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Investigation of Cytotoxic and Thrombolytic Effect of Ethanolic Extract of White Tea (*Camellia sinensis*).

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ABSTRACT

The present study was designed to investigate the cytotoxic activity of ethanol extract of *Camellia Sinensis* on brine shrimp and thrombolytic activity on human blood. Ethanolic extract of *Camellia Sinensis* was assessed with the Brine shrimp lethality bioassay was used to evaluate cytotoxicity and this extract assessed with human blood to evaluate thrombolytic effect. The Brine shrimp lethality bioassay was used to evaluate cytotoxicity. The extract showed remarkable cytotoxic activity, LC50 value of the extract was 5.312 µg/ml compared to vincristin sulphate, a reference drug. It also has evaluated as thrombolytic agent compared to streptokinase. It has significant thrombolytic effect. These findings demonstrate that the bud & leaves extract of *Camellia Sinensis* have excellent cytotoxic activity and significant thrombolytic effect.

Keywords: *Camellia sinensis*, Cytotoxic, Thrombolytic, LC50, percent (%) of clot lysis.

INTRODUCTION

Camellia sinensis is mainly cultivated in tropical and subtropical climates, in areas with at least 127 cm. (50 inches) of rainfall a year. However, the clonal one is commercially cultivated from the equator to as far north as Cornwall on the UK mainland. Many high quality teas are grown at high elevations, up to 1500 meters (5,000 ft), as the plants grow more slowly and acquire more flavors.

White tea is the least processed form of tea, made of beautiful silver buds and select leaves which have been steamed and dried. Because of its minimal processing, white contains more nutrients than its black or green cousins, making it the mightiest of the teas, the ultimate health tea. [9]

MATERIALS AND METHODS

Plant material

The leaves and silver buds of *Camellia Sinensis* were collected from Botanical garden of Jahangirnagar University, Savar, Bangladesh and authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Cytotoxic test

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds.[2],[3] Here simple zoological organism (Artemia salina) wasused as a convenient monitor for the screening. The eggs of the brine shrimp, Artemia salina, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hr to mature shrimp called nauplii. The brine shrimp lethality bioassay was performed to predict the cytotoxic activity [2], [6] of the Camellia Sinensis. For experiment, The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations 90.91 µg/ml, 74.07 µg/ml, 56.60 µg/ml, 38.46 µg/ml, 19.60 µg/ml and 9.09 µg/ml, 7.40 µg/ml, 5.60 µg/ml, 3.85 µg/ml, 1.97 µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Vincristine sulphate was used as positive control. [4], [5] then matured shrimps were applied to each of all

experimental vials and control vial. After 24 hrs, the vials were inspected using a magnifying glass and the number of survived naupili in each vials was counted. The mortality end point of this bioassay was defined as the absence of control forward motion during 30s observation. [7] From this data the percent of lethality of the brine shrimp naupili for each concentration and control was calculated. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality [8] was plotted on the graph paper and the values of LC50 were calculated Using Microsoft excel 2007.

Thrombolytic activity [10]

The thombolytic activity of this extractive was evaluated by the method of Prasad and collaborators (2006) [10] using streptokinase as standard. The dry crude extract (10 mg) was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered. Aliquots (5 ml) of venous blood were drawn from healthy volunteers which were distributed in five different pre weighed sterile micro centrifuge tube (1 ml/tube) and incubated at 37° C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone). To each microcentrifuge tube containing preweighed clot, 100 µl aqueous solutions of different partitionates along with the crude extract was added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37° C for 90 minutes and observed for clot lysis. After incubation, the released of fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt of released clot /clot wt) × 100

RESULTS AND DISCUSSION

The Ethanolic extract of *Camellia sinensis* possesses cytotoxic activity. The LC50 values obtained from brine shrimp lethality bioassay was 5.3µg/ml (Table 1 and Figure 1) whereas Vincristine sulfate showed 0.52µg/ml.

The ethanolic extract of C. sinensis (EECS) showed significant thrombolytic activity with 14.36% (Table 2, Figure 2) of clot lysis. The positive control (Streptokinase) showed 48.86% and negative non thrombolytic control (distilled water) showed 2.5% of clot lysis.

Table 1 Cytotoxic activity of *Camellia Sinensis*

Concentration (µg/ml)	LogC	No. of nauplii taken	No. of nauplii dead	%Mortality	Probit	LC_{50} (µg/ml)
1.97	0.292	10	3	30	4.75	5.312
3.85	0.585	10	4	40	5	
5.67	0.753	10	5	50	5	
7.40	0.87	10	5	50	5	
9.09	0.959	10	6	60	5.25	
19.60	1.26	10	6	60	5.25	
38.46	1.585	10	7	70	5.52	
56.60	1.753	10	7	70	5.52	
74.07	1.87	10	8	80	5.84	
90.91	1.96	10	9	90	6.28	

Table 2 Thrombolytic Activity of Camellia sinensis

No.	Weight of Empty alpine tube (gm)	Weight of clot with tube(B)	Weight of tube with clot (gm)	Weight of clot (gm) (A)	Weight of tube with clot after lysis (gm)	Weight of lysis (gm) (B)	Weight of Clot after lysis (gm) (X=C-E)	Average % of clot lysis
1	0.8157	1.1634	0.3477	1.1172	0.3015	0.0462	13.2873	14.36
2	0.8176	1.2351	0.4175	1.167	0.3494	0.0681	16.3114	
3	0.8334	1.2068	0.3734	1.1657	0.3323	0.0411	11.0070	
4	0.8223	1.1926	0.3703	1.1389	0.3166	0.0537	14.5018	
5	0.8334	1.2782	0.4448	1.2146	0.3812	0.0636	14.2986	
6	0.8239	1.2341	0.4102	1.1702	0.3463	0.0639	15.5778	
7	0.8293	1.2356	0.4063	1.1966	0.3673	0.039	9.5988	
8	0.8162	1.3608	0.5446	1.2622	0.446	0.0986	18.1050	
9	0.8244	1.2499	0.4255	1.1701	0.3457	0.0798	18.7544	
10	0.8212	1.3099	0.4887	1.2507	0.4295	0.0592	12.1138	

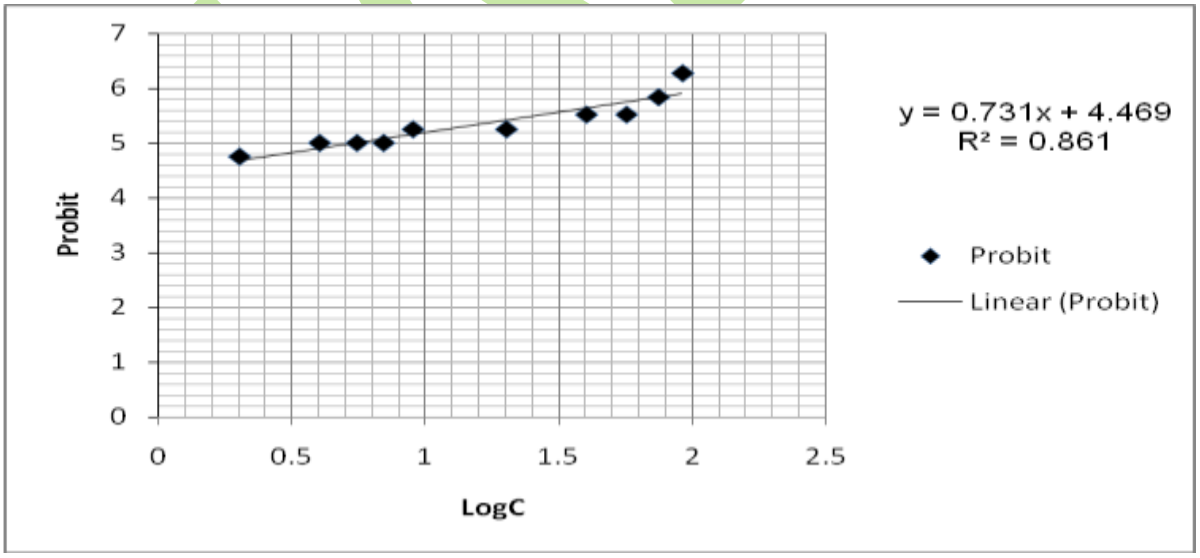

Figure1. Determination of LC_{50} value for extract of *Camellia Sinensis* leaves from linear correlation between log concentrations versus Probit value.

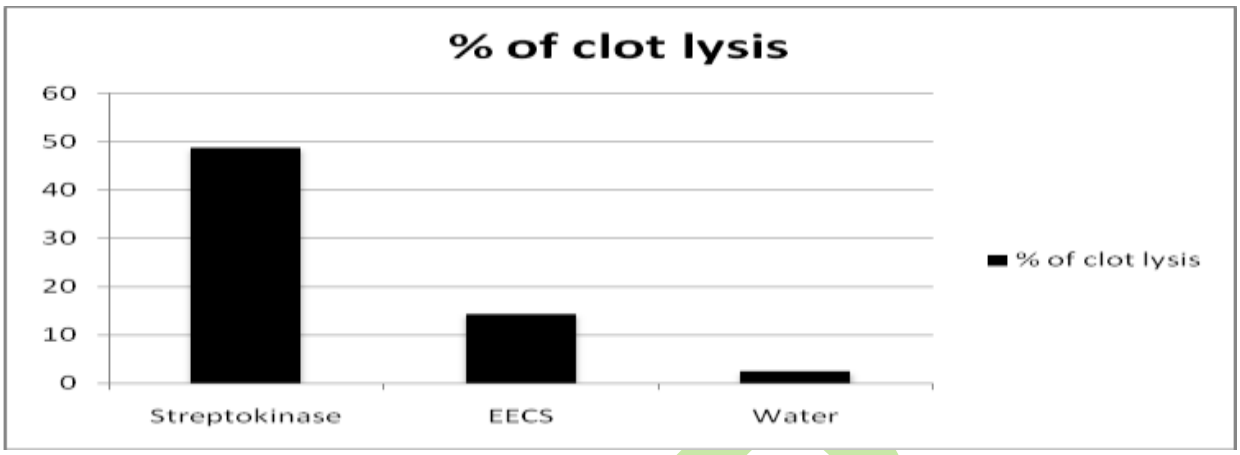

Figure2. Percent of clot lysis of ethanolic extract of *Camellia Sinensis*, Streptokinase, water.

CONCLUSION

From the above results, it is evident that the ethanolic crude extract of *Camellia sinensis* showed significant cytotoxic activities which suggest the presence of bioactive metabolites with biological properties such as antimalarial, anticancer etc. In thrombolytic study screening, the crude ethanolic extract of *C. sinensis* demonstrated significant thrombolytic activity in human blood specimen. The plant could be subjected for extensive chromatographic separation and purification processes to isolate bioactive lead compounds for the discovery of novel therapeutic agents.

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