Fibrinolytic effect of incubating human blood clots in Cinnamon Cassia using dose dependent : in vitro assays

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Abstract:

Introduction: Herbal medicine is the use of medicinal plants for prevention and treatment of diseases; it has less side effects and they have great effectiveness. This is an experimental comparative study was conducted in Port-Sudan city.

Aim: this study to determine fibrinolytic activity of *Cinnamon Cassia* aqueous extract at different concentrations *in vitro* .

Method: Standardized human whole blood clots were incubated at different concentrations of *Cinnamon Cassia* **(**0. 5, 2, 4, 8,and 16) %**.** The D-dimer (DD) levels and WB clot weight were measured for each concentration after 3 hours of incubation using I chroma II analyzer and sensitive balance. The data was analyzed by SPSS software using one way ANOVA analysis.

Results: The Overall, mean DD (ng/ml) levels were significantly different across samples incubated with *Cinnamon Cassia* aqueous extract at different concentrations , and the mean pre and post incubation WB clot weights (grams) were significantly decreased.

Conclusion: This study concluded that *Cinnamon Cassia* aqueous extract possesses fibrinolytic activity following *in vitro* incubation of WB clot at different concentrations. Therefore, further investigations for *Cinnamon Cassia* aqueous extract as a potential alternative fibrinolytict agent is needed.

Keywords: *Cinnamon Cassia*, Fibrinolysis, D-Dimer and Whole blood clot.

مستخلص:

الطب بالأعشاب هو استخدام النباتات الطبية للوقاية والعلاج ضد الأمراض في كل بلد فإنه يمتد من الطب التقليدي والشعبي لإستخدام مستخلصات عشبية موحدة ومموهة.الطب بالأعشاب هذه الأيام هو واحد من الطرق الشائعة لعلاج العديد من الأمراض لفعاليته وقلة آثاره الجانبية. هذه الدراسة مقارنة تجريبية أجريت في منطقة ولاية البحر الأحمر- بورتسودان تهدف إلى قياس نشاط تكسر الفبرين بواسطة مستخلص القرفة بتراكيز مختلفة في المعمل . تم تحضير جلطات دموية بشرية موحدة في تراكيز مختلفة من محلول مستخلص القرفة (8،4،2،1،0.5 و16)%جرام/مل بعد 0و3 ساعات حضانة , تم قياس مستويات ال دي دايمر ووزن جلطة الدم لكل تركيز باستخدام Ichroma والميزان الحساس. يتم تحليل البيانات بواسطة برنامج التحليل الإحصائي. وبصفة عامة كان متوسط مستويات ال دي دايمر (نغ/مل) مختلفا بشكل كبير عبر العينات التي تم تحضينها مع عدة تراكيز مختلفة من مستخلص القرفة , وكان متوسط وزن جلطة الدم (جرام) ما قبل وبعد التحضير كان هنالك فارق في الوزن. وخلصت هذه الدراسة إلى أن مستخلص القرفة بتراكيزه المختلفة يمتلك نشاط لتكسر الجلطة بعد الحضانة في المختبر ,لذلك ينبغي إجراء مزيد من الاختبارات في مستخلص القرفة ليصبح واحدا من المواد البديلة المكسرة للفبرين .

الكلمات المفتاحية: القرفة ، الفبرين، الجلطة الدموية، دي دايمر

Introduction

Thrombotic diseases such as myocardial, stroke and cerebral infarction which cause a serious consequences of the thrombus formed in blood vessels(1).Thrombolytic agents are used to dissolve the already formed clots in the blood vessels and as important means of establishing reperfusion, however, these drugs such as streptokinase (SK), rtPA and urokinase have serious complication which might lead to fatal consequences(2).In different country such as In India, streptokinase and urokinase were widely used due to the lower cost of SK, however, their use was associated with serious complication such as hemorrhage, severe anaphylactic reaction, lacks of specificity and immunogenicity (2-4). Herbal medicine was used since ancient times for the treatment of many diseases. The herbal products are often considered as safe compound because they are from natural orgin (3). There is increased research on traditional herbal medicines on the basis of their known effectiveness in the treatment of different illness for

which they have been traditionally applied (2).

Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet (5, 6), anticoagulant (7, 8), antithrombotic and thrombolytic activity (9). *Cinnamon* is a common spice used by different cultures around the world for several centuries. It is obtained from the inner bark of trees from the genus *Cinnamomum*, a tropical evergreen plant that has two main varieties; *Cinnamomumzeylanicum* (CZ) and *Cinnamon cassia* (CC) (also known as *Cinnamomumaromaticum/Chinese cinnamon).* In addition to its culinary uses, in native Ayurvedic medicine *Cinnamon* is considered a remedy for respiratory, digestive and gynaecological ailments (10, 11), from different parts of the world have demonstrated numerous beneficial health effects of Cinnamon, such as anti-inflammatory properties, anti-microbial activity, reducingcardiovascular disease, boosting cognitive function and reducing risk of colonic cancer (11).

There are many drugs used for the prevention and treatment of reduced fibrinolytic activity, such as streptokinase, urokinase and rtPA. However, these drugs have severe complications after treatment such as antigenicity , bleeding beside they are very expensive. *Cinnamon Cassia* has been reported as an anti-bacterial, anti-inflammatory, anti-viral, and anticancer activities mention earlier. Its thrombolytic effect on in vitro clot lysis not discovered yet. The *Cinnamon* source from the plant are likely to be saver , less antigenic and inexpensive. Therefore, this study was conducted to determine the fibrinolytic activity of *Cinnamon Cassia* aqueous extract at different concentrations on whole blood clot *in vitro* using DD and whole blood clot weight.

Materials and methods

Ethical consideration:

All volunteers were excused before taken the sample and encouraged by informing them the reasons and benefits of this study.

Whole blood (WB) clots preparation:

Standardized whole blood clots were prepared from (4.50) ml of venous blood derived from a healthy volunteer with blood group O positive (n = 15). The whole blood was then transferred into three different pre-weighed, sterile siliconized, plain glass tubes (12 × 75 mm). Each tube contained 1.50 ml whole blood without anticoagulant and the blood was allowed to clot at room temperature for 3 hrs to ensure complete clot retraction and separation of the clot from the edges of the tubes. After clot retraction the serum was carefully removed from the edges of the glass tube using a Pasteur pipette without disturbing the clot. Finally the weight of the tube together with the clot was determined using an electronic balance. The WB clot weight was estimated by calculating the difference between the weight of the tube containing the clot and the weight of an empty tube.

Preparation of Aqueous extract:

The *Cinnamon* bark (*cinnamomi cassia)* was pulverized and soaked in one volume of water (32 g of *Cinnamon* powder was soaked in 100ml of distilled water) for 48 hours at room temperature, and further dissolved. By sonication for 8 hours. The extract was filtered using sterile filter paper . The yield was about 100 ml, then all the extract were dissolved in a sterile beaker and sterilized by passing it through a 0.22-µm syringe filter.) then one ml of the preparation was added to one ml of normal pooled plasma to make serial dilution.

Preparation of normal pooled plasma (for normal control and as diluents:

Human pooled PPP was prepared and was strictly processed by

collecting 9.00 ml of WB into two tri-sodium citrate tubes (4.5 ml each) from participants with O blood group (n= 15). Immediately after blood collection, the samples were centrifuged at 1500×g for 15 min at room temperature. Then the supernatant was centrifuged at 1200 ×g for 15 min. The procedure was carried out according to the Clinical Laboratory Standardization Institute (CLSI) guidelines for coagulation tests.

WB clot lysis method for fibrinolytic Assessment of *Cinnamon Cassia* aqueous extract:

Different concentrations of *Cinnamon Cassia* aqueous extract (0.5,2,4,8,and 16%) were prepared using pooled PPP as the diluents. The WB clots were immersed in each dilution and incubated in temperature- controlled water bath at 37ºC for 0 and 3 hrs. After incubation, the pooled PPP was transferred into a microcentrifuge tube (bullet tube) using a Pasteur pipette after gentle shaking of the clot. The plasma was centrifuged at 1200 ×g for 5 min, and the supernatant was separated for DD measurement using coagulation analyzer as mentioned above. After removal of the plasma, the clots were weighted by calculating from the differences of the weight of the tube before incubation and the weight after incubation (WB clot weight = weight of tube containing WB clot before incubation – weight of tube containing WB after incubation) using analytical sensitive balance. Then the DD levels was measured by I-chroma II Automated immunoassay Analyzer.

Statistical analysis

Statistical analysis was carried by SPSS software and one way ANOVA test was applied followed by post-hoc comparison using Dunnett T3 for D-dimer and Paired t test was also applied for the clot weight analysis.

Results

A Total of (15) healthy adult subjects with group O were selected to prepare platelet poor plasma and whole blood clot according to inclusion criteria and (15) healthy adult subjects were included in this study for whole blood clot preparation.

Table (1): shows the means (SD) of DD level for different concentrations of *cinnamon* aqueous extract. The DD means level show gradually increasing between different concentrations of *cinnamon* aqueous extract until reach enzyme limit activity (plateau shape) then there was reduction in DD level as concentration increased, which indicates the effect of *cinnamon* aqueous extract on fibrinolysis.

Table (1): Descriptive statistics of DD level means (SD) level at different concentrations of *cinnamon* aqueous extract (n=7)

Concentrations of *cinnamon*	DD
	Mean (SD)
Base line	50.14 (1.07)
% 0.5	701.95 (143.14)
% 1	1066.13 (115.25)
% 2	1900.38 (372.03)
% 4	772.37 (125.77)
% 8	439.06 (89.57)
% 16	269.60 (61.68)

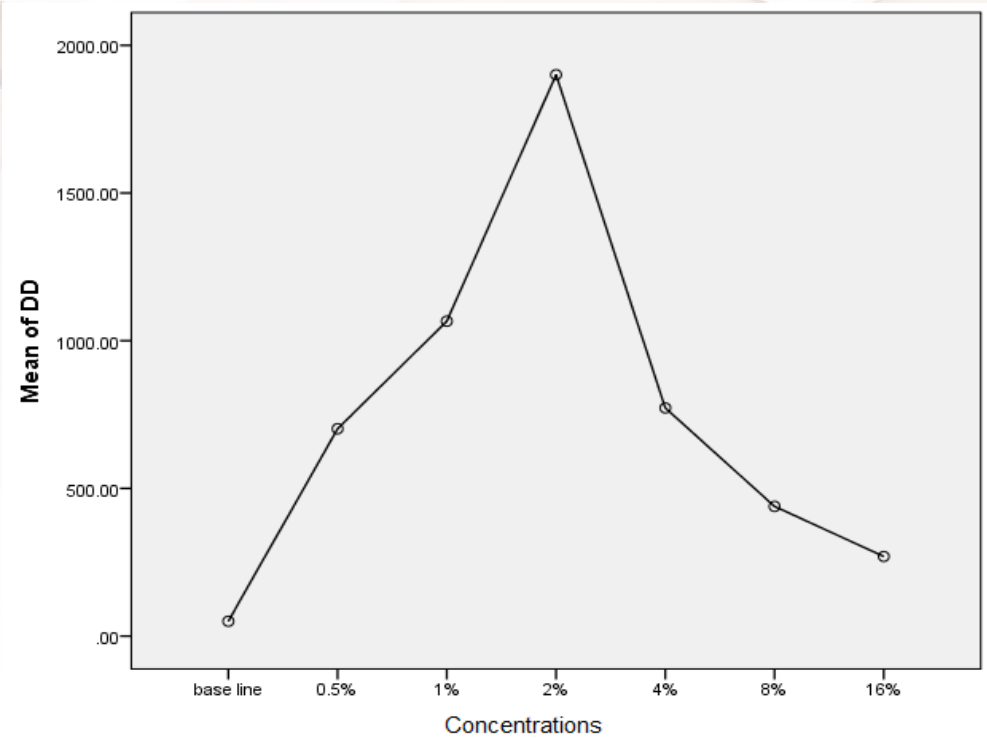

Figure (1): show the mean of DD level means (SD) level at different concentrations of *cinnamon* aqueousextract (n=7)

Table (2) Shows significant means differences (MD) of DD level between different concentrations of *cinnamon* aqueous extract base line vs %0.5, base line vs %1, base line vs %2, base line vs 4%l, base line vs 8%, base line vs %16, %0.5 vs %1, %0.5 vs 2%, 0.5% vs 8%, %0.5 vs% 16, 1% vs %2, 1% vs 4%, 1% vs 8%, 1% vs %16, %2 vs %4, %2 vs %8, 2% vs %16, %4 vs% 8 and 4% vs % 16($p<0.05$). Except for 0.5% vs 4% which showed insignificant MD ($p>0.05$). This indicates the effect of *cinnamon* aqueous extract on fibrinolysis at different concentrations on DD level.

Table (2): Comparison of means differences of DD level between different concentrations of *cinnamon* aqueous extract (n=7)

Concentrations	DD	
	MD (95% CI)	p-value
Base line vs % 0.5	-651.18 (-894.90, -408.72)	<0.001
Base line vs % 1	-1015.98 (-1296.82, -735.14)	<0.001
Base line vs % 2	-1850.24 (-2482.02, -1218.45)	<0.001
Base line vs % 4	-722.22 (-971.43, -473.02)	<0.001
Base line vs % 8	- 388.91 (-541.01, -236.81)	<0.001
Base line vs % 16	-219.45 (-324.198, -114.71)	<0.001
% 0.5% vs 1	-346.17 (-653.30, -75.04)	0.012
% 0.5% vs 2	-1198.43 (-1819.05, -577.81)	0.001
% 0.5% vs 4	-70.42 (351.46, -210.63)	> 0.950
% 0.5% vs 8	262.90 (17.75, 508.04)	0.033
% 0.5 % vs 16	432.36 (193.37, 671.34)	0.001
1% vs % 2	-834.26 (-1456.65, -211.86)	0.010
%1 vs %4	293.76 (5.46, 582.06)	0.045
1 vs 8%%	627.07 (368.34, 885.80)	<0.001
%1 vs %16	796.53 (536.16, 1056.90)	<0.001
% 2 vs 4%	1128.02 (506.63, 1749.40)	0.001
2 vs 8%%	1461.33 (837.10, 2085.69)	<0.001
2% vs 16%	1630.78 (1003.12, 2258.45)	<0.001
4% vs 8%	89.66, 576.95))333,31	0.007
4% vs 16%	502.77 (263.28, 742.25)	0.001
% 8 vs% 16	169.46 (13.53, 325.38)	0.030

One way ANOVA test was applied followed by post-hoc comparison using Dunnett T3. P value set as <0.05 is significant.

MD:meandiference

Table (3) showed the mean (SD) and mean differences of whole blood clot pre and post incubation with different concentration of *cinnamon* aqueous extract. The whole blood clot in post incubation with *cinnamon* aqueous extract was lower than pre and statistically significant at different concentrations of *cinnamon* aqueous extract ($p< 0.05$). Except for concentrations of 8% and 16% which showed insignificant value ($p>0.05$). The results indicated that the effect of the *cinnamon* aqueous extract on blood clot weight.

Table (3). Comparison of pre and post incubation of whole blood clot with *cinnamon* aqueous extract (n = 40)

Concentrations	Mean (SD)		MD (95% CI)	*P* value
	Pre	Post		
% 0.5	0.96 (0.21)	0.91 (0.21)	0.05 (0.03, 0.06)	<0.001
% 1	1.07 (0.12)	1.00 (0.10)	0.07 (0.03, 0.12)	0.006
% 2	0.97 (0.25)	0.87 (0.26)	0.10 (0.06, 0.14)	0.001
% 4	0.92 (0.22)	0.85 (0.25)	0.08 (0.02, 0.13)	0.012
% 8	0.92 (0.25)	0.84 (0.26)	0.08 (-0.01, 0.17)	0.059
% 16	0.91 (0.26)	0.81 (0.25)	0.10 (0.01, 0.18)	0.031

Paired t test applied p value set as 0.05. P-value <0.05 is significant

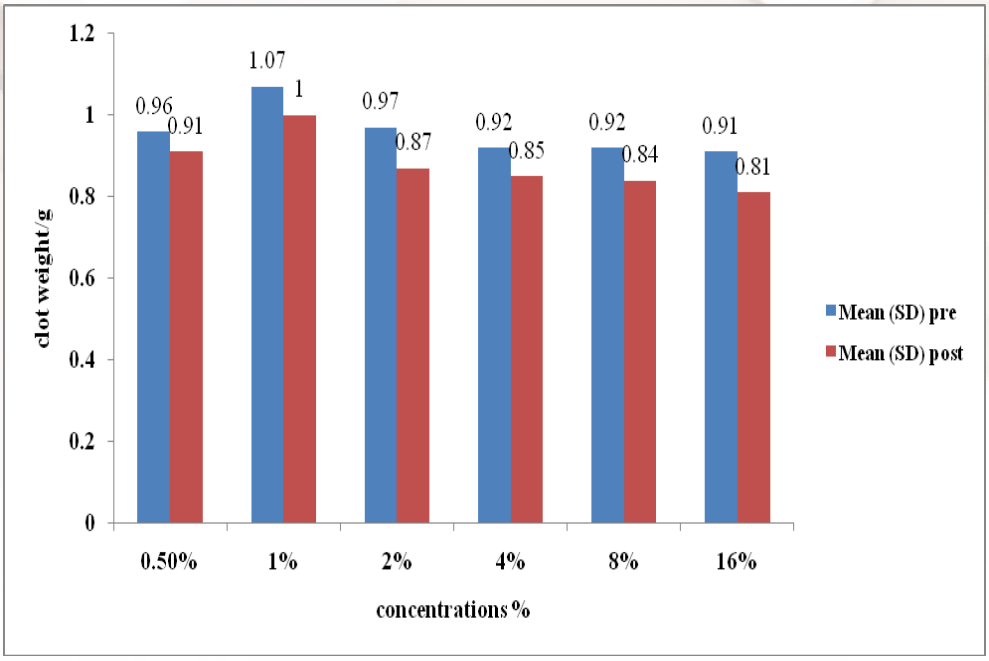

Figure (2): Comparison of pre and post incubation of whole blood clot with *cinnamon* aqueous extract (n = 7)

Discussion

Coronary heart disease (CHD) is the most common form of heart disease . It occurs when the arteries supplying blood to the heart narrow or harden from the build-up of plaque . Plaque is made up of fat, cholesterol and other substances found in the blood . This plaque build- up is also known as atherosclerosis. *Cinnamon* is the one of the most important herbal drugs and has been widely used in the world in a folk medicine , *cinnamon* has been traditionally applied to the treatment of inflammatory disorders , gastric diseases , antimicrobial , antiviral , anti oxidant , antitumor ,antihypertension , and antilipemic .This study aimed to to determine the fibrinolytic activity of *Cinnamon Cassia* aqueous extraction whole blood clot using *in vitro study* . The result of study showed that DD means level showed significant increasing between different concentrations of cinnamon aqueous extract (0. 5, 2, 4, 8,and

16) % , until reach enzyme limit activity (plateau shape) then there was reduction in DD level as concentration increased which indicates there was effect of *Cinnamon Cassia* aqueous extract on fibrinolysis.The result of WB clotting weight showed that there was reduction in clot weight pre and post which indicated there is significant difference. This study has been done by combination of two previous clot lysis studies by Parsat et al and Abuzar Elnager et al 2013. There is no previous study on the effect of *Cinnamon* and its thrombolytic effect on clot lysis which indicate that this study is novel.

Conclusion

This study concluded that *Cinnamon Cassia* extract possesses fibrinolytic
activity using DD level and clot weight.

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