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Effect of Toxic Extract from the Outercoat of the Fruit of *T. africana* on Intestinal D-Glucose Transport in Rat *in vivo*

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ABSTRACT

A saline extract solution from the outercoat of the fruit of T. africana *was prepared and tested for its total polyphenolic content and effect on intestinal* D-*glucose transport across rat ileum* in vivo. *Intestinal* D-*glucose transport was significantly reduced ($P < 0{\cdot}001$) by the presence of the outercoat-extract in the intestinal loop. This extract, which has appreciable polyphenolic content, caused both a reversible and a lasting inhibitory effect on glucose transport.*

Polyamide treatment of the extract decreased its ability to inhibit glucose transport but the inhibitory effect was not completely abolished by the treatment, further suggesting the presence of some other anti-absorptive factor(s) in the outercoat of the fruit of T. africana.

INTRODUCTION

A great deal of attention has been directed towards finding the effects of toxic principles of a wide variety of raw fruits fed to animals over the past several decades.

Significant impairment in the rate of growth (Jaffe & Vega, 1968; Cenarruzabeitia *et al.*, 1979; Moseley & Griffiths, 1979; Fowler, 1980), rate of intestinal absorption of sugars and amino acids (Griffiths & Moseley, 1980;

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Santidrian *et al.*, 1981*a,b*), rise in the non-protein nitrogenous fraction in skeletal muscle (Bello *et al.*, 1972) and several other physiologic functions have been reported when diets containing raw seeds as a source of protein were fed to growing animals. A number of heat-labile anti-nutritive factors, such as protease inhibitors (Warsy *et al.*, 1974), haemagglutinins (De Muelenaere, 1965), an anti-niacin factor (Guillaume, 1977) and polyphenolic compounds (Griffiths, 1979, 1981; Lawal *et al.*, 1987), have been identified.

The effects of polyphenols on the activity of digestive enzymes have been studied (Griffiths, 1981), but their effects on intestinal absorption mechanisms have not been extensively investigated. The primitive methods of handling and storage of the fruits of *Treculia africana* by farmers, in villages, may encourage contamination with the toxic extract of the outercoat of the fruit. The outercoat of *T. africana* contains polyphenols (Lawal *et al.*, 1987). Therefore, the work described in this paper was carried out to investigate the action of the extract of the outercoat of the fruit of *T. africana* on intestinal D-glucose transport, using an *in vivo* preparation of rat ileum.

Glucose was chosen because it is widely used in intestinal absorption studies, and represents the most important carbohydrate in nutrition.

MATERIALS AND METHODS

Sample collection

The mature fruits of *T. africana* were collected from different locations, (i) Ishara Remo and (ii) Ijebu-Ode, in Ogun State, Nigeria. The fruits were weighed.

Sample preparation

The outercoat of the fruit of *T. africana* was removed using a sharp knife.

Preparation of extract

One-hundred-and-fifty grams of the outercoat was pounded in a mortar into a homogeneous slurry. The slurry was macerated in 300 ml of ethanol:water (50:50) with continuous shaking. After 24 h, the extract was filtered, and the residue subjected to a second maceration of 30 min and filtered. The filtrates were combined, concentrated by rotary evaporation at 50°C, centrifuged at 15 000 rev min^{-1} for 20 min and the supernatant made up to 100 ml with distilled water.

The extracts were made free of polyphenols by treating them with 2% polyamide solution (a polyphenol complexing agent).

Absorption

Wistar-Strain rats (130–150 g) of either sex were starved overnight (with access to water only) anaesthetised with subcutaneous injection of 13% urethane (1 ml per 100 g) and placed on a heated operating table to maintain rectal temperature at 38°C. The rectal temperature was measured using a thermometer. A conventional intestinal loop technique described by Castellarnau *et al.* (1979), Mitjavila *et al.* (1970) and Sols & Ponz (1947) was employed. Briefly, laparatomy was performed and a 15–30 cm segment of distal ileum was cannulated at either end. The entry glass cannula was connected to a reservoir which was placed in such a way that a 10 cm water hydrostatic pressure was maintained throughout the experiment. The extremity of the exit cannula was introduced into a receptacle to collect the liquid not absorbed. This cannula was provided with a clamp for opening and closing.

The intestinal loop was washed by filling the reservoir connected to the entry cannula with physiological saline solution (0·9% NaCl) at 38°C. When the effluent fluid was translucent, the liquid in the reservoir was allowed to descend until a fixed level was reached. At this point the exit cannula was clamped, introduced into a volumetric flask and 10 ml of the solution (at 39°C) to be absorbed was placed in the reservoir connected to the entry cannula. The exit cannula was then opened and the liquid (10 ml) in the reservoir was allowed to descend to the fixed level. The exit cannula was then clamped and the time was noted. (All of the 10 ml test solution entered the loop.)

After 10 min, the reservoir connected to the entry cannula was filled with 30 ml physiological solution (at 38°C) and the clamp of the exit cannula was loosened, allowing the physiological solution to carry away what had not been absorbed into the volumetric flask. (The 30 ml of saline was adequate for washing out all unabsorbed solution.)

To carry out a second absorption experiment, the liquid in the reservoir was allowed to descend to the same fixed level and the exit cannula was clamped. Another 10 ml of test solution was placed in the reservoir and the procedure detailed above was repeated. Between five and seven successive absorption periods were carried out in the same animal.

The test solutions contain 0·9% NaCl, 2 mM D-glucose and 0·1 μCi ^{14}C-D-glucose with or without the extract from the outercoat of *T. africana*. All solutions were buffered to pH 7·4 and maintained at 38°C prior to use.

The undiluted extract solution was prepared by adding the extract

solution (obtained as described above), NaCl, D-glucose and ^{14}C-D-glucose to a final concentration of 0·9%, 2 mM and 0·1 μCi, respectively. This solution was used to make up the two-fold and four-fold dilutions by adding the desired volume to test solution without extract.

Radioactive samples were counted in a Packard Tricarb Liquid Scintillating Counter.

Sugar absorption was estimated as the difference between the total sugar present in the solution before (solution placed in the reservoir) and after (solution collected into the volumetric flask) passing through the intestinal loop, and was expressed in μmol cm^{-1} intestine. (As a check, the absorption was also calculated from recovery of radioisotope after washing the intestinal loop.)

Chemical analysis of the extract

Total polyphenolic content in each of the two extracts was evaluated by a modification of the Dadic (1976) method.

Statistics

Variation in results is expressed as the standard error (SE) of the mean; significance was tested using the student's *t*-test, while comparisons between absorption periods was by 'paired *t*-test'.

RESULTS AND DISCUSSION

Polyphenolic content

The polyphenolic content in the outercoat of the fruits obtained from the two locations did not differ significantly. The outercoat of the fruit obtained from Ishara-Remo had 3·4 ± 0·2 mg polyphenol per millilitre of extract while location (II), Ijebu-Ode, gave 3·2 ± 0·1 mg polyphenol per millilitre of extract. These values recorded are the mean values ± standard error.

Effect of extracts obtained from the outercoat of the fruit of *T. africana* on intestinal glucose transport

The results for glucose transport across rat ileum found in the presence and absence of the outercoat extracts are given in Table 1. Seven successive absorption periods were performed in the same animal and only one type of extract was assayed at any time.

TABLE 1

Effect of Extracts from the Outercoat of the Fruit of *T. africana* from Two Different Locations in Nigeria[a] on Intestinal Glucose Transport

	Absorption periods						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Extract content (%)	0	25	0	50	0	100	0
Intestinal glucose absorption (μmol D-glucose cm^{-1} per 10 min)[b]	$0{\cdot}22 \pm 0{\cdot}014$	$0{\cdot}15 \pm 0{\cdot}012$[c]	$0{\cdot}17 \pm 0{\cdot}013$[d]	$0{\cdot}09 \pm 0{\cdot}014$[c]	$0{\cdot}12 \pm 0{\cdot}013$[d]	$0{\cdot}06 \pm 0{\cdot}012$[c]	$0{\cdot}13 \pm 0{\cdot}014$[d]

[a] Location I, Ishara-Remo; Location II, Ijebu-Ode.

[b] Values are means $\pm$ standard errors.

[c] $P < 0{\cdot}001$, test (absorption in the presence of extract solution) compared with control (absence of extract solutions, Column 1).

[d] $P < 0{\cdot}001$, the data were compared with those obtained in the previous absorption. Number of independent estimates, $n = 7$.

For each extract non-diluted (100%), two-fold diluted (0·9% NaCl:extract saline solution, 50:50) and four-fold diluted (0·9% NaCl:extract saline solution, 75:25), saline extract solutions containing 2 mM glucose were prepared and assayed in the same intestinal loop. Glucose transport was measured before (first absorption period) during (2nd, 4th and 6th absorption periods) and after (3rd, 5th, and 7th absorption periods) exposure of the intestinal mucosa to physiological solutions containing the extracts.

In all the experimental conditions the presence of outercoat extracts in the intestinal loop (2nd, 4th, and 6th absorption periods) significantly ($P < 0·001$) diminished glucose transport. In each case, as the extract content in the luminal solution increased, a greater percentage decrease in glucose transport was observed.

No significant difference in the degree of inhibition was found when the intestinal mucosa was exposed to 'eight-fold' diluted extract solutions. After exposure of the intestinal epithelia to the extract solutions (3rd, 5th and 7th absorption periods), glucose absorption did not revert to control values although, for all three concentrations of extract, the observed glucose transport was significantly higher ($P < 0·001$) than that found in the previous period when extract was present in the luminal solution.

Thus part of the inhibitory effect caused by the extracts mentioned above could be eliminated by rinsing the intestinal loop with physiological solution.

The inhibition caused by the extracts from the outercoat of the fruit of *T. africana* was only partly reversible. Hence the lasting inhibition induced by these outercoat extracts could be ascribed to their polyphenolic content.

Effect of polyamide-treated extracts on glucose transport

In order to find out whether the polyphenolic compounds were responsible for the lasting inhibitory effect, extracts free of polyphenols were obtained by penetrating them with polyamide (a polyphenol complexing agent).

Five successive absorption periods were carried out in the same intestinal loop. One type of extract, with or without polyamide treatment, was assayed in each experiment. The extract solutions were not diluted. In the 1st, 3rd and 5th absorption periods, the intestinal loop was filled with physiological saline–glucose (2 mM) solution. In the 2nd absorption period, the loop was filled with physiological saline extract solution, previously treated with polyamide (and containing 2 mM glucose) and in the 4th absorption period, physiological saline extract solution containing 2 mM glucose was used. This experimental procedure allowed us to see how polyamide treatment of the extract solutions might modify their inhibitory power.

TABLE 2

Effect of Polyamide-Treated Extract of the Outercoat of the Fruit of *T. africana* on Intestinal Glucose Transport[a]

	Absorption periods				
	1	*2*	*3*	*4*	*5*
Extract content (%)	0	100 (+P)[b]	0	100	0
Intestinal glucose absorption (μmol glucose cm^{-1} per 10 min)[c]	0·23 ± 0·01	0·12 ± 0·01[d]	0·21 ± 0·02	0·03 ± 0·01[e]	0·10 ± 0·01

[a] From Location I (Ishara-Remo) or Location II (Ijebu-Ode).

[b] +P; extracts treated with polyamide.

[c] Values are means ± SE.

[d] $P < 0·001$, test compared with control (Column 1).

[e] $P < 0·001$, data from Column 4 compared with those in Column 2. Number of independent determinations = 5.

The results show (Table 2), that both polyamide-treated extracts and non-treated extracts significantly ($P < 0{\cdot}001$) inhibited intestinal glucose transport. The inhibition of glucose transport caused by each extract solution after pretreatment with polyamide was *smaller* than that observed in the presence of the same extract solutions without polyamide treatment.

Furthermore, the polyamide-treated extracts had very little effect on glucose transport measured after the extracts had been washed out from the intestinal loop (as can be seen by comparing the results corresponding to the 1st and 3rd absorption periods). Thus, treating the extract solutions to remove free polyphenolic compounds removed the persistent inhibitory effect. As in the first experiment (Table 1), the inhibition induced by extract solutions which had not been treated with polyamide was not completely removed by rinsing the intestinal loop.

Half the inhibition was due to polyphenols and appeared to be persistent, whilst the other half was due to other factors as yet unidentified.

Studies where the outercoat extract had been previously treated with polyamide confirmed this view. Thus, the inhibition caused by polyamide-treated extracts was reversible.

The polyphenolic compounds cannot act by complexing the glucose in the lumen of the intestine, since their inhibitory effect remained even after the intestinal loop was rinsed with physiological saline solution. Mitjavila *et al.* (1977) suggested that the inhibitory effect of tannic acid (a compound closely related to some polyphenols) on glucose transport could be due to a gross denaturing of the glycocalyx and of the brush border of the intestinal cells. On the other hand, Motilva *et al.* (1983) suggested that the polyphenols react at the brush-border (where the carriers for sugar transport are located), subtly modifying the membrane proteins and so impairing glucose transport without causing gross morphological changes visible under the microscope.

The other anti-absorptive factor(s) caused a very labile inhibition since its effect could be eliminated by rinsing the intestinal loop. However, further studies are being carried out to characterise its nature.

CONCLUSION

The results presented in this work suggest that the polyphenols, as well as other unknown factor(s) in the outercoat of the fruit of *T. africana*, may alter the absorptive capacity of the intestinal wall, and may reduce the amount of available glucose it contains.

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