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Oral hypoglycaemic, antihyperglycaemic and antidiabetic activities of Sri Lankan Broken Orange Pekoe Fannings (BOPF) grade black tea (*Camellia sinensis* L.) in rats

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A B S T R A C T

Ethnopharmcological relevance: Sri Lankan traditional practitioners recommend the consumption of black tea infusion (BTI) made from *Camellia sinensis* L. plant for regulation of glycaemia. However, they do not specify the grade of tea and their origin (i.e., agroclimatic elevation) and as such many prediabetics and milddiabetics use BOPF grade tea.
Aim of the study: This study examines the blood glucose lowering potential of Sri Lankan BOPF grade tea and its potency with respect to agroclimatic elevations.
Materials and methods: Unblended orthodox BOPF grade tea samples were collected from high-, mid- and low-grown agroclimatic elevations in Sri Lanka. Different concentrations of warm BTI (60, 120 and 480 mg/ml), tolbutamide (reference drug: 22.5 mg/kg body weight) and water (control) were orally administered to different groups of rats, and hypoglycaemic and antihyperglycaemic activities were assessed. Antidiabetic activity was determined using streptozotocin induced diabetic rats. Mechanisms of blood glucose lowering actions were investigated using several standards techniques.
Results: BTI exhibited significant ($P < 0.05$), dose-dependent and marked hypoglycaemic and antihyperglycaemic activities with quick onset. These effects did not differ with respect to agroclimatic elevation, although there were differences in the content of phyto-constituents. BTI also showed marked and quick antidiabetic activity. BTI inhibited intestinal glucose absorption and impaired α-glucosidase and α-amylase activities. BTI possessed insulinomimetic action, ability to improve insulin sensitivity and in vivo antioxidant activity. Notably, BTI was nontoxic.
Conclusions: BTI of Sri Lankan BOPF grade tea has oral hypoglycaemic, antihyperglycaemic and antidiabetic actions which are mediated via multiple mechanisms. This study also indicates that, BOPF grade tea of any agroclimatic elevations in Sri Lanka could be used in the regulation of glycaemia.

1. Introduction

Hot water infused brew of black tea [*Camellia sinensis* (L.) O. Kuntze (family: *Theaceae*)] is the most consumed beverage of the world after water (Mohamed and Zoysa, 2008). It is recorded that, globally about 3–5 billion cups are consumed daily (Modder and Amarakoon, 2002). Based on ethnomedical claims (Gomes et al., 1995; Anderson and Polansky, 2002; Modder and Amarakoon, 2002; Shoji and Nakashima, 2006) and epidemiological (He and Kies, 1994; Yang and Landau, 2000; Salazar-Martinez et al., 2004; Sharangi, 2009), clinical (Savage et al., 2003; Wu et al., 2003; Store and Baer, 2008) and experimental (Gomes et al., 1995; Abeywickrama et al., 2005; Cameron et al., 2008; Jayakody and Ratnasooriya, 2008; Kwon et al., 2008; Sharma et al., 2008; Kusano et al., 2008; Ratnasooriya et al., 2009; Abeywickrama et al., 2010) evidences, it is now considered as a medical preparation. Tea is manufactured from freshly harvested two or three of the topmost immature leaves and bud of the tea plant (Wijeratne, 2008). Based on the manufacturing techniques, there are three types of teas: green, oolong and black (Modder and Amarakoon, 2002). Globally, black tea is the most consumed variety (Anon., 2009).

In Sri Lanka, traditional medical practitioners often recommend heavy consumption (6–10 cups per day) of black tea brew for pre-

Abbreviations: ALT, alanine-transaminace; AST, aspartate-transaminace; BI, briskness index; BOPF, Broken Orange Pekoe Fannings; BTI, black tea infusion; BW, body weight; C, (+)catechin; DPPH, 1,1-diphenyl-2-picrylhydrazil; EC, (−)epicatechin; ECG, (−)epicatechin gallate; EGC, (−)epigallocatechin; EGCG, (−)epigallocatechin gallate; GA, gallic acid; TF, theaflavins; TPP, total polyphenols; TR, thearubigins; STZ, streptozotocin.

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diabetics and milddiabetics (Ediriweera and Ratnasooriya, 2009). However, these native physicians do not specify the grade of black tea and the origin (i.e., agroclimatic elevation) of black tea sample that should be used in their treatment regimen. Recently, hypoglycaemic, antihyperglycaemic and antidiabetic potential of Dust grade (grade containing smallest size leaf particles) Sri Lankan black tea was scientifically proven (Jayakody and Ratnasooriya, 2008). Many patients use 'Broken Orange Pekoe Fannings' (BOPF) grade of black tea (a grade containing medium size leaf particles) due to its easy availability and pleasant flavour and taste. However, neither the antidiabetic potential of Sri Lankan BOPF grade tea is scientifically proven nor its potency with respect to agroclimatic elevation of tea sample is known. This is worth examining since it is well recognized that pharmaco-theraputic potential of black tea infusion differs with many factors including country of origin, particle size (or grade of tea), brewing time and agroclimatic elevation of sample (Modder and Amarakoon, 2002; Wijeratne, 2008).

The aim of this study was to investigate the blood glucose lowering potential of tea infusion made from Sri Lankan BOPF grade black tea originating from three major agroclimatic elevations: low-grown (below 600 m, average mean sea level; amsl), mid-grown (between 600 and 1200 m, amsl) and high-grown (above 1200 m, amsl) and to investigate the possible mode of action using rat model.

2. Materials and methods

2.1. Black tea samples

Black tea, Broken Orange Pekoe Fannings (BOPF) grade manufactured using fresh two or three upper most tender leaves with bud of *Camellia sinensis* (L.) O. Kuntze (family: *Theaceae*) harvested in May 2006 was used in the present study. Black tea was obtained from the tea factories situated in randomly selected tea estates of three major agroclimatic elevations in Sri Lanka: high-grown (tea factory in NuwaraEliya District, 1382 m, amsl); mid-grown (tea factory in Kandy District, 820 m, amsl) and low-grown (tea factory in Kegalle District, 382 m, amsl). A similar manufacturing process of black tea production (orthodox) was used for processing in all three factories. Collected black tea samples (1 kg each) were packed immediately in moisture proof triple laminated aluminum foil bags and stored at −20 °C until use.

The composition of true to size particles defined for the BOPF grade tea was determined according to Samaraweera (1986) using a sieve shaker (Retsch AS 200, Retsch GmbH, Haan, Germany) fixed with standard set of sieves. The sensory quality of the tea samples was assessed organoleptically by professional tea tasters to verify their origin (i.e., the agroclimatic elevation).

2.2. Dosage of black tea infusion

Black tea infusion (BTI) was freshly prepared according to the international standards (Anon., 1980) [yield of tea solids (w/w) of BTI: 360, 430 and 440 mg/g for high-, mid- and low-grown, respectively]. Three different doses of high-grown BTI: 60, 120 and 480 mg/ml were made (equivalent to 1½, 3 and 12 cups, respectively for human). The volume of one cup is considered to be 170 ± 10 ml. However, only the high dose of BTI (480 mg/ml) of mid- and low-grown teas was used in this study. The doses of BTI selected were identical to what has been previously used for investigation of bioactivities of Sri Lankan BOPF grade black tea (Abeywickrama et al., 2010). Oral administration in experimental rats was performed with warm BTI (40 ± 3 °C) since at room temperature precipitation or creaming down of active ingredients occurs (Wickremasinghe and Perera, 1966).

2.3. Experimental animals

Healthy adult male rats of Wistar strain (210 ± 15 g, body weight) were used in this study. They were maintained in the animal house at the University of Colombo under standardized conditions (temperature at 30 ± 2 °C; photo period of 12 ± 1 h day light/dark cycle and relative humidity: 55 ± 5%) with free accesses to pelleted animal food (Ceylon Grain Elevators, Colombo, Sri Lanka) and drinking water *ad libitum*. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines (Anon., 2008) and the rules of the Department of Zoology, Faculty of Science, University of Colombo, Sri Lanka for Animal Experimentations.

2.4. Assessment of hypoglycaemic activity of BTI in normoglycaemic fasted rats

Sixty-three rats were randomly divided into seven groups (n = 9/group) and fasted for 16 h, with free access to water. These rats were orally administered by gastric intubation (5 ml/kg, bw) either with water (control) or different doses of BTI or tolbutamide (reference drug: State Pharmaceutical Corporation, Colombo, Sri Lanka) in the following manner: Group(1): water; Groups(2), (3) and (4): 60, 120 and 480 mg/ml of high-grown BTI, respectively; Groups(5) and (6): 480 mg/ml of mid- and low-grown BTI, respectively; and Group(7): 22.5 mg/kg bw dose of tolbutamide. Blood was collected from the tails of rats under mild ether anesthesia using aseptic precautions prior to the treatment and at hourly intervals of post treatment for 4 h. Serum was separated (at 27 °C) and glucose concentration determined in each sample (Fernandopulle et al., 1994).

The glucose content in blood and other extracted fluid samples was estimated colourimetrically (Varian Carry 50 Scan, Varian BV, Middleburg, Netherlands) at 500 nm using glucose oxidase enzymatic method as described by the manufacturer of test kit (Randox Laboratories Ltd., Antrium, UK). The glucose content was expressed as mg/dl of the used sample.

2.5. Evaluation of hypoglycaemic activity of BTI in normoglycaemic non fasted rats

The rats were randomly divided into seven groups (n = 9/group) and pre-treatment blood glucose levels were obtained. They were orally administered with either water or different doses of BTI or tolbutamide as described in Section 2.4. Blood samples were collected from the tails of treated rats at hourly intervals of post treatment for 4 h. Serum was separated (at 27 °C) and glucose concentration determined (Fernandopulle et al., 1994).

2.6. Assessment of antihyperglycaemic activity of BTI in rats

The rats were fasted for 16 h and randomly divided into seven groups (n = 9/group) and pre-treatment blood glucose levels obtained. They were orally administered either with water or different doses of BTI or tolbutamide as described in Section 2.4. One hour later, all rats in seven groups were orally loaded with 5 ml/kg bw of 50% (w/v) glucose (BDH Chemicals Limited, Poole, England) solution. Blood samples were collected from the tails of glucose loaded rats at hourly intervals for 4 h. Serum was separated (at 27 °C) and glucose concentration determined for the assessment of antihyperglycaemic activity in terms of oral glucose tolerance in rats (Ratnasooriya et al., 2004).