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Thrombolytic Potential of Aqueous and Methanolic Crude Extracts of *Camellia sinensis* (Green Tea): *In vitro* study

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Investigation of thrombolytic activity of *crude* extracts of *Camellia sinensis* (Green tea) using in-vitro thrombolytic model which is very simple, rapid and easy to do method. Both aqueous crude and methanolic extracts were studied however; the results of methanolic extract were more promising which exhibited maximum 95. 24% clot lysis as compared to aqueous one i.e. 90.34% at 800 µg/ml concentration in 72 hrs of incubation. Various concentrations of leaf extract i.e. 200µg/ml, 400µg/ml, 600ug/ml and 800µg/ml were tested at different time intervals including; 24hrs, 48hrs and 72hrs duration of incubation at 37^0 C for observing maximum clot lysis. The result indicated that concentrations of leaf extract enhanced the percentage of clot lysis in dose dependent manner. On the other hand, Streptokinase SK, a reference standard and water were used as a positive and negative control showed clot lysis maximum 96.63% and 41.32% in 72 hrs of incubation respectively. From results, it can be concluded that if further studies reveals the exact molecule from green tea diverse composition, an effective thromolytic candidate can be achieved for the improvement of the patients suffering from Atherothrombotic diseases.

Keyword: *Camellia sinensis,* Thrombolytic Activity, Green Tea, Atherothrombosis.

1. Introduction

Homeostasis maintains the integrity of circulatory system after damaging of the vascular channel[1]. Thrombus development is a critical event in the arterial diseases associated with myocardial infarction, anoxia, hypertension[2], stroke, and venous thromboembolic disorders that account for considerable number of deaths worldwide[3]. Currently all over the world; thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, alteplase, streptokinase etc. play a very important role in the management of patients with cerebral venous sinus thrombosis (CVST), is a common disorder that is often accompanied by critical morbidity and mortality[4-7]. Urokinase (UK), streptokinase (SK) are cheaply available but their use is not as such safe due to its immunogenicity factor (Jennings, 1996), high risk of hemorrhage, anaphylactic reaction and lack of specificity[1,8]. Because of the shortcomings in the existing thrombolytic agents, a number of researches are underway to improve the variants of these drugs for their better effective nature[9]. *Camiellia sinensis* (Green tea) has been consumed for centuries as a hot beverage and has got immense medicinal properties[10]. The plant of *Camiellia sinensis* is an inhabitant of Southeast Asian region

commonly known as green tea[11]. Since long time, green tea is being widely consumed in China, Pakistan, India, North Africa and Middle East[12]. Green tea has been shown to have a wide range of beneficial physiological and pharmacological effects[13]. Moreover; studies indicated that green tea possess significant anti-oxidant, anti-inflammatory, anti-carcinogenic, antiviral, anti-adhesive to cell surfaces, antiprotozoal, probiotic, anthelminthic, neuroprotective antimicrobial[11,14-20]. The purpose of the undertaken research study was to find out the thrombolytic potential of *Camiellia sinensis* (Green tea) by in vitro-method.

2. Material and methods

2.1 Preparation of aqueous extract:

The Green tea was purchased from the retail market in Karachi-Pakistan in reasonable price in large quantity were brought to the laboratory of Federal Urdu University of Arts, science and Technology (FUUAST)-Karachi-Pakistan for performing various biological activities. The extract was prepared in the concentration of 5% in distilled water. The aqueous extract was prepared in by boiling method of tea in water bath by constant agitation of for 15 minutes. Later, all the coarse suspended particles of tea were first removed by using strainer and then by passing via 0.22um filter (Sherwani *et al*., 2013). The extract was stored in refrigerator in small vials as aliquots till use.

2.2 Methanol Extraction:

The methanol extractions of the active ingredient of the leaves were carried out using Harbone method[21]. 25g of the grinded leaves were soxhlet extracted using 250ml of 95% methanol. The extraction lasted for six hours. The volatile oil obtained was concentrated by evaporation using water bath at 100°C.

2.3 In vitro Thrombolytic analysis:

The thrombolytic activity of *Camiellia sinensis* (green tea) was done by following the methods of using streptokinase (SK) as a standard reference[22-23].

2.4 Streptokinase (SK) Solution Preparation:

Commercially available lyophilized (Streptokinase) vial of 15, 00,000 I.U., was collected and then 5 ml of sterile distilled water was added and mixed properly. This suspension served as a stock from which 100µl (30,000 I.U) was used for *in vitro* thrombolysis[24-25].

Table 1: In vitro-thrombolytic activity of control (water) and standard (Streptokinase):

	Incubation time	Clot lysis
Standard	24hrs	79.32%
	48hrs	92.22%
	72hrs	96.63%
Control	24hrs	8.85%
	48hrs	27.65%
	72hrs	41.32%

Table 2: In vitro-thrombolytic activity of aqueous extract of *Camiellia sinensis* (Green tea):

Concentrations of crude leaf extract	Incubation time	Clot lysis
200ug/ml	24hrs	8.37%
	48hrs	24.44%
	72hrs	42.72%
400ug/ml	24hrs	12.55%
	48hrs	29.37%
	72hrs	56.57%
600ug/ml	24hrs	16.54%
	48hrs	43.68%
	72hrs	70.84%
800ug/ml	24hrs	21.73%
	48hrs	55.99%
	72hrs	90.34%

Table 3: In vitro-thrombolytic activity of methanolic extract of *Camiellia sinensis* (Green tea):

Concentrations of crude leaf extract	Incubation time	Clot lysis
200ug/ml	24hrs	10.77%
	48hrs	29.63%
	72hrs	47.42%
400ug/ml	24hrs	16.95%
	48hrs	36.77%
	72hrs	61.77%
600ug/ml	24hrs	21.24%
	48hrs	53.65%
	72hrs	77.34%
800ug/ml	24hrs	26.33%
	48hrs	67.79%
	72hrs	95.24%

2.5 Blood Collection:

Whole blood (4 ml) was drawn from healthy human volunteers (n = 10) by phlebotomist without a history of oral contraceptive or anticoagulant therapy[26,24]. 500 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots.

2.5 Bioassay:

2.5.1 Effect of crude leaf extract on clot lysis:

Blood sample (500µl) was distributed in pre weighed sterile micro centrifuge tubes and incubated at 37^0C for 90min for clot formation. After clot formation, the serum was finely and completely aspirated without disturbing the clot and the tubes were again weighed to determine the clot weight[27]:

(Clot weight = Weight of the tube containing clot – Weight of the empty tube).

Each eppendorf tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. On the other hand, as a positive control, 100 µl of SK and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the numbered control tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption[28]. Difference obtained in weight taken before and after clot lysis was denoted as percentage of clot lysis[29].

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

2.5.2 Maximum clot lysis observation with respect to concentration and incubation of time

Various concentrations of leaf crude extract of *Camiellia sinensis* (green tea) i.e. 200µg/ml, 400µg/ml, 600ug/ml and 800µg/ml were tested at various time intervals including; 24hrs, 48hrs and 72hrs duration of incubation at 37^0C for maximum clot lysis[28].

3. Results and discussion

Tea has a wide range of water soluble phytoconstituents[30]. Almost all the available thrombolytic agents still have significant shortcomings[31]. According to one of the reports, approximately, 30% of the pharmaceuticals are prepared from plants worldwide[32] and are considered to be less toxic and freer from side effects than the synthetic one[33]. Wonderful efforts have also been carried in recent past towards the exploration, discovery, designing and development of natural pro-ducts with antiplatelet[34], anticoagulant[35], antithrombotic[36] and thrombolytic activity of the plants[26]. As from the research findings of the undertaken study, it was clear concentrations of leaf extract enhanced the clot lysis process in an order of increasing dose along with the incubation time factor. Moreover, both nature of crude extracts aqueous and methanolic exhibited thrombolytic activity; nonetheless much significant results were obtained in methanolic extracts that pointed out the fact the treatment nature of extract preparation release different bioactive compounds. A study indicated that *Camiellia sinensis* (Green tea) contains flavonoids and some other related members like aflavins and the arubigins[37]. Other than flavonoids, green tea also possesses a rich amount of with cathechins[38] and poly phenols[39] that are expected to have biological activities. Some studies also pointed that as green tea is not fermented during processing preserving the enzymes and olive green color; on the contrary, black tea is fermented before drying. Fermentation can destroy some of the active components of black tea[40]. From our study, in case of aqueous *Camellia sinensis* (green tea) leaf extract maximum 90.34% clot lysis was achieved at 800 ug/ml concentration in 72 hrs of incubation as mentioned in Table No-2 while; methanolic extract had greater 90.24% clot lysis at 800 ug/ml concentration in 72 hrs of incubation as mentioned in Table No-3. However; streptokinase SK a reference standard and water were used as a positive and negative control that showed clot lyiss maximum 96.63% and 41.32% in 72 hrs of incubation respectively as indicated in Table No-

1. A number of studies in past have shown that green tea impairs blood clotting in man[41] as well in animals[42]. Similarly, other plants that also have thrombolytic activity that could be due to the wide range of composition like phytoconstituents including rich sources of alkaloids, flavonoids, tannins and terpenoids[43]. Not only plants, some reports like marine algae having a product called Seanol (phlorotannin – active compound), possessing the ability in promotion of dissolution of intravascular blood clot via antiplasmin inhibition[44]. Another remarkable achievement has been conducted in which thrombolytic agent fucoidan, a branched sulfated fucan extracted from brown seaweeds, having antithrombotic potential[45].

4. Conclusion

In conclusion, as literature that is already rich with beneficial effects of *Camiellia sinensis* (Green tea), an endeavor in our study to exploit the potential as thrombolytic agent was promising especially the methanolic version of tea extract. Further study ought to be conducted for the exploration of isolated molecule that can be effective, safer, cheaper, nontoxic enough for ameliorating the thrombosis conditions.

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