



A KINETIC STUDY FOR EX-VIVO INTESTINAL GLUCOSE UPTAKE ACTIVITY OF METHANOLIC EXTRACT OF *CANNA INDICA* FLOWER BY MODIFIED EVERTED GUT SAC TECHNIQUE

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Article Received on 09/11/2017

Article Revised on 30/11/2017

Article Accepted on 21/12/2017

ABSTRACT

Canna indica flower are traditionally used for the treatment of diabetes mellitus. Now a days everted rat gut sac technique was used to investigate the D-glucose uptake activity. In current study, modified Everted gut sac technique was performed with chick ileum instead of rat ileum. In this study the effect of methanolic extract of *canna indica* flower on glucose uptake by Everted guts was studied. The guts were mounted in a gut sac bath and different concentrations of glucose were added into mucosal fluid. Just before starting the experiment, the methanolic extract of *canna indica* flower (2mg/ml) was also added in the same compartment. After one hour of incubation, the glucose concentration in mucosal fluid and serosal fluid was measured by using photoelectric colorimeter. Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) were calculated in the presence and absence of the flower extract. It was observed that methanolic extract of *canna indica* flower significantly increases the D – glucose uptake by 0.11 mM/hr.

KEYWORDS: Everted gut sac, *canna indica* flower, Mucosal fluid, Chick ileum, D-Glucose.

INTRODUCTION

Diabetes mellitus is a debilitating and often life threatening disease with increasing incidence in rural populations throughout the world. It was postulated that diabetes is the most common chronic disorder affecting more than 176 million people worldwide, and this global figure has been set to double by the year 2030. Diabetes not only kills, but also one of the major causes of adult blindness, kidney failure, gangrene, neuropathy, heart attack and stroke.^[1] It is estimated that 25 % of the world population is affected by this disease.^[2] Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues, because the existing synthetic drugs have several limitations^[3] For instance, the available synthetic anti-diabetic agents produce serious side effects like hypoglycemia and hepato-renal disturbances.^[4] Medicinal plants play a great role in the traditional management of the disease due to their relative safety and low cost. Scientific investigation into some of these medicinal plants shows that they increase insulin secretion, enhances glucose uptake by adipose or muscle tissues, inhibit glucose absorption from intestine and glucose production from liver.^[5] However, very few of these medicinal plants have received scientific scrutiny despite the World Health Organization recommendations.

Canna indica L. (also known as saka siri, Indian shot) is a species of the *Canna* genus, belonging to the family Cannaceae. The flowers of *Canna indica* are brightly red. The appearance of red color is due to the presence of flavonoids, phenols and anthocyanin's.^[6] The biological activities of flavonoids have been extensively reviewed. Some of them have been found to possess anti-ischemic, anti-platelet, anti-inflammatory and anti-lipoperoxidant activities. Flavonoids have also been found to inhibit a wide range of enzymes involved in oxidation systems such as lipoxygenase, Monooxygenase. These biological activities are related to their antioxidative effects.^[7]

MATERIALS AND METHODS

Collection of plant material

The flowers of *Canna indica* were collected from the local region of Guntur. It was authenticated by Prof A. Ammani, Department of Botany, Acharya Nagarjuna University, Guntur and a voucher specimen was preserved in Department of Pharmacology, Chebrolu Hanumaih Institute of Pharmaceutical Sciences, Guntur for further reference.

Preparation of plant extract: The flowers were shade dried and pulverized. Powdered material of flowers is taken and extracted with 500ml of methanol by Soxhlet apparatus. The solvent was removed under reduced

pressure until the volume of extract reaches 20ml. Then the final extract is collected, dried and stored in a suitable container.

Phytochemical Screening

Phytochemical studies and qualitative tests were conducted for the methanolic extract of *Canna indica* flower to identify various phytoconstituents present in it. The results were indicated in table – 1.

Experimental design and surgical procedure

Fresh chick ileum was obtained from the slaughter house and immediately transported to the laboratory. The entire small intestine was removed quickly by cutting across the upper end of duodenum and the lower end of the ileum and by stripping the mesentery manually. 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, the segments of guts were blotted with a piece of Whatman filter paper no. 40 and weighed. One gram glass weight was passed through everted gut segment to empty the gut sac without any remnants. This was important to prevent peristaltic muscular contractions, which may otherwise alter the shape and internal volume of the sac. Then, the intestinal gut sacs were everted and 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.^[8]

Evaluation of intestinal glucose uptake under the influence of plant extract

Glucose (2g/L) was added to the medium just before the start of the 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 thread. The compartment containing the buffer in the sac was named serosal fluid compartment. The distended sac was placed inside a 30 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 extract on the uptake of glucose (substrates), glucose at varying concentrations was added into mucosal compartment fluid. The plant extract was also added in the same compartment in simulated gastric fluid. At the end of the incubation period of 60 minutes, the sacs were removed from the gut sac bath, blotted by a standardized procedure as described above and weighed. 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 weighed. 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.^[9] Glucose concentrations in both the compartments were measured using a commercially available glucose oxidase kit (Beacon Diagnostics Pvt. Ltd. Navsari, India). The amount of 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 and the results were indicated in table – 2.

Kinetic study on ex-vivo glucose uptake

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.^[10] 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 extract was 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 – 3.

Statistical analysis

The results were expressed in mean $\pm$ SEM. Statistical analysis was carried out by using paired t – test as in standard statistical Software Package of Social Science (SPSS) version 17.

RESULTS

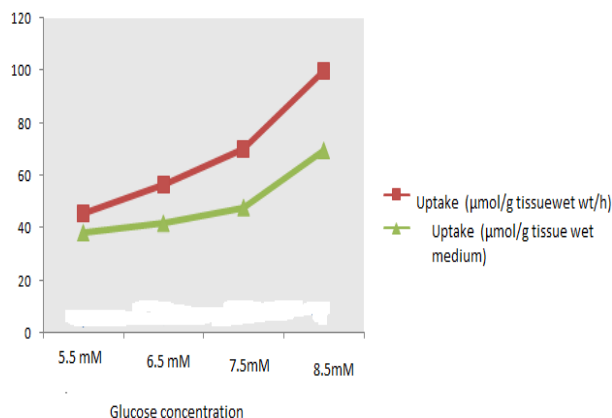
Table 1: Phytochemical screening of methanolic extract of *Canna indica* flower(MECI).

PHYTOCHEMICAL CONSTITUENTS	Methanolic extract of <i>Canna indica</i> flower(MECI)
Alkaloids	+
Glycosides	+
Flavonoids	+
Terpenoids	+
Carbohydrates	—
Saponins	—
Phytosterols	—
Fixed oils and fats	+
Phenols	+
Tannins	+

Table-2: Effect of MECI on the uptake of the varying concentrations of glucose by everted gut sacs of chick

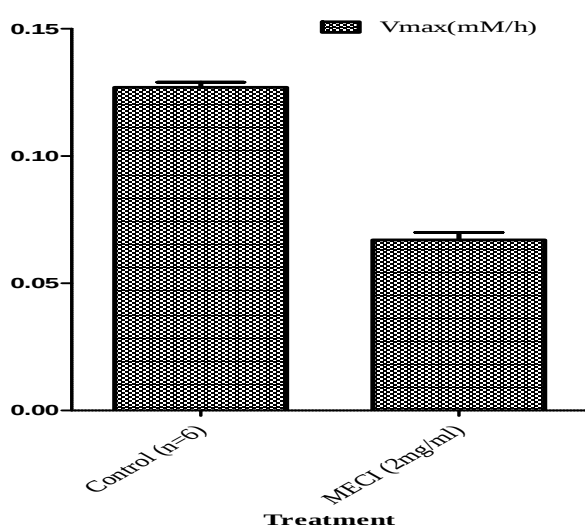
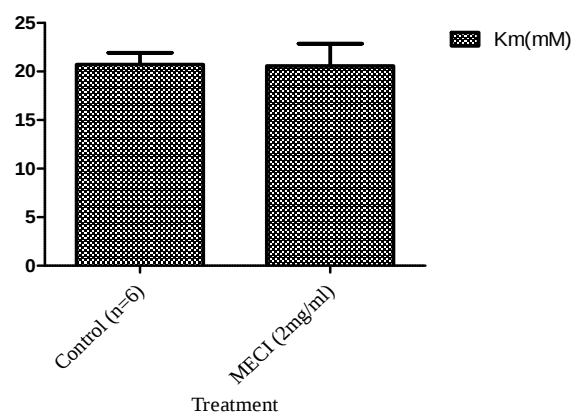
Glucose Concentration(mM)	Uptake ($\mu\text{mol/g tissue wet wt/h}$)	Uptake ($\mu\text{mol/g tissue wet medium}$)
5.5	45.4 $\pm$ 4.46	38.0 $\pm$ 2.46
6.5	56.6 $\pm$ 1.14	41.6 $\pm$ 1.22
7.5	70.0 $\pm$ 3.94	47.6 $\pm$ 1.81
8.5	99.9 $\pm$ 2.34	69.4 $\pm$ 2.47

The gut sacs were incubated in Kerbs-henseleit buffer (pH=7.4) at 37°C. Values are expressed as mean $\pm$ SEM of six experiments. *P<0.05.


Fig-1: Effect of MECI on the uptake of the varying concentrations of glucose by everted gut sacs of chick
Table 3: Effect of MECI Kinetic parameters of the transport of D-glucose at different concentrations (5.5-8.5) across the chick everted gut sacs.

Experiments	Vmax(mM/h)	Km(mM)
Control (n=6)	0.127	20.72
MECI (2mg/ml)	0.067	20.57

Km and Vmax values were obtained from Line weaver-Burk Plot.


Fig-2: Effect of MECI Maximal velocity (Vmax) of the transport of D-glucose at different concentrations (5.5-8.5) across the chick everted gut sacs.

Fig-3: Effect of MECI Michaelis – Menten constant (Km), of the transport of D-glucose at different concentrations (5.5-8.5) across the chick everted gut sacs.

DISCUSSION

The most challenging goal in the management of diabetes mellitus is to achieve blood glucose level as close to normal as possible. The importance of postprandial glucose control in the development of diabetic complications is widely recognized based on the direct stimulation of endothelial cells by glucose, supports the hypothesis that postprandial glucose in the early development spikes are important in the early development of both microvascular and macrovascular diseases.^[11] In the present study, ex-vivo glucose uptake activity of MECI might be attributed to delayed intestinal glucose absorption and/or increased glucose utilization by the intestine with reference to anaerobic glucose metabolism thereby creating decreased passage of glucose from mucosal side to serosal side of the intestine. Further studies were designed to understand the intestinal glucose uptake both in the presence and absence of the plant extract. The results obtained in the present ex vivo study envisaged that MECI significantly inhibits glucose absorption/transport in the everted chick gut and significantly reduced when compared to control. The Michaelis-Menten constant (Km) of the glucose uptake was calculated. Km is the affinity of the transferring enzyme for the substrate. In the present study it is simulated that Km is the affinity of glucose transporters i.e. GLUT2 and SGLT1 for glucose. The maximal velocity (Vmax) is regarded as the glucose uptake rate in the presence as well as in the absence of plant extract. The decrease in Vmax in the presence of

MECI indicated that the trans-membranal glucose transport was significantly decreased. However the Km remained unaltered in the presence as well as in the absence of the extract. This indicates that the MECI acts by bringing a non-competitive type of inhibition of transport of glucose at the level of small intestine. This might be due to the inhibition of glucose transporter proteins (GLUT2 and SGLT1) activity.

CONCLUSION

Based on the data obtained in the present study we propose that MECI possesses hypoglycemic property that inhibits the glucose transport at the site of intestinal brush border membrane in chicks.

ACKNOWLEDGEMENT

The authors are thankful to the management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences for providing facilities to carry out research work.

Conflict of Interest

The authors report no conflict of interest.

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