared using MS-Excel 2003.

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 gastrointestinal tract (Adiotomre *et al.*, 1990). To test the diffusion of

glucose *in vitro* our fabricated technique using ultra-fine filter paper perfectly simulated this model. As shown in Table 5 and Figure 1, Na-CMC significantly ($p<0.05$) inhibited the diffusion of glucose from different Na-CMC-glucose systems in a concentration-dependent manner through the ultra-fine filter paper.

Table 1. Amount of WSF of *A. auguata* obtained after filtration.

Amount of sliced leaves (gm)	Amount of distilled water (ml)	WSF of *A. augusta* after filtration (ml)
15	250	235 (Solution A)
30	250	232 (Solution B)
45	250	226 (Solution C)
60	250	222 (Solution D)
75	250	218 (Solution E)

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

Solution	Solution (ml)	Dried constituents (mg/ml)
Solution A	1	4
Solution B	1	9
Solution C	1	14
Solution D	1	19
Solution E	1	23

From the system H to L, the diffusion of glucose decreased with increasing concentration of Na-CMC as compared to the system G (control). In each system, maximum reduction of glucose diffusion (58.93 to 85.72%) was observed at 10 minutes and minimum reduction of glucose diffusion (7.08 to 29.3%) was observed at 300 minutes as compared to control. At 10 minutes diffused glucose from systems H to L was 11.5 to 4 mg/dL (41.07 to 14.28% of control). In the highest concentration of Na-CMC, significant reduction of glucose diffusion was found at 10 minutes. The results of this study showed that viscous WSF of ulatkambal significantly reduced the diffusion of glucose in a concentration-dependent manner as that of Na-CMC (Table 6 and Figure 2).

Increasing the concentration of WSF of ulatkambal from system M to Q decreased the diffusion of glucose significantly ($p<0.05$) as compared to the system G (control). In system M maximum diffusion of glucose (97.10%) was observed at 60 minutes and minimum diffusion (35.71%) was observed at 10 minutes. In system N maximum glucose diffusion (86.84%) was observed at 120 minutes and minimum diffusion (32.14%) was observed at 10 minutes. From system O to Q maximum glucose diffusion was observed at 300 minutes and minimum glucose diffusion was observed at 10 minutes. We found a significant difference in glucose diffusion between system N and O from 30 to 180 minutes. The system Q contained maximum amount of viscous constituents and thus the diffusion of glucose from this system was minimum. At 10 minutes, the diffusion of glucose from system M, N, O, P and Q were 10, 9, 7, 6.5 and 5.5 mg/dl, respectively. The diffusion of glucose from system Q was 19.64% of the control.

Table 3. Viscosity of different WSF of *A. auguata.*

Solution	Viscosity in cP (mPa.s)
Solution A	7.5
Solution B	17.5
Solution C	22.5
Solution D	30.0
Solution E	40.0

Previous studies reported that viscous water-soluble dietary fibers hampered diffusion of glucose and postponing the absorption and digestion of carbohydrates, thus lowered postprandial blood glucose (Baghurst *et al.,* 1996; Cameron-Smith *et al.,* 1997). In our laboratory, we found that both Na-CMC and WSF of *A. auguata* significantly ($p<0.05$) reduced the absorption of glucose administered orally in fasting rats. On the other hand, WSF of *A. auguata* significantly ($p<0.05$) reduced the absorption of metformin hydrochloride in alloxan-induced diabetic rats (Islam *et al.,* 2012). Khatun *et al.* (2010) reported that both Na-CMC and WSF of ladies finger substantially reduced the diffusion of glucose *in vitro*. Ou *et al.* (2001) reported that dietary fibers lowered postprandial serum glucose levels at least by three mechanisms. First, dietary fibers increase the viscosity of small intestinal juice and hinder diffusion of glucose; second, they bind glucose and decrease the concentration of available glucose in the small intestine; and third, they retard α-amylase action through encapsuling starch and the enzyme and might directly inhibit the enzyme. The decreased extent of diffusion of glucose by viscous WSF of ulatkambal across the ultra-fine filter paper was almost similar and consistent with our *in-vivo* study and also to that reported by Ou *et al.* (2001) and Khatun *et al.* (2010). Thus, it is clear that viscous aqueous extract of *A. augusta* have significant glucose entrapping capacity than that of

Na-CMC and this may be due to the presence of water-soluble fiber.

Although the situation for absorption in the small intestine could not be judged completely by the results of our study, but we can assume the possible reason. Ou *et al.*(2010) assumed that the possible mechanism may be the increase of the viscosity of the systems, retard the diffusion of glucose and/or adsorption of glucose on the dietary fibers which prevent its diffusion. Now we are trying to know the exact mechanism by which aqueous extract of *A. augusta* reduced the diffusion of glucose *in vitro*.

Table 4. Formulation of different mixture systems.

Glucose solution (ml)	Na-CMC solution (ml)	WSF of *A. augusta*	Distilled water (ml)	Designation of mixtures
10	0	0	10	Control solution of glucose (System G)
10	2	0	8	System H
10	4	0	6	System I
10	6	0	4	System J
10	8	0	2	System K
10	10	0	0	System L
10	0	10 ml of solution A	0	System M
10	0	10 ml of solution B	0	System N
10	0	10 ml of solution C	0	System O
10	0	10 ml of solution D	0	System P
10	0	10 ml of solution E	0	System Q

Table 5. Diffusion of glucose from different Na-CMC-glucose mixture systems at different time intervals.

Different systems	Diffusion of glucose (mg/dL)								
	10 min	20 min	30 min	60 min	90 min	120 min	150 min	180 min	300 min
System G	28±2.83	30±2.83	33±2.83	35±1.41	45±1.41	57±4.24	63±5.65	70± 5.65	99±1.41
System H	11.5±0.71	14±2.83	17±4.24	23±5.65	36±4.24	38±2.83	42±7.07	55±4.24	92±1.41
System I	9 ±0	11.5±1.41	16.5±3.53	21.5±3.53	28.5±2.12	34±2.83	38.5±2.12	45±1.41	85±1.41
System J	8.5±0.71	10.5±2.12	14.5±0.71	20.5±2.12	27.5±0.71	30.5±0.71	36.5±0.71	43±1.41	77±1.41
System K	5±1.41	10±1.41	12.5±2.12	19±1.41	24±2.83	29±1.41	35±1.41	41.5±2.12	74±1.41
System L	4±1.41	7±1.41	11±1.41	19±4.24	23±1.41	27±1.41	32.5±0.71	37±2.83	70±2.83

Values are expressed as mean ± S.D. of three experiments.

Table 6. Diffusion of glucose from different WSFUK-glucose mixture systems at different time intervals.

Different systems	Diffusion of glucose (mg/dL)								
	10 min	20 min	30 min	60 min	90 min	120 min	150 min	180 min	300 min
System G	28±2.83	30±2.83	33±2.83	35±1.41	45±1.41	57±4.24	63±5.65	70±5.65	99±1.41
System M	10±1.41	15.5±0.71	26±1.41	34±1.41	38±1.41	52.5± 0.71	56±2.83	64± 2.83	88±5.65
System N	9±1.41	14±1.41	24±1.41	30±1.41	36±1.41	49.5± 0.71	53±1.41	62±1.41	84±1.41
System O	7±0	11±1.41	16±1.41	19±1.41	26±2.83	33±2.83	40±1.41	48±4.24	77± 4.24
System P	6.5±0.71	10±1.41	14±1.41	16±1.41	22±1.41	28.5±o.71	38±2.83	46± 4.24	71± 2.83
System Q	5.5±0.71	8±1.41	12±0	15±1.41	20±1.41	26±1.41	36±2.83	44.5±0.71	71±1.41

Values are expressed as mean ± S.D. of three experiments.

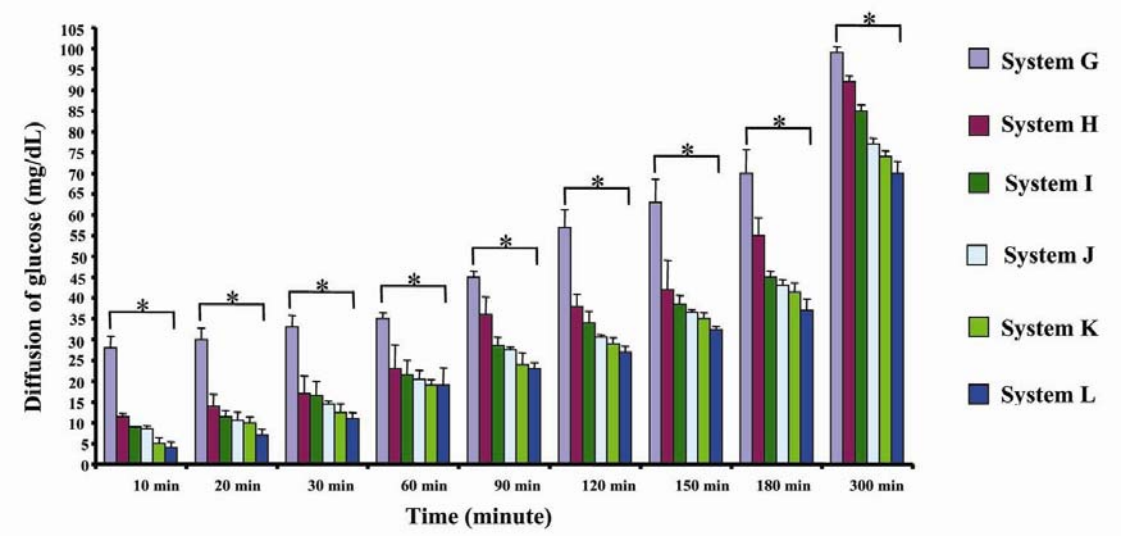

Fig. 1. Diffusion of glucose (mg/dL) from different viscous Na-CMC-glucose mixture systems at different time intervals**.** Data were presented as mean± S.D.; n=3, *p<0.05 compared to control (ANOVA followed by Dunnet's test using statistical software SPSS 15.0).

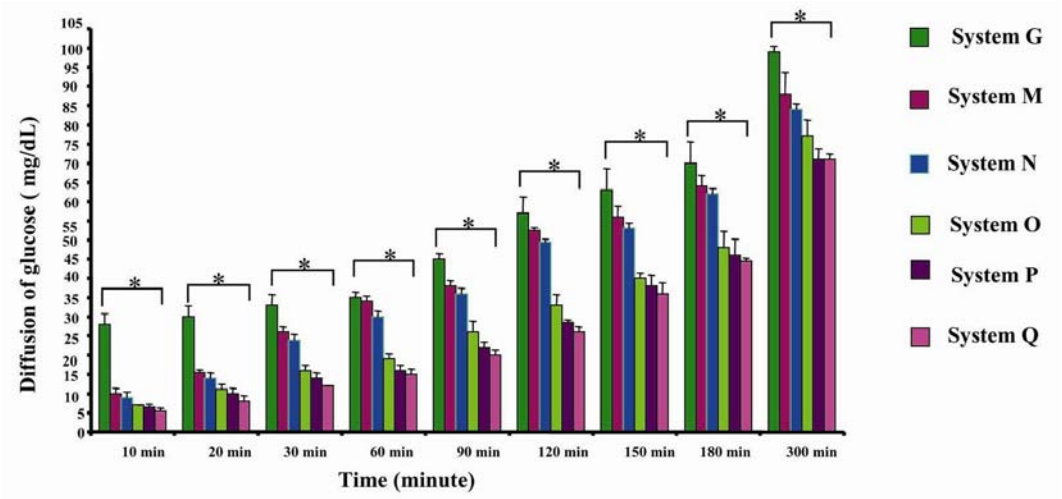

Fig. 2. Diffusion of glucose (mg/dL) from different viscous WSFUK-glucose mixture systems at different time intervals, where different systems contain different concentration of WSF of ulatkambal (system M, 4 mg/ml; system N, 9 mg/ml; system O, 14 mg/ml; system P, 19 mg/ml; system Q, 23 mg/ml) with a fixed conc. of glucose (8 mg/ml) solution. Data were presented as mean ± S.D.; n=3, *p<0.05 compared to control (ANOVA followed by Dunnet's test using statistical software SPSS 15.0).

Conclusion

In our study, we observed that viscous aqueous extract of *A. augusta* has significant (p<0.05) capacity to reduce the diffusion of glucose from different WSF-glucose systems as compared to control *in-vitro*. So, aqueous extract of fresh leaves of *A. augusta* may be potentially useful in controlling the postprandial blood glucose level in type 2 diabetic patient. More studies need to be performed on human to interpret the actual result of these interesting findings.

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