Effect of *Acacia catechu* on intestinal absorption of glucose in rats

Ngangom Gunindro*[1], Krishna Pramodini Devi[1] and Thokchom Imoba Singh[2]

[1]*Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur (India)*
[2]*Department of Pharmacology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur (India)*

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ABSTRACT

Effect of Acacia catechu on intestinal absorption of glucose was evaluated using ethyl acetate extract of black catechu, a product obtained from the heart wood of the plant in an in vivo model in rats. Two doses of 250 mg/kg and 500 mg/kg of the test substance given orally produced significant reduction of glucose absorption when compared with the control group, Presence of tannins and flavonoids in the extract could be responsible for reduction in the glucose absorption by affecting the Na^+, K^+ ATPases, the pumps providing the driving force for glucose entry inside the intestinal epithelium.

Key words: *Acacia catechu*, Black catechu, Glucose absorption, Flavonoids, Na^+, K+ ATPase

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INTRODUCTION

There has been considerable progress in the treatment of diabetes mellitus with oral hypoglycemic agents in the recent times, but the management of postprandial hyperglycemia (PPHG) in diabetic mellitus is often problematic than managing fasting hyperglycemia. PPHG is an independent risk factor for the development of macrovascular complications of diabetes mellitus, and even in absence of marked fasting hyperglycemia, it is a recognized risk factor for coronary artery disease [1]. Till today, the search for newer drugs for diabetes treatment continues because the existing synthetic drugs have several limitations [2] such as problematic side effects and decline in the response on long term use [3]. Medicinal plants have been increasingly used in most parts of the world for various effects such as hypolipidemic, hypoglycemic, antihypertensive, contraceptive, abortifacient, oxytocic, antimicrobial, and in the treatment of skin diseases [4]. More than 400 plant species have been reported for diabetes treatment [5]. However, the scientific and medical evaluations for efficacy have been done only for a few of these plants [6]. *Acacia catechu* Willd. (Family - Leguminosae) is such a plant reported to show hypoglycemic effects on experimental animal models of diabetes [7, 8, 9] but with poorly understood mechanisms. It is a small thorny tree of up to 12 m high, indigenous to India, Burma (Myanmar) and Thailand to China. In India, *Acacia catechu* is used as a multipurpose tree in forestation schemes in the dry parts [10].

In an attempt to evaluate effect of *Acacia catechu* on intestinal absorption of glucose, an important determinant of PPHG, the present study is carried out using an *in vivo* model of absorption study using ethyl acetate extract of black catechu (EBC). Black catechu (Synonyms – Kattha, Cutch), is a dry extract prepared from the heart wood of the tree by boiling with water and occurs as brittle black masses, which taste somewhat bitter initially and astringent afterwards. Commonly, it is used as an ingredient of betel leaf and paan masala. Black catechu contains essentially catechin isomers, acacatechin (2-10%), phlobatannin or catechutannic acid (25-33%), gum (20-30%), quercitrin, quercetin, catechu red and water [11]. Catechin and quercetin belong to flavonoids which are polyphenolic compounds [12].

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EXPERIMENTAL SECTION

The study was carried out in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur (India) after obtaining approval of the Institutional Animal Ethics Committee (No.1596/40/a/12/CPCSEA).

Animals

Twenty four healthy Wistar albino rats of either sex (12-14 wk), weighing between 110 and 150 g were obtained from the Animal house of RIMS, Imphal and divided into four groups (n = 6 in each group). The animals were acclimatized in standard polypropylene cages at the room temperature with 12 h light - dark cycle (light from 6 AM to 6 PM) and maintained on standard diet with free excess to water. The animals described as 'fasted' were deprived of food for 18 h, but allowed free access to water.

Preparation of test substance

Black catechu (Kattha in Hindi) was obtained from the local market. It was differentiated from Gambier (pale catechu), a closely alike product obtained from *Uncaria gambier* (Rubiaceae) by Gambier fluorescin test [11]. Using Soxhlet apparatus, powdered black catechu was defatted with petroleum ether (BP 40°C - 60°C), followed by extraction with 95% ethanol. Then, the ethanol extract was extracted with ethyl acetate [13]. At the end of extraction, the yield was 10.5%.

Phytochemical screening

The presence of flavonoids and tannins could be detected using standard tests in the extract. The presence of catechin was also confirmed [14].

Acute toxicity study

The median lethal dose (LD_{50}) of ethyl acetate extract of *Acacia catechu* in albino mice was reported to be 2500 mg/kg by intraperitoneal route [13]. In another study, the ethanol extract of black catechu was found to be safe at 2000 mg/kg given per orally (p.o) [9]. Therefore, we conducted the limit test of the test substance i.e. EBC in albino rats at one dose level of 2000 mg/kg p.o [15]. There was no mortality at 2000 mg/kg p.o and two doses of 250 and 500 mg/kg were chosen for the study.

Experimental setup

The fasted animals were divided into four groups of six in each and treated as follows:

1. Control: 2% gum acacia in D/W
2. Standard: Metformin (B. No.PT12011, Franco-Indian, India) –135mg/kg
3. Test 1: EBC – 250 mg/kg
4. Test 2: EBC – 500 mg/kg

The test substance (EBC) and metformin were suspended in 2% gum acacia in D/W. All the preparations were administered daily as single doses (1 ml/100g) per orally using gastric tubes for seven days. The dose of metformin for the rats was extrapolated from human dose (1.5 g/day) [16].

Absorption study

To evaluate the effect of EBC on intestinal absorption of glucose, the method of absorption study adopted by Das S *et al.* [17] was followed with some modifications. The fasted rats were anaesthetized with intraperitoneal injection of sodium pentothal (40 mg/kg). Through a midline abdominal incision in each rat, an intestinal loop of 10 cm in length from the pyloric end was made, care being taken to keep the vascular supply intact. 1 ml of 250 mg% D - glucose in normal saline at 37°C was introduced in the lumen of the intestinal loop using tuberculin syringe. After an absorptive period of 15 min, each rat was sacrificed and the loop was removed. The excised loop was weighed after stripping off the fat and mesentery, and cut open to recover the fluid left after absorption. The glucose absorption was calculated from the difference between the total amount of it introduced in the lumen and the amount recovered after the absorptive period. The absorption was expressed in mg/g dry weight of the tissue/h. The dry weight of the tissue segment was measured after dehydrating it with 95% ethyl alcohol for 24 h, followed by drying for 2 h at 120°C in the hot air oven.

Glucose estimation

Glucose was estimated by enzymatic colorimetric method using glucose kit (Human, Germany). The estimation was carried out as per the instructions provided by the kit manufacturer.

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Analysis of results

All the values were expressed as mean ± SD. The results were analyzed by one - way ANOVA (Analysis of variance) followed by Bonferroni test. P<0.05 was considered as statistically significant.

RESULTS

The dose of 2000 mg/kg p.o was found to be safe in the acute toxicity study. The glucose absorption (mg/g dry weight /h) in the control, metformin (standard), EBC - 250 and 500 mg/kg treated groups were 90.35 ± 5.26, 72.32 ± 4.63, 80.67 ± 2.73 and 76.87 ± 1.59 respectively. Metformin and EBC treated groups showed significantly reduced absorption (P<0.001) when compared with the control. There was significant difference in the absorption (p<0.01) between the EBC - 250 mg/kg and metformin treated groups. (Table 1)

Table 1: Effect of ethyl acetate extract of black catechu (EBC) on intestinal glucose absorption

Group (n = 6)	Treatment (1 ml/100 g p.o)	Glucose absorption (mg/g dry tissue wt/h)
Control	2% gum acacia (g.a) in D/W	90.35 ± 5.26
Standard	Metformin - 135 mg/kg in 2% g.a in D/W	72.32 ± 4.63[*]
Test 1	EBC - 250 mg/kg in 2% g.a in D/W	80.67 ± 2.73[†**]
Test 2	EBC – 500 mg/kg in2% g.a in D/W	76.87 ± 1.95[*]

All values are mean ± SD; n (animals in each group) = 6; [*] p< 0.001 compared with control; [†]p<0.01 compared with control; [**] p<0.01 compared with standard
(One - way ANOVA followed by Bonferroni test)

DISCUSSION

Animals in the same age group (12-14 wk) are used to minimize differences in the intestinal absorption. Fasting for 18 h prior to the absorption study is sufficient to keep the proximal segment of the small intestine empty [18]. The solvents like petroleum ether, ethanol and ethyl acetate are used for extractions of hypoglycemic principles from plants [19, 20]. The test substance in the present study is obtained by subjecting defatted black catechu to serial extraction with ethanol and ethyl acetate.

The highest capacity to absorb sugars in the intestine is in duodenum and upper jejunum [21]. In our study, glucose absorption is evaluated using proximal 10 cm of the small intestine from the pyloric end *in vivo*, and allowing an absorptive period of 15 min. Metformin is used as the standard drug. It an oral antihyperglycemic agent which decreases hepatic glucose output, inhibits absorption of glucose from gut, and increases glucose uptake by muscle and fat cells [22]. The inhibitory effect of metformin on intestinal glucose absorption is evident in the present study, and this observation supports the validity of the adopted study model. The test substance i.e. ethyl acetate extract of black catechu (EBC) produces significant reduction of intestinal glucose absorption when compared with the control. The dose of 250 mg/kg p.o of EBC produces less glucose absorption when compared with the standard.

The absorption of glucose is a two step process involving its uptake from intestinal lumen across the apical membrane into epithelial cell and a coordinated exit across the basolateral membrane. The uptake at the apical membrane is carried out by a membrane protein called Sodium/Glucose cotransporter (SGLT1), while exit at the basolateral membrane is through a facilitated sugar transporter called GLUT2. The uptake at the apical membrane is an active process, energized by electrochemical Na^+ gradient maintained by extrusion of Na^+ across the basolateral membrane by Na - K pump (Na^+, K^+ ATPase). The inhibition of membrane Na - K pump decreases apical membrane Na^+ gradient resulting to decline in the driving force for glucose entry into intestinal epithelium [23]. Tannins present in the plants exert inhibitory effect on many enzymes due to protein precipitation [24]. The flavonoids including quercetin [25] are known to inhibit membrane Na^+, K^+ ATPase [12, 26]. The presence of these chemicals in the extract (EBC) strongly suggests that it inhibits membrane Na^+, K^+ ATPases, which provide the driving force for glucose entry into intestinal epithelium. Therefore, it is concluded that *Acacia catechu* decreases intestinal absorption of glucose and hence, studies to explore the role of this plant in the management of postprandial hyperglycemia will be attractive.

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