The Effect of Trachyspermum Capsicum Aqueous Extract on Blood Homeostasis

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Abstract

T. capsicum from medicinal herbs is considered as a rich source of thymol with healing properties. Enzymatic coagulation factors stem from the liver. In the present study, 48 adult Wistar male rats were selected and divided randiomly into six groups, including control,witness, and 4 experimental groups. Bleeding time (BT), clotting time (CT), prothrombin time (PT) and activated partial thromboplastin time (PTT) were measured by wounding on the ears, slide and coagulometric methods, using the Dade Behring kits and four-channel-coagulometer, respectively. The resulting values were then analyzed. The results of the measurement of prothrombin time (PT) showed no statistically significant differences in the groups at $P \leq 0.05$ level. But the measure of activated partial thromboplastin time (PTT) revealed a significant difference between experimental group 2 and the control group. The average time of clotting represented a significant statistical difference in all experimental groups compared to the control group. Moreover, there was statistically significant difference between the mean value of bleeding time of experimental groups 1, 3 and 4 in comparison to the control group ($P \leq 0.05$). Based on the findings, it is concluded that an aqueous extract of 400 mg / kg. BW C. capsicum can be used to enhance the speed of coagulation. Since different doses of aqueous extract of T. capsicum show different results on the bleeding time, this extract can be used at low doses to enhance platelet activation and for people who are prone to blood clotting and thus prone to different strokes; the high doses of this extract prevent the formation of unwanted blood clots.

Keywords: *extract of T. capsicum, prothrombin time, thromboplastin time, bleeding time*

Introduction

The use of herbs for treatment purposes coincides with the history of human life. Although in the past half century, the use of chemical and synthetic drugs has been common among people, the severity of their harmful effects on marine life has been recognized and converted to herbal usages. It should be noted that the use of medicinal plants has historically been one of the most effective methods of treatment (Doosti,1978). Trachys (Trachyspremium capsicum) is a plant of the Apiaceae and herbaceous families (Amin,1991;Zagari,1988), aromatic, hairless and with erect stems to a height of 20 to 50 cm, with an 8-6 split. The plant is grown in the eastern regions of India, Iran, and Egypt (Ghasemi,2002;Hedge,1987), and its fruit is dry and ripe which is also called T. capsicum

(Hedge,1987; Zagari,1988). T. capsicum contains compounds such as thymol, Simon, a-pinene, D.pentene, γTerpinene, β-pinene, Sabinene, and Carvacrol. Its chemical composition of protein, fat, and cations include sodium, potassium, iron, calcium, magnesium, zinc, copper and cobalt (Ballba et al.,1973;Petal et al.,1979).

T. capsicum has been used orally for analgesic, anti-asthma, anti-nausea, mucus and locally used in the treatment of rheumatic pains and it has therapeutic effects on skin, nervous and urinary - genital diseases, as well as a diuretic, carminative and anti-worm (Nadkarinis,1976). Previous studies have shown that excessive consumption of this plant may cause yellowing the skin and blurred vision and its consumption has been prohibited for pregnant women and cardiovascular patients. Moreover, using T. capsicum for more than 3 weeks is very harmful as it increases bile and liver problems (Doosti,1978;Ameri Mahabad,1999). Enzymatic -coagulation factors stem from the liver. They are in the plasma as before enzymes and must be enabled to act. Tissue thromboplastin is a Lipoprotein which runs the external device of coagulation. In the absence of adequate amounts of vitamin K (such as coumarin poisoning), coagulation factors are released without adequate carboxylation. The activities of these factors are therefore inadequate and insufficient (Faranoush et al.,2006). Phospholipid cofactor platelets, which comes from platelet membranes, makes a structural path through which activation of clotting factors is greatly accelerated on its basis. Tissue thromboplastin is a phospholipid that forms a complex with a protein blend. Therefore, it is a lipoprotein. Anticoagulant such as EDTA, citrate, and oxalate make bonds with calcium to prevent clot formation (Majnoon Hosseini et al.,2001). X factor, activated in internal or external pathways by activated factor V through thrombin, converts prothrombin Pro-Enzymes (factor II) to thrombin. Thrombin accelerates domestic, shared or public routes by activating factor VIII, V; and thrombin major role is the conversion of solution fibrinogen to insoluble fibrin (Majnoon Hosseini et al.,2001). Vitamin K is necessary for thehepatic synthesis of prothrombin and coagulation factors of X, IX, VII (Razavi et al.,2015;Mohammadiha,1998). Any change in the internal or external factors routes of coagulation and/or blood platelet numbers and activity may alter blood homeostasis, resulting in an increase or decrease of the blood clotting process. Since there was no published research with respect to the effect of T. capsicum aqueous extract on the blood homeostasis, the present study was an attempt to investigate such effect.

Methods and Materials

In this study, 48 Wistar male rats weighing approximately 200 ± 20 g and aged between 2.5-3 months were used from Razi Vaccine and Serum Research Institute of Animal foster homes. The animals were transmitted and maintained to foster homes as laboratory animals in the Islamic Azad University of Kazeroun. The time used to be adapted to the environment of tested animals during the investigation with temperature 25 °±2 C was 12-hr light-dark cycle. In addition, in order to be adapted to environment, the tests were conducted one week after the arrival of the animals to the new situation. Standard extraction techniques were used to T. capsicum in order to prepare the purest extract. The seed was ground. To make the aqueous extract of T. capsicum, 1500 grams of the powder were mixed with distilled water, and then were put in a shaker device to completely dissolve it. After 48 hours, a metal cloth was used to make it smooth. At the same time, re-distilled water was added to the particles which were not

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crossed from the cloth for 24 hours. They were placed on a shaker and the procedure was repeated for this mixture. Finally, the supernatant filtrate liquid was poured in special Flatware pots and placed in an incubator to prepare the pure extract and water vapor. Setting the incubator temperature to 50 C^0 after 72 hours, the dry extract was cut from containers by spatula and was kept in a refrigerator (Behnam Rasuli et al.,2001).

The 48 mice under study were divided into 6 groups: control, witness, and four experimental groups. The experimental groups received different doses of an aqueous extract from T. capsicum during 32 days. The amount of distilled water for the control group, as well as the specific doses for each four experimental groups, was injected by insulin syringe intra-peritoneally. In order to evaluate the possible effects of T. capsicum extracts on body weight, all samples were weighed before the first intake of the extract and after the whole procedure. At the end of 32days, blood samples were collected in each group. Then, PTT, and PT tests were performed on the samples. Porothrombin time (PT) and activated partial thromboplastin time (APTT or PTT) measurement were carried out through coagulometer method using the Dade Behring kit and a set of four-channel-coagulometer. BT test was carried out with sores on the ears and CT test was performed by the slide method. The collected data were analyzed by SPSS software. ANOVA and Tukey tests were calculated and the level of significance for all tests was considered as $p \leq 0.05$.

Results

The results of this study are presented in Tables 1 and 2. The average prothrombin time (PT) in extract-treated groups has numerically declined as compared to the control group, but the average prothrombin time (PT) in none of the groups showed significant differences in comparison with the control group ($P > 0.05$).

The results of activated partial thromboplastin time (PTT) showed no significant statistical differences in the average time in the witness and control groups. The mean time for activated partial thromboplastin (PTT) in the experimental group 1 that received the extract increased numerically compared to the control group, and in other groups it reduced numerically. Moreover, in the experimental group 2 that received 400 mg /kg.BW of the T. capsicum aqueous extract, a significant statistical difference was observed in terms of relatively activated thromboplastin time ($P \leq 0.05$) compared to the control group.

The results of the clotting time (CT) in the control and witness groups showed no significant differences ($P>0.05$). The mean clotting time (CT) increased in the group receiving the extract compared to the control group so that all the experimental groups depicted a significant difference ($P \leq 0.05$) compared to the control group.

The results of the bleeding time (BT) demonstrated no statistically significant differences between the mean values of this parameter in the control group in comparison with the witness group. Moreover, the mean bleeding time (BT) in experimental groups 1 and 2 that received the extract showed a decrease as compared to the control group and an increase in the experimental groups 3 and 4. The changes in the experimental group 1 (dose as 200 mg / kg.BW), experimental 3 (600 mg / kg.BW), and experimental 4 (800 mg / kg.BW) revealed a significant difference in the mean bleeding time in the experimental group compared to the control group.

Table 1. The mean and standard deviation for prothrombin time, partial thromboplastin, bleeding time and blood coagulation of the various groups

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Parameter	Control (Minute)	Sham (Minute)	Experimental 1 (Minute)	Experimental 2 (Minute)	Experimental 3 (Minute)	Experimental 4 (Minute)
PT	17/33 ± 0/60	18/02 ± 1/08	17/27 ± 1/06	15/73 ± 1/18	16/55 ± 0/42	16/30 ± 0/67
PTT	37/42 ± 2/50	35/67 ± 1/76	38/86 ± 2/06	31 ± 1/18*	29 ± 2/68	32/75 ± 1/32
CT	1/22 ± 0/17	1/89 ± 0/31	3/10 ± 0/19*	2/83 ± 0/33	3/62 ± 0/31*	4/00 ± 0/20*
BT	1/60 ± 0/08	1/36 ± 0/08	1/31 ± 0/05*	1/41 ± 0/08	2/14 ± 0/12*	2/29 ± 0/04*

Table 2. Significant differences between prothrombin time, partial thromboplastin active, bleeding time and blood coagulation of the various groups compared with the control group.

Parameter	Experimental4	Experimental3	Experimental2	Experimental 1
PT	.293	.368	.256	.962
PTT	.194	.056	.043	.662
BT	.000	.004	.121	.015
CT	.000	.000	.002	.000

Discussion

In this study, the results did not show any significant statistical difference between the prothrombin time in any of the experimental groups compared to the control group. These findings indicate that the aqueous extract of T. capsicum has no significant effect on the activity of extrinsic pathway of blood coagulation (Table 2) (P> 0.05). However, the statistical analysis revealed a significant difference between the incidence of partial thromboplastin time active in the experimental group 2 compared to the control group at the end of the study period (Table 2) (P≤ 0.05).

It seems that the T. capsicum aqueous extract used with a dose of 400 mg/kg.BW decreases the time, but with an increase in the dose, this effect disappears. Thus, in order to accelerate coagulation pathway, a dose of T. capsicum extract 400 mg/kg.BW can be used.

Some previous studies highlighted the effects of vegetarian diets in reducing the clotting factor or increasing fibrinolysis. Such plants decrease the blood clot through decreasing the fibrinogen. Inhibition of platelet aggregation and other effects of some other plants can also increase prothrombin time (Noto et al,2002).

In this regard, Asghari et al. (2011) state that Allium Hirtifolium decreases serum fibrinogen and VII factor. So, taking this herb can reduce the risk of cardiovascular disease. Patients, for whom the changes in their coagulation factors are important, should consider this effect of Allium Hirtifolium.

The results of this study showed that there were statistically significant differences between the mean values of coagulation time in the experimental group compared to the control group. The difference between blood coagulation time in experimental group 1 in comparison with the control group is due to a decrease in duration of blood coagulation in experimental group 1. The increase in BT in groups 3 and 4 is enough to cause a significant difference between the amounts of time between the experimental groups compared to the control group. It seems that T. capsicum extract with a low dose (200mg / kg.BW) has a positive impact on platelet activation, while a high and very high dose of this extract has the opposite effect on platelet activation. The extract with low doses (200mg / kg.BW) can therefore be used to enhance the platelet activation. In people who are prone to blood clotting and thus prone to different strokes, the high doses of the extract (600, 800mg / kg.BW) prevent the formation of unwanted blood clots and high doses of the extract can be partially an alternative for aspirin. In cases where the aim is to maintain platelet activation in a natural level, 400 mg / kg.BW of this extract is the most appropriate dose for having no effect on platelet activation.

Flavonoids such as Quercetin, caempferol, and myricetin prevent platelet aggregation, so the flavonoids of T. capsicum can cause the prolonged bleeding time in groups 3 and 4 (Grenett et al.,2011).

In the process of coagulation, different factors affect each other so that even loss of a factor and its side effects depend on the other factors. Faranoush et al. (2009) point out that the severity of bleeding in patients with Factor XI deficiency is associated with the level of Factor V Brenner et al. (1997) investigated the predicting factors for bleeding in 45 families who lacked factor XI. Although the level of factors VIII and IX were related to the severity of bleeding, the level of factor VIII was not a predictor of the severity of bleeding

In general, the results of this study showed that different doses of aqueous extract of T. capsicum have different impacts on platelets as well as domestic and international routes of clotting activity. Therefore, when taking the extract, consumer properties should be taken into account and a proportional dose should be chosen.In this regard, Zomorodian et.al (2011) state that the main oil (net), A ecotype T. capsicum with high concentrations of thymol, show antimicrobial activity better than B ecotype.

The methanolic bark extract of Careyarboreal exhibits anticoagulant activities when compared to the standard warfarin Prolongation in PT. Moreover, prolongation of aPTT may be due to a decrease in coagulation factors such as V, VII, and X involved in extrinsic pathway, and also a decrease in coagulation factors such as VIII, IX, XI, XII (Varadharajan et al.,2010;Chan et al, 2007) .

The aqueous extract of leaves prolongs aPTT and thrombin time (TT). Some other investigations evaluated the bioactive compounds responsible for the anticoagulant activity as well as the determination of the coagulation factors affected (Hamid et al.,2010).Turmeric suppresses the ability of platelets to stick together to form clots, Curcumin prolongs aPTT, and PT significantly and inhibits thrombin and FXa activities. These anticoagulant effects

of curcumin are better than the other derivative of Curcuma longa. This is indicating that methoxy group in curcumin positively regulates the anticoagulant function of curcumin (Kim et al.,2012).

T. violence plant displays the best anticoagulant activity.In clinical tests of blood coagulation, PT is used to evaluate the overall efficiency of the extrinsic clotting pathway; a prolonged PT indicates a deficiency in coagulation factors V, VII, and X. On the other hand, aPTT is a test of the intrinsic clotting activity; a prolonged aPTT usually represents a deficiency in factors VIII, IX,XI, XII and V (Davison et al.,2012).

Terminalia Billerica fruits possess thrombolytic and antithrombotic activity in vitro; however in vivo clot dissolving properties and active component of Terminalia Billerica for clot lysis are yet to be discovered. (Waseem A Ansari, H Siddiqui, SatyaPrakash Singh., 2012). Regarding antithrombotic experiment, the clot is formed in a normal time or with a slight delay when normal saline is added to the control one, whereas the tube, to which Streptokinase is added, the clot is not formed and in case of extract solutions, significant delay in clot formation time is noted as according to concentration (Ansari et al.,2012).

Conclusion

As the findings in this study revealed, the aqueous extracts of T. capsicum did not show any significant impact on the activity of factors in extrinsic pathway of coagulation. Therefore, in order to accelerate coagulation pathway, 400 mg/kg. BW of aqueous extract of C. capsicum should be used. Since different doses of aqueous extract of T. capsicum manifest different results on the bleeding time, this extract can be used at low doses to enhance platelet activation and for people who are at risk of blood clotting and thus prone to different strokes. The high doses of the extract prevent the formation of unwanted blood clots and can be partially an alternative to aspirin.

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