Stimulatory effects of *Antidesma madagascariense* on D-glucose, L-tyrosine, fluid and electrolyte transport across rat everted intestine, comparable to insulin action *in vitro*

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ABSTRACT

Medicinal plants are believed to be an important source of potential therapeutic agents. This study investigates the effects of *Antidesma madagascariense* (AM) extract on the transport of D-glucose, L-tyrosine, fluid and electrolytes (Na^+ and K^+) across rat everted intestinal sacs. These sacs were mounted in an organ bath containing Krebs-Henseleit bicarbonate (KHB) buffer. Experimental findings showed that incubation with graded aqueous AM extracts above 0.375 mg/mL significantly ($P<0.05$) stimulated the mucosal disappearance and serosal appearance of glucose and fluid. The concentration of glucose accumulated in the intestinal tissues also increased significantly ($P<0.05$) compared to that found in the controls. Transport of the amino acid L-tyrosine was not significantly enhanced ($P>0.05$) when incubated with increasing concentrations of AM extract. Effects on electrolyte (K^+ and Na^+) transport were assessed. Na^+ uptake and transport was significantly enhanced ($P<0.05$) when incubated with 0.75 mg/mL AM extract; however, K^+ transport was not significantly enhanced ($P>0.05$). For comparison, insulin (1 and 2 units/mL) was incubated in the mucosal solution. Aqueous AM extract produced similar stimulatory effects on the transport of glucose, fluid and Na^+ as were found with insulin. It is hypothesised that bioactive phytochemicals such as flavonoids, alkaloids, leucoanthocyanins, phenols and saponins from AM leaf extract might interfere with the Na^+/glucose carrier, thereby enhancing the transport of glucose, Na^+ and fluid across rat everted intestinal sacs. Thus, AM may represent a possible alternative dietary supplement for the treatment of type 2 diabetes.

KEY WORDS: Antidesma madagascariense. Diabetes. Intestinal absorption. Intestines. Medicine, herbal.

Introduction

The World Health Organization (WHO) estimates that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary healthcare needs.[1,2] For instance, the use of herbs and medicinal plant products has become a mainstream phenomenon over the past two decades in many countries where herbs and phytomedicines (herbal remedies) have become one of the fastest growing segments in retail pharmacies and supermarkets.[3,4]

Available reports show that about 25% of all prescriptions sold in the USA are from natural products, while another 25% are from structural modifications of a natural product. In other reports,[5,6] it is proposed that three in 10 Americans use botanical remedies in any given year.

Modern pharmacopoeia comprise at least 25% of drugs derived from plants and of synthetic analogues built on prototype compounds isolated from plants.[7] Despite the availability of different approaches for the discovery of therapeutic agents, natural plant products remain one of the best reservoirs of new structural types.

At the same time, many people in developing countries (e.g., Taiwan, India, Pakistan, Mauritius) have begun to turn to alternative therapies as cheap sources of complex bioactive compounds, and evidence of the beneficial therapeutic effects of these medicinal herbs is seen in their continued use.[3,8,9]

One condition in which medicinal plants have been used extensively is diabetes mellitus.[10,11] This is a metabolic disorder of carbohydrate metabolism characterised by fasting elevation of blood glucose level.[12] While the cause of this elevation may be associated with either too little or too much insulin, the complications of chronically high serum glucose is devastating to the individual. If untreated, diabetes leads to severe complications such as atherosclerosis, retinopathy, neuropathy and ulceration and gangrene of the extremities

Prior to the introduction of therapeutic insulin, diet was the main form of treatment of this metabolic disease, and dietary measures included the use of traditional medicines mainly derived from plants. Indeed, the traditional use of Goat's rue (*Galega offinalis*) provided a basis for the development of metformin.[13,14] More than 1200 species of

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organisms have been used ethnopharmacologically or experimentally to treat symptoms of diabetes, and several reviews of plants with known antidiabetic activity or traditional use as antidiabetic remedies have been published.[15,16]

Thus, it would appear that traditional antidiabetic plants might provide a useful source of new oral hypoglycaemic compounds for the development as pharmaceutical agents or as simple dietary adjuncts to existing therapies. Although an orally active botanical substitute for insulin seems unlikely, new phytochemical molecules to stimulate endogenous insulin biosynthesis and secretion (and to promote insulin action) are realistic possibilities.[17]

Botanical dietary supplements often contain complex mixtures of phytochemicals that have additive or synergistic interactions. To this effect, several investigators suggest that the study of such traditional medicines might offer a natural key to unlock the diabetologist's pharmacy.

Nonetheless, the therapeutic approach offered by several traditional medicinal plant systems is more holistic and most of the reported effects are anecdotal, as few have received adequate scientific evaluation.[5,15]

The fundamental mechanisms behind the effects of these medicinal systems are unexplained. It is claimed that most medicinal preparations in traditional medicines contain a variety of synergistically acting phytochemicals that are thought to act on a variety of targets by various mechanisms.[18] Moreover, most have not yet received thorough medical and scientific evaluation, and the WHO Expert Committee on Diabetes Mellitus has emphasised that a methodical investigation of these purported remedies is required.[2]

In accordance these WHO recommendations, investigation of hypoglycaemic agents from plants, which have been used in traditional medicines, is of paramount importance. In the present study, a widely used medicinal plant *Antidesma madagascariense* (Euphorbiaceae) from Mauritius, which has documented antidiabetic properties, is investigated for possible effects on glucose, amino acid, fluid and electrolyte (Na^+ and K^+) transport across everted intestinal sacs *in vitro*.

Materials and methods

Preparation of plant materials

Antidesma madagascariense (Euphorbiaceae) leaves were collected and deposited at the Herbarium collection of the Department of Chemistry, Faculty of Science, University of Mauritius. The leaves were oven-dried for several hours or air-dried in a drying cabinet for four to five days until a constant mass was obtained. The dried plant material was homogenised to a fine powder in an electrical food grinder and stored in well-sealed plastic containers.

Extraction

Crude aqueous extracts were used for *in vitro* transport studies. Powdered materials (10 g) were extracted to exhaustion with 50 mL water in a Soxhlet apparatus for 5 h. The solvent was then distilled under reduced pressure and temperature (40^0C) to provide a crude plant extract. These were concentrated under vacuum using a rotary evaporator (Buchi rotavapor R-114, Switzerland) that ensures evaporation of bulky solutions to small-volume concentrates without bumping at temperatures of 70–100^0C. The resultant concentrate was measured and the gummy material collected in water for examination. Percentage yield was calculated and the paste-like suspension was diluted in water for further experiments.

Animals

Male Swiss albino rats weighing 100–250 g (10–15 weeks) were maintained on commercial feed and tap water ad libitum throughout the study. They were housed in standard

Table 1. Effects of graded concentrations of AM leaf extract (0.375–6.0 mg/mL) on transport of D-glucose, L-tyrosine and fluid across rat everted intestinal sacs.

Concentration of AM leaf extract added to medium (mg/mL)	D-glucose transport (μmol/g tissue [wet])			L-tyrosine transport (μmol /g tissue [wet])			Fluid transport (mL/g tissue [wet])		
	Mucosal disappearance	Gut wall content	Serosal appearance	Mucosal disappearance	Gut wall content	Serosal appearance	Mucosal disappearance	Gut wall content	Serosal appearance
0	77.5±2.87	10.7±1.14	66.8±3.10	13.9±1.44	1.2±0.13	12.7±1.16	1.05±0.05	0.19±0.02	0.85±0.05
0.375	78.3±4.80 (+1.3)	12.8±1.47 (+19.6)	65.5±2.70 (–1.9)	12.7±1.26 (–8.6)	1.3±0.13 (+8.3)	11.4±1.52 (–12.6)	1.18±0.06* (+18.0)	0.21±0.03 (+10.5)	0.90±0.06 (–5.9)
0.75	94.1±3.82* (+21.4)	17.8±1.83* (+66.4)	76.3±2.85 (+14.2)	13.3±1.15 (–5.7)	1.0±0.08* (–16.7)	12.3±1.33 (–3.2)	1.36±0.06* (+29.5)	0.24±0.05* (+26.3)	1.09±0.05* (–28.4)
1.50	97.5±4.00* (+25.8)	19.6±0.97* (+83.2)	77.9±3.90 (+16.6)	14.7±1.12 (+5.8)	1.1±0.06 (–8.3)	13.6±1.33 (+7.1)	1.39±0.04* (+32.4)	0.22±0.04 (+15.8)	1.10±0.04* (+29.4)
3.0	98.3± 4.44* (+26.8)	18.6±2.36* (+73.8)	79.7±4.37* (+19.3)	14.0±1.04 (+0.7)	1.2±0.20 (–)	12.8±1.29 (+0.8)	1.38±0.07* (+31.4)	0.23±0.02* (+21.9)	1.12±0.04* (+31.8)
6.0	98.7±3.72* (+27.4)	20.4±1.34* (+90.7)	78.3±3.78* (+17.2)	13.4±1.43 (–3.6)	1.1±0.07 (–8.3)	12.3±1.24 (–3.2)	1.44±0.06* (+37.1)	0.26±0.03* (+36.8)	1.16±0.07* (+36.5)

Results expressed as mean ± SEM of seven observations in each group.
*$P<0.05$ from the control without AM leaf extract added to the mucosal solution.
% inhibition (–) or stimulation (+) of transport compared to the control experiment in parentheses.

environmental conditions with 12-h light and 12-h dark exposure. Prior to the experiments the animals had free access to water and food. Investigations using experimental animals were conducted in accordance with internationally accepted principles for laboratory animal use and care.

Everted intestinal sac preparation

Everted rat intestine has been used widely as a suitable *in vitro* model for the study of intestinal transfer of nutrients and drugs.[19–24] The rats were killed after overnight fasting. The abdomen was opened by a midline incision. The whole of the small intestine was removed by cutting across the upper end of the duodenum and the lower end of the ileum and manually stripping the mesentery. The small intestine was washed out carefully with cold normal oxygenated saline solution (0.9% [w/v] NaCl) using a syringe equipped with a blunt end. The mid-portion of the small intestine from each animal was used in order to minimise transport variability.[21]

Intestinal segments (10 ± 2 cm) were everted according to the conventional technique described by Wilson and Wiseman,[19] with modifications.[23–25] The everted intestine was placed in glucose-saline (0.9% [w/v] NaCl) at room temperature in a flat dish. A thread ligature was tied around one end to facilitate subsequent identification and to check for perforation. After weighing, the empty sac was filled with 1 mL Krebs-Henseleit bicarbonate (KHB) buffer ($NaHCO_3$ 25 mmol/L; NaCl 118 mmol/L; KCl 4.7 mmol/L; $MgSO_4$ 1.2 mmol/L; NaH_2PO_4 1.2 mmol/L; $CaCl_2$ 1.2 mmol/L; Na_2EDTA 9.7 mg/L. Unless otherwise stated, glucose (10 mmol) was added to the medium just before the start of the appropriate experiment. The pH was maintained at 7.4.

A 1-mL blunt-ended syringe was used to measure accurately the amount of fluid introduced, and the filled intestinal sac was then slipped carefully off the needle and the loose ligature on the proximal end was tightened. After weighing, the distended sac was placed inside an organ bath containing 50 mL of the same incubation medium (mucosal solution).

The organ bath was surrounded by a water jacket maintained at 37–40^{0}C and placed in metabolic shaker at 100–110 shake/min. A gas mixture of 95% O_2 and 5% CO_2 was bubbled through the external incubation medium during the incubation period.[9]

Intestinal transport studies

The everted intestinal sacs were incubated for 30 min, as described previously.[20] At the end of the incubation period, the sac was removed from the organ bath, blotted using a standardised procedure using Whatman paper and then weighed again. The serosal fluid was drained through a small incision into a test tube. Gentle pressure was applied in order to empty the sac completely, after which the serosal and mucosal fluids were measured. The empty sac was weighted again. The terms used in expressing the transfer capacity are those reported by Obatomi *et al*.[26]

Biochemistry

Serosal or mucosal testing solutions (0.5 mL) were transferred to separate centrifuge tubes and 0.5 mL trichloroacetic acid (2% w/v) was added. The solutions were centrifuged at 3000 rpm for 10–20 min to sediment cell debris. Supernatant (0.5 mL) was transferred to clean tubes and the final volume made up to 1 mL with distilled water.[24] D-glucose, L-tyrosine, electrolytes and inorganic phosphate transport was evaluated by measuring the increase in concentration of the compounds inside and outside the intestinal sacs after 30-min incubation using standard protocols.

D-glucose was measured using a commercially available glucose oxidase kit (Boehringer Mannheim, Mannheim, Germany). L-tyrosine in the incubating buffer solution was determined as described by Lowry *et al*.,[27] with slight modifications.[24] Sodium and potassium in the serosal and mucosal solutions were determined by atomic absorption against standard concentrations of the electrolytes. The quantity of Na^+ and K^+ absorbed (mucosal disappearance) and transported (serosal appearance) were calculated from the respective changes in the volume of the fluids, and expressed as the amount/g of wet tissue, as described previously.[9]

Measurement of fluid transport

The method of Obatomi *et al*.[26] was used. Initial serosal volume was determined as the difference between the weights of the empty and full everted sacs prior to incubation. The final serosal volume was calculated by subtracting (after incubation) the weight of the empty sac from that of the filled sac. Mucosal fluid transfer was expressed in terms of the reduction in volume of fluid on the mucosal side during the course of the experiments. Serosal fluid transfer was reflected in the increase in the volume inside the sac. Gut fluid uptake was determined by measuring the increase in the volume of fluid in the gut wall. Uptake and release of fluid were expressed as mL/g wet tissue.[28]

Control experiments

For comparison, insulin (1 and 2 units/mL) was incubated in the mucosal solution, as described by Fromm *et al*.[29] Transport of the amino acid L-tyrosine was investigated as it has been reported[30] not to interact with glucose transporters, and also to investigate any possible selectivity of the stimulatory effects of AM extract. In each series of experiments, a parallel control strip was included from the same rat under the same incubation conditions and in same medium but without the plant extract, unless otherwise stated. For experiments performed where everted sacs were incubated with different chemicals, a corresponding volume of water was added to minimise any variation.[9,28]

Statistical analysis

All data were expressed as the mean ± SEM for seven intestinal segments in each group. Difference in mean ± SEM between the control and experimental groups were assessed using the one-way analysis of variance (ANOVA) test. $P<0.05$ was considered statistically significant. Manipulation and statistical analyses were performed using Excel software (Microsoft 2000) and SPSS (version 10.0) for Windows 2000.

Results

Table 1 shows the effects of graded concentrations (0.375-6.0 mg/mL) of AM leaf extract on the transport of D-glucose, L-tyrosine and fluid. Data obtained show that aqueous extract of AM significantly enhanced ($P<0.05$) the uptake of D-glucose and fluid transport. Only AM concentrations above

Table 2. Effects of insulin on transport of D-glucose, L-tyrosine and fluid across rat everted intestinal sacs.

Concentration of insulin added to the to medium (units/mL)	D-glucose transport (μmol/g tissue [wet])			L-tyrosine transport (μmol /g tissue [wet])			Fluid transport (mL/g tissue [wet])		
	Mucosal disappearance	Gut wall content	Serosal appearance	Mucosal disappearance	Gut wall content	Serosal appearance	Mucosal disappearance	Gut wall content	Serosal appearance
0	78.9±3.42	11.2±1.58	67.7±2.39	15.2±1.77	1.7±0.17	13.5±1.72	1.14±0.08	0.23±0.05	0.90±0.05
1	99.7±4.63*	28.8±2.11*	70.9±3.19	16.3±1.23	2.0±1.03	14.3±1.13	1.42±0.10*	0.33±0.06*	1.01±0.02
2	103.7± 4.79*	30.3±2.53*	73.4±3.47	17.1±1.38	1.4±0.77	15.7±1.28	1.55±0.13*	0.37±0.12*	1.11±0.08*

Results expressed as mean ± SEM of seven observations in each group.
*$P<0.05$ from the control experiments (without insulin).

0.375 mg/mL produced significant ($P<0.05$) stimulatory effects on the mucosal uptake of glucose. However, the serosal appearance of D-glucose was enhanced only at high concentrations (3.0–6.0 mg/mL) of AM, unlike mucosal disappearance and gut wall content.

At 0.375 mg/mL leaf extract, only mucosal disappearance of fluid was statistically enhanced ($P<0.05$), unlike the gut wall and serosal appearance. Above 0.375 mg/mL AM extract, mucosal disappearance, gut wall content and serosal appearance of fluid were significantly enhanced ($P<0.05$), except for the wall content ($P>0.05$) at AM concentration of 1.5 mg/mL.

L-tyrosine transport was not significantly enhanced ($P>0.05$) with increasing graded concentrations of AM, except at 0.75 mg/mL for the gut wall concentration of L-tyrosine. A slight but statistically insignificant inhibitory effect was observed on the mucosal disappearance and serosal appearance of L-tyrosine when incubated with 0.375, 0.75 and 0.6 mg/mL leaf extract.

The percentage stimulation of D-glucose, L-tyrosine and fluid transport were calculated from values obtained from the experimental group (with plant extract) compared to the controls. Increasing concentrations of AM extract (0.375 to 6.0 mg/mL) increased the percentage stimulation of glucose and fluid transport.

To investigate the possible mechanisms involved, insulin was used as a control (Table 2). Incubation with 1 and 2 units/mL insulin increased the uptake of glucose and fluid significantly ($P<0.05$). These stimulatory effects were comparable to the action of the aqueous AM leaf extract on the absorption and transport of these nutrients. However, transport of the amino acid L-tyrosine was not significantly affected ($P>0.05$). Also, the amount of glucose accumulating in the intestinal tissues (gut wall content) was significantly higher ($P<0.05$) when insulin was added to the mucosal solution.

Transport of Na^+ and K^+, with or without AM extract and in the presence of 1 unit/mL insulin was also compared. The results are summarised in Table 3. Comparison of 1 unit/mL insulin and 0.75 mg/mL AM extract were the minimal stimulatory concentrations chosen. Leaf extract (0.75 mg/mL) and insulin (1 unit/mL) concentrations chosen appeared to have a similar stimulatory effect on the transport of D-glucose, fluid and Na^+. However, transport of L-tyrosine and K^+ was not significantly enhanced ($P>0.05$) when insulin was incubated with the everted intestinal sacs.

Discussion

The pathophysiology of type 2 diabetes involves impairment in insulin action on target tissue, a deficiency in insulin secretion, or both. Insulin sensitivity is determined by the ability of insulin to promote glucose uptake and utilisation. Thus, in insulin-resistant conditions, there is decreased glucose clearance in response to insulin. Decreased insulin-mediated glucose clearance seen in type 2 diabetes has been demonstrated in humans at risk of developing diabetes, including those with hypertension, hyperlipidaemia, a strong history of disease, or who are obese.[31–33]

In the present study, an aqueous extract of AM, a medicinal plant common to Mauritius, was found to possess significant stimulatory effects on the uptake, tissue accumulation and transport of D-glucose, fluid and Na+ across rat everted intestinal sacs. However, only concentrations above 0.375 mg/mL significantly stimulated absorption of glucose and fluid. In contrast, the transport amino acid L-tyrosine was not affected by the AM extract, indicating that the inhibitory compound acts on the glucose-active sites of the enterocytes.

The concentration of glucose retained in the intestinal tissues increased significantly with increasing concentrations of the plant extract. This could be attributed to a possible effect on a glucose-trapping system in the enterocytes by glucose phosphorylation (hexokinase). It might also be speculated that bioactive compounds in the AM extract increased the rate of metabolism or increased the rate of conversion of glucose into other respiratory metabolites.[34]

It is generally accepted that glucose is actively transported across the brush border into enterocytes by the high affinity Na^+/glucose co-transporter SGLT 1. The energy required for this is obtained by coupling glucose transport to the Na^+ and electrical gradients across the membrane. The second step is the downhill transport of glucose from the enterocytes across the basolateral membrane into the bloodstream.[35,36]

As the present study found that AM extract enhanced the transport of glucose and Na^+ across rat everted intestinal sacs, it is possible that that bioactive phytochemicals in AM leaf extract interact with the Na^+/glucose co-transporter in enterocytes, thereby enhancing the uptake of glucose and Na^+ across the intestine. In addition to its involvement in glucose transport, SGLT 1 behaves as a molecular water

pump.[37–39] It is widely accepted that the net transport of fluid from the mucosal to serosal side of the intestine depends on the active transport of Na^+ (i.e., if electrolytes transport is enhanced through membranes then water transport is also enhanced). This effect, called solvent drag, is increased if glucose is absorbed more rapidly and could be responsible for the increased Na^+ absorption.

It has been demonstrated that SGLT1 transports 210 water molecules with each glucose and two Na^+ molecules.[39] Interestingly, transport of fluid, glucose and Na^+ in the present study was also enhanced when incubated with increasing concentrations of AM leaf extract. Therefore, the increased absorption and transport capacity across the intestine induced by the AM extract was probably related to an increase in SGLT 1 activity.

Findings from the present study also showed that the stimulatory effect of the leaf extract on glucose, fluid and Na+ were comparable to the action of insulin *in vitro*. Insulin regulates glucose homeostasis primarily through suppression of hepatic glucose production and the stimulation of peripheral glucose uptake. It is also reported to increase glucose absorption in everted intestinal sacs. In studies of the isolated rabbit intestine in a Ussing chamber, insulin increased the net absorption of 3-*0*-methylglucose.[33] In addition, insulin is reported to regulate Na^+/glucose co-transporter activity in rat small intestine,[40] and that it significantly stimulates the active uptake of glucose across rat small intestine.[41]

Ducluzeau *et al*.[32] suggest that the stimulation of glucose metabolism in muscle and adipose tissue, which is ultimately responsible for post-absorptive blood glucose clearance, is the primary relevant clinical action of insulin. In the present study, it is important to note that AM extract stimulated entry and transport of glucose and Na^+ across the rat everted intestine. Thus, if bioactive phytochemicals cross the small intestine and reach muscle, adipose or kidney tissue then AM intake can exert similar stimulatory effects, comparable to the action of insulin, as observed in the everted rat intestine in the present study.

Antidesma madagascariense possesses an array of phytochemicals (e.g., flavonoids, alkaloids, leucoanthocyanins, phenols and saponins).[42] These biologically active phytochemicals have been shown to have therapeutic value.[14,15] Thus, it can be hypothesised that the observed effects on the transport of glucose, fluid and electrolytes demonstrated in the present study are due to the presence of these phytochemicals in the leaf extract.

In conclusion, results from the present study support use of the aqueous AM extract as a traditional dietary antidiabetic remedy. If these *in vitro* animal studies can be extended to the *in vivo* situation, AM administration may prove useful as an adjunct to conventional therapy, in order to reduce the dosage of insulin in the management of type 2 diabetes.

The properties and structures of the potentially useful phytochemicals responsible for the effects observed here merit further investigation as the basis for a potential oral hypoglycaemic agent. □

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Table 3. Effects of AM leaf extract (0.750 mg/mL) and insulin (1 unit/mL) on transport of sodium and potassium across rat everted intestinal sacs.

Transport of sodium and potassium (μmol/g tissue [wet])	Control (without AM and insulin)		0.75 mg/mL AM in mucosal solution (mg/mL)		1 unit/mL insulin in mucosal solution	
	Mucosal disappearance	Serosal appearance	Mucosal disappearance	Serosal appearance	Mucosal disappearance	Serosal appearance
Na^+	130.5±9.79	95.1±5.44	157.9 ±13.56*	114.8±10.26*	160.3±15.39*	128.7±11.29*
K^+	63.9±2.59	50.2±2.36	68.3±4.25	52.5±3.51	71.3±4.69	54.6±2.58

Results expressed as mean ± SEM of seven observations in each group.
*P<0.05 from the control experiments (without AM extract or insulin) and with 1 unit/mL insulin.

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