

SHORT COMMUNICATION

***In vitro* thrombolytic activity of Sri Lankan black tea, *Camellia sinensis* (L.) O. Kuntze**

W.D. Ratnasooriya*, T.S.P. Fernando and P.P. Madubashini

Department of Zoology, Faculty of Science, University of Colombo, Colombo 03.

Revised: 11 June 2007 ; Accepted: 03 September 2007

Abstract: This study examined the thrombolytic potential of black tea [*Camellia sinensis* (L.) O. Kuntze.] *in vitro*, using Sri Lankan high grown Dust grade No: 1 black tea and goat blood. Different concentrations of tea were made in isotonic saline (2.5, 5, 10 and 20 mg/mL) and added to preclotted blood and the clot lysis activity was determined (in terms of weight and expressed as % of lysis) following 90 minute incubation at 37 °C. The results showed that tea induced mild to moderate (121-520%) and significant ($p < 0.05$) clot lysis activity. This effect was dose-related. However, the clot lytic activity of black tea was markedly lower than streptokinase (0.1 mg/mL). It is concluded that Sri Lankan black tea possesses fast acting mild to moderate thrombolytic activity *in vitro* when tested on goat blood.

Key words: Black tea, *Camellia sinensis* (L.) O. Kuntze, clot lysis, Dust grade tea, thrombolytic

INTRODUCTION

Tea, which is produced from tender shoots of the *Camellia sinensis* (L.) O. Kuntze (Family: Theaceae) plant, is the most consumed beverage in the world besides water. Based on the manufacturing process, there are three major types of tea: black (fully aerated or fermented); green (unaerated or unfermented) and oolong (partially aerated or semi fermented)^{1,2}. Several studies have shown that tea, especially the black and green types, impair blood clotting in man and animals¹⁻³. Further, it is hypofibrinogenic and shown to inhibit platelet aggregation in rabbits both *in vivo* and *in vitro*^{1,2,4,5}. As such, tea is claimed to be cardioprotective^{1,2}. However, less attention has been focused on clot lysing activity of tea. Since tea contains a variety of water soluble phytoconstituents,^{1,6} it is possible that tea may affect thrombolysis. Thus, the aim of this study was to examine the thrombolytic potential of black tea *in vitro*. This was tested using Sri Lankan

Dust grade No: 1 black tea. Black tea was selected as it accounts for 70% of global tea production and 80% of global tea consumption^{1,2}. Dust grade tea was selected since it is the most commonly consumed type of tea in Sri Lanka.

MATERIALS AND METHODS

Dust grade No: 1 tea was manufactured (on 11-08-2005) at the St. Coombs Estate tea factory (1362 m above mean sea level) of the Tea Research Institute, Talawakelle, with its own green leaves, using the orthodox-rotorvane manufacture technique. Tea samples were packed in triple laminated aluminum foil bags and stored at -20 °C until use. Black tea brew/infusion was made according to the ISO standards⁷: 2 g of tea in 100 mL boiling water and brewed for 5 min. This was then freeze dried and used to make different concentrations in isotonic saline: 2.5, 5, 10 and 20 mg/mL.

Goat blood was collected from the Colombo Municipal Slaughter house, Dematagoda and immediately citrated using 3.1% sodium citrate solution⁸. Thousand microlitres of this blood was added to preweighed microcentrifuge tubes. Two hundred microlitres of 2% calcium chloride was then added to each of these tubes⁴, mixed well and incubated at 37 °C for 45 min for clotting to occur. Clot lysis activity was determined as described in previous studies⁹. Briefly, after clot formation, serum was aspirated out without disturbing the clot and each of these tubes was weighed again to determine the weight of the clot. Thousand microlitres of different concentrations of the tea extract, 2.5 mg/mL (n = 52), 5 mg/mL (n = 77), 10 mg/mL (n = 80) and 20 mg/mL (n = 50) or saline (n = 66) or 0.1 mg/mL of streptokinase (ZLB Behring,

*Corresponding author

Masburg, Germany), reference drug (n = 90) was added to tubes with clots. All the tubes were incubated at 37 °C for 90 min. The fluid left was then carefully removed and the tubes were weighed again. The difference in weight before and after clot lysis was expressed as % clot lysis. The results are expressed as means ± SEM. Statistical comparisons were made using one-way ANOVA with Tukey's family error post hoc test. Significance was set at p < 0.05. Dose dependencies were determined by the regression coefficient (r).

RESULTS

The results are summarized in Table 1. As shown, all four concentrations of black tea induced significant (p < 0.05) clot lysis activity *in vitro*, compared to control: 2.5 mg/mL by 121 %; 5mg/mL by 374 %; 10mg/mL by 519 %; and 20 mg/mL by 314 %. This effect shows a dose-related trend (r² = 0.59; p < 0.05). Streptokinase (0.1 mg/mL) evoked a huge and a significant (p < 0.05) clot lysis activity (by 1117 %).

Table 1: Effect of Sri Lankan high grown Dust grade No: 1 tea on blood clot lysis of goat blood *in vitro* (mean ± SEM)

Concentration of tea and controls	n	% Blood clot lysis
0.9% NaCl solution	66	1.88 ± 0.17
2.5 mg/mL	52	4.15 ± 0.42*
5 mg/mL	77	8.91 ± 0.42*
10 mg/ mL	80	11.64 ± 2.70*
20 mg/mL	50	7.29 ± 0.77*
Streptokinase (0.1 mg/mL)	90	22.89 ± 1.58*

* p < 0.05 compared to control, one-way ANOVA with Tukey's family error post hoc test

DISCUSSION

This study examined the thrombolytic potential of black tea *in vitro* using goat blood. The test model used is a newly developed cheap and simple technique which can be performed with limited facilities available in countries like Sri Lanka. But this technique is validated, sensitive and reliable⁹. Further, a large number of blood samples were tested in this study. Goat blood was used due to easy availability in large quantities and because physiology and biochemistry of thrombolytic mechanisms of different mammals are almost identical^{10,11}. The results show, for the first time, that black tea possesses mild

thrombolytic activity. This is an important finding which may have important implications in cardiovascular health. In addition, this finding may indicate the possibility of developing novel thrombolytic agent/s from tea.

Thrombolytic activity of black tea was rapid and showed a dose related trend (in terms of regression analysis) indicating that the effect is genuine and is selective. However, the thrombolytic activity of the highest dose (20 mg/mL) was lower than that produced by the penultimate dose (10 mg/mL). This could be due to the existence of a bell-shaped dose response curve¹². Alternatively such an effect may result from desensitization¹².

Previous studies have shown that tea impairs coagulation of blood of animals and in man both *in vitro*^{1,3} and *in vivo*¹ and is claimed to be cardioprotective^{1,2}: lowers fibrinogen and impairs platelet aggregation^{1,2,5}. This study showed that black tea has thrombolytic activity as well. Together, these studies indicate that black tea has the ability both to impair thrombus formation and to facilitate thrombus dissolution.

Acknowledgement

This investigation received financial support from the NSF under the grant number NSF/Fellow/2005/01. Thanks are also due to Dr. Ziyad Mohamed, former Director of the Tea Research Institute of Talawakelle.

References

1. Modder W.W.D. & Amarakoon A.M.T. (2002). *Tea and Health*. pp. 81-93, Tea Research Institute of Sri Lanka, Talawakelle.
2. Amarakoon T. (2004). *Tea for Health*. pp. 1-37, BT Options (Pvt) Ltd., Colombo.
3. Ratnasooriya W.D., Amarakoon A.M.T., Fernando T.S.P., Ranatunga R.A.A.R. & Abeywickrama K.R.W. (2007). *In vitro* anticlotting activity of Sri Lankan high grown black tea (*Camellia sinensis*). *Sri Lanka Journal of Tea Science* 72(1):23-29.
4. Mitane Y.S., Miwa M. & Okada S. (1990). Platelet aggregation inhibitors in hot water extracts of green tea. *Chemical and Pharmaceutical Bulletin* 38: 790-793.
5. Tijburg L.B.M., Mattern T., Folts J.D., Weisgerber U.M. & Katan M.B. (1997). Tea flavonoids and cardiovascular disease: a review. *Critical Reviews in Food Science and Nutrition* 37(8): 771-785.
6. Balentine D.A., Wiseman S.A. & Bouwens C.M. (1997). The chemistry of tea flavonoids. *Critical Reviews in Food Science and Nutrition* 37(8): 693-704.
7. International Organization for Standardization (1980). *Tea- Preparation of liquor for use in sensory tests: ISO 3103*, pp. 1-4. Geneva, Switzerland.

8. Ratnasooriya W.D. & Ranatunga K.W. (1975). A plant extract that prevents clotting of mammalian blood. *Ceylon Journal of Science* **11**(2): 77-82.
9. Prasad S., Kashyap R.S., Deopujari J.Y., Purohit H.J., Tori G.M. & Dagainawala H.F. (2006). Development of an *in vitro* method to study clot lysis activity of thrombolytic drugs. *Thrombosis Journal* **4**(2): 1-4.
10. Keeton W.T. (1980). *Biological Science*. pp. 257-307, W.W. Norton and Company, New York, USA.
11. Hickman C.P., Roberts L.S. & Hickman F.M. (1984). *Integrated principles in Zoology*. pp. 611-638, Mosby College Publishing, St. Louis, USA.
12. Rang H.P., Dale M.M. & Ritter J.M. (1995). *Pharmacology*. pp.331-350, Churchill Livingstone, London, UK.