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The effect of the aqueous stem bark extract of *Kokoona zeylanica* Thw. on the blood glucose level of mice

P.D. Dayananda, W.B. Yapa and W.D. Ratnasooriya

Department of Zoology, University of Colombo, Colombo 03, Sri Lanka
Address correspondence: wdr@zoology.cmb.ac.lk

ABSTRACT

This study examined the blood glucose lowering potential of the aqueous stem bark extract (ABE) of Sri Lankan endemic plant - *Kokoona zeylanica* (Family: Celestraceae). This was tested in normoglycaemic mice using three oral doses (1800, 2500 and 3000 mg/kg of body weight). The results show a moderate but dose dependant acute hypoglycaemic activity with a fairly rapid onset (4 h). However, it did not have a hypoglycaemic effect in non fasted mice or in fasted mice (treated consecutively for 30 days). ABE did not improve the glucose tolerance test indicating the lack of antihyperglycaemic activity. However, ABE improved the sucrose tolerance test indicating the α-glucosidase inhibitory activity, which appears to be the main mechanism of hypoglycaemic activity. ABE was well tolerated (in terms of overt clinical signs of toxicity, hepatotoxicity and renotoxicity), and it did not provoke a change in the lipid profile. It is concluded that the ABE of *K. zeylanica* has a safe, moderate and acute hypoglycaemic activity

KEY WORDS: *Kokoona zeylanica,* Hypoglycaemia, Diabetes, Toxicity

INTRODUCTION

Kokoona zeylanica Thw. (Family: Celestraceae) is a large tree (20 - 35 m high) branched towards the top with a rough outer bark which is corky and grey in colour and an inner bark which is bright yellow or dark orange in colour. Leaves are obovate, cuneate at base, rounded or letuse at apex with serrated margins entirely or faintly. Flowers are in axillary panicles, bisexual and dull yellowish brown in colour, (1). *Kokoona* species occur in the Annamallay hills in India and in Sri Lanka. *Kokoona zeylanica* is endemic to Sri Lanka, which is rather rare in the country and found in forests (Pelawatte, Pasdun Korale, Ratnapura, Ambagamuwa, Udugama and Hiniduma) in moist regions between 1000 - 4000 feet altitude (2, 3).

According to phytochemical studies, the water extract has revealed the presence of alkaloids, saponins, terpenes and tannins in the inner stem bark of *K. zeylanica* (4). Twelve D:A-friedo-oleanane triterpenes have been isolated from the hot benzene extract of the inner stem bark of *K. zeylanica* (5). Zeylasterone, pristimerin (6), minor triterpene and celastranhydride (7) are also recorded from the outer stem bark of *K. zeylanica* from the light petroleum extract.

In the traditional medicine, the inner bark is used as a treatment for snakebites, swollen joints, eye diseases, framboesia pimples and skin diseases. The inner bark may also be used to lighten the colour of the skin and for removing marks from the face. It is often used as a snuff for severe headaches and the oil from the seed is used as a leech repellent (2). *K. zeylanica* is also claimed to be useful as a treatment for diabetes mellitus (2, 8, 9). Several of the multiplant decoctions for diabetes mellitus also contain the inner stem bark of *K. zeylanica* as one of the components (8). However, so far its effect on blood glucose level has not been scientifically investigated. The aim of this study was to examine the hypoglycemic potential, and the toxic effects of the aqueous stem bark extract of *K. zeylanica*.

MATERIALS AND METHODS

Plant collection authentication

The stem bark of *K. zeylanica* was purchased from D. Peris & Sons Ltd. Drug Merchants, Gabo's Lane, Pettah, Colombo and was identified and authenticated by Dr. M. Chandrasena of the Institute of Indigenous Medicine, University of Colombo. A voucher specimen of the leaves and bark (WDR/kokun) was deposited at the museum of the Department of Zoology.

Preparation of the ABE of *K.zeylanica*

The outer gray bark of the *K. zeylanica* was removed and the pieces of inner bark were dried in the shade for two days and powdered using a mechanical grinder (Sumeet Master No: 864, Sumeet machines Ltd, Nasik, India). The powder (750 g) was mixed with tap water and then refluxed with 4 l of tap water for two days in a round bottom flask fitted to a Leibig's condenser.

The yellowish colour solution was filtered using a centered filter, freeze dried (10 g, yield 1.3%) and stored airtight in a refrigerator (+4 ºC). The freeze dried powder was dissolved in distilled water (DW) to obtain the required dosages in 1 ml solution. The doses used (1800, 2500 and 3000 mg/kg) were identical to doses that have been used previously to investigate antinociceptive activity of aqueous stem bark extract (ABE) of *K. zeylanica* (5).

Experimental animals

Healthy, adult ICR mice (Weight 25 - 40 g) purchased from the Medical Research Institute, Borella, Sri Lanka and maintained at the animal house of the Department of Zoology, University of Colombo were used in this study. Mice were housed under standard animal house conditions (temperature: 28 - 31ºC, photoperiod: approximately 12 h natural light per day and relative humidity: 55 - 60%) with continuous access to pelleted food (Vet House Ltd, Colombo, Sri Lanka) and tap water. The research was conducted in accordance with the internationally accepted principles for animal use and care and guidelines and rules of the Faculty of Science, University of Colombo for animal experimentations. All surgical interventions were done under ether anesthesia using aseptic precautions.

Investigation of the effect on fasting blood glucose level

Thirty mice were fasted for 16 h (water was allowed *ad libitum*). Mice were randomly divided into five equal groups (n = 6 per group) and treated orally in the following manner: group 1 with 1 ml of Distilled water (DW), group 2 with 3000 mg/kg of ABE, group 3 with 2500 mg/kg of ABE, group 4 with 1800 mg/kg of ABE, and group 5 with 3 mg/kg of hypoglycaemic drug glibenclamide (State Pharmaceutical Corporation, Colombo, Sri Lanka) in 1 ml of DW. The mice were observed for any apparent increase in activity after the administration. Blood was collected from the tail of the each mouse 1 h prior to the treatment and at 2 and 4 h of post treatment under the mild ether anesthesia, using aseptic precautions. The blood was allowed to clot at room temperature (28ºC - 31ºC) and centrifuged at 3000 rpm and the serum was separated. Glucose concentration of the serum was determined as pre manufacturer's instructions using Randox glucose oxidase assay kit (Randox Labs, Antium, UK) and a spectrophotometer (V500, Jasco Corp, Tokyo, Japan) at 550 nm.

Investigation of the effect on random blood glucose level

Twelve mice were randomly divided into 2 equal groups (n = 6 per group) and treated orally in the following manner: group1 with 1 ml of DW and group 2 with 3000 mg/kg of ABE. Blood samples were collected from tail 1 h prior to the treatment and 2 and 4 h of post treatment. Serum was separated and glucose levels were determined.

Investigation of the effect of ABE on glucose tolerance

Twelve mice were fasted for 16 h (water was allowed *ad libitum*). Mice were randomly divided into 2 equal groups (n = 6 per group) and treated orally in the following manner: group 1 with 1 ml of DW and group 2 with 3000 mg/kg of ABE. One hour later, both groups were orally treated with 3 g/kg of glucose in 1 ml of DW (10). Blood samples were collected from the tails immediately prior to the treatment and at 1, 3 and 5 h after the glucose administration. Serum was separated and glucose levels were determined (11).

Investigation of the effect of ABE on Sucrose Tolerance

Twelve mice were fasted for 16 h (water was allowed *ad libitum*). Mice were randomly divided into 2 equal groups (n = 6 per group) and treated orally in the following manner: group1 with 1 ml of DW and group 2 with 3000 mg/kg of ABE. One hour later, both groups were orally treated with 2 g/kg of sucrose, in 1 ml of DW (13). Blood samples were collected from the tails immediately prior to the treatment and at 1, 3 and 5 h after the glucose administration. Serum was separated and the glucose levels were determined (11).

Investigation of chronic administration of ABE on fasting blood glucose level

Twelve mice were randomly divided into 2 equal groups (n = 6 per group) and treated orally in the following manner: group1 with 1 ml of DW per day and group 2 with 3000 mg/kg of ABE per day for 30 consecutive days. These mice were subsequently used to test the effect of ABE: on diaphragm uptake of glucose, on glycogen content of the liver and skeletal muscle and to investigate the possible side effects and toxicological effects of ABE. Once a week these mice were fasted for 16 h (water was allowed *ad libitum*). Blood samples were collected from tail 1 h prior to the treatment and 2 and 4 h of post treatment. Serum was separated and the glucose levels were determined (13).

Investigation of the effect of ABE on diaphragm uptake of glucose

The two groups of mice were taken (n = 6 per group) and treated orally in the following manner: group 1 with 1 ml of DW per day and group 2 with 3000 mg/kg

of ABE per day for 30 consecutive days. After 30 days, mice were sacrificed with chloroform; diaphragms were removed and washed with Tyrode ringer (14) immediately. Diaphragms were incubated at 37ºC (CO_2 incubator, Sanyo Electric Co. Ltd., Tokyo, Japan) in BSA (2%, W/V) supplemented Tyrode Ringer buffer containing 1 g/l glucose for 30 minutes after the incubation, each diaphragm was removed from the incubation medium, blotted off extra buffer and weighed. Aliquots (10 µl) of the incubation medium were removed and assayed for glucose concentration. 10 µl of the incubation medium was removed and assayed for the pre glucose concentration prior to the experiment. The glucose uptake by diaphragm was expressed as mg/dl (15).

Investigation of the effect of ABE on glycogen content of the liver and skeletal muscle

The two groups of mice were taken (n = 6 per group) and treated orally in the following manner: group 1 with 1 ml of DW per day and group 2 with 3000 mg/kg of ABE per day for 30 consecutive days. After 30 days, mice were chloroformed and portions of their livers and the gastronemous muscles were removed and the glycogen content was determined using a spectrophotometric method (16).

Investigation of the effect of ABE on lipid profile

The two groups of mice were taken (n = 6 per group) and treated orally in the following manner: group 1 with 1 ml of DW per day and group 2 with 3000 mg/kg of ABE per day for 30 consecutive days. After 30 days blood samples were collected from the tails and serum was separated. Total Cholesterol, HDL & LDL cholesterol, triglycerides concentration in the serum were determined using Randox kits and the spectrophotometer.

Investigation of the toxicological effects of ABE

The two groups of mice were taken (n = 6 per group) and treated orally in the following manner: group 1 with 1 ml of DW per day and group 2 with 3000 mg/kg of ABE per day for 30 consecutive days. During the treatment period the animals were observed daily for overt signs of clinical toxicity (diarrhoea, salivation, lachrymation, ataxia, convulsions, exopthalmia, loss of fur, change of fur colour, food and water intake, abnormal behaviour patterns and stress) within the first hour of treatment every day. In addition, the food and water intake and the body weights of these mice were determined weekly.

After 30 days the body weights of the mice were recorded and blood samples were collected from the tails and serum was separated. Creatinine, urea, SGOT and SGPT concentration in the serum were determined using Randox kits and a spectrophotometer.

Analysis of data

The data are represented as means ± SEM. Mann - Whitney U test was used for the statistical comparison (using Minitab 11 for Windows). A probability level of 0.05 or less was used.

RESULTS

Effect of ABE on fasting blood glucose level

As shown in Table 1 both the high dose (28%) and the mid dose (13.9%) of ABE reduced the fasting blood glucose level significantly ($P< 0.05$) at the 4 h after the administration. However, there was no significant effect at the 2 h after the treatment. On the other hand, lower dose had no significant effect on the blood glucose level. However, the effect was dose dependent ($P<0.05$, $r^2 = 0.91$). The reference drug (Glibenclamide) significantly, suppressed the blood glucose level by 23.5% ($p<0.05$) at the 2 h and by 28.8% ($p< 0.01$) at the 4 h after the administration.

Effect of ABE on random blood glucose level

The high dose of ABE did not significantly ($P> 0.05$) suppress the random blood glucose level in the serum at the 2 h and 4 h after the administration as compared with the control. (Random blood glucose level; control vs. treatment; 2 h after the treatment: 132.83 ± 3.39 vs. 129.50 ± 4.22 mg/dl; 4 h after the treatment: 133.00 ± 1.21 vs. 129.67 ± 4.86 mg/dl).

Effect of ABE on glucose tolerance

As shown in the Table 2 the high dose of ABE did not significantly ($P> 0.05$) suppress the increase of serum glucose level at the 1 h, 2 h and 3 h after the oral load of glucose as compared with the control.

Effect of ABE on sucrose tolerance

As shown in the Table 3 the high dose of ABE significantly ($P< 0.05$) suppressed the increase of serum glucose level by 13% at the 1 h after an oral load of glucose as compared with the control. However, there was no significant effect at the 3 h and the 5 h after the oral glucose challenge.

Effect of chronic administration of ABE on fasting blood glucose level

As shown in the Table 4 the chronic administration of high dose of ABE did not significantly ($P> 0.05$) reduce the fasting blood glucose level on the 1, 2, 3, 4 week during the treatment as compared with the control.

Effect of ABE on diaphragm uptake of glucose

The high dose of ABE did not significantly ($P> 0.05$) increase the uptake of glucose by the diaphragm as compared with the control. (Glucose uptake per gram of diaphragm; control vs. treatment; 60.5 ± 25.6 vs.

Table 1. The effect of oral administration of the aqueous stem bark extract of K. zeylanica on fasting blood glucose level

Treatment	Glucose concentration (mg/kg) (Means ± SEM, n = 6 per group)		
	Pre - treatment	Post - treatment	
		2h	4h
Control	85.77 ± 5.34	80.25 ± 6.46	81.17 ± 3.80
ABE			
3000 mg/kg	87.77 ± 5.54	76.20 ± 3.44	58.32 ± 7.50*
2500 mg/kg	90.78 ± 5.23	80.57 ± 4.51	69.83 ± 1.66*
1800 mg/kg	77.50 ± 4.72	70.43 ± 4.73	75.18 ± 6.54
Glibenclamide			
3mg/kg	82.75 ± 3.97	61.43 ± 3.96*	57.75 ± 5.00**

*As compared to control *p< 0.05, **p<0.01 ; ABE = aqueous stem bark extract*

Table 2. The effect of the aqueous stem bark extract of K. zeylanica on oral glucose tolerance test

	Glucose concentration (mg/kg) (Means ± SEM, n = 6 per group)			
		Post - treatment		
Treatment	Pre - treatment	1h	3h	5h
Control	93.50 ± 5.12	162.83 ± 8.48	134.7 ± 11.4	122.67 ± 9.73
ABE (3000 mg/kg)	92.50 ± 4.15	153.83 ± 3.52	125.17 ± 7.80	122.83 ± 6.37

ABE = aqueous stem bark extract

Table 3. The effect of the aqueous stem bark extract of K. zeylanica on oral sucrose tolerance test

	Glucose concentration (mg/kg) (Means ± SEM, n = 6 per group)			
Treatment	Pre - treatment	Post - treatment		
		1h	3h	5h
Control	97.00 ± 6.92	144.00 ± 6.72	104.00 ± 3.52	102.83 ± 7.15
ABE (3000 mg/kg)	93.67 ± 4.98	125.17 ± 8.34*	106.83 ± 7.73	103.7 ± 10.3

*As compared to control *p< 0.05 ; ABE = aqueous stem bark extract*

Table 4. The effect of the chronic administration of the ABE of K. zeylanica on fasting blood glucose level of mice

	Glucose concentration (mg/kg) (Means ± SEM, n = 6 per group)			
Treatment	1 Week	2 Week	3 Week	4 Week
Control	101.83 ± 7.15	90.42 ± 4.88	90.53 ± 3.60	85.88 ± 2.75
ABE (3000 mg/kg)	94.83 ± 6.91	89.82 ± 2.84	91.83 ± 4.98	83.73 ± 2.66

ABE = aqueous stem bark extract

Table 5. The effect of the chronic administration of the ABE of K. zeylanica on lipid profile of mice

	Cholesterol concentration (mg/dl) (Means ± SEM, n = 6 per group)		
Treatment	HDL cholesterol	LDL cholesterol	Total cholesterol
Control	65.67 ± 7.07	12.79 ± 1.2	106.6 ± 10.1
ABE (3000 mg/kg)	71.85 ± 9.44	14.39 ± 1.45	111.4 ± 11.3

ABE = aqueous stem bark extract

Table 6. The toxicological effects of the aqueous stem bark extract of K. zeylanica

		(Means ± SEM, n = 6 per group) # of weeks				
	Treatment	1	2	3	4	5
Food intake (g)	Control	0.066 ± 0.003	0.063 ± 0.005	0.054 ± 0.002	0.045 ± 0.006	0.042 ± 0.005
	ABE (3000 mg/kg)	0.060 ± 0.003	0.056 ± 0.004	0.045 ± 0.004	0.051 ± 0.003	0.048 ± 0.005
Water intake (ml)	Control	0.143 ± 0.007	0.11 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.12 ± 0.004
	ABE (3000 mg/kg)	0.128 ± 0.006	0.11 ± 0.01	0.09 ± 0.02	0.11 ± 0.01	0.13 ± 0.01
Body weight (g)	Control	33.25 ± 1.13	34.41 ± 0.94	35.01 ± 0.90	36.47 ± 1.08	36.76 ± 0.97
	ABE (3000 mg/kg)	35.25 ± 1.44	36.6 ± 1.24	37.37 ± 1.07	37.32 ± 1.20	37.83 ± 1.20

ABE = aqueous stem bark extract

Table 7. The toxicological effects of the aqueous stem bark extract of K. zeylanica on the kidney and liver functions

		(Means ± SEM, n = 6 per group)
Parameter	Treatment	
SGOT concentration	Control	15.58 ± 0.95
(U/L)	ABE (3000 mg/kg)	21.25 ± 4.35
SGPT concentration	Control	10.50 ± 1.63
(U/L)	ABE (3000 mg/kg)	11.17 ± 1.38
Creatinine concentration	Control	1.167 ± 0.519
(mg/dl)	ABE (3000 mg/kg)	1.125 ± 0.437
Urea Concentration	Control	20.5 ± 4.78
(mg/dl)	ABE (3000 mg/kg)	19.98 ± 2.08

ABE = aqueous stem bark extract

65.7 ± 19.0 mg/dl).

Effect of ABE on glycogen content of the liver and skeletal muscles

The high dose of ABE did not significantly (P> 0.05) increase the glycogen content both in the liver and the skeletal muscle as compared with the control. (Glycogen content in the liver; control vs. treatment; 1.30 ± 0.05 vs. 1.41 ± 0.05 mg per 100 g of liver and glycogen content of the skeletal muscle; control vs. treatment; 1.31 ± 0.07 vs. 1.37 ± 0.78 mg per 100 g of liver).

Effects of ABE on lipid profile

The results obtained for the possible side effects are summarized in Table 5. As shown, the treatment with the high dose of ABE did not significantly (P> 0.05) change the concentration of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides in the serum as compared with the control.

Toxicological effects of ABE

Oral treatment of high dose of ABE for consecutive 30 days did not induce any overt signs of clinical toxicity or stress in acute or chronic terms. As shown in Table 6, the treatment with the high dose of ABE did not significantly (P> 0.05) affect the food and water intake, body weight as compared with the control. As shown in Table 7, the treatment with the high dose of ABE did not significantly (P>0.05) change the concentration of SGOT, SGPT, creatinine and urea as compared with the control.

DISCUSSION

This study examined the blood glucose lowering potential of ABE of *K. zeylanica*, the endemic plant, in Sri Lanka using a mouse model. The results show that ABE possesses an acute, moderate, oral hypoglycaemic activity (in normoglycaemic fasted mice). This hypoglycaemic effect had a fairly rapid onset (within 4 h) and was dose dependant, indicating a genuine hypoglycaemic action mediated via phytoconstituent/s present in the extract. Terpenoids and alkaloids have been shown to reduce blood glucose level (17) and these phytoconstituents are present in ABE, possibly indicating that ABE induce hypoglycaemia is mediated via these constituents. The hypoglycaemic ativity of ABE was comparable to glibenclamide at the 4th hour, however, glibenclamide produce hypoglycaemia also at the 2nd hour after the treatment.

However, ABE did not induce hypoglycaemia in

normoglycaemic non-fasted mice. A similar result has been reported with the stem bark extract of *Cassia fistula* (11). Further, ABE did not have an antihyperglycaemic activity (in terms of oral glucose tolerance test). Presence of hypoglycaemic activity without antihyperglycaemic activity as seen in this study, is also reported with *Salacia reticulata* (18).

The ABE did not impair food intake, and it also did not promote physical activity of treated mice such as locomotary behaviour, rarerings or auto grooming. Therefore, these cannot account for blood glucose lowering activity of ABE.

An increase in glycogen synthesis can reduce blood glucose level (19). However, this mode of action is unlikely to be operative with ABE as it failed to increase glycogen content in the liver and skeletal muscles. The increase in entry of glucose into tissues can produce hypoglycaemia (19) but this mechanism is unlikely, as ABE did not increase the intake of glucose into the diaphragm. This may be due to the inability of ABE to increase insulin release from pancreas and/or due to the lack of insulinomimic activity as evident from the glucose tolerance test: ABE failed to inhibit the rise in blood glucose concentration following an oral glucose challenge.

The ABE inhibited the rise in blood glucose level following an oral sucrose challenge. This indicates that ABE reduced blood glucose level by inhibiting intestinal α-glucosidase activity, as some plant extracts such as *Salacia reticulata* (18) and synthetic drugs like voglibose (20). Further, ABE induce expulsion of soft stools. Production of soft stools is reported as a side effect of α-glucosidase inhibitors (21). This appears to be the main mechanism by which ABE induces hyperglycaemia. Possibility also exists that ABE reduce blood glucose level by impairing intestinal glucose absorption, as the extract was fairly viscous. Several viscous plant extracts are claimed to inhibit intestinal glucose absorption by fiber effect (22, 23).

The ABE was well tolerated even with chronic administration and there were no overt signs of toxicity, hepatotoxicity (in terms of SGOT and SGPT levels or alteration in liver weights), or renotoxicity (as judged by serum urea and creatinine levels). Further, there were no adverse effects on food and water intake, or body weight. In addition, ABE did not affect the lipid profile (serum triglycerides and total cholesterol, HDL and LDL cholesterols).

In conclusion this study scientifically shows for the first time safe, oral hypoglycaemic potential of the stem bark extract of *K. zeylanica* – endemic plant of Sri Lanka. The results also provide support for the traditional claim that *K. zeylanica* stem bark has blood glucose lowering properties.

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