

Soluble Dietary Fiber from Aloe Vera and Lady's Finger; Effect on Glucose Absorption in Type-2 Diabetic Model Rats

F. Afrin¹, M. S. Hossain^{1*}, M. T. Amin¹, M. M. Islam², B. Rokeya³

¹Department of Pharmacy, Noakhali Science & Technology University, Sonapur, Noakhali-3814, Bangladesh

²Department of Pharmacy, Daffodil International University, Dhaka, Bangladesh

³Department of Pharmacology, Bangladesh University of Health Sciences (BUHS), Bangladesh

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Abstract

Despite considerable progress in diabetes mellitus by conventional synthetic drugs, the search for natural anti-diabetic plant products is going on. The effects of soluble dietary fibre (SDF) of Aloe Vera and Lady's Finger on glucose absorption were evaluated by the gut perfusion method in Type-2 diabetic model rats. SDFs were extracted from Aloe Vera and Lady's Finger by enzymatic digestion method. Both the SDF extracts of Aloe Vera and Lady's Finger and glucose (control) suspended in buffer solution at a dose of 1.25 g/kg were perfused at a rate of 0.5 mL/min for 30 min through the gut. The % of glucose absorption observed for up to 30 min. The % of glucose absorption for Aloe Vera and Lady's Finger during the 30 min was 52.98 ± 5.67 and 57.74 ± 4.81 , respectively, compared to control 67.74 ± 8.62 . The p-value (0.061) for Aloe Vera was quite closer to the level of significance. On the other hand, Lady's Finger had a non-significant effect on glucose absorption ($p=0.145$) in Type-2 diabetic model rats' gut. The results suggested the therapeutic potential of the SDF of Aloe Vera and Lady's finger, which suppressed postprandial hyperglycemia after glucose ingestion.

Keywords: Aloe Vera; Glucose absorption; Lady's Finger; Soluble dietary fiber (SDF); Type-2 diabetes.

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1. Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from defective insulin secretion, insulin action, or both [1,2]. Between two diabetes mellitus, Type-2 diabetes is the most typical form of diabetes and characterize by disorders of insulin secretion and insulin resistance [3,4]. Insulin deficiency leads to chronic hyperglycemia, leading to severe diabetic retinopathy, diabetic neuropathy, nephropathy, cardiovascular complications, and

* Corresponding author: pharmasalim@yahoo.com

ulceration [5-10]. 7 % of the Western countries' population affected by Type-2 diabetes, and globally it affects 5-7 % of the world's population [11].

Five classes of oral hypoglycemic agents are approved for the treatment of diabetes. If diet and exercise fail to achieve adequate glycemic control, oral therapy prescribe in patients. The drug category includes sulfonylurea, biguanide, an alpha-glucosidase inhibitor, thiazolidinedione, and meglitinide. Although the initial response may be right, these hypoglycemic agents may lose their effectiveness in a significant percentage of patients. These drugs have various side effects, such as sulfonylurea have limited absorption capacity and cause of weight gain due to hyperinsulinemia [12,13]. Biguanide causes weakness, fatigue, lactic acidosis. The alpha-glucosidase inhibitor may cause diarrhea, while thiazolidinediones may increase LDL-cholesterol level, coronary heart disease, and heart attacks [14]. Insulin is usually added to an oral agent when glycemic control is suboptimal at a maximal oral medication dose. Weight gain and hypoglycemia are common side effects of insulin [15]. Vigorous insulin treatment may also carry an increase in atherogenesis [16]. The limitation of currently available oral anti-diabetic agents, either in terms of efficacy or safety, coupled with the emergence of the disease into a global epidemic, has encouraged alternative therapy to manage diabetes more efficiently and safely.

Complementary and alternative therapy (CAM) is a treatment that is neither widely taught in medical schools nor commonly practiced in hospitals. Recently the use of CAM worldwide is increasing highly [17]. CAM for diabetes has become increasingly popular in the last several years. Alternative therapies with anti-diabetic activity have been researched relatively extensively, particularly in India. Dietary fiber is the edible part of the plants or analogous carbohydrates resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances [16]. Dietary fiber intake provides many health benefits. A generous intake of dietary fiber reduces the risk of developing the following diseases: coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal disorders [18-22]. Furthermore, increased consumption of dietary fiber improves serum lipid concentrations, lowers blood pressure, improves blood glucose control in diabetes, promotes regularity, aids in weight loss, and appears to improve immune function [23-25].

Several research works have been performed on SDF from different natural resources. It suggested that it can reduce blood glucose levels and increase the absorption of several minerals, including iron, zinc, calcium, and magnesium, reducing LDL cholesterol levels, improving gut function, etc. But the impact of SDF from Aloe Vera and Lady's Finger on glucose absorption level in the gut is not clear. In the present study, we aim to determine the impact of SDF from Aloe Vera and Lady's Finger on Type-2 diabetes model rat's glucose absorption level in gut.

2. Materials and Methods

2.1. Chemicals and reagents

Protease, termamyl or α -Amylase, amyloglucosidase solution, streptozotocin, and other chemicals collected from Sigma Aldrich; Merck, Germany.

2.2. Dietary fiber collection

For this present investigation, good quality, green and fresh Lady's Finger (2 kg), and Aloe Vera (5 kg) were bought from Karwan Bazar of Dhaka city. Only the edible parts of the vegetables are used for analytical purposes.

2.3. Fiber extraction

2.3.1. Isolation of mucilage

Blend parts of Lady's Finger and Aloe Vera mucilage were collected in a separated and previously washed, dried, and weighed weighing bottle. Then these blended contents of Lady's Finger and Aloe Vera were filtered through a pre-cleaned filter cloth. Filtered parts of Lady's Finger and Aloe Vera were poured into four times its absolute ethanol volume 98.9% (pro-analysis, Merck K GAA, Germany) in a beaker separately.

After 72 h, the supernatant portion was collected, and also the precipitates were separated by centrifugation at 5000 rpm for five min (2655 g RCF). Lady's Finger's supernatant portion and the depositions were re-dissolved in a minimum amount of distilled water with stirring and subjected for freeze-drying (HETOSICC Freeze Drier, Denmark). The same procedure followed for Aloe Vera. This freeze-dried material is termed parent mucilage (PM). Parent mucilage for both Lady's Finger and Aloe Vera was then subjected to enzyme treatment for starch and protein removal.

2.3.2. Isolation of SDF from mucilage

The parent mucilage (5.197 g for Lady's Finger and 4.734 g for Aloe Vera) for both Lady's Finger and Aloe Vera was suspended in phosphate buffer (0.1 M; pH 7.0) in a well stoppered conical flask. Protease (0.5 mg) was added to remove protein from the sample, and it was agitated in an ultrasonic bath for 2 min, shake in an incubator at 60°C for three h. The suspension was then dialyzed for 16 h in distilled water by changing the water several times. The dialysate concentrated and freeze-dried. After cooling the enzyme-treated protein and starch, free material was collected.

The freeze-dried sample was again suspended in an acetate buffer (0.1 M; pH 5.0). Terminal (heat-stable alpha-amylase) (100 μ L) was added to it, and the mixture was heated at 96°C for 1 h. The material was cooled, and then amyloglucosidase (300 μ L) was added to it, and the mixture was shaken for 16 h in a thermostatic shaker water bath (GFL England) 60 °C. After cooling, the enzyme-treated mix was centrifuged at 2500 rpm for

15 min. There are two phases obtained, liquid phase and residue. The liquid phase was collected by decantation; water (50 mL) was added to the residue, ultrasonicated for 5 min, centrifuged at 2500 rpm for 15 min. The process repeated one more time. The combined liquid phase was dialyzed for 48 h in distilled water by changing the water several times. The dialysate was reduced to a small volume (10 mL) and freeze-dried. This freeze-dried material was termed as SDF as 3.67 g for Lady's Finger and 3.46 g for Aloe Vera.

2.4. GUT perfusion experiment

This technique was used to study the effect of the SDF of Aloe Vera and Lady's Finger on intestinal absorption of glucose in rats [25]. The *in-situ* rat gut technique uses a single rat, which served as its control in evaluating the glucose absorption rate profile of SDF from Aloe Vera and Lady's Finger.

2.5. Experimental rats

A total number of six adult Long Evans Type-2 rats weighing 170-210 g were included in the study. The animals bred at the Bangladesh University of Health Sciences (BUHS) animal house, Dhaka, Bangladesh, maintained at a constant room temperature of 22 ± 5 °C with humidity of 40-70 % and natural 12 h day-night cycle. All of the rats were kept in plastic cages having dimensions of 30 × 20 × 13 cm, and softwood shavings were employed as bedding in the cages. The rats were fed on a standard laboratory pellet diet and water supplied ad libitum standard rat. The influence of circadian rhythm was avoided by starting all experiments at 8:30 am. The experiments were conducted according to the ethical guidelines approved by the Bangladesh Association for Laboratory Animal Science.

2.6. Design of the Study

Type-2 diabetes was induced in the rat by a single intraperitoneal injection of Streptozotocin (STZ) in citrate buffer (10 mL), at a dose of 90 mg/kg of body weight into the rat pups (48 h old, average weight 7 gm) as described by Bonner-Weir *et al.*, [26]. STZ injection to neonates led to the injury of the pancreas, destroying the functional β -cells. At the age of 3 months, when an oral glucose tolerance test (OGTT) was done, the remaining β cells could not cope with the load, which reflected in the postprandial rise of serum glucose level at 30 min (Table-1). The surge was significant among all rats compared to the baseline value. These rats were selected based on this experiment, and a gut perfusion study was carried out with these Type-2 diabetic model rats. Diabetic model rats with blood glucose level >7.00 mmol/L, at fasting conditions, were selected to study the effects of Aloe Vera and Lady's Finger's SDF. The SDF of Aloe Vera and Lady's Fingers were dissolved in Krebs's solution [27] at a dose of 25 mg/mL so that the amount of extract in the perfused intestine is equivalent to the dose of 1.25 g/kg.

Rats fasted for 36 h were anaesthetized with sodium pentobarbital (50 g/kg body weight) and kept in the dark and silent place for sleeping. Aloe Vera and Lady's Finger's SDF in Kreb's solution supplemented with glucose was passed through the duodenum's beginning just after the stomach. The perfusate was collected from the catheter set at the end of the duodenum (40 cm). The control group (basal) was perfused only Kreb's solution that was supplemented with glucose. The system was set at a constant temperature of 37 °C in an incubator to maintain the rat's body temperature, and the perfusion rate was 2.5 mL/5 min. Collecting tubes were placed into the "collector," which was fixed to move after every 5 min. Fractions were collected by the Bio-rad model 2110 fraction collector. The perfusion time was 30 min. The result was expressed as a percentage of unabsorbed glucose, calculated from the amount of glucose in the solution before and after the perfusion.

2.7. Sample collection

At first, the GUT of Type-2 diabetes rats washed out by the Kreb buffer solution for 10 min. Then Buffer-glucose solution was passed through the GUT for 30 min. The first sample was discarded. Finally, the cannula is connected to the solution of the SDF of Aloe Vera with Buffer-glucose solution. Fractions for the next 30 min of perfusion were collected. This procedure was repeated for the SDF of Lady's Finger.

2.8. Biochemical procedure

Glucose concentration is estimated by the glucose oxidase (GOD/POD) method (Sera Pak, USA). The absorbance is measured by microplate ELISA Reader (Bio-Tek EL-340, USA). Before determining glucose concentration by the GOD-POD method, the samples were diluted ten times [28].

2.9. Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) Software for Windows version 20 (SPSS Inc, Chicago, Illinois, USA). Values were expressed as Mean±SD (Standard Deviation), analysis of variance (ANOVA, Bonferroni Post Hoc Test), and independent sample 't'-test was done as the test of significance $p \leq 0.05$ was considered as the minimal level of statistical significance.

3. Results and Discussion

To generate a rat model mimicking human Type-2 diabetes with impaired insulin secretion and insulin resistance, we used STZ injection (90 mg/kg body weight) to 48 h old pulps. STZ injection to neonates led to the injury of the pancreas, destroying the functional β -cells. At the age of 3 months, when an oral glucose challenge (500 mg/kg body weight) was done, the remaining β cells could not cope with the load, which reflected in the postprandial rise of serum glucose level at 30 min (Table 1). The surge

was significant among all rats compared to the baseline value. These rats were selected based on this experiment, and a gut perfusion study was carried out with these Type-2 diabetic model rats.

Table 1. Check values of STZ induced Type-2 diabetic rats after three months of STZ injection.

Number of animals	Fasting glucose level (0 min) mmol/L	After oral glucose load (30 min) mmol/L
n=6	7.71±0.55 (100%)	14.82±1.35 (192%)
Paired Samples T-test (0 min Vs. 30 min)		
P=0.000		

This Table shows the significant rise in serum glucose levels after 30 min of glucose loading.

GUT perfusion experiment was performed for Aloe Vera and Lady's Finger's SDF in Streptozotocin (STZ)-induced Type-2 diabetic model rats. The effect of SDF of Aloe Vera and Lady's Finger on glucose absorption was expressed as the percentage of absorbed glucose, calculated from the amount of glucose in solution before and after the perfusion.

The experiment results showed that % of absorbed glucose (absorbed glucose content) in Type-2 diabetic model rats almost the same for control (Glucose), Aloe Vera, and Lady's Finger during the first 10 min of perfusion. However, with time, the percentage of absorbed glucose decreased for Aloe Vera and Lady's Finger, both compared to control due to SDF's presence in it. It may be probably due to Aloe Vera and Lady's Finger's SDF, which may inhibit glucose absorption by trapping glucose inside its gummy gel and, consequently, slows down the glucose absorption while moving through the digestive tract. The result is presented in Fig. 1. Our findings are similar to the findings reported by Rajasekaran *et al.* who indicated that an Aloe Vera gel extract was effective in lowering hyperglycemia levels in STZ-induced diabetic rats, and by Indah *et al.* for Lady's Finger, which has hypoglycemic effects in response to *Abelmshus Esculentus* Treatment using STZ-Induced Diabetic Rats [29,30].

Again, during a total time of 30 min of perfusion with glucose, the percentage of glucose absorption for the control group is higher than Aloe Vera and Lady's Finger. Additions of Aloe Vera and Lady's Finger's SDF to the glucose perfusate resulted in decreased absorbed glucose content. In this case, Aloe Vera's SDF showed a better result than the SDF of Lady's Finger. The level of significance for Aloe Vera ($p=0.061$) is quite similar to the significant result ($p<0.05$), whereas for Lady's Finger, the value deviates much ($p=0.145$) from the level of significance ($p<0.05$). Results are presented in Fig. 2.

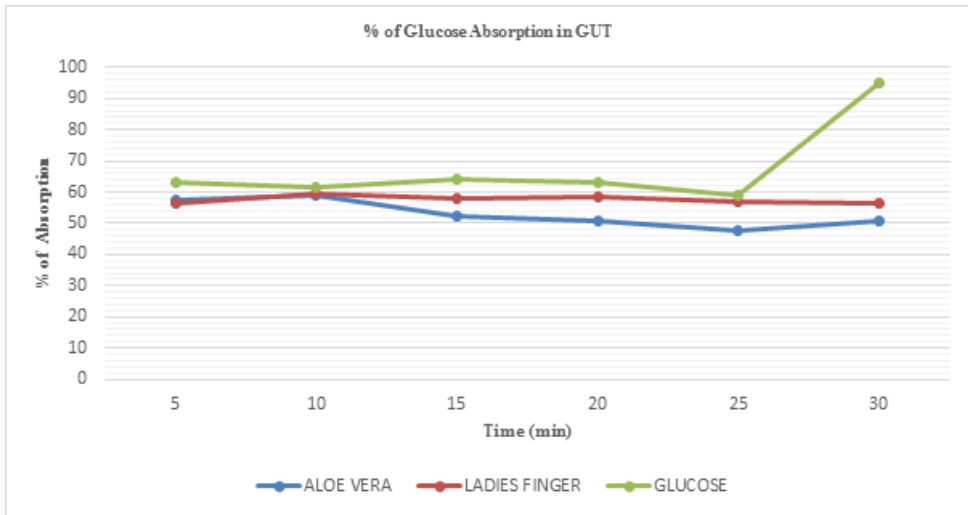


Fig. 1. Percentage of absorbed glucose in the gut of Type-2 diabetic rat.

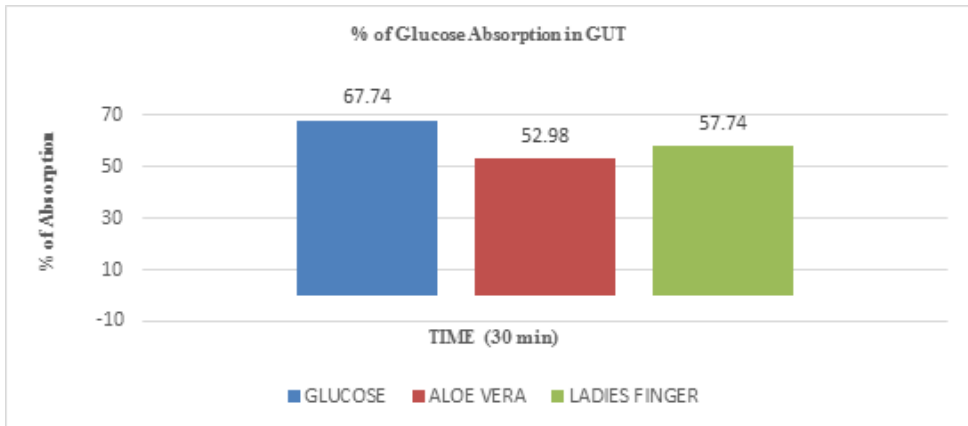


Fig. 2. Graph comparing the % of total glucose absorption in the gut at a total time of 30 min in a group of control rats vs. Aloe Vera vs. Lady's Finger.

The present study was undertaken to investigate the effect of SDF of Aloe Vera and Lady's Finger on glucose absorption in the gut of STZ induced Type-2 diabetic model rats by using the gut perfusion technique. The results demonstrated that Aloe Vera and Lady's Finger's SDF showed a gradual decrease in glucose absorption in rat's gut. Reduction of absorbed glucose content in the gut is noticed when given with a simultaneous glucose load and SDF of Aloe Vera and Lady's Finger separately in Type-2 diabetic model rats. It indicates that the SDF may interfere with intestinal glucose absorption by various mechanisms [31].

One mechanism postulated that the SDF might trap glucose inside its gummy gel. Inside the gel, glucose might shield from digestive enzymes and less likely to reach the intestines' wall. Consequently, glucose is absorbed into the bloodstream more slowly and blunting the sharp spike in blood glucose. Another explanation for soluble fiber's effect on blood glucose is that, for nutrients to be absorbed into the intestines, they must first cross an unstirred water layer covering the intestines' surface. Soluble fiber thickens this layer, making it more resistant to the movement of nutrients diffusing into the body. The theory also explains why blood glucose levels rise more slowly when consumed with soluble fiber. The present study confirms this effect because when the SDF of Aloe Vera and Lady's Finger was given along with glucose solution, it decreased glucose absorption in the gut during in-situ perfusion of the small intestine the glucose solution in the control group of rats. Our results agree with A. Okyar *et al.*, who reported that Aloe Vera leaf pulp extract is efficient in Type-1 and Type-2 diabetic rats [32]. Another hypoglycaemic effect of Aloe species was made by Agarwal, who prescribed a diet plan containing Aloe Vera leaves to five thousand heart disease patients twice daily for five years and reported marked decreases in blood sugar levels [33]. Subsequently, Ghan-nam *et al.*, using the plant dried sap, revealed a hypoglycaemic agent that lowered alloxan-diabetic mice's blood sugar levels [34]. These findings were confirmed by Ajabnoor [35]. Aloe Vera juice's chronic effect was investigated in combination with glibenclamide in diabetic patients, and the results also supported its hypoglycaemic effect [36,37]. Apart from this, Lady's Finger with peel and seed powder supports the anti-diabetic potential in diabetic rats [38]. Our findings that the consumption of SDF from Aloe Vera and Lady's Finger exerts an anti-diabetic effect reduces the gut's glucose absorption rate. Moreover, dense water-soluble dietary fibers hamper glucose diffusion and postponing the absorption and digestion of carbohydrates, resulting in lower postprandial blood glucose levels [39].

4. Conclusion

In conclusion, the present study has demonstrated that Aloe Vera's SDF showed relatively closer to significant ($p=0.061$) inhibition of glucose absorption. In contrast, the Lady's Finger's SDF affects glucose absorption, but the significance level deviates much ($p=0.45$). SDF of both mucilages traditionally used in the treatment of Type-2 diabetes mellitus. The result obtained from gut perfusion demonstrates, more conclusively, SDF of Aloe Vera and Lady's Finger can be useful in diabetic treatment. However, further studies are required to isolate the active principle(s) and elucidate the underlying mechanism of glucose absorption of the SDF of Aloe Vera and Lady's Finger to explore SDF's role as a potential anti-diabetic agent.

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