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GLYCAEMIC REGULATORY PROPERTIES OF SRI LANKAN LOW GROWN ORTHODOX ORANGE PEKOE BLACK TEA (*Camellia sinensis*)

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ABSTRACT

This study examined the glycaemia regulatory potential of Sri Lankan low grown orthodox Orange Pekoe (O.P.) grade black tea brew (BTB) which was made according to ISO 3103 specifications. Different doses of BTB [223 (equivalent to 1.5 cups), 446 (equivalent to 3 cups) and 1339 mg /kg (equivalent to 9 cups)]were orally administered to normoglycaemic rats (N= 9-12/group) and their fasting, random and post parandial (after glucose and sucrose challenges) blood glucose levels were determined using standard procedures. The effect of BTB on amylase (N=3) and glucosidase activities (N=6) were also assessed spectrophotometrically *in vitro*. The results showed that, BTB impaired the rise in blood glucose level following both oral glucose and sucrose challenges. *In vitro* studies showed that BTB possess mild (but dose-dependent) antiamylase activity but no anti-glucosidase activity. It is concluded that Sri Lankan low grown orthodox O.P. grade black tea has the potential to be used as a beverage to regulate glycaemia /dysglycaemia by suppressing post parandial hyperglycaemia.

Keywords: Black tea, Orange pekoe, *Camellia sinensis*, Glycaemia, Dysglycaemia, Hyperglycaemia.

INTRODUCTION

Globally, diabetes mellitus is one of the biggest public health problems placing a substantial burden on healthcare services [1]. It has already reached pandemic proportions: over 300 million people worldwide have diabetes and this number probably would rise to 500 million within a generation [1]. Recent estimates suggest that the prevalence of diabetes is rising sharply in developing countries in South Asia/Indian subcontinent (which is home to almost one quarter of world’s population) including Sri Lanka [2]. Studies have also shown that progression of diabetes is more aggressive in South Asians in comparison to other ethnic groups [2]. Surprisingly, in Sri Lanka, an estimated 20% of all adults are suffering from dysglycaemia and 11% from type 2 diabetes [3].

In the western medicine, there are several drugs available for the treatment of diabetes which exert there therapeutic effects by different mechanisms [4]. On the whole, these allopathic drugs are efficacious but produce undesirable side effects [4]. Also, some of these drugs are expensive and out of reach for many people living in developing countries [1]. Further, adequate low temperature storage facilities are limited in many developing countries [1]. Hence, the search for new, highly active, cheap therapeutics which are heat resistant and patient friendly continues to slow down the progression of diabetes [1]. Some of these studies focus on plant based materials which are already used as anti-diabetics in Ayurveda, traditional and folk medicines [5]. Interestingly, more than 800 plants in the Indian subcontinent alone are claimed to possess anti-diabetic properties, although, in many, their alleged effect is not supported by solid scientific evidence and have not been characterized for their mechanism of action [5].

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In this context, we started a program of research to investigate glycaemia regulating potentials of Sri Lankan black tea, which is manufactured from fresh 2-3 top most tender leaves and unopened buds of evergreen plants of *Camellia sinensis* (L.) O. Kuntze (Family: Theaceae): since Sri Lankan traditional medical practitioners often recommend heavy daily consumption (6-10 cups) fresh and warm black tea brew (BTB) as a beverage for pre diabetics and mild diabetics [6]: it is sugar and preservative free [7]; is the most consumed beverage besides water [7]; and accounts for 80% of global tea consumption [7]. As of now, we have examined dysglycaemia regulatory abilities and anti-diabetic potential of two grades of Sri Lankan orthodox black tea, namely, Dust No.1 (low grown) [8] and Broken Orange Pekoe Fannings (BOPF) (low, mid and high grown) [9]. Parenthetically, there are three agro-climatic elevations in Sri Lanka, low (up to 600m above average mean sea level: amsl), mid (between 600-1200m, amsl) and high (above 1200m, amsl) [10].

The aim of this study was to assess the glycaemia regulatory potential of Sri Lankan low grown orthodox Orange Pekoe (O.P.) grade of black tea using both *in vivo* and *in vitro* techniques. Such a study is extremely valuable since it is well recognized that pharmaco-therapeutic potential of BTB defers with many factors including agro-climatic elevation and particle size (grade of tea) [10,11]. O.P. grade tea is a whole leaf variety, in contrast to Dust No.1 and BOPF, which are broken leaf varieties [12].

MATERIALS AND METHODS

Source of tea

Top most immature leaves and buds of *C. sinensi s*plucked from the plantation of St. Jochims tea estate of the Tea Research Institute, Hedallana, Ratnapura, Sri Lanka (29 m above mean sea level: low grown) (Latitude $6^0$42"57.96" Longitude $80^0$22" 46.2") during November – December 2011 were used to process O.P. grade black tea by orthodox-rotovane technique at the estate factory. The sieve analysis of the sample has shown that 83.5% of tea particles were true size (1400-2000 µm) and typical for the grade [13]. Moreover, orgenoleptic profile analysis conducted by the professional tea tasters at the Tea testing unit of Sri Lanka Tea Board has confirmed that the sample used can be accepted as well made high quality O.P grade Sri Lankan black tea [13]. Tea samples were packed in triple laminated aluminium foil bags (1 kg each) and stored at -20^0C until use.

Experimental Animals

Healthy adult Wistar rats (200-225g) purchased from the Medical Research Institute, Colombo, Sri Lanka were used. They were kept under standardized animal house conditions (temperature: 28-31^0C, photoperiod: approximately 12 h natural light per day, relative humidity: 50-55%) at the animal house of the Department of Zoology, University of Colombo. All the animals were acclimatized for 14 days prior to the experiment. The rats had free access to pelleted food (CIC Feed Pvt. Ltd., Ekala, Sri Lanka) and domestic tap water. The experiments were conducted in accordance with the internationally accepted laboratory animal use and care guidelines and rules of the Faculty of Science, University of Colombo, for animal experimentation. Ethical clearance was obtained from Institute of Biology, Sri Lanka (ERC I0BSL 109/11/13).

Preparation of black tea brew (BTB)

BTB was made according to the ISO standards (ISO 3103); adding 2g of O.P. grade black tea to 100 ml of boiling water and brewed for 5 min [14]. This contained 36.1% (w/v) tea solids in water. For *in vivo* studies, 3 different doses were appropriately made: 1339 mg/kg (equivalent to 9 cups, 1 cup = 150 ml) of BTB in 3 ml of water was prepared by adding 10 g of O.P. grade black tea to 30 ml boiling water and brewing for 5 min. Then, 446 mg/kg (equivalent to 3 cups) and 223 mg/kg (equivalent to 1.5 cups) doses of BTB were prepared by diluting appropriately with boiling water. For *in vitro* studies BTB was freeze dried.

Evaluation of the effect of BTB of O.P. grade black tea on fasting blood glucose level

Forty two rats were fasted overnight for 16 h, but water was allowed *ad libitum*. Using aseptic precautions, under light ether anesthesia, 50 µl 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 (3 ml of distilled water, N=9), 2 (223 mg/kg of BTB, N=9), 3 (446 mg/kg of BTB, N=9), 4 (1339 mg/kg of BTB, N=9) and 5 (Tolbutamide, 22.5 mg/kg, N= 6). Blood samples were collected from the tails of the rats immediately prior to treatment and at hourly intervals for 4 h and fasting blood glucose levels of rats were determined [8] using a glucometer (Accu-check Active, Roche, Mannheim, Germany).

Evaluation of the effect of BTB of O.P. grade black tea on random blood glucose level

Thirty rats were randomly divided into 5 groups and their pre treatment blood glucose levels were determined using a glucometer (Accu-check Active, Roche, Mannheim, Germany). Then, different doses of O.P. tea or distilled water (control) were orally administered as follows (N = 6 / group); Group 1: 3 ml of water, Group 2: 223 mg/kg dose of O.P. tea, Group 3: 446 mg/kg dose of O.P. tea, Group 4: 1339 mg/kg dose of O.P. tea and Group 5: 22.5 mg/kg dose of Tolbutamide. Using aseptic precautions, under light ether anesthesia, 50 µl of blood was collected from the tails of these rats, at hourly intervals, for 4 h and their blood glucose levels were determined using the glucometer [8].

Evaluation of the effect of OP Grade black tea on the oral glucose tolerance test

Fifty rats were fasted for 16 h and their pretreatment blood glucose levels were determined using a glucometer. The rats that had fasting blood glucose level in between 70 – 120 mg/dl were selected and they were randomly divided in to 5 groups. Afterwards, O.P. grade black tea or water or Tolbutamide were orally administered as follows; Group 1: 3 ml of water (N = 10), Group 2: 223 mg/kg dose of O.P. tea (N = 11), Group 3: 446 mg/kg dose of O.P. tea (N = 12), Group 4: 1339 mg/kg dose of O.P. tea (N = 11) and Group 5: 22.5 mg/kg dose of Tolbutamide (N = 6). One hour later, all the rats were orally administered with 5 ml/kg b.w. of 50% (w/v) glucose (BDH Chemicals Limited, Poole, England) solution. Blood samples were obtained using aseptic precautions under light ether anesthesia and their blood glucose levels were determined at one hour intervals for 3h post treatment using a glucometer [8].

Evaluation of the sucrose tolerance activity of O.P. grade black tea

Eighteen rats were fasted for 16 h and their pretreatment blood glucose levels were determined using a glucometer. The rats were randomly divided in to two groups and one group was orally administered with 3 ml of water (N = 9) and the other group with 446 mg/kg dose of O.P. tea (N = 9). One hour later, 5 ml/kg dose of 40% (w/v) sucrose (BDH Chemicals Limited, Poole, England) solution was orally administered to all the rats. Their post treatment blood glucose levels were determined for 3 h, at hourly intervals, using a glucometer [8].

Investigation of anti-amylase activity of O.P grade black tea *in vitro*

The anti-amylase assay was performed according to Bernfeld [15] with modifications. Briefly, 50 µl from BTB (for initial screening, N=3), 40 µl of starch (1% w/v) and 50 µl of enzyme (5 µg/ml) in a total reaction volume of 1 ml in 100 mM sodium acetate buffer (pH 6.0) were incubated at 40 ºC for 15 min. Then 0.5 ml of DNS (3,5-dinitrosalicylic acid) reagent was added and placed in a boiling water bath for 5 min and allowed to cool in a water bath containing ice. Absorbance was taken at 540 nm using 96-well micro plate reader (SpectraMax Plus, Molecular Devices, USA). Control experiments were conducted in an identical way replacing BTB with 50 µl distilled water. For sample blank incubations (to allow for absorbance produced by the BTB extract) the enzyme solutions were replaced with acetate buffer and the same procedure was carried out. For dose response relationship studies, BTBs which had the highest anti-amylase activity at screening were selected. The freeze dried BTBs of 12.5, 25, 50, 100 and 200 µg/ml were used in the assay (N=6). Acabose was used as the positive control. (concentrations: 6.25, 12.5, 25, 50 and 100µg/ml, N=6). Anti-amylase activity (inhibition %) of each BTB was calculated using the following equation.

Inhibition (%) = [Ac - (As-Ab)/ Ac] * 100

Where, Ac is the absorbance of the control, Ab is the absorbance produced by BTB (sample blank) and As is the absorbance in the presence of BTB.

Investigation of anti-glucosidase activity of O.P. grade black tea *in vitro*

Anti-glucosidase assay was performed according to Matsui et al [17] with modifications (N-6). Briefly, reaction mixtures containing 4 Mm p-nitrophenyl – α-D-glucopyranoside (substrate), 50 mU /ml of α- glucosidase enzyme, 40 µl of different concentrations of BTB (100,200 and 400 µg/l) in total reaction volume of 0.1 in 50 mM sodium acetate buffer (p^H 5.8) were incubated at 37^0 C for 30 min using 96-well microplate reader. The reaction was stopped by addition of 50 µl of 0.1 Na_2CO_3 and the absorbance was recorded at 405 nm. Acarbose was used as the positive control (concentrations: 6.25,12.5,25,50 and 100µg/ml, N=6).

Anti-glucosidase activity (inhibition %) of each BTB was calculated using the following equation.

Inhibition (%) = [Ac - (As-Ab)/ Ac] * 100

Where, Ac is the absorbance of the control, Ab is the absorbance produced by BTB (sample blank) and As is the absorbance in the presence of BTB.

Statistical analysis

Data is represented as Mean ± SEM. Mann – Whiney U-test was used to analyse *in vivo* results.IC_{50}values were computed and regression analysis made using Microsoft Excel 2007 package. Significance level was set at P<0.05,

RESULTS

Blood glucose lowering activity of O.P. grade black tea

The results obtained with BTB on fasting blood glucose level are summarized on Table 1. As shown, all the three doses (223, 446, 1339 mg/kg) tested failed to significantly (P > 0.05) lower the fasting blood glucose level at hourly intervals up to 4h following oral administration. On the other hand, Tolbutamide significantly, (P<0.05) impaired the random blood glucose level from 1 h post treatment upto 4h post treatment.

Evaluation of the effect of BTB of O.P. grade black tea on random blood glucose level

The results obtained with BTB on random blood glucose level are summarized on Table 2. As shown, all the three doses (223, 446, 1339 mg/kg) tested failed to significantly (P > 0.05) lower the random blood glucose level at hourly intervals up to 4h following oral administration.

Evaluation of the effect of OP Grade black tea on the oral glucose tolerance test

The results of glucose tolerance test are summarized in the Table 3. As given, 223 mg/kg dose of BTB significantly (P<0.05) reduced the blood glucose of rats at 2h (by 29 %) and 3h (by 16.%) post treatment of high dose of glucose while, 446 mg/kg dose of BTB reduced (by 18%) the blood glucose level only at 3h post treatment. In contrast, 1339 mg/kg dose of BTB did not significantly (P>0.05) change the blood glucose levels of rats. Tolbutamide, the standard drug, significantly (P<0.05) reduced the blood glucose level of rats at 1h (by 22%), 2h (by 30%) and 3h (by 32%).

Evaluation of the sucrose tolerance activity of O.P. grade black tea

The results of sucrose toleranceactivity of BTB are summarized in the Table 4. As depicted, 446 mg/kg dose of BTB significantly (P<0.05) reduced (by 10%) the blood glucose level of rats at 1h post treatment of oral administration of high dose of sucrose. However, 446 mg/kg dose of BTB did not significantly (P> 0.05) alter the blood glucose level at 2h and 3h post treatment

Investigation of anti- amylase activity of O.P grade black tea *in vitro*

Tables 5 and 6 summarizes the results obtained with *in vitro* anti-amylase activity. As shown, BTB evoked a dose dependent (r^2 = 0.96; P<0.05). amylase inhibitory activity with and IC_{50} value of 952.32 ±69.23.µg/ml) However, this activity appears to be mildIncontrast, the anti-amylase activity of the reference drug , acarbose, range from 10.18 to 44.86% with an IC_{50} value 100.11 ± 6.79 µg/ml.

Table 1. Effect of O.P. grade black tea on fasting blood glucose lowering activity in rats (Mean ± SEM)

Treatment			**Fasting blood glucose level (mg/dl)**		
Pre treatment		**1h post treatment**	**2h post treatment**	**3h post treatment**	**4h post treatment**
Control / (water)(N = 9)	97.63 ± 4.54	88.35 ± 3.95	77.13 ± 2.37	79.67 ± 2.54	80.73 ± 4.98
1.5 cups / (223 mg/kg)(N = 9)	101.16±3.57	91.67 ± 4.83	85.98 ± 5.46	83.44 ± 3.68	81.5 ± 4.55
3 cups / (446 mg/kg)(N = 9)	89.58 ± 3.93	87.58 ± 2.55	83.31 ± 5.38	82.56 ± 5.61	80.69 ± 3.64
9 cups / (1339 mg/kg) (N = 9)	91.48 ± 3.62	92.89 ± 3.18	87.95 ± 3.79	84.5 ± 3.71	79.78 ± 4.32
Tolbutamide	86.58 ± 2.54	68.53 ± 1.58*	58.86 ± 2.14*	65.92 ± 2.60*	72.46 ± 1.05*

*- (P < 0.05), Mann-Whitney - U test (compared to the control)

Table 2. Effect of O.P. grade black tea on random blood glucose lowering activity in rats (Mean ± SEM)

Treatment			**Random blood glucose level (mg/dl)**		
Pre treatment		**1h post treatment**	**2h post treatment**	**3h post treatment**	**4h post treatment**
Control / (water)(N = 6)	126.27 ± 2.33	123.82 ± 5.69	117.64 ± 6.24	122.96 ± 8.91	118.75 ± 3.0
1.5 cups / (223 mg/kg)(N = 6)	122.67 ± 2.42	129.57 ± 4.6	135.89 ± 4.33	129.59 ± 2.73	126.97 ± 1.79
3 cups / (446 mg/kg)(N = 6)	118.46 ± 2.94	128.08 ± 4.58	125.97 ± 3.41	126.57 ± 3.72	122.66 ± 5.51
9 cups / (1339 mg/kg) (N = 6)	122.47 ± 2.35	117.56 ± 3.15	125.68 ± 2.68	118.75 ± 1.83	120.26 ± 3.86

Table 3. Effect of O.P. grade black tea on glucose tolerance activity in rats (Mean ± SEM)

Treatment		**Blood glucose level (mg/dl)**		
Pre treatment		**1h post treatment**	**2h post treatment**	**3h post treatment**
Control / (water)(N = 10)	93.26 ± 7.14	133.67 ± 4.75	110.76 ± 6.99	93.99 ± 3.98
1.5 cups / (223 mg/kg)(N = 11)	91.67 ± 2.45	125.94 ± 3.7	86.07 ± 4.58*	78.72 ± 2.76*
3 cups / (446 mg/kg)(N = 12)	90.35 ± 3.58	125.38 ± 4.67	90.35 ± 3.55*	88.55 ± 3.60
9 cups / (1339 mg/kg) (N = 11)	90.36 ± 6.11	126.93 ± 4.62	115.78 ± 5.92	108.07 ± 4.93
Tolbutamide(N = 6)	81.78 ± 1.96	104.33 ± 3.85*	77.87 ± 7.56*	64.04 ± 3.69*

*- (P < 0.05), Mann-Whitney - U test (compared to the control)

Table 4. Effect of O.P. grade black tea on sucrose tolerance activity in rats (Mean ± SEM)

Treatment		**Blood glucose level (mg/dl)**		
Pretreatment		**1h post2h post treatment**		**3h post treatment**
Control / (water)(N = 9)	94.41 ± 3.49	116.66 ± 2.64	85.99 ± 1.67	92 ± 2.64
3 cups / (446 mg/kg) (N = 9)	95.41 ± 4.17	105.23 ± 2.82*	93.6 ± 2.4	92 ± 2.59

Table 5. Amylase inhibitory activity of O.P. grade black tea *in vitro* (Mean ± SEM, N = 3)

Concentration µg/ml	% Inhibition
25	- 5.60 ± 3.44
50	- 2.49 ± 1.91
100	3.07 ± 4.94
200	5.20 ± 2.23
400	17.67 ± 0.77

Table 6. Amylase inhibitory activity of Acarbose *in vitro*.(Mean ±SEM, N +3)

Concentration µg/ml	% Inhibition
100	44.86 ± 2.34
50	27.02 ± 1.76
25	18.46 ± 1.65
12.5	14.92 ± 0.61
6.25	10.18 ± 0.99

Table 7.Glucosidase inhibitory activity of O.P. grade black tea *in vitro* (Mean ± SEM; N = 6)

Concentration µg/ml	% Inhibition
100	0.15 ± 0.41
200	0.32 ± 1.38
400	0.42 ± 0.88

DISCUSSION

This study examined the glycaemia regulation potential of Sri Lankan low grown orthodox O.P. grade black tea, which is a whole leaf variety [12]; most widely used in the blending of tea [12]. This was evaluated using both *in vivo* (in terms of impairing fasting blood glucose level, random blood glucose level, post prandial blood glucose level and blood glucose level following an oral sucrose challenge) and *in vitro* (as judged by anti-amylase and anti-glucosidase activities). Unfortunately, we could not use streptozotocin induced rat diabetic model which is an obvious limitation of this study. The black tea sample used was garden fresh, unblended and typical for the O.P. grade and agro-climatic elevation [in terms of sieve analysis [13], organoleptic analysis, phytoconstituent analysis [13] and quantity of water extractable in BTB [13]: most of the other studies have used blended teas of multiple origins with no specifications of agro-climatic elevations [17,18]. Five minutes brewing time was employed in making the BTB: as extraction of most of the water soluble phytochemicals including flavonols, theaflavins and thearubigins are completed within 4 min [19]. Further, fresh and warm (37-40^{0}C) BTB was used for oral treatment as cooling results in precipitation of important phytochemicals such as caffeine and flavonoids [19], and because traditional physicians recommend to use fresh and warm BTB as a beverage for pre diabetics and mild diabetics [6]. Also, in *in vivo* studies, oral administration to normoglycaemic rats (fasted and non-fasted) was selected to simulate human consumption [8-9] and the *in vitro* tests used are well established, widely used and validated assays [15,16].

The results show, for the first time, that Sri Lankan low grown orthodox O.P. grade black tea has blood glucose level lowering properties: *in vivo* tests, blood glucose level was impaired in both glucose and sucrose tolerance test whilst only mild anti-amylase activity was evident *in vitro* assays. Impairment in rise in blood glucose level in the oral glucose tolerance test suggests that the Sri Lankan O.P. grade tea has the ability to increase insulin secretion from β cells of the pancreas or to increase insulin sensitivity and/or insulinomimetic activity [8,9]. Indeed, polyphenols in black tea such as theaflavins and thearubigins are shown to possess insulinomimetic activity [20]. Further, this action suggests that this tea has anti-hyperglycaemic activity as well [8,9]. In complete contrast, BTB could have inhibited the glucagon release to mitigate the elevation of post prandial blood glucose level [8]. But, we have no experimental evidence to support this notion.

As pointed out earlier, BTB of O.P. grade tea inhibited the rise in blood glucose level mildly following an oral sucrose challenge. This suggests that it has mild anti-glucosidase activity [8]. Mild *in vivo* anti-glucosidase activity may be due to low theaflavin content in the O.P. tea [21]; since, theaflavin is reported to be the most effective component in black tea responsible for inhibition of Glycosidase enzyme [22]. Further, the extraction procedures used can influence Glycosidase activity of black tea [22]. Glucosidase enzyme is also involved in absorption of glucose in the small intestine [23] and inhibition of its activity, as seen in this study, could play an important role in reducing rise in blood glucose level following both oral glucose challenge and oral sucrose challenge. However, no anti-glucosidase activity was evident in *in vitro* assay, although some investigators have shown marked anti-glucosidase activity *in vitro* with black tea (grade not specified) [22,23]. The discrepancy between this study and other *in vitro* studies [22,23] may be because they have used high concentrations of black tea extracts (in mg levels), differences of pH and differences in the enzyme sources (unspecified) [22,23].

In this study there was no reduction in fasting and random blood glucose levels, and only a mild impairment in blood glucose levels following oral glucose and sucrose challenges was evident. On the other hand, strong impairments in these parameters are reported in Sri Lankan high grown orthodox Dust No.1 black tea [8] and Sri Lankan orthodox BOPF grade black tea belonging to all the three agro-climatic elevations (low, mid and high) [9]. This is rather puzzling and unexpected result since all the 3 black tea grades (O.P., Dust No.1 or BOPF) have essentially the same phytoconstituents [9, 21,24]. Nevertheless, several reasons can be put forward to explain this discrepancy. It is well known that bioactivities of black tea are dependent on different factors including country of origin, agro-climatic elevation, tea brewing conditions, quality of water used in making the tea brew, temperature of the tea brew, processing method, age of leaf, harvesting season, climatic changes, wind velocity, particle size (grade of tea) of the manufactured tea and the composition of phytoconstituents in the tea leaf and in the tea brew [10-11]. However, the discrepancy between this study and our previous studies with Dust No.1 [8] and BOPF [9] cannot be attributed to the country of origin of the tea samples: all tea samples were from Sri Lanka. Neither could it be attributed to the processing method as all the tea samples were made using orthodox rotavane technique [8,9]. It is also unlikely to be due to differences in brewing conditions, quality of water used (as distilled water was used) or temperature of the tea brew as these were almost identical. Differences in agro-climatic elevations cannot be a reason for the difference in blood glucose levels observed in this study and in our previous studies [8,9]: since no difference in severity of blood glucose lowering capacity was evident with BOPF grade tea belonging to the three agro-climatic elevations of Sri Lanka [9]. Unfortunately, we have no information on age of leaf, climatic changes and wind velocity; therefore, no comment can be made regarding their influence with this study and our previous studies [8,9].

On the contrary, the differences in the particle size between the tea samples (O.P., Dust No.1 or BOPF) is likely to play a critical role in evoking the differences in the lowering of blood glucose levels in this study and our previous studies [8,9]. Dust No.1 and BOPF are broken leaf varieties whose particle sizes are small (Dust No.1 and BOPF 300-500µm and 500-800µm respectively) whilst O.P. is a whole leaf variety whose particle size is comparatively large (1400-2000µm) [8,9,24]. It is well recognized that, larger the particle size, lower would be the surface to volume ratio [25] and therefore slower would be the release rate of water soluble constituents. This argument is reinforced further by the observation that bioactivities such as diuretic [26] and anxiolytic activities [27] of O.P. grade tea are weaker than that of Dust No.1 [28] and BOPF [29].

Theaflavins and thearubigins are two characteristic polyphenols in black tea [7]. Studies have shown that theaflavins and thearubigins are mainly responsible for blood glucose lowering potential of black tea: such as enhance insulin sensitivity/activity [1]; inhibition of amylase and glucosidase activities [22,23]; and insulinomimetic activity [20]. Theaflavins, thearubigins and total polyphenol content of O.P. grade tea is reported to be appreciably low [21] compared to Dust No.1 [24] and BOPF [9]. Thus, this maybe the other main reason, if not the sole reason, for the weak blood glucose lowering properties displayed in this study (both *in vivo* and *in vitro*). Further, it is now known that there are four types of theaflavins in black tea [22], although, it is still unknown which type contributes most to the blood glucose lowering properties of black tea. It is possible that the theaflavin type which contributes most to the blood glucose lowering activities may be minimal in the O.P. grade black tea accounting for its mild blood glucose lowering potentials. Some investigators have made claims that thearubigin/theaflavin ratio has a bearing on absorption of phytoconstituents in the intestine [29] and O.P. tea has a high ratio compared to Dust No.1 [8] and BOPF [9]. This could be another reason that can be attributed to the mild glycaemic regulatory activity observed in this study.

The O.P. grade tea in this study also displayed a mild anti-amylase activity *in vitro* in agreement with other types of black teas [23]. Mammals possess two types of amylase enzymes having different pH optimas; salivary and pancreatic [30]. In this study, bovine pancreatic amylase was used as the enzyme source which may not be homologues to human pancreatic enzyme. This mild anti-amylase activity of BTB would likely to contribute for the suppression of post prandial hyperglycaemia evident in this study. Interestingly, inhibition of amylase activity is now considered as an attractive biochemical strategy in the management of post prandial hyperglycaemia in type 2 diabetes with low side effects [23]. Excessive inhibition of amylase activity leads to abdominal distention, flatulence, meteorism and possibly diarrhoea [23].

Reactive oxygen species are linked with pathogenesis of type 2 diabetes mellitus [31] and antioxidants shown to have a therapeutic value in alleviating this condition [31]. We have previously demonstrated that BTB of O.P. grade possesses marked antioxidant activity *in vitro* [21]. Obviously, this antioxidant action can play an important role in regulation of glycaemia. What is more, we have shown, for the first time, that O.P. grade black tea has anti-glycation (inhibition of Advance Glycation End-products/AGEs formation and AGEs cross-link breaking) activity *in vitro*[32]. Accumulation of AGEs is implicated in diabetic complications [33]. Hence, the presence of anti-glycation activity in BTB [32] is yet another valuable feature which could have in the preventive effects on diabetic complication.

CONCLUSION

In conclusion, this study shows, for the first time, the ability of Sri Lankan low grown orthodox O.P. grade black tea to impair rise in blood glucose level following oral challenges of glucose and sucrose, and to impair amylase activity *in vitro*. These features together with its antioxidative [21] and anti-glycation [32] activities suggest that it is sensible to be consumed has a cheap and safe, plant based beverage for regulation of dysglycaemia and to lower the risk of developing diabetes. It could be an effective strategy because of likelihood of high compliance and absence of side effects [23].

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REFERENCES

1. Jankun J, Al-Senaidy A, Skrzypczak-Jankun E. Can drinking black tea fight diabetes; literature review and theoretical indication. *Europian Journal of Immunology*, 37, 2012, 167-172.
2. Jayawardena R, Ranasinghe P, Byrne NM, Soares MJ, Katulanda P, Hills AP. Prevalence and trends of the diabetes epidemic in South Asia: a systematic review and meta-analysis. *BMC Public Health,* 12, 2012, 380-403.
3. Katulanda P, Constantine GR, Mahesh JG, Sheriff R, Seneviratne RDA, Wijeratne S. Matthews, DR. Prevalence and projections of diabetes and pre-diabetes in adults in Sri Lanka—Sri Lanka Diabetes, Cardiovascular Study (SLDCS). *Diabetic Medicine*, 25, 2008, 1062-1069.
4. Anonymous. Diagnosis and classification of diabetes mellitus. *Diabetic care,* 13, 2010, 562-569.
5. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology*, 81, 2002, 81-100.
6. Ediriweera ERHSS, Ratnasooriya WD. A review on herbs used in treatment of diabetes mellitus by Sri Lankan ayurvedic and traditional physicians. *Ayurveda*, 30, 2009, 373-391.
7. Modder WWD, Amarakoon AMT. Tea and Health, Tea Research Institute, Talawakelle, Sri Lanka. 2002, 1-182.
8. Jayakody JRAC, Ratnasooriya WD. Blood glucose level lowering activity of Sri Lankan black tea brew (*Camellia sinensis*) in rats. *Pharmacognosy Magazine,* 4, 2008, 341.
9. Abeywickrama KRW, Ratnasooriya WD, Amarakoon AMT. Oral hypoglycaemic, antihyperglycaemic and antidiabetic activities of Sri Lankan Broken Orange Pekoe Fannings(BOPF) grade black tea (*Camellia sinensis*, L.) in rats. *Journal of Ethnopharmacology*, 135, 2011, 278-286.
10. Zoysa AKN. Climatic requirements and soil In: Zoysa AKN (ed) Hand Book on Tea. Tea Research Institute of Sri Lanka, Talawakelle, 1st ed., Sri Lanka, 2008, 10-15.
11. Wijeratne M A. Harvesting of tea. *Hand book on tea. Talawakelle, Sri Lanka: Tea Research Institute of Sri Lanka*, 2008, 94-104.
12. Anonymous. Statistical Bulletin, Sri Lanka Tea Board, Colombo, Sri Lanka, 3-20.
13. Ratnasooriya WD, Muthunayake TBS. Anticlotting properties of Sri Lankan low grown Orthodox Orange Pekoe grade black tea (*Camellia sinensis* Linn). *World Journal of Pharmaceutical Science,* 2, 2014, 1-7.
14. Anonymous. ISO 3103: Tea-Preparation of liquor for use in sensory tests: ISO 3103: 1530 International Organization for Standardization, Geneva, Switzerland. 1997, 1-4.
15. Bernfeld P. Amylases, alpha and beta. *Methods in Enzymology*, 1955, 149-158.
16. Matsui T, Veda J, Oki T, Sugita K, Terahara N, Matsumoto. alpha - glucosidase inhibitory action of natural acylatedanthocyanins: Survey of natural pigments with potent inhibitory activity. *Journal of Agriculture and Food Chemistry*, 49, 2001, 1948-1951.
17. Gomes A, Vedasiromoni JR, Das M, Sharma RM, Ganguly DK. Anti-hyperglycemic effect of black tea (*Camellia sinensis*) in rat. *Journal of Ethnopharmacology,* 45, 1995, 223-226.
18. Anderson RA. Polansky MM. Tea enhances insulin activity. *Journal of Agricultural and Food Chemistry*, 50, 2002, 7182-7186.
19. Peterson J, Dwyer J, Jacques P, Rand W, Prior R, Chui K. Tea variety and brewing techniques influence flavonoid content of black tea. *Journal of Food composition and analysis*, 17, 2004, 397-405.
20. Cameron AR, Anton S, Melville L, Houston NP, Dayal S, McDougall GJ, Stewart D, Rena G. Black tea polyphenols mimic insulin/insulin-like growth factor-1 signalling to the longevity factor FOXO1a. *Aging Cell*, 7, 2008, 69-77.
21. Ratnasooriya WD. An assessment of potential health benefits of Sri Lankan black tea by studying its bioactivities II. National Science Foundation of Sri Lanka Final Report (Grant No: NSF/Fellow/2011/01), 2014.

22. Uchida N, Yoshimoto N, Nakamura A, Takagi A, Honda K, Moto M, Katsuraya K, Hashizume N. Difference of inhibitory effect of α-glucosidase by tealeaves species and extraction condition and effect of black tea on postprandial blood glucose level elevation in ICR mice. *Journal of Analytical Bio-Science,* 36, 2013, 193-202.
23. Kwon Y, ApostolidisE,Shetty K. Inhibitory potential of wine and tea against α-Amylase and α-Glucosidase for management of hyperglycemia linked to type 2 diabetes. *Journal of Food Biochemistry*, 2008, 32, 15-31.
24. Ratnasooriya WD. An assessment of potential health benefits of Sri Lankan black tea by studying its bioactivities I. National Science Foundation of Sri Lanka Final Report (Grant No: NSF/Fellow/2000/01), 2008.
25. Ruch TC, Patton HD, Howell WH. *Physiology and Biophysics*. 19th edn, WB Saunders, London, 1966, 1-23.
26. Ratnasooriya WD, Muthunayake TBS, Indeesha EK, Ratnasooriya CDT. Oral diuretic activity of Sri Lankan low grown Orange Pekoe grade black tea (*Camellia sinensis* L.) in rats. *International Journal of Bioassays*, 2, 2013, 286-293.
27. Ratnasooriya WD, Muthunayake TBS, Indeesha EK, Ratnasooriya CDT. Sri Lankan Orange Pekoe grade black tea impairs anxiety. *International Journal of Bio Bioassays*, 1, 2012, 217-223.
28. Ratnasooriya WD, Fernando TSP, Ranatunga RAAR. Anxiolytic activities of hot water brew of Sri Lankan black tea (*Camellia sinensis* L.) in rats. *Australian Journal of Medical Herbalism*, 19, 2007, 178-184.
29. Chen L, Lee MJ, Li H, Yang CS. Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metabolism and Disposition*, 25, 1997, 1045-1050.
30. Barret KE, Barman SM, Boitano S, Brooks HL. Ganong's review of medical physiology. 23rd edn. McGrew Hill edition Private Limited. New Delhi, India. 2010, 429-479.
31. Fujiwara T, Ohsawa T, Takahashi S, Ikeda K, Okuno A, Ushiyama S, Horikoshi, H. Troglitazone, a new antidiabetic agent possessing radical scavenging ability, improved decreased skin blood flow in diabetic rats. *Life Sciences*, 63, 1998, 2039-2047.
32. Ratnasooriya WD, Abeysekera WK, Muthunayake TB, Ratnasooriya CD. *In vitro*Antiglycation and Cross-Link Breaking Activities of Sri Lankan Low-Grown Orthodox Orange Pekoe Grade Black Tea (*Camellia sinensis* L). *Tropical Journal of Pharmaceutical Research*, 13, 2014, 567-571.
33. Vasan S, Foiles P, Founds H. Therapeutic potential of breakers of advanced glycation end product–protein crosslinks. *Archives of Biochemistry and Biophysics*, 419, 2003, 89-96.