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Blood Glucose Level Lowering Activity of Sri Lankan Black Tea brew (*Camellia sinensis*) in rats

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ABSTRACT

This study examined the blood glucose level lowering ability of black tea brew (BTB) of Sri Lankan *Camellia sinensis* (L.) O.Kuntze (Family*: Theaceae)*. This was tested in normoglycaemic and streptozotocin induced diabetic rats using high grown Dust grade No: 1 tea (doses used: 84 mg /ml, equivalent to 1.5 cups; 168 mg/ml, equivalent to 3 cups; and 501 mg/ml, equivalent to 9 cups, orally). The results show that BTB possess promising and significant ($P < 0.05$) hypoglycaemic (both in normoglycaemic fasted rats and non fasted rats), antihyperglycaemic (in terms of improved glucose tolerance test), and antidiabetic (when tested in streptozotocin induced diabetic rats) activities. BTB (only highest dose tested) also exhibited α-glucosidase inhibitory activity (in terms of sucrose tolerance test) inhibited glucose absorption from the lumen of small intestine and possessed marked antioxident activity *in vitro* (in terms of DPPH assay). On the other hand, the highest dose failed to increase insulin output and glycogen content in liver and skeletal muscle. It is concluded that BTB made from Sri Lankan black tea has blood glucose lowering activity in both normoglycaemic and diabetic status.

KEY WORDS- *Camellia sinensis*, antidiabetic, hypoglycaemia, antihyperglycaemia, diabetes, black tea

INTRODUCTION

Today diabetes mellitus is a common disease. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025 (1). In the allopathic system of medicine, there are five classes of oral drugs, namely, sulphonylurea, repaglinide, biguanides, thiazolidinediones and α-glucosidase inhibitors used in the treatment of diabetes mellitus (2). Although, these drugs are highly effective they are expensive and posses adverse side effects (2). On the other hand, in Ayurvedic and other traditional systems of medicine there are several relatively inexpensive herbs that are claimed to have safe antidiabetic potentials (3, 4). However, efficacy of many of these have not been scientifically proven. In addition, there are few other herbs which are claimed by folklore as having antidiabetic properties but not mentioned in Ayurvedic medicine. Black tea brew (BTB) and green tea brew of *Camellia sinensis* (Family: *Theaceae)* is one of these. There are several studies showing antidiabetic potential of green tea brews of *C. sinensis* (5, 6) but scientific studies on this aspect of black tea brew are limited (7, 8). No studies have been reported so far on blood sugar lowering properties of BTB made from Sri Lankan teas. It is important to investigate this in BTB made from Sri Lankan tea since final chemical composition of tea brew and hence its pharmacological properties of tea brew is known to vary with several factors such as country of origin, geographical background of soil, the cultivating method, the collection season, the age of the leaves, grades of tea, brewing conditions of time and temperature (5, 9). Further, some scientists have emphasized that more research on blood sugar lowering properties of tea are needed before its antidiabetic potentials are confirmed (6).

The aim of this study was therefore to investigate the blood glucose lowering activity of black tea brew made from Sri Lankan teas. This was examined in rats using Sri Lankan high grown Dust grade No:1 tea which is drunk widely by the Sri Lankans. If potent activity is found it would be a cheap radily available orally active herbal source that could be beneficial in diabetes mellitus.

MATERIALS AND METHODS

Experimental Animals

Healthy, adult male Wistar rats weighing 200-225 g purchased from the Medical Research Institute, Colombo, Sri Lanka were used in this study. The animals were housed in plastic cages in the animal house, Department of Zoology, University of Colombo, under standardized conditions (temperature: 28-31 ^{o}C, photoperiod: approximately 12 h natural light per day, relative humidity: 50-55%). The animals were fed with

pelleted food (Ceylon Grain Elevators, Colombo, Sri Lanka) and clear drinking water *ad libitum*. Except at the time of experimental procedure the animals were handle only during cage cleaning. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines and rules of the Faculty of Science, University of Colombo, Sri Lanka, for animal experimentations.

Source of Tea

Two or three topmost immature leaves and buds of *C. sinensis* plucked from the plantation of St. Coomes tea estate of the Tea Research Institute, Talawakelle, Sri Lanka (1382m above sea level; high grown) in August 2005, were used to process Dust grade No: 1 black tea by orthodox – rotorvare technique at the estate factory. The tea samples were packed in triple laminated aluminium foil bags (1kg each) and stored at –20 ^{0}C until use. Further, these tea samples selected was pure, unblend and typical to the grade as confirmed by sieve analysis, organoleptic profile and physical and chemical analysis.

Preparation of Black Tea Brew (BTB)

BTB was made by adding 2 g of tea sample to 100 ml boiling water and brewed for 5 min (10). [Yield 43.7 % (w/w)] for oral treatment to rats. Four doses of BTB (84 mg/ml, equivalent 1.5 cups; 167 mg/ml, equivalent to 3 cups; 501 mg/ml and equivalent to 9 cups.) were made in 2 ml of water. The volume of 1 cup is considered to be 170 ml.

Estimation of crude fiber content

Crude fiber content of the tea sample (n = 3) was determined as described by the International Standard Organization (ISO), Switzerland (11). The results were expressed as a % (w/w).

Effect of BTB on fasting blood glucose level

Thirty rats were fasted overnight for 16 h, but water was allowed *ad libitum*. Using aseptic precautions, under light ether anaesthesia, 0.5 ml of blood was collected from their tails. Immediately afterwards, these rats were randomly divided into 5 groups and treated orally in the following manner: 1 (1 ml of distilled water (DW), n = 6), 2, (501 mg/ml of BTB, n = 6), 3 (167 mg/ml of BTB, n=6) and 4 (84 mg/ml of BTB, n = 6) and 5 (Tolbutamide, 22.5 mg / kg, n = 6). Blood samples were collected from the tail of the rats immediately prior to treatment and at hourly intervals for 4 h. Serum was separated and glucose concentration determined using glucose oxidase assay kit (Randox Laboratories, Antrium, UK) and a spectrophotometer (V 500, Jasco Cooperation, Tokyo, Japan) (12).

Effect of BTB on random blood glucose level

Twelve rats were randomly divided into two equal groups. Group 1 (n = 6) was orally treated with 1 ml of DW and group 2 (n = 6) with the 501mg/ml of BTB. Blood samples were collected from tails 1h prior to treatment and at hourly intervals for 4 h. Serum was separated and glucose levels were determined as before (12).

Effect of BTB on oral glucose tolerance

To ascertain the effects of BTB during acute glycaemia, a glucose tolerance test was performed. Thirty rats were fasted for 16 h and randomly assigned into 5 groups. These rats were then orally treated in the following manner: 1 (1 ml of DW, n = 6), 2 (501 mg/ml of BTB, n = 6), 3 (167 mg/ml of BTB, n = 6), 4 (84 mg/ml of BTB, n = 6), 5 (22.5 mg/ml of tolbutamide, n = 6). One hour later, all these rats were orally loaded with 5 ml/kg of 50% (w/v) glucose solution. Blood samples were collected from the tails of these rats immediately prior to treatment and at hourly intervals for 4 h after glucose administration. Serum was separated and glucose concentration determined as before (13).

Effect of BTB on oral sucrose tolerance

Twelve rats were fasted for 16 h and were randomly divided into two equal groups (n = 6 / group). One group was orally administered with 1 ml of DW and the other with the 501 mg/ml of BTB. One hour later, these rats were orally treated with sucrose solution (2 g/ kg). Samples of blood were collected from the tails of these rats immediately prior to treatment and at hourly intervals for 4 h after sucrose administration. Serum was separated and glucose concentration determined as before (13).

Effect of the BTB on blood glucose levels of streptozotocin-induced diabetic rats

Streptozotocin (STZ) (Wako Chemicals, New York, USA) was dissolved in 0.1M cold citrate buffer (PH = 4.5), Immediately afterwards, 60 mg/kg dose of STZ was injected into the tail vain under mild ether anesthesia with aseptic precautions. Seventy two hours later, blood samples were collected from the tails of these rats and glucose levels were determined. Twelve rats having blood glucose level > 150 mg/ml and showing polydipsia and polyuria were selected. These rats were assigned randomly into two equal groups (n = 6/group). One group was orally treated with the 501 mg/ml of BTB and other with 1 ml DW daily for 14 consecutive days. Blood samples were then collected from tails of

these rats and serum glucose levels were determined on days 1, 7 and 14 of treatment and day 7 post treatment (13).

Effect of BTB on glucose absorption from intestine

Twelve rats were fasted for 16 h and divided equally into two random groups (n = 6 / group). Then 501 mg/ml of BTB was orally administered to one group and 1 ml of DW to the other group. Thirty minutes later, 10 ml/kg of 50% glucose solution was given orally. Following 2 h, these animals were sacrificed and their small intestines were exposed. 50 ml of DW was then infused from one cut end of the intestine and the content was collected at the other end. This was centrifuged at 3000 rpm for 5 min and glucose content of the supernatant was then estimated using Randox kit and spectrophotometer (13).

Effect of BTB on liver and skeletal muscle glycogen content

Twelve rats were assigned into two equal groups. One group was orally administrated daily with the 501 mg/ml of BTB and other with 1 ml of DW for 30 consecutive days. On day 1 post treatment, these rats were etherized and portions of their livers and gastrocnemius muscles were removed and blotted free of blood. Glycogen content was determined using a spectrophotometer as described in detail by Borst et.al. (14). General food intake, locomotory behaviours, rearing and autogrooming activities were observed daily upto 2h following BTB administration.

Effect of BTB on insulin level in blood

Twelve rats were randomly divided into two equal groups. Group 1 (n = 6) was orally treated with 1 ml of DW and group 2 (n = 6) with the 501 mg/ml of BTB. One hour later, blood samples were collected from their tails. Serum was separated and insulin levels were determined (15).

Estimation of in vitro antioxident activity of BTB

This was done using 750 µl of freshly prepared 1-1-diphenyl-2-picrylbhydrazyl (DPPH) solution. Briefly, three concentrations of BTB (84,167 and 501 mg/ml) were made and 750 µl of these samples (n = 6) were added to equal volume of DPPH and incubated at 30^0 C for 5 min. Absorbance was then measured at 517 nm using a spectrophotometer. The percentage of the DPPH radical scavenged by BTB was calculated and the antioxident activity was expressed as Trolox in µg / litre (16).

Statistical analysis

The data were expressed as the mean ± SEM. Statistical analysis was performed using Mann-Whitney t-test. Significance was set at $P \leq 0.05$. Linear regression analysis was performed to assess dose-dependencies.

RESULTS

Estimation of crude fiber content

The percentage of crude fiber content of the tea sample was 9.20 ±0.04%.

Effects on fasting blood glucose level

The results are summerized in Table1. As shown, in normoglycaemic fasted rats the low dose of BTB did not significantly ($P > 0.05$) reduced the blood glucose level. On the other hand, both the mid dose (1^{st} h by 17%; 2^{nd} h by 17% and 3^{rd} h by 11%) and the high dose (1^{st} h by30%; 2^{nd} h by 10% and 3^{rd} h by 12%) significantly ($p < 0.05$) impaired the blood glucose level upto 3 h.

Effect on random blood glucose level

As shown in Table 2, the highest dose of BTB significantly reduced the random blood glucose level only at 1h (by 11%).

Effect on oral glucose tolerance test

As shown in Table 3, all three doses of BTB significantly ($P < 0.05$) reduced the increase in blood glucose level provoked by the glucose challenge upto 3 h: low dose (1^{st} h by 34%; 2^{nd} h by 30% and 3^{rd} h by 28%); mid dose (1^{st} h by 36%, 2^{nd} h by 32% and 3^{rd} h by 26%) and high dose (1^{st} h by 44%; 2^{nd} h by 23% and 3^{rd} h by 25%). In contrast, tolbutamide was effective throughout the 4 h period: (1 h by 37.5 %; 2h by 31.5 %; 3h by 25.5% and 4h by 20%).

Effect on oral sucrose tolerance

As shown in Table 4, the high dose of BTB significantly ($P < 0.05$) impaired the rise in blood glucose level up to 3 h following sucrose challenge. 1h (by 40 %; 2h by 35 % and 3h by 26 %).

Effect on blood glucose level of streptozotocin diabetic rats

As shown in Table 5, 501 mg/ml of BTB significantly ($P <0.05$) impaired the fasting blood glucose level of streptozotocin diabetic rats on days 1 (by 21 %), 7 (by 20 %) and 14 (by 24 %) of treatment.

Effect on glucose absorption from intestine

As shown in Table 6, 501 mg/ml of BTB markedly (by 75%) and significantly ($P < 0.05$) inhibited the glucose absorption from the luman of the intestine.

Effect on glycogen content in liver and skeletal muscle

Chronic treatment of 501 mg/ml of BTB did not significantly ($P > 0.05$) alter the glycogen content of both liver (control vs treatment: 166.01 ± 0.65 vs 158.61 ± 4.83 µg / 100mg) and gastrocnemius muscle (control vs treatment: 174.05 ± 1.24 vs ± 168.23 ± 1.48

µg/100 mg). Further, there was no apparent reduction in food intake and increase in locomotory activity, rearings and autogrooming activity.

Effect of BTB on insulin level

The 501 mg/ml of BTB failed to increase blood insulin level of normoglycaemic rats. (insulin level in both control and treated groups were less than < 2 microIU / ml of blood).

Estimation of in vitro antioxident activity of BTB

The antioxident activity of BTB samples is given in Table 7. As shown, BTB exhibited dose-dependent (r^2 = 0.83, P < 0.05) antioxident activity *in vitro*.

DISCUSSION

This study examined the blood glucose lowering potential of BTB of *Camellia sinensis* made from Sri Lankan high grown Dust grade No:1 black tea using rats. The results show that the BTB possess promising hypoglycaemic (both in normoglycaemic fasted and non fasted rats.), antihyperglycaemic (in terms of improvement of glucose tolerance test) and antidiabetic (when tested in streptozotocine induced diabetic rats) activities. This is an important finding with wide health implications since today tea is the second most consumed beverage in the world (5). Further, this finding also scientifically justifies the claim of Sri Lankan folklore that black tea is beneficial for diabetic patients. The blood glucose lowering activity of BTB was rapid: hypoglyceamic (within 1 h), antihyperglycaemic (within 1 h) and antidiabetic (within 1 day). Further, the hypoglycaemic and antihyperglycaemic effects of BTB were dose - dependent suggesting a genuine effect which is possibly receptor mediated. It is noteworthy that due to high price of streptozotocin dose-dependent studies on antidiabetic effects were not undertaken which is a limitation of this investigation. Simultaneous possession of hypoglycaemic, antihyperglycaemic and antidiabetic activities by BTB is an interesting feature since not many herbal extracts used in treatment of diabetes have all these three activities together (4).

BTB did not impair food intake. It also did not promote physical activity of the treated rats such as locomotory behaviour, rearings or autogrooming. Therefore, these cannot account for blood glucose lowering activity of BTB. An increase in glycogen synthesis can reduce blood glucose level (17). However, this mode of action is unlikely to be operative with BTB as it failed to increase glycogen content of liver and skeletal muscles. BTB impaired the blood glucose level in streptozotocin-induced diabetic rats. Phenolic structures usually have antidiabetic potentials (5). Black tea is rich in flavonoids such as catechins, theaflavins, thearubigins and flavonols (5,9) which can account for the antidiabetic action. Insulin is secreted by the ß cells of the pancreas and streptozotocin at the dosage used in this study irreversibly destroys the ß cells (18). This suggest that BTB excerts its blood glucose lowering activity not directly through insulin but via other extrapancreatic mechanisms. This is further supported by the fact that BTB did not increase serum insulin level following its administration. A similar results have been reported with oolong tea using sterptozotocin induced diabetic and Kk-Ay diabetic mice (5). On the other hand, BTB caused a marked improvement in the glucose tolerance test as reported with bio tea (8). This suggest that, BTB has insulinomimetic activity. Interestingly, it is claimed that epicatachin in tea is able to mimic insulin (19). In complete contrast, BTB could have impaired glucogon release to reduce the blood glucose level but we have no experimental evidence to support this notion.

BTB inhibited the rise in blood glucose level following an oral sucrose challenge. This indicates that BTB reduced blood glucose level by inhibiting intestinal α-glucosidase activity (20) as some antidiabetic herbal extracts such as *Salacia reticulata* (21) and synthetic drugs such as acabose (2). In this regard, it is of interest to note that catechin in tea is shown to inhibit α-amylase activity in human small intestine (22) and some polysaccharides and polyphenols in black tea to impair amylase, sucrase and maltase activities in the small intestine of rats (23). Thus, this is likely to be one of the main mechanisms of reducing blood glucose level in this study.

BTB caused a profound impairment of glucose absorption from the lumen of the small intestine. This blocking activity can be implicated with its blood glucose lowering action. BTB contain crude fiber and fiber is known to inhibit intestinal glucose absorption (4): so called 'fiber effect' (4). Interestingly, green tea is also shown to inhibit intestinal glucose absorption in rats (6). Alternatively, BTB could inhibit intestinal glucose absorption by impairing Na^+-glucose cotransporter as reported with green tea polyphenols in rabbit intestinal epithelial cells (24).

Oxygen free radicals are now known to be involved in the pathogenesis of diabetes mellitus (25) and antioxidents have therapeutic value in alleviating this condition (25). The BTB had marked antioxident activity i*n vitro* as is shown by others (16) which may be attributed to its flavonoides (5, 9). Thus, it is

Table 1. Effect of Black tea brew (BTB) on blood glucose levels of fasted rats

Treatment	*Dose (mg/ml)*	*Glucose Concentration (mg/dl)*				
		Pre-treatment	*1 h*	*2 h*	*3h*	*4 h*
Control	DW	91.42 ± 1.40	86.28 ± 1.46	81.05 ± 1.07	83.15 ± 1.27	79.98 ± 0.75
BTB	501	84.22 ± 1.36	60.64 ± 0.73*	77.51 ± 2.37*	73.68 ± 1.94*	83.06 ± 1.71
	167	84.30 ± 1.90	71.68 ± 2.15*	67.28 ± 1.80*	74.08 ± 1.32*	82.27 ± 1.47
	84	93.08 ± 1.68	82.95 ± 1.90	73.39 ± 1.33	78.15 ± 1.47	78.31 ± 1.31
Tolbutamide	22.5 mg/kg	85.11 ± 1.25	47.85 ± 1.10*	62.15 ± 0.88*	64.07 ± 1.03*	73.80 ± 2.30

*As compared to control *P < 0.05, DW = Distilled water (means ± SEM, n = 6)*

Table 2. Effect of Black tea brew (BTB) on random blood glucose levels of rats

Dose (mg/ml)	*Glucose Concentration (mg/dl)*				
	Pre treatment	*Treatment*			
		1 h	*2 h*	*3 h*	*4 h*
Control (DW)	131.29 ± 2.76	134.70 ± 3.31	129.98 ± 2.34	130.28 ± 2.26	125.57 ± 3.75
BTB (501)	139.28 ± 5.58	120.72 ± 3.20*	134.05 ± 2.56	134.76 ± 4.18	131.80 ± 3.50

*As compared to control *P < 0.05, DW = Distilled water (means ± SEM, n = 6)*

Table 3. Effect of Black tea brew (BTB) on oral glucose tolerance test

Dose (mg/ml)	*Glucose Concentration (mg/dl)*				
	Pre treatment	*Treatment*			
		1 h	*2 h*	*3 h*	*4 h*
Control (DW)	83.80 ± 1.30	113.01 ± 1.86	101.14 ± 0.79	99.04 ± 2.14	89.62 ± 2.40
BTB 501	78.33 ± 1.22	63.01 ± 1.22*	78.31 ± 1.25*	74.14 ± 1.60*	84.92 ± 1.52
167	80.12 ± 1.75	72.13 ±1.62*	69.40 ± 1.72*	73.81 ± 1.70*	87.96 ± 1.95
84	74.45 ± 1.27	74.32 ± 1.50*	71.2 ± 0.78*	71.60 ± 1.46*	85.02 ± 1.65
Tolbutamide (22.5 mg/kg)	85.90 ± 0.65	70.62 ± 0.59*	69.28 ± 1.20*	73.72 ± 1.05*	71.48 ± 1.24*

As compared to control *P < 0.05, DW = Distilled water *(means ± SEM, n = 6)*

Table 4. Effect of Black tea brew (BTB) on oral sucrose tolerance test.

Dose (mg/ml)	*Glucose Concentration (mg/dl)*				
	Pre treatment	*Treatment*			
		1 h	*2 h*	*3 h*	*4 h*
Control (DW)	86.64 ± 1.63	122.05 ± 3.96	105.30 ± 1.83	95.35 ± 2.20	76.97 ± 1.91
BTB (501)	82.38 ± 1.56	73.55 ± 0.78*	68.62 ± 1.38*	70.45 ± 1.38*	77.84 ± 1.83

As compared to control *P < 0.05, DW = Distilled water *(means ± SEM, n = 6)*

Table 5. Effect of Black tea brew (BTB) on blood glucose level of streptozotocin-induced diabetic rats.

Dose (mg/ml)	*Glucose Concentration (mg/dl)*				
	Pre treatment	*Treatment*		*Post treatment*	
		Day 1	*Day 7*	*Day 7*	*Day 14*
Control (DW)	164.91 ± 3.04	169.30 ± 1.90	165.46 ± 4.17	157.47 ± 1.13	153.97 ± 3.99
BTB (501)	167.65 ± 5.12	133.81 ± 7.87*	132.08 ± 6.40*	119.16 ± 3.57*	159.75 ± 4.31

*As compared to control *P < 0.05, DW = Distilled water (means ± SEM, n = 6)*

Table 6. Effect of Black tea brew (BTB) on glucose absorption from intestine of rats

Dose mg/ml	*Glucose Concentration (mg/dl)*
Control (DW)	8.47 ± 0.48
BTB (501)	14.78 ± 0.29*

*As compared to control *P < 0.05, DW = Distilled water (means ± SEM, n = 6)*

Table 7. In vitro antioxidant activity of Black tea brew as determined by DPPH assays

Dose mg/ml	Antioxident activity (Trolox equivalents µg/L)
501	2685 ± 6.0
167	3572 ± 86.52
84	3923 ± 6.5

(means ± SEM)

possible that this antioxident activity of BTB contributes to its antidiabetic action.

Increase in lipogenesis can reduce blood glucose level (17). Flavonol, myricetin present in black tea is known to stimulate lipogenesis and glucose uptake through cell membranes in rat adipocytes *in vitro* (26). Such an action of BTB could contribute to the reduction of blood glucose level in this study. Furthermore, black tea is shown to stimulate glucose metabolism in cells (5, 27) and this mode of action is also likely to be operative in this study in reducing blood glucose level. Further, in present study, the possibility that BTB may reduced renal glucose resorption (17) and improve insulin sensitivity at receptor level as proposed for some sulponylureas (2) and plant extracts (28) cannot be completely ruled out.

In conclusion, this study for the first time, shows the hypoglycaemic, antihyperglycaemic and antidiabetic potentials of BTB made from Sri Lankan black tea. Further, it adds strength to the claim made by Sri Lankan folklore that BTB of *Camellia sinensis* is beneficial for diabetes mellitus.

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