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Effect of Aqueous Fruit Extract of *Xylopia Aethiopica* on Intestinal Fluid and Glucose Transfer in Rats

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Summary: Intestinal fluid and glucose absorption was studied in jejunal and ileal segments in *Xylopia aethiopica* fed rats using inverted sac technique. Thirty male Wistar rats were assigned into three groups of 10 rats each; control, 100mg/kg and 200mg/kg *Xylopia aethiopica* treated groups. The control group received normal rat chow and water while the low dose and high dose groups received oral administration of *Xylopia aethiopica* extract at doses of 100mg/kg and 200mg/kg body weight respectively in addition to daily rat chow and water intake for 28 days. The results showed significant reduction and increase in fluid transfer in the jejunum and ileum respectively ($P<0.01$) compared with control. 100mg/kg increased gut fluid uptake in the ileum while 200mg/kg treatment reduced uptake in jejunum compared with control. Both doses had significantly increased jejunal and ileal glucose transfer. Gut glucose uptake was increased in jejunum and ileum of *Xylopia aethiopica* treated groups. Both doses increased the crypt depth but significantly decreased the villus height in the ileum ($P<0.05$). In conclusion, increased ileal gut fluid uptake may be beneficial in diarrheal state while an enhanced glucose uptake implies that glucose substrate may be made available to cells for synthesize of ATP for cellular activities.

Keywords: *Xylopia aethiopica,* Glucose, Absorption, Jejunum, Ileum, Rat.

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INTRODUCTION

One of the physiological processes of gastrointestinal tract is absorption. The products of digestion as well as drugs and water are transported across the epithelial membrane of the small intestine. Compounds with high lipid solubility are transferred faster through the lipid of the cell membrane while water soluble substance diffuse through water-filled pores in the cell membrane (Smith and Morton, 2001). Absorption is high in the duodenum than in the ileum. The upper small intestine has been identified as possible sites of hydrolysis and absorption of many flavonoids, (Spencer *et al*,2003). In vitro and insitu models, intestinal segments have given knowledge of fluid and glucose absorption as well as transport mechanism in rat and *in vivo* absorption in humans (Amidon *et al*, 1995; Lennernas *et al*, 1997). Inverted sac technique has not only been used to determine fluid transfer but also regional variation in absorption in the different part of gastro- intestinal tract (Stephens *et al*, 2002; Mohamedally *et al*, 2006). Lindahl *et al*, (1998) using in situ single pass perfusion, studied the effective permeability of drugs and glucose in each region of jejunum and ileum.

Xylopia aethiopica (Fleischer *et al*, 2008; Ameyaw and Owusu Ansah, 2007; Kouninki *et al*, 2005; Asekun and Adeniyi, 2004) is used as spice in many parts of West Africa. In Nigeria, not only is it used as spice, but in some delicacies as "isi-ewu" (igbo), "obeata" (Yoruba). Some medicinal properties attributed to the plant include, cure for dysentery, stomach or back pain and bulimia (Abbiw, 1990, Ameyaw and OwusuAnsah, 2007). Glucose uptake is mainly performed by sodium -dependent glucose transporter and the activity of this carrier has been reported to be inhibited by polyphenols (Kobayashi *et al*, 2000). *Xylopia aethiopica* fruit has polyphenols and in spite of this, it is medicinally used to manage gastrointestinal ailments. Here we studied fluid and glucose absorption in *Xylopia aethiopica* fed rats using inverted sac technique.

MATERIALS AND METHODS

Experimental animals

Male albino rats obtained from the Department of Physiology animal house, University of Calabar, Nigeria, were acclimatized for 7 days before the experiments, after approval for the study was obtained from the College of Medical Sciences Ethical Committee. The rats were weighed and separated into 3 groups housed in plastic cages of ten animals each, they were under constant temperature of 28^0c ± 2^0c and relative humidity of 50 ± 5%, and 12h dark/light cycle. The animals were given rats chow (Pfizer, Ltd, Nigeria) and drinking water for 28 days. In addition, low and high dose test groups received 100mg/kg and 200mg/kg body weight of *Xylopia Aethiopica* extract respectively *p.o* once daily.

Extract preparation

Dried fruit material of *Xylopia Aethiopica*was purchased from Watt market, Calabar, Nigeria and authenticated at the Department of Botany, University of Calabar, Nigeria. The dried crude extract was prepared by pulverizing and macerating 1kg/10L of water (w/v) for 12h and then filtered with Whatman No 1 filter paper. The filtrate was dried in an aerated oven at 45^oc to a constant weight (Obiefuna *et al*, 1994). The resulting dark brown extract was stored in a sample container for the experiments.

Preparation of inverted sac for fluid and glucose absorption

After an overnight fast, the animals were sacrificed and a midline incision 3-4 cm was made along the lineaalba and the intestine was removed and washed in physiological saline. Segments I, II, III, and IV each 10cm long (2 jejunum and 2 ileum) were cut out for making of sacs as described by Wilson and Wiseman (1954), and modified by Adeniyi and Olowokorun (1987).

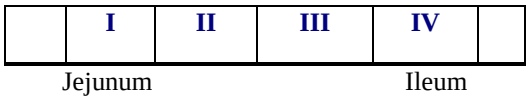

Fig.1
Four segments for sac making.

Each sac was made by tying the distal end of the segment with the dried thread having a standard length (10cm). From the end, a rod was used to push the end inwards thereby inverting the sac (mucosa end out, serosa in). The sac was then filled with 1ml of Kreb's bicarbonate solution (serosa fluid) and the free end was tied afterward with a similar thread. Forty millimeters (40ml) of the Kreb's bicarbonate solution was placed in incubating flasks labeled I,II,III and IV respectively and each flask was aerated using 95% Oxygen and 5% carbon dioxide gas mixture (Air Liquid Nigeria, PLC) in Gallenkamp shaker bath (England) for 30 minutes. The sacs were immersed in the aerated fluid for 2 minutes before they were further incubated for 28minutes. After incubation, the sacs were blotted and weighed as follows; W_1 = weight of dish + 2 ligatures; W_2 = weight of dish + 2 ligatures + empty sac; W3= weight of dish + 2 ligatures + initial weight of full sac; W_4 = weight of dish + 2 ligatures + final weight of full sac; W_5 = weight of dish + 2 ligatures + final weight of empty sac

Using the Ames/MBI blood analyzer, glucose reagent kits for glucose the concentrations in the Kreb's bicarbonate solution and intestinal segments before and after incubation as well as the concentration in the segments of sacs after incubation were determined. The fluid and glucose transfer was expressed as ml/g sac/30minutes and mg/g sac/30minutes respectively, according to Parsons *et al*, 1958; Barry *et al*, 1961; AdeniyiOlowokorun, 1987 and Obembe *et al*, 2010. Fluid and glucose transfer was determined as a measure of volume transferred by a unit wet weight of intestine for a given period. The mucosal fluid transfer (MFU), serosal fluid transfer (SFT) and gut fluid uptake (GFU) were determined by using the results from the weighing as follows;

Initial wet weight (IWW) = $W_2 - W_1$
Initial Serosal Volume (ISV) = $W_3 - W_2$
Final Serosal Volume (FSV) = $W_4 - W_5$
Serosal Fluid Transfer (SFT) = FSV - ISV
Gut fluid uptake (GFU) = $W_5 – W_2$
Mucosal fluid transfer (MFT) = SFT + GFU

MFT, SFT and GFU and were expressed as volume/g sac/30 minutes. The terms used for the glucose transfer are mucosal glucose transfer (MGT), serosal glucose transfer (SGT) and gut glucose uptake (GGU). MGT is the amount of glucose that disappears from the mucosal fluid while the SGT is the amount of glucose that enters the serosal fluid. GGU is the difference in glucose concentration between mucosal and serosal fluids after incubation. The value includes glucose metabolized and those found in the gut wall at the end of the experiment. (Obembe et al, 2010).

Histological preparation of jejunum and ileum

Appropriate portions of jejunum and ileum from the sacrificed rats were cut and placed in normal saline. They were cut open to empty its content, and

then rinsed in normal saline, and fixed in 10% formalin solution. The tissues were dehydrated in alcohol and cleared in xylene. They were embedded in paraffin wax and thin sections of 5 microns were cut and stained with haematoxylin for 15 minutes, differentiated with 1% acid alcohol and counter stained in eosin for 2 minutes and mounted with dextrenepolysterene xylene (PDX) (Drury and Wallington, 1994). The sections were then viewed under the microscope and photomicrographs were taken.

Determination of villus height and crypt depth

The villus height and crypt depth was determined by the method described by Kik (1991). The measure of the distance from the crypt opening to the tip of the villus and the villus and measure from the base of the crypt to the level of the crypt opening are villus height and crypt depth respectively. Five villi from the segments were selected from each rat in the three groups for microscopic analysis, using × 40 magnifications. Images of jejunum and ileum were captured and displayed on a high resolution 18" colour monitor using Moticam 352 mounted on a Motic Digital Microscope DMB3-233 (JAPAN). The software motic image plus 2.0mL was used to measure the villus height and crypt depth.

Statistical Analysis

Results are presented as mean ± SEM. Data were analyzed using one way ANOVA then followed with a post hoc test (LSD). P values of less than 0.05 were adjudged to be significant statistically.

RESULTS

Fluid uptake in jejunum and ileum in control and *Xylopia Aethiopica*-fed rats.

The mean jejunal mucosal and serosal values for fluid transfer in the test groups was significantly (P<0.001) lower than in control. The high dose group had significantly (P<0.001) lower jejunal gut fluid uptake compared with control. On the other hand, ileal mucosal and serosal fluid transfer was significantly (p<0.001) increased compared with control. However, only the gut fluid uptake was significantly (P<0.01) higher in the low dose group than in control (Table 1)

Glucose uptake in jejunum and ileum in control and *Xylopia Aethiopica* fed rats.

The values of jejunal glucose concentration before incubation in the mucosal and serosal layers for control, low dose and high dose are shown in Table 1. There was no significant difference among the different groups before incubation. After incubation, jejunal mucosal glucose transfer in both doses was significantly (P<0.01) higher compared with control. The serosal glucose transfer was only increased in the high dose group (p<0.05). Jejunal gut glucose uptake in the low dose group was significantly (p<0.01) higher compared with control but there was no significant difference in the high dose group. Similarly, the values of ileal mucosal glucose transfer after incubation in the test groups were significantly (P<0.05) higher than in control. Gut glucose uptake was significantly (P<0.05) higher in the low dose group than in control (Table 2).

Table 1:
Fluid transfer in jejunum and ileum in *Xylopia Aethiopica*fed rat (mg/g/sac/30min).

Group	Body weight range	Jejunum			Ileum		
		Serosal fluid transfer	Mucosal fluid transfer	Gut fluid uptake	Serosal fluid transfer	Mucosal fluid transfer	Gut fluid uptake
Control	180-220	0.77±0.27	1.04±0.01	0.28±0.08	0.29±0.10	0.45±0.13	0.17±0.03
Low dose (LD)	180-220	0.20±0.6***	0.49±0.11**	0.28±0.08	0.39±0.17**	0.87±0.12***	0.48±0.12**
High dose (HD)	180-220	0.27±0.10***	0.46±0.11**	0.18±0.02**	0.38±0.09**	0.58±0.09**	0.20±0.05

** P<0.01, ** *P<0.001 vs. control. Values are mean ± SEM for 20 sacs in 5 rats, n = 10

Table 2
Glucose transfer in jejunum in Xylopia Aethiopica fed rats (Mmol/dl/sac/30min)

Group	Body weight (g)	Glucose Conc. Before incubation		Glucose Conc. After Incubation		Glucose uptake
		Mucosa	Serosa	Mucosa	Serosa	
Control	180-220	0.55 ± 0.22	0.57 ± 0.12	0.59 ±0.02	0.43 ±0.06	0.16 ±0.06
Low dose (LD)	180-220	0.52 ± 0.22	0.53 ± 0.12	0.69 ±0.01**	0.35 ±0.02	0.34±0.02**
High dose(HD)	180-220	0.50 ± 0.20	0.52 ± 0.20	0.68 ±0.01**	0.58 ±0.02*	0.10 ±0.09

** P<0.01, *P<0.05 vs. control. Values are mean ± SEM for 20 sacs in 5 rats, n = 10.

Table 3.
Glucose transfer in ileum of *Xylopia Aethiopica* fed rats, mol/dl/sac/30min)

Group	Body weight (g)	Glucose Conc. Before incubation		Glucose Conc. After Incubation		Glucose uptake
		Mucosa	Serosa	Mucosa	Serosa	
Control	180-220	0.20±0.07	0.25±0.05	0.35±0.06	0.22±0.02	0.13 ±0.0.8
Low dose (LD)	180-220	0.22±0.06	0.26±0.04	0.54±0.12*	0.28±0.01	0.26 ± 0.04*
High dose(HD)	180-220	0.23±0.04	0.27±0.01	0.47 ±0.06*	0.32±0.06	0.15 ±0.07

** P<0.01, *P<0.05 vs. control. Values are mean ± SEM for 20 sacs in 5 rats, n = 10.

Table 4:
Villus height, depth and crypt depth/villus height rations in *xylopia aetthiopica* fed rats

Groups	Villus Height		Crypt depths		Crypt depth/villus height	
	jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Control	1.65 ±0.42	1.41 ±0.19	1.00 ±0.32	1.00 ±0.16	0.71 ± 0.04	0.59 ±0.05
Low dose	1.50 ±0.28	1.07 ±0.18	0.86 ±0.11	0.53 ±0.04*	0.60 ±0.07	0.53 ±0.07
High dose	1.65 ±0.25	0.75 ±0.19*	1.07 ±0.13	0.63 ±0.13*	0.67 ±0.05	0.53 ±0.05

*P<0.05 vs control, values are in mean ± SEM for 20 sacs in 5 rats, n = 10

Villus height, depth and crypt depth/villus height rations in *xylopia aetthiopica* fed rats

In table 4, jejunal and ileal villus height and crypt depth in control, low dose and high dose groups in mm are shown. There was no significant difference in jejunal villus height among the groups but ileal villus height was significantly (P<0.05) lower in the high dose group than in control.The jejunal crypt depth was not significantly different in control and test groups but ileal crypt depth in test groups were significantly (P<0.05) lower than in control. There was no significant difference in the crypt depth and villus height ratios in the control and test groups.

DISCUSSION

The effect of *Xylopia Aethiopica* on intestinal fluid and glucose transfer was investigated using inverted

sac technique in jejunal and ileal segments in male albino Wistar rats. The jejunalserosal and mucosal fluid transfer (SFT and MFT) of the test groups were significantly reduced compared with control but the ilea serosal and mucosal fluid transfer were significantly higher compared with control. The gut fluid uptake (GFU) in jejunum was significantly reduced but in the ileum it was significantly raised. These effects might be caused by the extract or regional variation in fluid absorption in the gastrointestinal tract.

Reduced jejunal gut fluid uptake may be due to inhibition caused by some phytoconstituents in the plant (Jodoin *et al,* 2002). Regionally, the jejunum is known for its high fluid absorption, but the results have shown a reduction in absorption of fluids. Several factors may be responsible, particularly the activity of protein transporters. It has been reported that p-glycoproteins (Berginc *et al,* 2009) and multidrug resistant protein 2 (Cermark *et al,* 1998; Rodiguez-Proteur *et al*, 2006) which are ATP-dependent multi-drug transporter proteins expressed in the small intestine constitute potential biochemical barrier that limits the rate and extend of absorption in the small intestine (Deferme and Augustijns, 2003) and that these proteins efflux drugs back to the intestinal lumen (Abdukard *et al*, 1999). This may explain the reduced absorption of fluid in the high dose *Xylopia Aethiopica* - treated group. Furthermore, it is believed that the upper small intestine is the site of hydrolysis and absorption of flavonoids (Spencer *et al,* 2003) and that high flavonoids concentration inhibit metabolism and transport process (Kuo *et al,* 1998). *Xylopia Aethiopica*has been reported to contain flavonoids and polyphenols (Okwari, 2010). Therefore, the reduced gut fluid uptake in the jejunum when compared with ileum may be attributed to high concentration of flavonoids.

The jejunal and ileal mucosal glucose transfer (MGT) as well as the gut glucose uptake (GGU) were significantly raised in low dose *Xylopia Aethiopica* treated group compared with control. This suggests that the extract at low dose enhanced the absorption of glucose in the jejunum and ileum. Actively transported sugars are known to be moved from mucosal to serosal sides against a concentration gradient following highly specific transport mechanisms (Davenport, 1984). Intestinal glucose uptake is mainly performed by the sodium dependent glucose transporter (SGLUT-1) (Kobayashi *et al*, 2000; Walgren *et al*, 2000; Sanchez de Medina *et al*, 1997). Regional variation in absorption is said to be masked by different p-glycoproteins expression in the small intestine which have been confirmed by immunoblotting studies (Spencer *et al*, 2003). It has been reported that a rise in glucose level may be the result of an uptake into β-cells facilitated by an insulin-independent glucose transporter protein (Thoren, 2001). Therefore the uptake of glucose at both jejunum and ileum enhanced by the extract provide a substrate from which cells can synthesize ATP to maintain cellular activity.

The jejunal villus height (VH) was not significantly different but ileal VH was only significantly decreased in the high dose *Xylopia Aethiopica* treated group compared with the control. The crypt depth (CD) was significantly decreased in the ileum compared with the control. Decreased VH and CD in the ileum suggest reduced absorptive and secretive activity of the small intestine (Kelly *et al*, 1998). Damage affecting the VH and CD in the high dose *Xylopia Aethiopica* treated group may be caused by the extract as plant phytochemical compound can alter activity of important transport proteins and significantly modify the physiological properties of the small intestine (Berginc *et al*, 2006).

In conclusion, the aqueous fruit extract of *Xylopia Aethiopica* reduced jejunal fluid uptake but increased ileal mucosal and serosal fluid uptake in the rats. It may be possible that the extract has effect on expression of transport proteins although its mechanism is not known. The enhanced uptake of glucose at both jejunum and ileum provides substrate from which cells can synthesis ATP to maintain cellular activity.

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