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In vitro anticlotting activity of Sri Lankan high grown black tea (*Camellia sinensis* L. O. Kuntze)

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ABSTRACT

This study examined the blood anticlotting potential of Sri Lankan black tea using high grown Dust No 1, BOPF and BOP grades and citrated shed goat blood. Different concentrations of these three grades of tea (0, 0.125, 0.25, 0.625, 1.25, 2.5 mg/ml) were made in isotonic saline (0.9% NaCl, w/v) using freeze dried tea samples. The results showed that the highest dose of all tea grades significantly ($P < 0.05$) prolonged the calcium-induced *in vitro* clotting time upto 24 h (the longest time investigated). A similar anticlotting activity was also evident with different concentrations of decaffeinated Dust No 1 and BOPF samples (decaffeinated BOP not investigated). It is concluded that Sri Lankan black tea possess strong blood anticoagulant activity at least, *in vitro*.

Key Words: *Camellia sinensis*, Sri Lankan black tea, anticlotting, blood clotting, BOP, BOPF, Dust No: 1

INTRODUCTION

Tea, which is made from tender shoots of *Camellia sinensis* L. O. Kuntze (Family: Theaceae) plant is currently the most consumed beverage in the world besides water (Modder and Amarakoon, 2002). Based on the method of processing there are three major types of teas: black (fully aerated), green (un aerated) and oolong (partially aerated). Of these, black tea accounts for about 78% of global tea consumption (Anon, 2002).

The ancients believed that tea has many health benefits. In recent times scientists have undertaken a considerable amount of scientific research and confirmed many of these beneficial effects. The health benefits shown so far, especially of green and black tea, include antioxidative, antiinflammatory, anticarcinogenic, antiangiogenic, antiarteriosclerotic, antidiabetic, antiobesitic, antiaging antibacterial, antiviral, hypocholesterolaemic, anticlotting and promotion of immune function (Dufresne and

Farnworth, 2001; Modder and Amarakoon, 2002; Amarakoon, 2004). *Helicobacter pylori* growth inhibiting activity *in vitro* (O'Mahony *et al.*, 2004); mild *in vitro* and *in vivo* (in rats) antioxidant activity (Abeywickrama *et al.*, 2005); an improvement of lipid profile (Abeywickrama *et al.*, 2004); and anti bacterial activity against *Candida albicans* isolates (Udagama *et al.*, 2000) etc.

Although, Sri Lankan black tea is acclaimed to be one of the best in the world and drunk in more than 125 countries and accounts for about 19% of global tea consumption (Anon, 2002), published studies on its bioactivities are limited. Hence, a program of research was initiated to investigate the bioactivities and potential health benefits of Sri Lankan black tea. This is important because it is now well recognized that several factors such as country of origin, soil characters, method of cultivation, environmental pollution, the elevation of the tea plantation, the age of the leaves, harvesting season, technological process during tea production, the grade of the tea, brewing conditions of time and temperature, the amount and type of water used in brewing, influence the fluid composition of tea brew (Wickramanayake, 1996; Balentine *et al.*, 1997; Modder and Amarakoon, 2002; Gramza *et al.*, 2006) and hence its pharmacological effects. In this study, *in vitro* blood anticlotting activity of Sri Lankan high grown Dust grade No: 1, BOPF and BOP grades of tea, was investigated using citrated goat blood.

METERIALS AND METHODS

Dust grade No:1, Broken Orange Pekoe Fannings (BOPF) and Broken Orange Pekoe (BOP) grades of black tea that was manufactured at St. Coomes Estate tea factory (1362 m above mean sea level) of the Tea Research Institute, Talawakelle, Sri Lanka, using orthodox-rotovane manufacture technique were used. Decaffeinated Dust No. 1 and BOPF were also used. 50g of each type of tea sample in 400 ml boiling water and steeped for 5 min. Tea brew obtained after filtration was further concentrated using a rotary evaporator. Concentrated brew was then freeze dried and stored at 4 °C in brown colored airtight containers to prevent absorption of moisture, breakdown of lipids, microbial activity and conversions of catechins to theaflavins and thearubigins. These freeze dried samples were used to make different concentrations in isotonic saline (NaCl, 0.9% W/V): 0.125, 0.25, 0.625, 1.25, 1.375 and 2.5 mg/ml.

Goat blood (arterial) was collected from the Colombo Municipal slaughterhouse, Dematagoda, Sri Lanka, and immediately citrated using 3.1% sodium citrate solution (Ratnasooriya and Ranatunga, 1975). Citrated blood and tea brew mixtures were made in clean glass tubes by mixing 4 ml of blood in 1ml of different tea brews (Ratnasooriya and Ranatunga, 1975). Similarly, 4 ml of blood was mixed with 1ml of isotonic saline and was used as the control. 1.0 ml of different blood - tea brew mixtures (treatments)

or blood saline mixture (control) was pipetted out into small, clean dry glass tubes (10 mm diameter, 5 cm height), 0.2 ml of 2% calcium chloride added from an eppendoff pipette and the tubes were stoppered immediately. The content of each tube was thoroughly mixed and the calcium induced clotting time was determined by tilting each tube every 30 sec. until a firm clot is formed (Ratnasooriya and Ranatunga, 1975). If the blood did not clot by 10 min. it was considered as unclotted. These unclotted samples were left for 24 h and reexamined for the appearance of a firm clot by tilting.

The results are expressed as means ± SEM. Statistical comparisons were made using Mann-Whitney U-Test. Significance was set at $p \leq 0.05$.

RESULTS

As shown in Table 1, all concentrations of black tea brews of Dust grade No: 1, BOPF and BOP up to 1.25 mg/ml did not significantly ($P > 0.05$) change the calcium induced clotting time, compared to the control. In contrast, no clotting was observed up to 10 min. with 2.5 mg/ml concentrations of all these types of tea brews. These effects were highly significant ($P < 0.001$). Similarly, all the concentrations of decaffeinated Dust grade No: 1, and BOPF did not induce a significant ($P > 0.05$) change with the calcium induced clotting time except at the highest concentration tested (2.5 mg/ml) where no clotting was observed up to 10 min. Further, in all these unclotted samples (at 10 min.) no firm clot was evident even after 24 h.

DISCUSSION

This study examined the anticlotting potential of Sri Lankan black tea (Dust No: 1, BOPF and BOP) *in vitro* using large number of citrated shed goat blood samples. These particular tea grades were selected as these have a high demand among Sri Lankan tea drinkers. Goat blood was used due to its easy availability and because amongst mammals blood clotting mechanisms are essentially similar (Rang *et al.*, 1995). Therefore, the results obtained are meaningful and similar to humans.

The results show, for the first time that, three grades of Sri Lankan black tea, namely, Dust No: 1, BOPF and BOP possess potent anticlotting activity *in vitro*. A remarkable feature of this anticlotting action was that it was not dose-dependent but exhibited an all or none type of response lasting for a long period of time (at least upto 24 h): only the highest concentration prevented clotting. Such drug responses although rare are reported with certain phamacophoes. For example, *in vitro* anticlotting action of aqueous root extracts of *Terminalia glabra* on citrated bovine blood (Ratnasooriya and Ranatunga, 1975), inhibition of human sperm motility *in vitro* by melatonin (Ratnasooriya *et al.*, 1994) and induction of analgesia in rats with aqueous extracts of

Psychotria sarmentosa (Ratnasooriya and Dharmasiri, 1999). Absence of dose-response relationship may possibly indicate the lack of receptor mediation in inducing the anticlotting action. The results show the potential of Sri Lankan black teas to inhibit blood clot formation and also the possibility of developing a cheap agent/drug to store blood samples in blood banks.

The precise mode of the tea-induced anticlotting mechanisms were not investigated in this initial study but some putative mechanisms could be postulated. The clotting of blood involves two pathways: the extrinsic and the intrinsic or contact (Rang *et al.*, 1995). The intrinsic pathway is usually activated when the shed blood comes in contact with an artificial surface such as glass under *in vitro* conditions (Rang *et al.*, 1995). Since this study was conducted *in vitro* it suggests that the anticlotting was mediated via interference of the intrinsic pathway. But, it dose not altogether excludes the mediation of the extrinsic pathway *in vitro* conditions. *In vivo* effects of Sri Lankan teas on blood clotting are also being studied and the results obtained so far indicate that BOPF grade tea affects blood clotting.

Decaffeination of Dust No:1 and BOPF grades of tea did not suppress the anticlotting action in this study. This indicates that anticlotting mechanism in this study is not mediated via caffeine. However, in a recent study caffeine consumption has shown to inhibit platelet aggregation, possibly by up regulation of adenosine A_{2A} receptors (Duffy *et al.*, 2001). Furthermore, decaffeination of Sri Lankan black tea but not Sri Lankan green tea has shown to eliminate the diuretic potential almost completely (Ratnasooriya *et al.*, 2006).

Vitamin K is critically important in the formation of blood factors II (prothrombin), VII, XI and X (fibrinogen). Drugs such as warfarin which prevents the reduction of vitamin K which is necessary for its action as a cofactor of the carboxylase enzyme is known to induce anticlotting action (Rang *et al.*, 1995). But, such a mode of action is unlikely to be operative here as this mechanism occurs, only *in vivo* and would not, have any effect on clotting of shed blood.

Tea is known to lower fibrinogen (which is essential for clotting) level in blood (Greenwell, 1999), and therefore impairs clotting. But these mechanisms too operate only under *in vivo* conditions (Rang *et al.*, 1995) and therefore cannot account for the anticlotting action evident in this study.

Irrespective of the pathway of clotting platelets play a vital role in the thrombus formation (Rang *et al.*, 1995). Tea is rich in polyphenols (Balentine *et al.*, 1997; Modder and

Amarakoon, 2002; Amarakoon, 2004) and tea polyphenols are known to inhibit both platelet activation (Gramza *et al.*, 2006) and aggregation (Modder and Amarakoon, 2002; Amarakoon, 2004; Gramza *et al.*, 2006) and thereby causes prolongation of the clotting time. Such a mechanism is likely to be operative in this study. Further, tea has a strong antioxident activity (Modder and Amarakoon, 2002; Amarakoon, 2004; Abeywickrama *et al.*, 2005) and antioxidants are shown to delay blood clotting (Greenwell, 1999; Felţen, 2004) possibly by inhibition of platelet function. This mode of action is also likely to play a substantial role in the present study.

Drugs like heparin prevents clotting both *in vitro* and *in vivo* primarily by impairing the activity of thrombrin to prevent clot formation. In addition, it also has a negative influence on platelet aggregation (Rang *et al.*, 1995). Although, we do not have definitive evidence such a mode of action may be possible in view of the ability of tea to inhibit production of thromboxane (Greenwell, 1999). Additional experiments are needed to verify these mechanisms.

In conclusion this study for the first time, show that Sri Lankan high grown black teas (Dust No: 1, BOPF and BOP) have strong blood anticlotting activity *in vitro*.

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Table 1. Effect of different concentrations of black tea brew (Dust No:1 ,BOP and BOPF) and decaffeinated black tea brew (Dust No:1 and BOPF) on calcium-induced clotting time of goat blood in vitro (mean ± SEM; sample number (N) are given in parenthesis)

Concentration (mg/ml)	Clotting time (min)				
	Dust No: 1	**BOP**	**BOPF**	**Decaffeinated Dust No:1**	**Decaffeinated BOPF**
0 (control)	2.06 ± 0.004(60)	2.06 ± 0.004(60)	2.06 ± 0.004(60)	2.06 ± 0.004(50)	2.06 ± 0.004(50)
0.125	2.21 ± 0.011(50)	2.25 ± 0.009(60)	2.24 ± 0.007(60)	2.21 ± 0.011(50)	2.11 ± 0.009(50)
0.250	2.28 ± 0.010(50)	2.25 ± 0.012(60)	2.11 ± 0.011(60)	2.15 ± 0.009(50)	2.11 ± 0.009(50)
0.625	2.36 ± 0.009(50)	2.48 ± 0.007(60)	2.27 ± 0.009(60)	2.21 ± 0.009(50)	2.17 ± 0.009(50)
1.250	2.98 ± 0.022*(50)	3.04 ± 0.026*(60)	3.04 ± 0.018*(60)	2.67 ± 0.009(50)	2.43 ± 0.030(50)
2.500	> 10 (50)	> 10 (60)	> 10 (60)	> 10 (50)	> 10 (50)

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