AEGLE MARMELOS LEAF EXTRACT INHIBIT UPTAKE OF GLUCOSE ACROSS RAT EVERTED GUT SACS *IN VITRO*

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Summary

Aegle marmelos is used in many parts of the world, including India as a medicinal plant for its hypoglycemic property. An everted gut sac technique was used to investigate the inhibitory effect of *Aegle marmelos* on the uptake of D(+)-glucose *in vitro*. Everted gut sacs were incubated in a erlenmeyer flask for 1 hour and aqueous extract of *Aegle marmelos* leaves (3.62 mg/mL) were added to the mucosal medium at varying glucose concentrations. The aqueous extract of the leaves were found to inhibit primarily the uptake of glucose.

Keywords:*Aegle marmelos*, Hypoglycemic, Aqueous extract

Introduction

Type 2 diabetes mellitus affects approximately 215 millions people worldwide. It is currently clear that aggressive control of hyperglycemia in patients with type 2 diabetes can attenuate the development of chronic complications such as retinopathy and neuropathy. To date, Type 2 diabetes relies mainly on several approaches intended to suppress the hyperglycemia, which include reducing gut glucose absorption.

Hypoglycemic activity has been reported in many plants during the last 20 years (1). *Aegle marmelos* is one among them. Prominent examples included aegeline, a hydroxyl amide alkaloid from *Aegle marmelos* leaves, which suppressed both blood glucose and plasma triglyceride levels (2).

Aegle marmelos is commonly known as 'bael' in India (3).It is a spiny tree belonging to the family of *Rutaceae*, widely used in Indian ayurvedic medicine for the treatment of diabetes mellitus. It is an indigenous tree found in India, Myanmar, Pakistan and Bangladesh. The leaves, roots, bark, seeds and fruits are edible and has medicinal values. The medicinal properties of this plant have been described in the Ayurveda. In fact, as per Charaka (1500 B.C) no drug has been longer or better known or appreciated by inhabitants of India than bael (4).

The leaves of *Aegle marmelos* are astringent, a laxative, and an expectorant and are useful in treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhea, dysentery, heart palpitation, and asthmatic complications (3). It has been claimed that the leaves of *Aegle marmelos* posses contraceptive efficacy (5). Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* were reported to have a cardio tonic effects in mammals (6). *Aegle marmelos* leaf extract was found to be a potential antioxidant drug, which reduces the blood sugar level in alloxan induced diabetic rats (7). This study was therefore undertaken to assess the possible biological properties of *Aegle marmelos* leaf extract on glucose transport across rat everted gut sac *in vitro*.

Materials and methods

Preparation of the extracts from *Aegle marmolos*

The *Aegle marmelos* leaves were collected from Vellore District of Tamilnadu, India during the month of September to December. Fresh leaves were washed with distilled water and shade dried. The shade dried leaves were powdered in an electrical blender and stored at 5°C until further use. The powdered leaves were taken 10 g each and mixed with 20ml of distilled water and were stirred magnetically at room temperature. The residue was removed by filtration and the aqueous extracts were used for experiments.

Experimental design and surgical procedure

Adult male Swiss *Albino* rats weighing 100-150g were housed at room temperature and were used in this experiment. Animals were maintained on commercial feed and tap water. Before each experiment, the animals were starved for twelve hours but allowed for tap water use. Rats were sacrificed by cervical dislocation. The abdomen was opened by a midline incision. The entire small intestine was removed quickly by cutting across the upper end of the duodenum and the lower end of the ileum, and by stripping the mesentery manually (8). The small intestine was then washed out with normal saline solution (0.9% w/v NaCl) using a syringe equipped with blunt end.

Preparation of Everted gut sacs

Intestinal segments (10±2 cm) were everted according to the method described by Wilson & Wiseman (9). The sacs were filled with 0.5 ml of the incubation medium (serosal fluid) and were placed in 25 ml Erlenmeyer flasks with 5 ml of the same medium (mucosal fluid). After oxygenation of the flasks with 100% O_2 for 1 min, they were tightly stoppered and kept in a shaker (90-110 oscillations/min) for 1 h at room temperature. The incubation medium was a Krebs-Henseleit bicarbonate buffer (KHB).

The composition of the buffer was (mM/L): $NaHCo_3$ 25; NaCl 118; KCl 4.7; $MgSO_4$ 1.2; $CaCl_2$ 1.2; and Na_2EDTA 9.7 mg/L. Glucose (5.5 mM) was added to the medium just before the start of appropriate experiments.

Effects of *Aegle marmelos* on the uptake of glucose transport

For studying the effect of the plant extract on the uptake of glucose (substrate), glucose was added into mucosal compartment fluid. The leaf extract was also added in the same compartment (3.62 mg/ml). At the end of the incubation period (1 h), the sacs were removed from the flask and these sacs were emptied and the serosal fluid from the sacs was used for the estimation of glucose. Similar estimations were also performed on samples of mucosal fluid in the flasks. The initial serosal fluid content was determined as the difference between the weight of the empty and the filled everted sac before incubation, and the final serosal fluid content was calculated by subtracting the weight of the empty sac from that of the filled sac, after incubation.

Glucose concentrations were measured using a commercially available glucose oxidase kit (Lifechem ^{TM} – Glucose-LR). The loss of glucose from the mucosal fluid assumed to represent the glucose taken up by the intestine, and the rise in glucose in serosal fluid, the glucose released. The difference is attributable to the glucose retained in the tissue. Uptake and release of glucose were expressed as µM/g tissue wet weight/h.

Control experiments

In each series of experiments, control everted gut sacs derived from the same rat in a buffer containing no substrate were run in parallel. The controls were run either with or without plant extract and results were corrected accordingly.

Statistical analysis

The difference between the mean± S.E.M. between the controls and the experimental groups were examined using the one way analysis of variance (ANOVA) test. P value less than 0.05 were considered as significant. All data were analyzed using Excel. The analysis of variance at 95% level of confidence was used to test for the significant differences in the concentration of intestinal glucose absorption.

Results

Incubation of the rat everted intestinal sacs with *Aegle marmelos* extracts resulted in the inhibition of transport of glucose. With varying concentration of substrate (glucose 5.5 – 8.5 mM), it was found that *Aegle marmelos* significantly inhibited the uptake of glucose ($p<0.05$).The intestine were incubated in Kerbs-Henseleit buffer (pH=7.4) at 37ºC. n=number of sacs used. Values are expressed as mean±S.E.M. of six experiments. (NS) = not statistically significant.

Table1.Effects of *Aegle marmelos* on the uptake of the varying concentrations of Glucose

Glucose concentration in the medium (mM)	Uptake (µmol/g Tissue wet wt/h) Control (n = 6)	Uptake (µmol/g Tissue wet wt/h) AM (3.62 mg/ml) (n = 6)	p-value
5.5	35.2±0.51	17.8±0.52	p<0.05
6.5	40.3±0.52	19.5±0.52	p<0.05
7.5	46.4±0.51	20.7±0.54	p<0.05
8.5	53.2±0.54	24.9±0.61	p<0.05

AM- *Aegle marmelos*, Significant-p<0.05

Discussion

The present findings show that aqueous extracts of *Aegle marmelos* inhibits glucose absorption significantly. Earlier studies (10) have shown that aqueous extract of *Aegle marmelos* leaves (1 gm/kg for 30 days) significantly controlled blood glucose, urea, body weight, liver glycogen and serum cholesterol of alloxanized (60 mg/kg) rats were compared to controls and this effect was similar to insulin treatment (10,11). Therefore it is possible that the active compounds of *A.marmelos* leaf extract decrease blood glucose level by inhibiting the absorption of the glucose from the alimentary tract.

Our findings would tend to indicate that glucose transport was significantly decreased in the presence of aqueous leaf extract of *A.marmelos*. Therefore, it is most probable that active phytochemicals in the leaves of *A.marmelos* binds on the glucose transporters thus may lead to wash out of glucose from the body. The later may be responsible for the hypoglycemic phenomena noted by various investigators in animals after the *Aegle marmelos* leaf extract was administered (11). Based on this data obtained in this study, regarding the mechanism of action, we can propose that *Aegle marmelos* aqueous extracts may possess hypoglycemic properties that inhibit the glucose transport at the site of intestinal brush border membranes. This study provides evidence for a biochemical mechanism which reveals inhibition of glucose uptake effect of *Aegle marmelos* leaf extract in the rat small intestine.

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