ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Suppression of Gut Glucose Absorption and Enhancement of Gut Fluid Absorption in Alloxan - Induced Diabetic Rats Treated with Crude *Aloe vera* Gel

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ABSTRACT

This study was aimed at determining the effect of treatment of type 1 diabetes mellitus (T1DM) with crude *Aloe vera* gel on gut fluid and glucose absorption which are determinants of the degree of post-prandial hyperglycemia. Twenty four male albino Wistar rats weighing 180 - 200 g were randomly assigned 1 of 4 groups (n = 6) thus, control, diabetic untreated (DM) group, diabetic group treated (DMT) with crude *Aloe vera* gel at a dose of 0.4 ml/100g body weight per oral route and control group treated (CT) with crude *Aloe vera* gel at a dose of 0.4 ml/100g body weight per oral route. All animals had access to food and tap water *ad libitum*. After 21 days of administration, animals were sacrificed by cervical decapitation. Determination of fluid transfer and glucose uptake was done using appropriate methods. Histology of small intestine was also done using standard techniques. Serosal fluid transfer was significantly ($P < 0.05$) lower in DMT group compared with control. Gut fluid uptake was significantly ($P < 0.05$) higher in DMT group compared to control and CT group. Mucosal and serosal glucose concentrations before incubation were significantly ($P < 0.05$) lower in DM group compared to control. Mucosal glucose concentration after incubation was significantly ($P < 0.05$, $P < 0.05$, $P < 0.01$) increased in DM, DMT and CT group respectively, compared to control. Serosal glucose concentration after incubation was significantly ($P < 0.01$, $P < 0.001$, $P < 0.01$) increased in DM, DMT and CT group respectively, compared to control. Gut glucose transfer was significantly ($P < 0.05$, $P < 0.05$, $P < 0.01$) lowered in DMT group compared to control, DM and CT group respectively. We therefore conclude that crude *Aloe vera* gel potentiates the effect of T1DM in increasing gut fluid uptake. Crude *Aloe vera* gel also reduces gut glucose transfer in DM group, which is a beneficial effect to reduce post-prandial hyperglycemia.

Keywords: *Aloe vera*, diabetes mellitus, glucose absorption, gut fluid uptake, gut glucose uptake

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ISSN: 0975-8585

INTRODUCTION

Diabetes mellitus (DM) remains the most common disorder of carbohydrate metabolism. Diabetes mellitus is a metabolic disorder of multiple etiologies that is characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism [1]. DM may result from defects in insulin secretion, insulin action or both [1]. The efficacy of absorption of digested food materials is potentially affected by altered motility of the small intestine observed in DM [2], and by alterations in the transport mechanisms facilitating nutrient uptake across the intestinal membrane [3].

The handling of ingested glucose by the gut is important in the regulation of postprandial glucose concentrations and, hence glycaemic control**.** At the level of the liver, handling of glucose is mediated by the glucose gradient between portal venous and hepatic arterial blood [4]; accordingly, high systemic blood glucose levels may favour a decreased availability of absorbed glucose to the systemic circulation. During hepatic glucose uptake, glycogenolysis and gluconeogenesis are concurrently suppressed; limiting the increase in systemic glucose [5,6]. Impairment of this suppression contributes to postprandial hyperglycemia in DM, especially in patients with Type II Diabetes Mellitus (T2DM). One therapeutic approach for treating DM is to decrease the magnitude of absorption of glucose in the small intestine, thus reducing post-prandial hyperglycaemia [7,8,9].

In the world today, a greater percentage of the population depend on plant materials for treatment of various ailments, DM inclusive. *Aloe vera* is a perennial plant that belongs to family Liliaceae, having over 350 species [10]. *Aloe vera* is used therapeutically in three basic forms: Aloe gel - which is obtained by slicing the *Aloe vera* leaf; Aloe latex - which is obtained from the inner surface of the *Aloe vera* leaf and Aloe whole leaf - which is obtained by blending the entire leaf, thus, mixing the gel and latex. A large number of biological activities have been demonstrated in *Aloe vera* gel use, to explain its purported health benefits, including anti-inflammatory [11], anti-microbial and anti-proliferative [11], lipid and glucose lowering [12,13], immuno-stimulatory and antioxidant functions. *Aloe vera* latex which is obtained from the inner part of the skin of the leaves has been reported to contain anthraquinones and possess laxative effect [14]. A number of potentially active ingredients in the latex and gel of *Aloe vera* have been identified, though not much is known about their possible mechanisms of action. Following wide range of reports linking DM to malabsorption and the use of *Aloe vera* in the management of DM, this study seeks to determine the effect of treatment of T1DM with crude *Aloe vera* gel on fluid and glucose absorption.

MATERIAL AND METHODS

Plant Material and Preparation of Crude *Aloe vera* gel

Aloe vera leaves with length between 40 and 50 cm were obtained from a mature *Aloe vera* plant in University of Uyo botanical garden and was authenticated by the Chief Herbarium officer of Botany Department of University of Calabar, Calabar, Nigeria. Clean water was used

to rinse the leaves to remove debris and sand. The leaves were then dried with a clean piece of cloth. Using a knife, the leaves were sliced longitudinally to expose the gel. The gel was gently scraped into an electric blender to homogenize. The dose of the crude extract used for this study was 0.4 ml/100g body weight [15].

Animal Preparation and Protocol

Twenty four (24) male albino Wistar rats weighing 150 - 180 g were used for this study. The animals were obtained from the animal house of Department of Physiology, University of Calabar. After 14 days of acclimatization, the animals were randomly assigned one of four groups such that each group contained six (6) rats, thus; Group 1 - control; group 2 - diabetic untreated group (DM), group 3 - diabetic treated group (DMT) and group 4 - control treated group (CT). The animals were placed in well ventilated metabolic cages and exposed to 12/12 hours light/dark circle. All animals had access to food and tap water *ad libitum.*

Treatment with Crude *Aloe vera* gel

Crude *Aloe vera* gel administration commenced after 14 days of habituation. The gel was administered to DMT and CT group at a daily oral dose of 0.4 mg/100g for 21 days. Administration of crude *Aloe vera* gel was facilitated by the use of a syringe and orogastric tube.

Induction of Type 1 Diabetes Mellitus

Type 1 DM was induced by intraperitoneal administration of 100 mg/kg alloxan after 24 hours fast. Diabetes was confirmed 48 hours after alloxan administration using a glucose meter (IMFOMED IMPEX, INDIA) and test strips. Blood used for this purpose was obtained by pricking the distal end of each animal's tail. Rats with fasting blood glucose level $\geq$ 180 mg/dl 48 hours after alloxan administration were considered diabetic.

Determination of Fluid Absorption by the Small Intestine

The animals in the different groups were sacrificed by cervical decapitation after 21 days of administration of crude *Aloe vera* gel. Absorption of fluid and glucose by the everted sac technique of Wilson and Wiseman [16] as modified by Adeniyi and Olowokoorun [17] was employed in this study. Four segments (I, II, III and IV) of length 10 cm each were cut out in a manner shown in figure 1 for sac making.

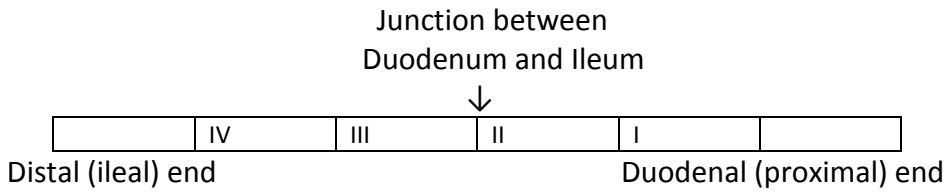

Figure 1. Diagram showing sac making technique

Each sac was made by tying the distal end of the segment with a dry thread having a standard length, inverting the sac (mucosa surface out, serosal surface in) before filling it with 1ml Krebs bicarbonate solution (serosal fluid), and tying the other end with a similar thread. 40 ml of standard Krebs bicarbonate solution was the mucosa fluid used, and was put in incubating flasks labeled I, II, III, and IV, respectively, and each flask was aerated using a 95% O_2, 5% CO_2 gas mixture in a Gallenkamp Shaker bath for 30 minutes. The sacs were then immersed in the aerated fluid and aerated further for 2 minutes after which there were incubated for another 28 minutes. After incubation, the sacs were blotted and weighed thus;

W1 = Weight of empty dish + 2 ligatures
W2 = Weight of empty dish + 2 ligatures + empty sac
W3 = Weight of empty dish + 2 ligatures + initial weight of full sac
W4 = Weight of empty dish + 2 ligatures + final weight of full sac
W5 = Weight of empty dish + 2 ligatures + final weight of empty sac

The units for fluid transfer employed in this study are those of Parsons *et al* [18]. Where, fluid transfer was determined as a measure of volume transferred by a unit wet weight of intestine for a given period. The mucosal fluid transfer (MFT), serosal fluid transfer (SFT) and gut fluid uptake (GFU) were determined using the results from the weighing as shown below;

Initial wet weight (IWW) = W2 – W1
Initial serosal volume (ISV) = W3 – W2
Final serosal volume (FSV) = W4 – W5
Serosal fluid transfer (SFT) = (W4 – W5) – (W3 – W2)
Gut fluid uptake (GFU) = W5 – W2
Mucosal fluid transfer (MFT) = SFT + GFU

SFT, GFU and MFT were expressed as volume/g sac/30minutes, where serosal fluid transfer is defined as a change in serosal lumen after incubation. Gut fluid uptake (GFU) is defined as increase in the fluid content of the intestine tissue owing to the increase in the water content of intestinal tissue and the swelling of the epithelial cells. Mucosal fluid transfer (MFT) is the decrease in the volume of fluid on the mucosal side during absorption.

Determination of Glucose Absorption by the Small Intestine

The terms used for glucose transfer are mucosal glucose transfer (MGT), serosal glucose transfer (SGT) and gut glucose uptake (GGU). MGT is the amount of glucose lost from the mucosal fluid. SGT is the amount of glucose that entered the serosal fluid after incubation. The GGU represents the amount of glucose metabolized and those found in the gut wall at the end of experiment. A glucose kit (Ames blovel analyzer glucose kit UK) for blood and glucose was used. The concentrations of glucose in Krebs bicarbonate solution and intestine segments before and after incubation as well as the concentration in the lumen of the sac after incubation were determined. The units for glucose transfer are same as for fluid transfer. The

physiological solution was bubbled continuously with 95:5 per cent oxygen, carbondioxide mixture, the pH was between 7.35 and 7.40 and the temperature was maintained at 37^{o}C.

Histology of Small Intestine

Small intestine tissue sections were obtained using standard method and treated with haematoxilin and eosin stains. Other stains used included silver, reticulum, trichrome and Periodic Acid Schiff (PAS). Permanent preparations using routine biopsy method [18] was used.

Statistical analysis

All results are presented as mean ± standard error of mean (SEM). The One - way analysis of variance (ANOVA) was used to analyze the data collected followed by the post hoc multiple comparison (Least significant difference procedure). *P* =.05 was considered significant. Computer software, SPSS version 17.0 and excel analyzer were used for the analysis of results in this study.

RESULTS

Fluid Absorption by the Small Intestine

Serosal Fluid Transfer (SFT)

The mean values for serosal fluid transfer in the different experimental groups were 1.02 ± 0.02, 0.83 ± 0.10, 0.75 ± 0.10 and 0.96 ± 0.07 ml/g sac/30 minutes for control, DM, DMT and CT group respectively. SFT was significantly ($P < 0.05$) lower in DMT group compared with control. Table 1.

Mucosal Fluid Transfer (MFT)

The mean values for mucosal fluid transfer in the different experimental groups were 1.14 ± 0.04, 1.19 ± 0.07, 1.20 ± 0.03 and 1.10 ± 0.02 ml/g sac/30 minutes for control, DM, DMT and CT group respectively. There was no significant difference in MFT in the groups studied. Table 1.

Gut Fluid Uptake (GFU)

The mean values for gut fluid uptake in the different experimental groups were 0.075 ± 0.01, 0.107 ± 0.01, 0.147 ± 0.05 and 0.061 ± 0.01 ml/g sac/30 minutes for control, DM, DMT and CT group respectively. GFU was significantly ($P < 0.05$) higher in DMT group compared to control and CT group. Table 1.

Table 1.0 Fluid transfer in rat intestine in the different experimental groups

Groups	Serosal Fluid Transfer (ml/g sac/30mins)	Mucosal Fluid Transfer (ml/g sac/30mins)	Gut Fluid Uptake (ml/g sac/30mins)
Control	1.02 ± 0.02	1.14 ± 0.04	0.075 ± 0.01
DM	0.83 ± 0.10	1.19 ± 0.07	0.107 ± 0.01
DMT	0.75 ± 0.10 [a]	1.20 ± 0.03	0.147 ± 0.05*
CT	0.96 ± 0.07	1.10 ± 0.02	0.061 ± 0.01

Values are presented as mean ± SEM. [a]$P < 0.05$ vs Control.

Intestinal Segments Glucose Transfer

Mucosal and Serosal Glucose Concentration before Incubation

The mean values for both mucosal and serosal glucose concentrations before incubation in the different experimental groups were 132.2 ± 13.18, 106.9 ± 4.83, 113.7 ± 2.68 and 117.9 ± 3.45 mg/g sac/30 minutes, for control, DM, DMT and CT group respectively. Mucosal and serosal glucose concentrations before incubation were significantly ($P < 0.05$) lower in DM group compared to control. Table 2.

Mucosal Glucose Concentration after Incubation

The mean values for mucosal glucose concentration after incubation in the different experimental groups were 112.0 ± 2.93, 125.2 ± 5.09, 124.2 ± 0.36 and 129.4 ± 4.35 mg/g sac/30 minutes, for control, DM, DMT and CT group respectively. Mucosal glucose concentration after incubation was significantly ($P < 0.05$, $P < 0.05$, $P < 0.01$) increased in DM, DMT and CT group respectively, compared to control. Table 2.

Serosal Glucose Concentration after Incubation

The mean values for serosal glucose concentration after incubation in the different experimental groups were 99.7 ± 2.02, 113.6 ± 3.44, 115.5 ± 0.91 and 113.9 ± 2.71 mg/g sac/30 minutes, for control, DM, DMT and CT group respectively. Serosal glucose concentration after incubation was significantly ($P < 0.01$, $P < 0.001$, $P < 0.01$) increased in DM, DMT and CT group respectively, compared to control. Table 2.

Gut Glucose Uptake (GGU)

The mean values for gut glucose uptake in the different experimental groups were 12.3 ± 1.73, 12.0 ± 2.43, 8.7 ± 1.00 and 15.5 ± 1.78 mg/g sac/30 minutes, for control, DM, DMT and CT group respectively. GGU was significantly ($P < 0.05$, $P < 0.05$, $P < 0.01$) lowered in DMT group compared to control, DM and CT group respectively. Table 2.

ISSN: 0975-8585

Table 2.0 Glucose transfer in rat intestine in the different experimental groups

Groups	Glucose Concentration Before Incubation (mg/g sac/30mins)		Glucose Concentration After Incubation (mg/g sac/30mins)		Gut Glucose Uptake (mg/g sac/30 minutes)
	Mucosal	Serosal	Mucosal	Serosal	
Control	132.2 ± 13.18	132.2 ± 13.18	112.0 ± 2.93	99.7 ± 2.02	12.3 ± 1.73
DM	106.9 ± 4.83 [a]	106.9 ± 4.83 [a]	124.2 ± 0.36 [a]	113.5 ± 1.68 [b]	12.0 ± 2.43
DMT	113.7 ± 2.68	113.7 ± 2.68	125.2 ± 5.09 [a]	115.5 ± 0.91 [c]	8.7 ± 1.00 [a,d,e]
CT	117.9 ± 3.45	117.9 ± 3.45	129.4 ± 4.35 [b]	113.9 ± 2.71 [b]	15.5 ± 1.78

Values are presented as mean ± SEM.
[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ vs Control, [e]$P < 0.001$ vs CT, [d]$P < 0.05$ vs DM

Histology of the Small Intestine

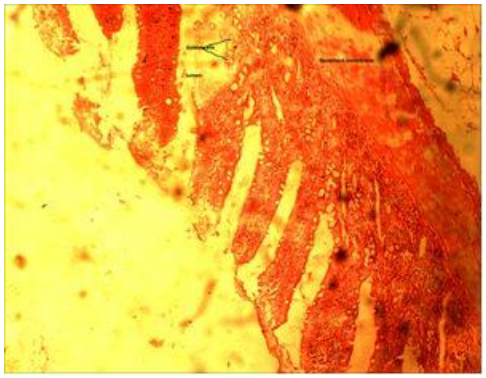

Plate 1

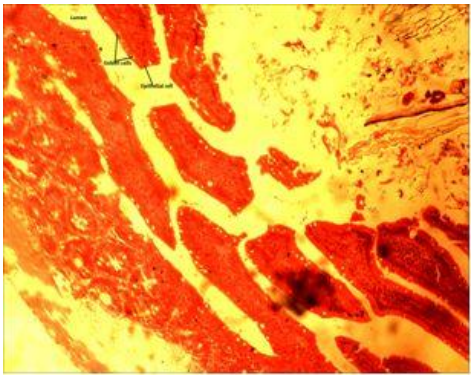

Plate 2

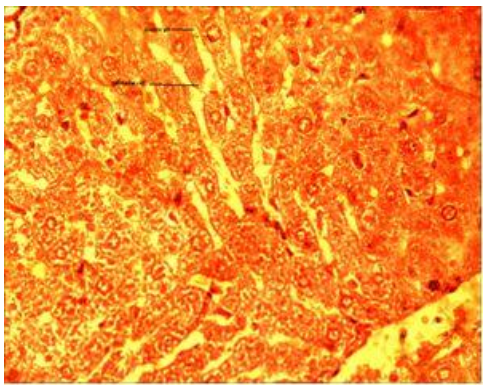

Plate 3

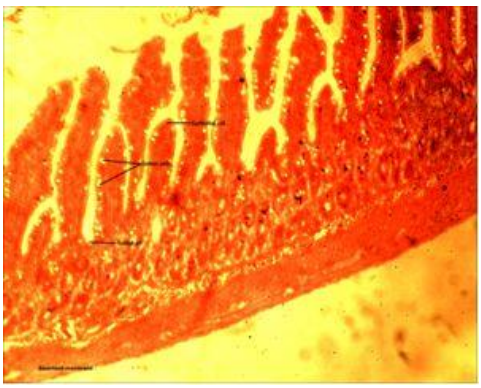

Plate 4

Plate 1 - Control
Plate 2 - diabetic untreated (DM) group
Plate 3 - diabetic treated (DMT) group
Plate 4 - control treated (CT) group

Results obtained from histological analysis of the small intestine did not show any marked damage in structures in any of the groups.

ISSN: 0975-8585

DISCUSSION

Diabetes mellitus (DM) is a group of metabolic disease with increasing incidence in rural populations the world over. Reports from our previous studies [13,15] had shown the beneficial effect of crude *Aloe vera* gel in reducing fasting blood glucose levels in diabetic animals.

Mucosal and serosal glucose concentrations before incubation were significantly reduced in DM group compared to control. Contrary to Dyer *et al* [19] and Fedorak *et al* [20] who both reported that diabetic animals exhibit increased capacity for glucose uptake from the gut, results obtained in our study showed that gut glucose uptake in the DM group was not significantly different from control, but was significantly reduced in DMT group compared to control, DM and CT group respectively. It can therefore be deduced that crude *Aloe vera* gel reduced gut glucose absorption as a mechanism of reducing post-prandial hyperglycemia, thus reducing blood glucose concentration. Increased glucose uptake in CT group did not translate into increased blood glucose concentration [13] as excess glucose is converted to glycogen and stored in the liver, unlike diabetes mellitus where gluconeogenesis is the order of the day. Thus, increased glucose uptake in CT group confirms the observation of Nna *et al* [13] who reported that normal animals administered crude *Aloe vera* gel gained weight without a concomitant increase in food intake. This effect may equally be related to the enzymes contained in crude *Aloe vera* gel. Aloe gel contain enzymes like alkaline phosphatase, amylase, carboxypeptidase, catalase, cellulase, lipase and peroxidase, some of which are involved in the breakdown of food sugars and dietary fats.

Serosal fluid transfer was significantly lower in DMT group compared with control, while gut fluid uptake was highest in DMT group, followed by DM group. It is a well-known fact that absorption of fluid is usually passive and follow solutes like glucose and sodium. Ironically, despite the reduced glucose absorption recorded in DMT group compared to DM group, gut fluid uptake was highest in the DMT group, followed by DM group. Intestinal fluid uptake was lowest in CT group. These findings clearly show that diabetes mellitus strongly affects fluid uptake. However, the effect of *Aloe vera* gel on fluid uptake in diabetes mellitus is compensatory to augment the dehydration secondary to osmotic diuresis occasioned by hyperglycemia.

Results of histological examination did not show any noticeable defect in structures of the small intestine in the groups studied.

CONCLUSION

On the basis of the results obtained from this study, we therefore conclude that crude *Aloe vera* gel potentiates the effect of T1DM in increasing gut fluid uptake. Crude *Aloe vera* gel also reduces gut glucose transfer in DM group, which is a beneficial effect to reduce post-prandial hyperglycemia.

ISSN: 0975-8585

ACKNOWLEDGEMENT

Authors hereby acknowledge the kind assistance of the laboratory staff of Department of Physiology, College of Medical Sciences, University of Calabar.

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