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Research Article

Inhibitory Effect of *Eugenia jombolina* and *Momordica charantia* on Uptake of *L*-Tyrosine and *D*-Glucose Across the Rat Intestinal Sac - *In Vitro*

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ABSTRACT

In recent years there has been a renewed interest to screen plant extracts, juices, combinations of two or more plant drugs for a possible beneficial uses in diabetes. *Momordica charantia* (MC) commonly known as karela and *Eugenia jombolina* (EJ), have been reported to have antiviral, antidiabetic, antitumor activities etc. The inhibitory effects of aqueous fruit extract of MC and aqueous stem bark extract of EJ on the transport of *d*-glucose, *l*-tyrosine was studied across rat-everted intestine in vitro. Krebs–Henseleit bicarbonate buffer was used as mucosal medium to incubate the gut sacs. Increasing concentrations (1.5–12 mg/ml) of MC and EJ extract were incubated in the mucosal solution. The serosal appearance and mucosal disappearance of d-glucose and l-tyrosine were significantly inhibited with increasing concentrations of MC, EJ bark extracts and their combination (50:50). The aqueous extracts of the selected plants were found to inhibit primarily the uptake of glucose in a dose-dependent manner and uptake of tyrosine was affected only at higher concentration of extract especially in case of MC. Most importantly, the study has shown inhibition of the active transport of *d*-glucose, *l* -tyrosine across rat intestine which may be effect of saponins and tannins in MC extract and EJ stem bark extract respectively. Results demonstrate tolerability and efficacy of herbal combinations of two plants that seem to act differently but synergistically to regulate glucose-homeostasis.

Keywords: *d*-glucose, *Eugenia jambolina*, Intestinal uptake**,** *l*-tyrosine, *Momordica charantia*, Stem bark

INTRODUCTION

Diabetes mellitus is composed of a myriad of derangements on carbohydrate, lipid and protein metabolism, which are associated to an absolute or relative deficiency of insulin secretion/action. Oral anti-diabetic drugs used for diabetes mellitus type 2 that works by preventing the digestion of carbohydrates. Carbohydrates are normally converted into simple sugars (monosaccharide) which can be absorbed through the intestine. Major factors that can increase blood glucose levels include glucose absorption by the small intestine (after ingesting a meal) and the production of new glucose molecules by liver cells. Major factors that can decrease blood glucose levels include the transport of glucose into cells for use as a source of energy or to be stored for future use and the loss of glucose in urine an abnormal event that occurs in diabetes mellitus. *Momordica charantia* fruit (MC) is a member of Cucurbitaceae, commonly known as karela, bitter gourd or bitter melon and has a long history of use as an Ayurvedic sugar lowering agent where the plant extract has been referred as vegetable insulin[1]. However, MC has been found to be very useful in various diseases like arthroscleroses, hypertension, cardiovascular problems and hyperlipidemia[2]. In addition, some compounds like polypeptide-p have been isolated from fruits of MC involved in lipid metabolism[3] and transport, compound polypeptide-p has been reported against human immunodeficiency virus (HIV)[4,5]. *Eugenia jombolina* from Myrtaceae family is one of the plants for the treatment of various diseases. Most of parts of *Eugenia jambolana* are used in traditional system of medicine in India and well explored for pharmacological activity except bark. According to Ayurvedic system of medicine, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic, antidiabetic and in good for bronchitis, asthma, dysentery, blood impurities and ulcers. Stem bark of *Eugenia jambolana* reported to posses' kaempferol and its 3-o-glycoside, β-sitosterol-D glucoside, sucrose, gallic acid, ellagic acid, gallotannin and ellagitannin from alcoholic extract[6,7] and myricetine in small amount have been reported from stem bark of *Eugenia jambolana*[8]. The *Eugenia jombolina* stem bark (EJ), the fruit, the seed as well as the leaves are utilized in the treatment of insulin dependent diabetes mellitus[9]. Mahomoodally et al. (2006, 2007) have recently investigated the effects of MC fruit extract on the transport of glucose, amino acid and fluid across rat

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intestinal segments under varying concentrations but the exact mechanism is still unclear[10,11]. The present study describes the inhibitory effect of EJ Bark in combination with MC crude extract on intestinal absorption of *d*-glucose. Transport of an amino acid exhibiting high affinity for the common sugar transport system was measured in the presence of EJ, MC, and combination of both (EJMC), using same graded concentrations as reported.

MATERIAL AND METHODS

Preparation of Extracts from stem bark of EJ and MC Fruit

Fresh green MC whole fruits were obtained from a local farm and bark of EJ was collected from area nearby Baramati (Maharashtra, India). The fruits were first washed properly before seeds were removed from the fruit slices and were washed several times with distilled water to remove traces of seeds. Fruit slices were dried and finely powdered store in well sealed containers. EJ Bark was washed to remove debris dried by similar way as that of MC and powdered in mechanical pulverizer. Powder MC and EJ were than extracted with water by cold maceration for 48 hrs water was then distilled off under reduced temperature and pressure 40°C. The extracts were consternated in vacuum using a rotary evaporator (Rotavap) to get semisolid mass of MC and EJ freeze-dried to get powdered extract the resultant the gummy material of MC and dry powder of EJ collected in appropriate solvent for examination. Percentage yields were calculated and suspensions were prepared in the distilled water for further experiments.

Preparation and Incubation of Everted Gut Sacs

The in vitro model of Everted intestine (gut) of rat is a suitable for the study of intestinal transference of nutrients and drugs and has been widely used[10-13]. Adult male Wistar albino rats weighing 200 - 250 g maintained on commercial standard environmental conditions with 12 h light and 12 h dark exposure at animal house of department were used for the study. Prior to experiments animals had free access to water and food. After overnight fasting enthausized with diethyl ether, 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 with normal saline solution (0.9% w/v NaCl) using a blunt end syringe, same portion of the intestine from each animal were taken to avoid and minimize variation in transport of ions. Intestinal segments (5±1 cm) were then everted according to the method described by Wilson and Wiseman[14]. The weight of each sac was recorded and filled with prepared Krebs–Henseleit bicarbonate (KBH) buffer. The composition of the KBH buffer was (mM/l): $NaHCO_3$ 25; NaCl 118; KCl 4.7; $MgSO_4$ 1.2; NaH_2PO_4 1.2; $CaCl_2$ 1.2; and Na_2EDTA 9.7 mg/l. Glucose (2 g/l) was added to the buffer and same was used as medium for experimental procedure and pH of medium maintained at 7.4 throughout the study[11,15]. After preparation of sacks each sack weighed and placed in the organ bath containing prepared medium maintained surrounded by a water jacket maintained at 37-40°C and placed in metabolic shaker at 37-40°C and 100-110 shakes/min. The incubation medium was continuously being provided with 95% oxygen with air, after 30 min sac were removed from medium blotted by standardized and weighted again and noted down. The mucosal, serosal fluid transfer of glucose and tyrosine was determined by using autoanalyser (Erba) and spectrophotometric method. The amount of *l*-tyrosine and *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 and tyrosine are expressed as µmol/g tissue. The aqueous MC, EJ extracts *per se* and their combination EJMC (50:50) was incubated in the mucosal solution in the organ bath. The active transport of d-glucose and l-tyrosine were evaluated by measuring the concentration of both compounds inside and outside the intestinal sacs after 30 min of incubation. *l* -tyrosine in the incubating buffer solution was determined as described by Lowry et al. & Mahomoodally et al, with some modifications[16,11]. Glucose was measured using a commercially available glucose oxidase kit. The terms 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. For every experimental part, a parallel control gut sacs were prepared from the same rat under same incubation conditions as with the plant extract. Control guts sac was incubated in same medium without the plant extract, instead of extract corresponding volume of water was added to minimize any variation.

Statistical evaluation

All data were expressed as mean ± S.E.M. for five intestinal sacs in each group. The differences were compared with the control and experimental group using the one-way analysis of variance (ANOVA) test. $p < 0.05$ considered as significant.

RESULTS

The present results showed that concentrations ranging from 1.5 to 12mg/ml of MC significantly ($p< 0.05$) inhibited the uptake of *d*-glucose and *l*-tyrosine. It was also observed that the concentration of *d*-glucose and *l*-tyrosine accumulated or metabolized by the enterocytes (gut wall content) was higher ($p < 0.05$) than the control at 12mg/ml of MC. Results are mentioned as absorption (mucosal disappearance) and transport (serosal appearance) across inverted intestinal sacs of rat are depicted in Table 1. At higher concentrations 6 & 12 mg/ml of EJ (Table 2) did not have a significant inhibition (41.94 ± 0.86, 33.71 ±2.47) of d-glucose absorption and transport compared to MC (36.06 ± 1.53, 22.40 ± 1.46) but combination shows significant dose dependant inhibition. The inhibition of *d*-glucose

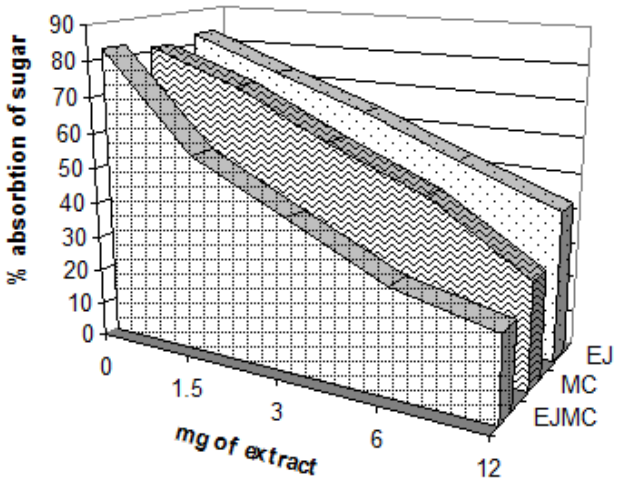

Figure 1: Percentage uptake (absorption) of sugar with EJ, MC and EJMC across the rat intestinal sac.

transport increased gradually with combination of both drugs (81 to 30 %) than the MC fruit extract (83 to 24 %) (Fig.1). Additionally, *l*-tyrosine absorption and transport was significantly ($p < 0.05$) inhibited at 3, 6 and 12 mg/ml MCEJ compared to the control experiments. On the other hand, accumulation of *l*-tyrosine in the gut wall of the rat intestine was significantly different ($p < 0.05$) only at concentration of 6 mg/ml of EJMC compared to MC and control. (Table1 & Table 3)

DISCUSSION

The results of the present study demonstrate that inhibition of sugar and amino acid was evaluated and confirmed. Quantification of sugar and amino acid represent the effect of MC, EJ and its combination EJMC on the sugar metabolism through the intestinal rout. Glucose retained within intestinal wall is difficult to quantify since glucose is rapidly metabolized by enterocytes[17]. It is worth mentioning that EJMC reduced both mucosal disappearance and serosal appearance of glucose when compared to each of EJ and MC *per se*. MC and EJ have been found to possess a wide range of bioactive phytochemicals such as saponins, polyphenolic compounds like tannins and flavonoids have been isolated. Bioactive saponins, tannins constituents of natural medicines have been found to suppress the transfer of glucose from the stomach and the small intestine and inhibit glucose transport at the brush border membrane[18,19]. It is also reported that EJ is toxicologically safein a preclinical studies[20]. Furthermore, Johnson et al. (1986) reported that saponins constituents of herbs reduce the permeability barrier to Na+ at enterocytes, it is most probable that MC extract inhibit the uptake of by inhibiting the Na+-glucose co-transporter (SGLT1)[21]. The SGLT1 is a high affinity glucose transporter that couple's glucose transport to an inwardly directed Na+ gradient[22-24] thus, release the electrochemical gradient and removing the driving force for sugar transport. Furthermore, these polyphenolics like (+) catechin, (-) epicatechin, (-) epigallocatechin and epicatechin gallate, tannic acid have been shown to possess potent S-GLUT-1-mediated inhibition of glucose and antihyperglycemic activity. The manipulation of S-GLUT-1-mediated transport along with α-amylase and α -glucosidase inhibitory activity by plant phenolics makes them very exciting candidates in the control and management of hyperglycemia[25]. Said et.al. (2007), suggested that tannins in olive and walnut leaves are supposed to act to regulate glucose-homeostasis α -glucosidase inhibitors thus reducing the absorption of carbohydrates in the gut[26]. Such an effect was evidenced in our experiments with the inverted intestine segment. Thus, the observed inhibitory effect of MC and EJ in the present study might be due to the bioactive compounds such as saponins and tannins present in MC fruit and EJ bark.

CONCLUSION

Results from the present investigations tend to indicate that MC fruit and EJ bark extract inhibits the uptake of d-glucose, l-tyrosine across rat inverted intestinal sacs and at same time increasing metabolism of glucose in enterocytes. Hence, MC with EJ bark can a potential alternative drug therapy of PPHG, since the most challenging goal in the management of NIDDM patients is to achieve blood glucose level as close to normal as possible. Results obtained in the present study demonstrate safety, tolerability and efficacy of EJMC combinations seem to act differently but potently than individual EJ or MC to regulate glucose-homeostasis. Our study tends to validate the combined ethnobotanical use of MC fruits and EJ bark in traditional medicines. However, further pharmacological and chemical

Table 1: Effects of increasing concentrations of aqueous MC fruit extract *per se* (1.5–12 mg/ml) on transport of d-glucose and l-tyrosine across rat inverted intestinal sacs

Concentration of MC extract in medium (mg/ml)	d-Glucose transport (µM/g tissue wet weight)			l-Tyrosine transport (µM/g tissue wet weight)		
	Mucosal disappearance	Gut wall content	Serosal appearance	Mucosal disappearance	Gut wall content	Serosal appearance
0	73.98 ± 2.63	14.03 ± 2.42	59.95 ± 2.64	15.90 ± 2.06	1.32 ± 0.22	14.58 ± 2.41
1.5	69.82 ± 3.01	16.19 ± 2.04	53.63 ± 3.05	14.82 ± 2.37	1.41 ± 1.28	13.41 ± 1.54
3	62.09 ± 1.54 [a]	18.54 ± 1.59 [a]	43.59 ± 1.16[a]	13.63 ± 1.55	1.68 ± 1.16	11.95 ± 1.69[a]
6	58.37 ± 2.06 [a]	22.31 ± 2.31 [a]	36.06 ± 1.53 [a]	11.52 ± 3.07 [a]	2.19 ± 1.40[a]	09.33 ± 1.88[a]
12	49.22 ± 1.80 [a]	27.82 ± 0.93 [a]	22.40 ± 1.46 [a]	8.64 ± 1.04[a]	3.11 ± 0.20[a]	05.53 ± 0.28[a]

Table 2: Effects of increasing concentrations of aqueous EJ stem bark extract *per se* (1.5–12 mg/ml) on transport of d-glucose and l-tyrosine across rat inverted intestinal sacs

Concentration of EJ extract in medium (mg/ml)	d-Glucose transport (µM/g tissue wet weight)			l-Tyrosine transport (µM/g tissue wet weight)		
	Mucosal disappearance	Gut wall content	Serosal appearance	Mucosal disappearance	Gut wall content	Serosal appearance
0	78.72±1.90	13.10±1.21	65.62±1.99	16.08±0.76	1.39±0.14	12.91±1.42
1.5	72.32±2.06	14.37±1.73	57.95±2.40	15.01±1.41	1.89±0.30	11.82±0.64
3	65.31±1.40	14.84±1.23	50.47±0.03 [a]	12.82±0.83 [a]	2.07±0.30[a]	9.05±0.56 [a]
6	59.44±2.83 [a]	17.50±1.36 [a]	41.94±0.86 [a]	10.03±1.48 [a]	2.23±0.24[a]	8.70±0.66 [a]
12	54.61±2.88 [a]	21.90±1.43[a]	33.71±2.47[a]	10.88±0.68 [a]	2.09±0.28[a]	8.23±0.55 [a]

The results are expressed as mean ± S.E.M of five observations in each group, [a] $p < 0.05$ from the control without extract added to the mucosal solution.

Table 3: Effects of increasing concentrations of EJMC (1.5–12 mg/ml) on transport of d-glucose and l-tyrosine across rat inverted intestinal sacs

Concentration of MCEJ extract in medium (mg/ml)	d-Glucose transport (µM /g wet tissue weight)			l-Tyrosine transport (µM /g wet tissue weight)		
	Mucosal disappearance	Gut wall content	Serosal appearance	Mucosal disappearance	Gut wall content	Serosal appearance
0	79.56±1.99	13.13±2.02	66.43±2.12	16.30±0.86	1.32±0.22	14.98±1.37
1.5	70.61±2.08	25.57±1.73 [a]	44.86±2.51 [a]	12.82±1.24	1.66±0.30	11.16±0.42
3	62.33±1.29 [a]	29.10±1.18 [a]	34.23±0.79 [a]	10.03±0.54 [a]	1.87±0.41[a]	08.16±0.35[a]
6	51.03±1.87 [a]	26.93±0.61 [a]	24.10±2.09 [a]	09.82±1.27 [a]	2.53±0.33[a]	07.29±0.81[a]
12	41.95±2.58 [a]	22.22±1.36 [a]	19.27±0.93 [a]	09.09±0.84	2.00±0.20[a]	07.09±0.28[a]

The results are expressed as mean ± S.E.M of five observations in each group. [a] $p < 0.05$ from the control without extract added to the mucosal solution.

investigations are needed to identify the exact mechanism of the inhibitory effect and to isolate the bioactive principle involved.

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