

A Kinetic Study for In-vitro Intestinal Uptake of Monosaccharide across Rat Everted Gut Sacs In the Presence of Some Antidiabetic Medicinal Plants

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Citation

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Abstract

Enicostemma hyssopifolium (EH), *Eugenia jambolana* (EJ), *Tinospora cordifolia* (TC), *Gymnema sylvestre* (GS), and *Trigonella foenum* (TF) are traditional antidiabetic medicinal plant used in India. An everted rat gut sac technique was used to investigate the effect of aqueous extracts of these plants on kinetic parameters of D (+) glucose. Everted guts were mounted in a gut sac bath and aqueous extracts were added to the mucosal medium at varying substrate concentrations. Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) were calculated in the presence and absence of drug. It was observed that aqueous extract of EJ, TC, GS and TF significantly reduced V_{max} of D (+)-glucose uptake by 40.09, 28.67, 83.67 and 34.5 $\mu\text{M hr}^{-1}$ respectively, whereas K_m remained unaltered suggested a non-competitive type of inhibition was present.

INTRODUCTION

Diabetes mellitus (DM) is a debilitating and often life threatening disease with increasing incidence throughout the world. It was postulated that DM is the most common chronic disorder affected more than 176 million people worldwide, and this global figure has been set to double by the year 2030, Tiwari & Madhusudaan [1]. The treatment of DM relied heavily on dietary measures, which included the use of traditional plant therapies. Several reviews on plants with known antidiabetic activity or with traditional use as antidiabetic remedies have been published, Ajganonkar [2], Oliver-Bever [3], Bailey & Day [4]. Traditional antidiabetic plants might provide a useful source for developing new oral hypoglycemic compounds as pharmaceutical entities or simple dietary adjuncts to the existing therapies. Studying such traditional medicines might offer an alternative and natural key to unlock diabetologists' pharmacy. On the other hand, suggested mechanisms describing therapeutic effects of several traditional medicinal plant systems are holistic, Handa et al [5]. Most of the reported hypoglycemic plants are anecdotal, and only few have received adequate scientific evaluation. The fundamental mechanisms of these medicinal systems are still unexplainable using modern tools, Rahman & Zaman [6]. 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 modes and mechanisms, Tiwari & Madhusudaan [1]. In this study, we investigated the effect of *Enicostemma hyssopifolium*, Maroo et al [7], Srinivasan et al [8], vasu et al [9], Vijayvargia [10]. *Eugenia jambolana*, Achrekar et al [11], Grover et al [12], Mallick [13], Ravi et al [14], Kedar et al [15], *Tinospora cordifolia*, Gupta et al [16], Stanely et al [17], Wadood et al [18], Raghunathan [19], *Gymnema sylvestre*, Shanmugasundaram et al [20], Baskaran et al [21], Okabayashi et al [22], Sughira et al [23], and *Trigonella foenum* Khosla et al [24], Ravikumar & Anuradha [25], Ribes et al [26], Vats et al [27], a well-documented hypoglycemic plants on the kinetic uptake of D (+)-glucose in in-vitro everted gut sac model as described by Wilson & Wiseman [28].

MATERIALS AND METHODS

PREPARATION OF THE AQUEOUS EXTRACTS

Dried aerial parts of EH, seeds of EJ, stem of TC, leaves of GS and seeds of TF were purchased from local market of Ahmedabad, Gujarat. Air dried plant materials were ground to powder, 100g of each was extracted with 500 mL water by maceration. The water was evaporated under vacuum at 50°C. Extracts were diluted in distilled water to use in experiments.

EXPERIMENTAL DESIGN

Adult male Swiss albino rats weighing 150-180 g and housed at temperature 25 ± 2 °C were used in this study. Animals were maintained on commercial feed and tap water ad libitum. Before each experiment, the animals were starved for 12 hours but allowed for tap water ad libitum use. Rats were sacrificed by severe blow on the head against a hard surface. 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, Barthe et al [29]. 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 (5 ± 1 cm) were everted according to the method described by Wilson & Wiseman. After being everted, segments of guts were blotted with a piece of whatman filter paper no. 40 and weighted. A 1 g glass weight was fixed and tied to the end of the everted gut segment to make an empty gut sac. This was important to prevent peristaltic muscular contractions, which may otherwise alter the shape and internal volume of the sac. The 1 g glass weight was the minimum weight to secure the above-mentioned conditions. After weighing, the empty sac was filled with 0.5 mL of Krebs-Henseleit bicarbonate buffer (KHB). The composition of the buffer was: NaHCO_3 25 mM/L; NaCl 118 mM/L; KCl 4.7 mM/L; MgSO_4 1.2 mM/L; NaH_2PO_4 1.2 mM/L; CaCl_2 1.2 mM/L; and Na_4EDTA 9.7 mg/L. Glucose (2g/L) was added to the medium just before the start of the appropriate experiment. The pH was maintained at 7.4. The sac was filled with a blunted-ended syringe and then the needle was slipped off carefully, and the proximal end of the sac was tightly tied with the thread. The compartment containing the buffer in the sac was named serosal fluid compartment. The distended sac was placed inside a 40 mL KHB bath (mucosal fluid compartment) and mounted. This gut sac bath was placed in a carbon dioxide incubator adjusted at 5 % CO_2 and 37°C. For studying the effect of the plant extracts on the uptake of glucose (substrates), glucose at varying concentrations was added into mucosal compartment fluid. The plant extracts were also added in the same compartment after digestion in 2 mL of simulated gastric fluid (5 mg/mL). At the end of the incubation period (60 min), the sacs were removed from the gut sac bath, blotted by a standardized procedure as

described above and weighted. The serosal fluid was drained through a small incision into a test tube. The emptied sac was shaken gently to remove the adhered fluid and the tissue was weighted. The final serosal volume was determined by subtracting (after incubation) the weight of the empty sac from that of the filled sac. The gut fluid uptake was determined by measuring an increase in the volume of fluid in the gut wall. Glucose concentrations in both the compartment were measured using a commercially available glucose oxidase kit (Span Diagnostics Ltd. Sachin, India). The amount D (+)-glucose transported from the mucosal compartment was characterized as 'uptake' while the serosal gain of the substances is treated as 'release'. Uptake and release of glucose was expressed as mM/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 acid digested plant extracts and results were corrected accordingly.

DATA ANALYSIS

All experiments were carried out in triplicate. Comparison of D (+)-glucose uptake difference between the controls and experimental groups were examined using paired t - test for mean \pm SEM. In terms of enzyme kinetics, glucose transported per hour was analogue to the velocity of transfer, in other words, to the concentration difference of glucose between compartments at the beginning and end of an experiment. The Michaelis – Menten constant (K_m), which is the affinity of the transferring enzyme for the substrate, and maximal velocity (V_{max}), which is the rate of transfer reaction, in the presence as well as in the absence of studied plant extracts were determined from the differences of uptake and release values using the Michaelis-Menten and Lineweaver-Burk Plots in Microsoft Excel. Any difference with p values less than 0.05 were considered as statistically significant. Mean \pm SEM of K_m and V_{max} values were presented Table 1.

RESULTS

Biochemical parameters of D (+)-glucose transport across rat everted small intestines in vitro are shown in graphical forms in Figures. The K_m and V_{max} were calculated in the absence as well as in the presence of plant extracts in the mucosal solution and are shown in Table 1. Data analysis revealed

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that V_{max} for glucose uptake decreased by 40.09, 28.67, 83.67, and 34.5 $\mu\text{M hr}^{-1}$ in the presence of the aqueous EJ seed extract, TC stem extract, GS leaf and TF, respectively. The apparent K_m remained unaltered in the all case of the studied plant extracts.

Figure 1

Table 1: Biochemical parameters obtained for the effect of plant extracts on the transport of D (+)-glucose at different concentrations (5-20 mM) across the rat everted gut sacs.

Experiments	EH		EJ		TC		GS		TF	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
V_{max} ($\mu\text{M hr}^{-1}$)	108.2	112.1	144.1	103.2	98.09	69.42	170.7	87.03	171.9	137.4
K_m (μM)	24.75	24.39	27.02	27.26	22.26	22.22	53.34	53.37	45.81	45.94

The everted gut sacs were incubated in Krebs- Henseleit buffer (pH= 7.4) at 37°C.

Figure 2

Figure 1: Effect of extract on D (+)-glucose uptake across rat everted gut sac. ns: not significant, * $p < 0.05$

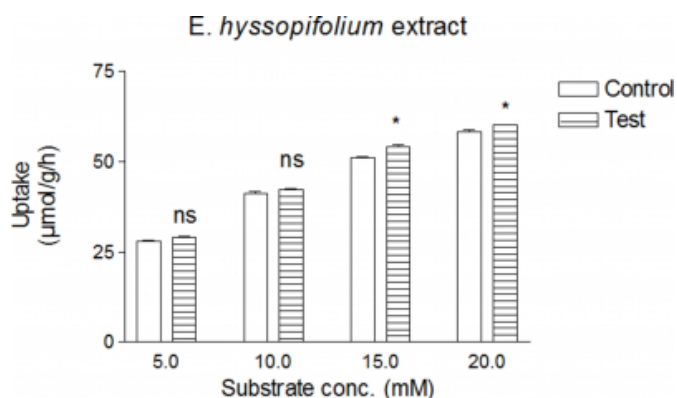


Figure 3

Figure 2: Effect of extract on D (+)-glucose uptake across rat everted gut sac. * $p < 0.05$, *** $p < 0.0001$

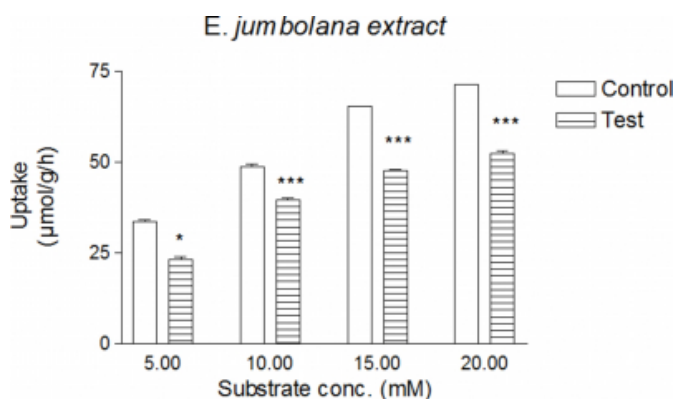


Figure 4

Figure 3: Effect of extract on D (+)-glucose uptake across rat everted gut sac. ** $p < 0.001$.

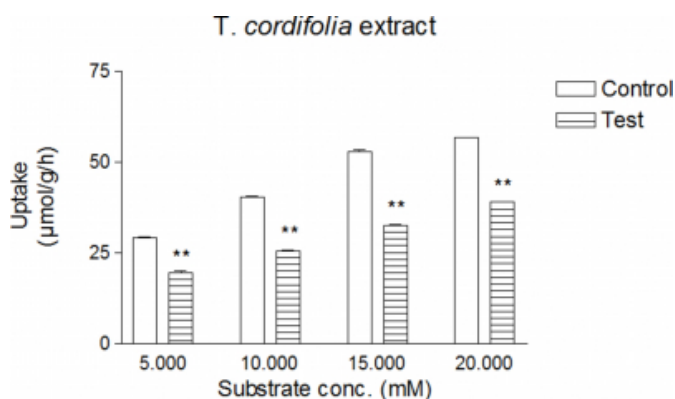


Figure 5

Figure 4: Effect of extract on D (+)-glucose uptake across rat everted gut sac. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.

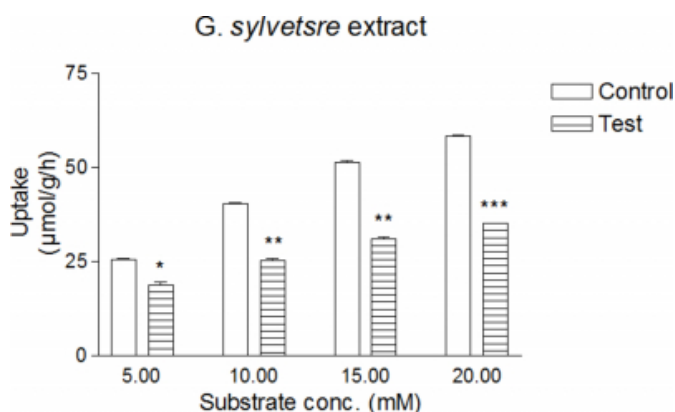
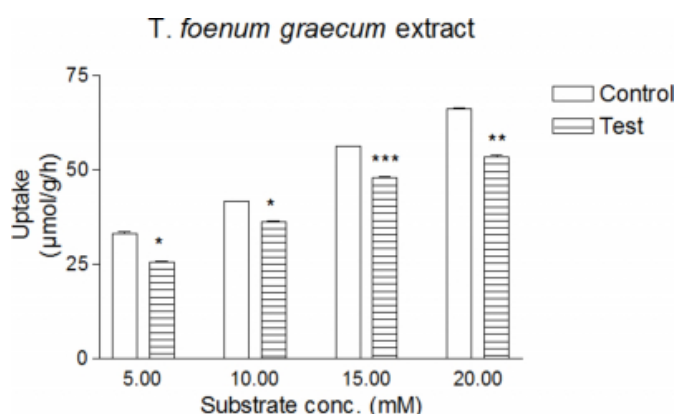


Figure 6

Figure 5: Effect of extract on D (+)-glucose uptake across rat everted gut sac. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$.



DISCUSSION

The most challenging goal in the management of patients with DM is to achieve blood glucose level as close to normal as possible. Unfortunately, postprandial hyperglycaemia (PPHG) or hyperinsulinaemia are independent risk factors for the development of vascular complications in DM patients Tiwari & Madhusudaan [1]. Mechanisms playing role in release and transport of glucose across the intestinal brush border membrane down to the blood stream have attracted much attention recently as potential targets to control PPHG. In this category, majority of recent studies reported the potential use of antidiabetic medicinal plants on inhibition of glucose transport. Drugs that reduce PPHG by suppressing the absorption of carbohydrate are effective in prevention and treatment of non-insulin dependent DM. Alpha-glucosidase inhibitors are used to establish greater glycemic control over hyperglycemia in diabetes mellitus type 2, particularly with regard to PPHG. They may be used as monotherapy (acarbose, miglitol, voglibose) in conjunction with an appropriate diabetic diet and exercise, or they may be used in conjunction with other anti-diabetic drugs. They may also be useful in patients with diabetes mellitus type 1; however, this use has not been officially approved by the Food and Drug Administration.

Our findings would tend to indicate that glucose transport was significantly decreased in the presence of the aqueous extract of EJ seeds, TC stem, GS leaf, and TF seeds which caused a decrease in the V_{max} . Only the aqueous extract of aerial parts of EH did not decreased the glucose absorption in vitro rather it increases the V_{max} . Since the aqueous extract of aerial parts of EH found to contain high amount of reducing sugars in free form it may presume that it could

cause enhancement of glucose uptake in vitro. However, the K_m remained unaltered in the presence as well in the absence of these extracts. This indicates that these extracts act by bringing a non-competitive type of inhibition of glucose at the level of the small intestine. Glucose transport through biological membranes requires specific transport proteins. Active transport of glucose through the apical membrane of intestinal and kidney epithelial cells depends on the presence of secondary active Na^+ /glucose symporters, SGLT-1 and SGLT-2, which concentrate glucose inside the cells, using the energy provided by co-transport of Na^+ ions down their electrochemical gradient, Hediger & Rhoads [31]. Passive transport (facilitated diffusion) of glucose through the cellular membrane is otherwise catalyzed by glucose carriers (protein symbol GLUT), Henderson et al [32]. GLUT 2, a transmembrane carrier protein of GLUT family, located in basolateral membrane of small intestine, is very efficient carrier of glucose at small intestine level. A thiazolidinedione class of drug, rosiglitazone, activated the GLUT2 mRNA level in the liver, Im et al [33] and thereby reducing blood glucose level. Encouraging data are available concerning effects of flavonoids on inhibiting GLUT 2 and their hypoglycemic effect, Kwon et al [34], Song et al [35], Johnston et al [36].

Although our findings are promising, uncertainties remain. Some investigators suggested that flavonoids decreased glucose uptake by a sodium-dependent pathway via the sodium-dependent glucose transporter 1 SGLT1. It is most probable that active phytochemicals in the seeds of EJ, stem of TC, leaf of GS and seeds of TF binds on the glucose transporters thus may lead to wash out of glucose from the body. The latter may be one of the mechanisms for the hypoglycemic phenomenon after administration of these extracts noted by various investigators in animals or subjects.

In conclusions, our study provides evidence for a biochemical mechanism which carries blood glucose lowering effect of EJ seeds, TC stem, GS leaf and TF seeds in intestine via non-competitive inhibition. However, further kinetic data on carrier-mediated transport of D (+)-glucose is needed.

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