Effects of *Erythroxylum macrocarpum* (Erythroxylaceae), an endemic medicinal plant of Mauritius, on the transport of monosaccharide, amino acid and fluid across rat everted intestinal sacs *in vitro*

Mohamad Fawzi Mahomoodally¹, Ameenah-Gurib Fakim², *Anwar Hussein Subratty*¹

¹Department of Health Sciences, ²Department of Chemistry, Faculty of Science, University of Mauritius, Reduit, Mauritius ( *author for correspondence*)

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**Abstract**

The use of herbal medicine is widespread and growing. *Erythroxylum macrocarpum* (Erythroxylaceae) (EM) is an endemic medicinal plant of Mauritius. Here, the effects of crude aqueous extract of EM leaves on D-glucose, L-tyrosine and fluid absorption, gut wall content and transport across rat everted intestinal sacs were investigated *in vitro*. Rat everted intestinal sacs were incubated with increasing graded concentrations of EM (1.5-12 mg/ml) in the mucosal solution. D-glucose absorption and transport was not significantly affected (p>0.05), whilst L-tyrosine transport was inhibited at 6 and 12 mg/ml of EM. The fluid absorptive capacity of the small intestine was decreased in the presence of 6 and 12 mg/ml of EM in the incubating buffer. It is hypothesised that active phytochemicals such tannins, phenols and alkaloids in the EM extract decreased the permeability of the enterocytes membrane and the energy independent transport of fluid. The present data also tend to validate the diuretic effect of EM.

**Keywords:** *Erythroxylum macrocarpum*, traditional medicine, diuretic, phytochemicals

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Mauritius’un endemik tibbi bitkisi *Erythroxylum macrocarpum*’ın (Erythroxylaceae); *in vitro*’da ters çevrilmiş şişan bağırskak keselerinde monosakkarit, amino asit ve sıvı taşınmasını etkileri

**Özet**

Bitkisel ilaç kullanımı çok yaygın ve giderek artmaktadır. *Erythroxylum macrocarpum* (Erythroxylaceae) (EM) Mauritius’da endemik bir tibbi bitkidir. Burada *in vitro*’da EM yapraklarının ham sıvı ekstrelerinin D-glukoz, L-tirozin ve sıvı absorpsiyonuna bağırskak çeper içeriğine ve şişan bağırskakların ters çevrilmiş keselerinde taşınmasına etkisi araştırıldı. Şiğan bağırskakların ters çevrilmesi ile elde edilen keseler mukosa çözeltisinde EM nin giderek artan konsantrasyonlarında (1.5 – 12 mg/ml) inhibe edilirler. D-glukoz absorpsiyonu ve taşınması kayda değer bir şekilde etkilenecekten (p>0.05), L-tirozin taşınması 6 ve 12 mg/ml EM mevcudiyetinde azaldı. Taniner, fenoller ve alkaloidler gibi aktif fitokimyasalların EM ekstrelerinde enterosit membran permeabilitesini ve enerjiye bağlımsız sıvı taşınmasını azalttığı hipotezi ortaya konmuştur. Bu araştırımda elde edilen veriler aynı zamanda EM nin diüretik etkisini de ortaya koymıştır.

**Anahtar sözcüklar:** *Erythroxylum macrocarpum*, geleneksel ilaç, diüretik, fitokimyasallar
Introduction

Herbal medicine is the oldest and most widely used form of medicine in the world today. Scientific interest in the use of herbal medicines has increased in recent years as plants offer exemplary source for drugs discovery (Mathews et al., 1999; Austin, 1998; Wayne, 1998). The World Health Organisation (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs (Alison and Flatt, 1991; Blumental et al., 1998). For instance, the use of herbs and medicinal plant products has become a mainstream phenomenon over the past two decades in the United States where herbs and phytomedicines have become one of the fastest growing segments in retail pharmacies and supermarkets (Austin, 1998; Mathews et al., 1999). In Asian regions such as India and China, western medicines have rediscovered many of these traditional medicines as cheap sources of complex bioactive compounds (Philipson, 1994). Modern pharmaceuticals are typically oral dosage forms containing single synthetic chemicals, which have potent clinical activity. On the other hand, medicinal plants, unlike pharmacological drugs have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of their individual constituents. It is a known scientific fact that a significant number of many potent drugs used today trace their origins to plants (Hostettmann, 1999). For instance, the famous cardiac stimulant digitalis and the antidiabetic drug metformin from the plant *Glega officinalis*.

The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years (Mathews et al., 1999). In Mauritius, the local population has a long-standing tradition in the use of herbal medicine. Many indigenous and endemic plant species of Mauritius have been used in folkloric medicine to treat various ailments of man (Sussman, 1980; Wong Ting Fook, 1980; Gurib-Fakim et al., 1993, 1996a, b, c). Available reports tend to show that indigenous folk-medicinal plant preservation and study is vital because such plants are fully adapted to local environments and to conditions compared to introduced species (Qin and Xu, 1998; Hong et al., 2004). Pharmacologically active compounds and phytochemicals isolated from such endemic and indigenous plants used in folk medicine have been the center of interest during the last few decades (Farnsworth, 1994; Benzi and Ceci, 1997). In addition, it is also of importance to preserve and maintain knowledge about medicinal uses of plant resources of Mauritius, which is characterized by a rich flora of exotic, endemic and indigenous plant species. Currently, several kinds of extracts from various exotic, endemic and indigenous plants are sold as decoctions or "tisanes" in several markets across Mauritius to treat minor ailments (Gurib-Fakim et al., 1993,1996a, b, c). This native herbal folk medicinal practice forms an essential part of the heritage of the local pharmacopoeia of Mauritius. Nonetheless, only a few medicinal plants have been validated for their medicinal virtues and most of the reported medicinal plants are anecdotal and few have received adequate scientific evaluation. The fundamental mechanisms of these medicinal systems are still unexplainable using modern tools.

In the present study, an endemic medicinal plant, *Erythroxylum macrocarpum* (Erythroxylaceae) EM that forms part of the local pharmacopoeia of Mauritius was studied. The crude aqueous extract of EM was used to investigate its effect on the transport of D-glucose, L-tyrosine and fluid across rat everted intestinal sacs in *vitro*.

Materials and methods

Collection of EM

Leaves of EM used in the study were collected from Conservation Management Areas (Maccabe forest) situated in the upper humid region of Petrin. The Curator of the National Herbarium, at the Mauritius Sugar Industry Research Institute (MSIRI) confirmed the identity of the plants. Voucher specimens were deposited at the Herbarium collection of the Department of Chemistry, Faculty of Science, University of Mauritius.

Preparation of the extracts from EM leaves

The plant materials (leaves) were oven dried for several hours or air dried in a drying cabinet for 4 to 5 days until constant mass was obtained. The dried plant materials were homogenized in an electrical food grinder to a fine powder and stored in well-sealed
plastic containers. Crude aqueous extracts were used for in vitro transport studies across rat intestine. Powdered (10 g) plant materials were extracted to exhaustion with 50 mL of water in a Soxhlet apparatus for 5 hours. The solvent was then distilled off under reduced pressure and temperature (40°C) to afford crude plant extract. The extracts were concentrated in vacuo using a rotary evaporator (Model Buchi rotavapor R-114, Switzerland) that ensures evaporation of bulky solutions to small volume concentrates without bumping at temperatures between 70 to 100°C. The resultant concentrate was measured and the gummy material collected in the appropriate solvent (water) for examination. Percentage yield was calculated and the paste-like suspension was diluted in the extraction solvent (water) for further experiments.

Animals

Male Swiss albino rats weighing 100-150 g maintained on commercial feed and tap water ad libitum were used throughout the study. They were maintained in standard environmental conditions with 12hr light and 12hr dark exposure. Prior to experiments 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

After overnight fasting, rats were killed by a severe blow on the head against a hard surface. 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 blunt end. The mid-section of the small intestine from each animal was used in order to minimize the transport variability of the segments (Tandon et al., 1993). Intestinal segments (10 ± 2 cm) were then everted according to the method described by Wilson and Wiseman (1954). Briefly, a stainless steel rod (350 mm, 1.5 mm) was used to push the ileal end of the gut into the gut lumen until it appeared at the duodenal opening of the intestine on the rod. The everted intestine was then slipped off the steel rod and placed in glucose-saline at room temperature in a flat dish. A thread ligature was tied around one end of the everted intestine to facilitate subsequent identification. After weighing, the empty sac was filled with 1 ml of Krebs-Henseleit bicarbonate (KHB) buffer. The composition of the buffer was (mM/l): NaHCO3 25; NaCl 118; KCl 4.7; MgSO4 1.2; NaH2PO4 1.2; CaCl2 1.2; and Na2EDTA 9.7 mg/l. Glucose (10 mM) was added to the medium just before the start of the appropriate experiments. The pH was maintained at 7.4. A 1 ml blunted-ended syringe was employed for the accurate measurement of the amount of fluid to be introduced into the intestinal sacs. The filled intestinal sac was then slipped off the needle carefully 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°C and placed in metabolic shaker at a frequency of 100-110 shake/min. The external incubation medium was continuously bubbled with a gas mixture of 95 % O2 and 5% CO2 during the whole incubation period.

Intestinal transport studies

At the end of the incubation period (30 min), the sac was removed from the organ bath, blotted by a standardized procedure and weighted again. The serosal fluid was drained through a small incision into a test tube. So as to empty the sac completely, gentle pressure was applied, after which the serosal and mucosal fluids were measured. The empty sac was weighted again. The weight of the empty sac before and after the incubation did not differ significantly. The terms and units used in expressing the transfer capacity in the present experiment are those employed by Obatomi et al., (1994).

Effects of EM on transport of D-glucose and L-tyrosine

Varying concentrations of aqueous EM extract (1.5-12 mg/ml) together with 10 mM D-glucose and 2 mM L-tyrosine were incubated in the mucosal solution in the organ bath. The active transport of D-glucose and L-tyrosine was evaluated by measuring the increase in concentration of both compounds inside and outside
the intestinal sacs after 30 mins of incubation. L-tyrosine in the incubating buffer solution was determined as described by Lowry et al. (1951) with modifications. Glucose was measured using a commercially available glucose oxidase kit (Boehringer Mannheim, GmbH, Mannheim, FRG). The term used for D-glucose and L-tyrosine transfer are mucosal glucose transfer, serosal glucose transfer and gut glucose uptake. Mucosal glucose transfer is the amount of glucose that disappeared from the mucosal fluid while serosal glucose transfer is the amount of glucose that entered the serosal fluid. Gut glucose uptake is the difference in glucose concentration between the mucosal and serosal fluid after incubation. This value includes glucose metabolized and those found in the gut wall at the end of the experiment. The same protocol was used to calculate transport of L-tyrosine. Uptake and release of D-glucose and L-tyrosine are expressed as µmol/g tissue wet weight (Mahomoodally et al., 2004a,b,c,d). All chemicals were purchased from Sigma (UK).

**Effects of EM on transport of fluid**

The initial serosal volume was determined as the difference between the weights of the empty and filled everted sac 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. The mucosal fluid transfer was expressed in terms of the diminution in the volume of fluid of the mucosal side during the course of the experiments. The serosal fluid transfer was reflected in the increase in the volume inside the sac and the gut fluid uptake was determined by measuring the increase in the volume of fluid in the gut wall. Uptake and release of fluid are expressed as ml/g tissue wet weight. All chemicals were purchased from Sigma (UK).

**Control experiments**

In each serie of experiments, a parallel control strip was included from the same rat under same incubation conditions as with the plant extract. Control guts were incubated in same medium without the plant extract.

**Statistical evaluation**

All data were expressed as mean ± SEM for seven intestinal segments in each group. The difference between the mean ± SEM between the control and experimental group were assessed using the One Way Analysis of Variance (ANOVA) test. P values of less than 0.05 were considered statistically significant. Statistical analyses were performed using Excel software (Microsoft 2000) and SPSS version 10.0 for windows 2000.

**Results**

Table 1 illustrates the effects of crude aqueous extract of EM on the absorption (mucosal appearance), tissue concentration (gut wall content) and transport (serosal appearance) of D-glucose, L-tyrosine and fluid across rat everted intestinal (gut) sacs.

<table>
<thead>
<tr>
<th>Concentration of EM leaf extract added to the medium (mg/ml)</th>
<th>D-glucose transport (µM/g tissue wet wt)</th>
<th>L-tyrosine transport (µM/g tissue wet wt)</th>
<th>Fluid transport (ml/g tissue wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal disappearance</td>
<td>Gut wall content</td>
<td>Serosal appearance</td>
<td>Mucosal disappearance</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>0</td>
<td>80.1±3.15</td>
<td>11.6±1.44</td>
<td>68.5±3.04</td>
</tr>
<tr>
<td>1.5</td>
<td>78.3±4.03</td>
<td>12.6±1.48</td>
<td>65.7±3.44</td>
</tr>
<tr>
<td>3.0</td>
<td>78.7±4.89</td>
<td>13.8±1.19</td>
<td>64.9±3.01</td>
</tr>
<tr>
<td>6.0</td>
<td>76.3±5.31</td>
<td>13.5±1.29</td>
<td>62.8±2.66</td>
</tr>
<tr>
<td>12.0</td>
<td>74.9±3.58</td>
<td>11.0±1.39</td>
<td>63.9±4.15</td>
</tr>
</tbody>
</table>

The results are expressed as means ± S.E.M of seven observations in each group. *p< 0.05 from the control without EM added to the mucosal solution.
Data from the present study shows that increasing graded concentrations of EM (from 0.5 to 12 mg/ml) in the mucosal solution did not have any significant effect on the transport of D-glucose. However, a slight inhibitory effect on D-glucose transport, though not significant, was observed with increasing concentrations of EM in the incubating buffer from 78.3±4.03 to 74.9±3.58 for 1.5 and 12.0 mg/ml of EM respectively. L-tyrosine absorption and transport were inhibited in the presence of 6 and 12 mg/ml of EM. Gut tissue concentration of L-tyrosine was significantly inhibited (p <0.05) at 12 mg/ml of EM in the incubating buffer. On the other hand, serosal appearance (transport), gut wall content (tissue swelling) and mucosal appearance (absorption) as detected by changes in the volume and weight of the intestine were not inhibited (p>0.05) with increasing concentrations of EM from 0.5 to 3 mg/ml in the mucosal solution. However, higher concentrations of EM (6 and 12 mg/ml) crude aqueous extract in the incubating mucosal solution significantly inhibited the transport of fluid across rat everted intestinal sacs compared to the control. Mucosal disappearance, serosal appearance and gut wall swelling of the enterocytes was inhibited (p<0.05) at 12 mg/ml of EM compared to the control experiments.

Discussion

There is a general consensus centered towards the fact that water is transported across small intestine in the absence of any hydrostatic or osmotic gradients. That is, fluid transport occurs in the absence or even against significant external osmotic or hydrostatic gradients, but is secondary to active solute transport (Zeuthen, 2000; Wright et al., 2002; Menild et al., 1998). Experimental findings from the present investigation showed that 6.0 and 12.0 mg/ml of the crude aqueous extract of EM had a significant inhibitory effect on the absorption, transport and tissue swelling of the rat small intestine. It has been reported that cotransporters of the symport-type behave as molecular pumps, in which a water flux is coupled to the substrate flux movements (Zeuthen, 2000). It has also been shown that water is cotransported with Na⁺ and sugar via conformational changes of the SGLT 1 binding proteins at the level of the enterocytes (Loo et al., 2002). These authors also demonstrated that 260 water molecules are directly coupled to each sugar molecule transported. Interestingly, in the present study it was observed that a high concentration of EM (12 mg/ml) tend to inhibit the transport of glucose across rat everted intestinal sacs, however, values obtained for glucose transport were not statistically significant. On the other hand, the amino acid, L-tyrosine, was sharply inhibited at a concentration of 12 mg/ml. Therefore, it can be hypothesized that the difference in solute concentrations, hence osmosis, across the everted small intestine could justify the inhibitory effects on fluid transport of EM. Furthermore, it is possible that bioactive phytochemicals in EM crude leaf extract have altered the permeability of the plasma membrane of the enterocytes, hence inhibiting the energy independent transport of fluid, osmosis, across the small intestines. We have recently found that EM contains several classes of active phytochemicals such as alkaloids, leucoanthocyanins, tannins, and phenols (Mahomoodally et al., 2005d). These classes of phytochemicals have been reported to reduce the permeability barrier to Na⁺ at enterocytes, thus discharging the electrochemical gradient and removing the driving force for fluid transports across the small intestine (Johnson et al., 1986). Hence, the inhibitory effects of EM on fluid and L-tyrosine transport might be justified on the presence of these secondary metabolites. On the other hand, it can be hypothesized that similar inhibitory effects would operate if these active phytochemicals reach the kidney tubules. Thus, reabsorption of fluid would be inhibited in the proximal, distal and collecting duct of the nephron. Consequently, reabsorption of water would be inhibited resulting in the production of large volume of urine, hence having a diuretic effect.

In conclusion, the observed inhibitory effects of EM on fluid absorption and transport might to some extent validate the diuretic effect of EM reported by the traditional practitioners of Mauritius (Gurib-Fakim et al., 1993, 1996a, b, c). However, further in-depth studies using selective inhibitors and in vivo models should be used to validate the pharmacological claims of EM and its underlying biochemical mechanism(s). The properties and structure of the potentially useful phytochemicals responsible for the observed effects merit further investigation.

Acknowledgement

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References


