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Oral hypoglycaemic effect of *Canthium coromandelicum* leaf extract on Wistar rats

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Abstract

Canthium coromandelicum (Burm. F), are trees found in dry scrub and monsoon forests in Sri Lanka and Southern India. Immature leaves have been a traditional food accompaniment to rice among the people living in Sri Lanka where the plant is frequently found. The present study was designed to investigate the possible oral hypoglycaemic activity of the leaf extract in rats. Percentage decreases of serum glucose levels of 15.4 % - 25.7 % were observed at doses of 15-30 g/ kg body weight following a glucose challenge. This is indicative of acute hypoglycaemic (anti-hyperglycaemic) activity of the leaf extract. Oral glucose challenge on the 8th and 15th day following repeated administration of leaf extract (20g/kg body weight) for 7 and 14 days did not suppress the fasting or the post-prandial serum glucose levels. It is concluded that the observed acute hypoglycaemic (anti-hyperglycaemic) effect is possibly due to inhibition or reduction of intestinal glucose absorption mediated by soluble dietary fibre (SDF; 1.2% DM) and pectin (38% of SDF) in the leaf. This will explain the absence of a chronic effect. This study provides evidence for its traditional recommendation as a functional food in diabetes.

Key words: *Canthium coromandelicum,* antihyperglycaemic activity, normoglycaemic rats, aqueous extract

Introduction

Diabetes mellitus (DM) and related complications are a major health problem worldwide. The reports of Diabetic Association of Sri Lanka indicate that 12 % of the urban population and 7 % of the rural population are already suffering from diabetes with more people becoming victims. The prevalence of Type II diabetes is higher compared to Type I. The management of diabetes Type II

at its initial stage is mainly by dietary control. The recommendations of the WHO committee on DM are to investigate hypoglycaemic agents of plant origin. In this respect investigations carried out in Sri Lanka have shown that there are many medicinal plants which are known to have anti-diabetic effect with proven scientific efficacy (Fernando, 1990, Tissera, 2001). However, these plants are mainly used in preparation of decoctions and few medicinal plants known to have anti-diabetic activity are edible (Serasinghe *et al.*, 1990) and could be used in dietary control.

Canthium coromandelicum (Burm. F), commonly referred to as *Kara* (Sinhala) are trees found in dry scrub and monsoon forests in Sri Lanka and Southern India. According to folklore the leaves made into a dry curry (*mallum*) have been used as a possible treatment for many ailments including hyperglycaemia in generations past. Interestingly the leaves have also been frequently used as a food during periods of food scarcity (during the Second World War). As reported data was scarce information was gathered from Ayurvedic Center, Maharagama, Sri Lanka. From the data we found that the leaves of this plant are not used by the indigenous practitioners as an anti-diabetic pharmacophore in Sri Lanka, but some practitioners recommend leaves to be included in the diet of diabetic patients. With this knowledge and as there were no scientific reports to indicate the hypoglycaemic efficacy the current study was undertaken to evaluate the possible oral hypoglycaemic activity of an aqueous leaf extract of *Canthium coromandelicum.*

Marerials and Methods

Plant material

Fresh young *Canthium coromandelicum* leaves as used in curry preparations, collected from Padukka area in the Western province of Sri Lanka were used throughout the study. The plant was identified and authenticated by Dr B M P Singhakumar (Department of Forestry, Faculty of Applied Sciences, University of Sri Jayewardenepura) and a voucher specimen was deposited at the Department of Biochemistry, Faculty of Medical Sciences.

Soluble dietary fibre and pectin content of leaves

The leaves in bulk were dried in a hot air oven at 40°C (3 hrs) and ground to obtain a powder. Moisture content of the leaves was determined by drying the leaves at 105°C until constant weight (AOAC, 1984) and soluble dietary fibre by enzymatic digestion (Asp *et al.*, 1983). Pectin content in soluble dietary fibre was determined by the Carbazole reaction (Dekker and Richards, 1972).

Preparation of leaf-extract for evaluation of hypoglycaemic activity

Canthium coromandelicum leaves were washed and air-dried. Appropriate amounts were weighed, cut into pieces and blended with distilled water (house

hold blender). The normal concentration of the water extract was 4 g/ mL (corresponds to an edible portion of 20 g/kg BW).

Experimental animals

Male Wistar rats, (8 weeks) procured from Medical Research Institute, Colombo were maintained under standard conditions in the Animal House, University of Sri Jayawardenepura, Nugegoda, Sri Lanka. The animals were given a standard WHO diet (Sabourdy, 1988) throughout the study with water provided *ad-libitum*. The plant extract, glucose and distilled water were administrated using a Sondi needle.

Effect of a single and different doses of leaf extract on glucose challenge

Test and control groups of five rats each (body weight =183 ± 24 g) were subjected to an overnight fast (14 hrs). Fasting blood glucose was determined as below. The test group was given the plant extract at a dose of 20g/ kg body weight and the control group 1 mL of distilled water. After 30 minutes a glucose load (3g/ kg body weight) was administered to all rats. Venous blood was drawn after 90 minutes (200 µL) for determination of blood glucose using glucose oxidase kit. Only the 90 min post glucose load blood glucose was determined according to.WHO criteria (Kumar and Clark, 1994).

Rats (body weight 200 ± 56 g) were divided into 5 groups with 5 rats in each group. After an overnight fast the 4 test groups were administrated with a dose of leaf extract corresponding to 15g/ kg body weight, 20g/ kg body weight, 25g/ kg body weight, 30g/ kg body weight respectively. The control group was administrated 1 mL of distilled water. Blood glucose was determined by the same method as described above.

Multiple-dose effect of feeding leaf extracts (long term effect)

Twelve rats (body weight 305±14 g) were divided into test and control groups each containing 6 animals. The test group was administrated a leaf extract at a dose of 20g/ kg body weight for 7 days. The control group was administrated 1 mL of distilled water. Fasting blood glucose and the blood glucose level after a glucose load (3g/kg body weight) without administration of the leaf extract were determined on day 8. Oral feeding of leaf extract and distilled water was further continued for another 7 days. On the 15th day the serum glucose levels were determined following administration of a load of glucose without administration of leaf extract.

Statistical analysis

The results are presented as mean ± SD. The results of control and test groups were compared using the students t-test at 95% confidence interval using Microsoft Excel 98 package.

Results and Discussion

The dry matter of the leaves contained 1.2 ± 0.2 % soluble dietary fibre of which 38 % was pectin. The moisture content of the leaves was 70.4 ± 1.1 (DM 29.6%). According to the fibre analysis *Canthium coromandelicum* leaves contain considerable amount of soluble dietary fibre and pectin. Pectin reduces the uptake of glucose from the gut (Kim, 2003).

Effect of a single dose and different doses of leaf extract on glucose challenge

In the single-dose experiment (dose 20g/kg BW) the test group showed a significant ($p= 0.005$) decrease in serum glucose level compared to that of the control in the glucose challenge test. The mean percentage decrease of serum glucose level was 18.4 %. The fasting glucose levels of control and test were 89.3 ± 16.6 and 82.4 ± 7.0 mg/dL whereas the post serum glucose of control and test were 117.1 ± 2.6 and 95.6 ± 6.7 mg/dL respectively. A similar effect was observed for 15, 25 and 30 g/ kg BW of the leaf extract (percentage decrease 15.4 - 25.7) thus indicating a possible anti-hyperglycaemic effect on normoglycaemic Wistar rats after a glucose challenge.

Multiple-dose effect

Oral glucose challenge carried out on the 8th day and 15th day following repeated administration of the extract (20g/kg body weight) did not suppress the fasting glucose levels or the post glucose serum glucose levels (Table1).

Table 1. Multiple dose effect of the leaf extract of *Canthium coromandelicum*

Blood glucose levels	Test (mean ± SD)		Control (mean ± SD)	
	8th day	15th day	8th day	15th day
Fasting (mg/dL)	82 ± 16^{a}	78 ± 20^{a}	82 ± 18^{a}	93 ± 10^{a}
Post serum (mg/dL)	111 ± 15^{b}	121 ± 7^{b}	111 ± 16^{b}	128 ± 11^{b}

n=5; $^{a, b}$ Values with same superscript in the same row are not significantly different ($p < 0.05$)

Unlike in the case of lowering blood sugar by insulin like factors or by an effect on receptors, decrease in blood glucose by decreasing rate of absorption is usually compensated by gluconeogenesis. If the decrease is due to above mentioned reasons, both long and short-term effects will cause a decline in blood sugar. Since there is no hypoglycaemic effect of the leaf extract on

administration of multiple doses it can be assumed that the observed acute hypoglycaemic (anti-hyperglycaemic) effect could be attributed to inhibition of intestinal glucose absorption. The aqueous leaf extract was a viscous solution with a gummy consistency and therefore it is possible that the inhibition of intestinal glucose absorption is mediated via the soluble dietary fibre and the pectin present in the leaf thus indicating that the effect is transient.
In conclusion this study shows for the first time that *C. coromandelicum* has oral anti-hyperglycaemic potential. Thus *C. coromandelicum* leaf could be considered as a functional food for people with diabetes.

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