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The Physiological Changes in Blood Coagulation Parameters Induced by the Therapeutic Doses of the Chinese Danshen Plant (*Salvia miltiorrhiza*) in Male Guinea Pigs (*Cavia porcellus*)

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Abstract: The present experimental physiological study on male Guinea pigs aims to examine the effects of the Chinese Danshen roots aqueous extract on the blood coagulation parameters. Animals in two treated groups (G_2) and (G_3) were given daily two therapeutic doses for 15 days (21 and 43 mg kg⁻¹, respectively). The current results of the haematological measurements showed that Danshen roots extract induced marked significant inhibitory effects on the blood coagulation parameters, mainly under the influence of the high therapeutic dose (43 mg kg⁻¹). Under this dose, significant increases were recorded in the means of Prothrombin time, INR and APTT (66.80±1.8 sec, 11 and 56.60±2.7 sec, respectively) compared to the control group. On the other hand, significant decreases were recorded in means of serum Fibrinogen level and Fibrin degraded products (1.65±0.12 g L⁻¹ and 0.63±0.05 mg L⁻¹, respectively) compared to the control group. Similarly, significant decreases were observed in the means of the following coagulation parameters:- Factor (IX), Factor (VIII), Anti-thrombin (III), Protein C and protein S (29, 46, 57, 61 and 24%, respectively) compared to control group. In conclusion, the results can be considered as an indicator proving the hypocoagulative effect of the active compounds of Danshen roots on blood coagulation process. Hence, this useful therapeutic effect can be used in the treatment of thrombosis cases in human patients. But, further pharmacological and toxicological studies should be performed to examine the therapeutic safety of Danshen roots.

Key words: Danshen roots, *Salvia miltiorrhiza*, blood coagulation, vascular thrombosis

INTRODUCTION

Danshen (*Salvia miltiorrhiza*, Family Lamiaceae) is a traditional Chinese medicinal plant (Ody, 2001). This plant is still used up today in Chinese herbal medicine to treat number of diseases such as coronary arteriosclerosis, hypertension, hepatitis, stroke and ischemic apoplexy (Chan *et al.*, 2004; Adams *et al.*, 2006). It is also used to improve and nourish the blood circulation and to remove blood stasis (Liu *et al.*, 2000; Xie and Mao, 2001; Lam *et al.*, 2006). Danshen plant has different names in China including Red Sage, Chinese Sage, Chinese *Salvia* (Chan *et al.*, 2004). According to Ody (2001), the therapeutic part of this plant is its crude dried red roots. In regard the active therapeutic compounds of Danshen roots, previous investigators reported the presence of the following therapeutic compounds in the Danshen roots:- Tanshinone (IIA), Tanshinone (IIB), Isotanshinone, Rosmarinic acid, Ursolic acid, Salvianolic acid and Cryptotanshinone (Zou *et al.*, 1993; Che *et al.*, 2004; Lam *et al.*, 2006; Zhang *et al.*, 2006). According to earlier studies, some of these compounds are believed to cause vasorelaxative effect on the coronary arteries and improvement of the

blood circulation to the heart (Steinkamp *et al.*, 2007; Lee *et al.*, 2008). Specialists of Chinese herbal medicine believe that the aqueous extract of Danshen roots has a unique therapeutic anticoagulative effect on the blood coagulation process, this anticoagulative effect can help in treating cases of vascular thrombosis in humans (Ody, 2001; Zhang *et al.*, 2006). Some earlier studies attempted to investigate this anticoagulant therapeutic effect of Danshen roots (Cheng, 2000; Pan *et al.*, 2005). But, it is very important to mention that there are still some disagreement and uncertainty between the investigators in regard this useful therapeutic effect of the Chinese Danshen. For instance, Yu *et al.* (1997) have uncertainty about this effect and they oppositely believe that treatment with Danshen roots can cause hypercoagulation and vascular thrombosis in human patients. In contrary, others believe that Danshen roots have anticoagulative effect and it can be used to remove the vascular thrombus (Ody, 2001; Izzo *et al.*, 2005). However, the therapeutic safety and the effectiveness of Danshen roots have not been confirmed yet through systematic scientific researches, for this reason, the USA Food and Drug Administration did not approve the therapeutic use of this plant in USA due to

the lack of sufficient scientific evidences regarding the therapeutic effectiveness and safety of this plant (Bristol *et al.*, 2008). Therefore, the present experimental study on adult male Guinea pigs aims to investigate the physiological effects of two therapeutic doses of the aqueous extract of Danshen roots (*Salvia miltiorrhiza*) on the blood coagulation parameters, in order to confirm or reject the claim that Danshen root produce anticoagulative effect on the blood coagulation process.

MATERIALS AND METHODS

Preparation of Danshen roots extract's and the doses:

Fresh Danshen roots (*Salvia miltiorrhiza*) have been obtained directly from (Southeast Medicine Logistic Company for the Chinese medicinal herbs), in Quanzhou City, China through private friend. At the same time, few samples of the leaves and flowers have been obtained as well for the taxonomy purpose. In Jeddah, first of all the taxonomical position and the scientific name of Danshen samples have been examined and confirmed in the herbarium of the Biological Sciences Department, Faculty of Science in King Abdul Aziz University. The scientific name of the Danshen roots was found to be correctly (*Salvia miltiorrhiza*). The method used to prepare the aqueous extract of Danshen roots and the two tested therapeutic doses was exactly as that described by Ajarem (1990) which is a regular well known method used in the herbal medicine researches. Two calculated therapeutic doses were prepared from the above extract (first dose:- 21 mg of Danshen roots powder kg^{-1} of animal body weight, while the second : 43 mg of Danshen roots powder kg^{-1} of animal body weight). These tested therapeutic doses were selected according to the earlier studies of Ody (2001) and Chen and Chen (2004) with little modification.

Animals and treatment: Twenty four adult male Guinea pigs (*Cavia porcellus*) (Dunkin Hartely strain) weighing 700-750 g were used in this study. The animals have been obtained from the animal house unit of King Fahed Medical Research Center in King Abdul Aziz University in Jeddah. Each animal was housed in a wide proper plastic cage and kept under constant normal temperature (22°C), 12 h dark/light cycle (Al-Tayib, 2004). Animals were given distilled water and diet daily. Prior the experiment start, animals were left for two weeks to acclimatize. After the acclimatization period, animals were divided in-to 3 groups (8 animals in each), the first group (G_1) was the control group, whereas the second group (G_2) and the third group (G_3) were treated groups, in which each animals was given orally a daily dose of

(21 and 43 mg kg^{-1} , respectively) using gastric feeding tube, for a continuous treatment period of 15 days.

Collection of blood samples: At the end of the treatment period, each animal of the three groups was anesthetized by inhalation of drops of Diethyl Ether in a piece of cotton. Blood samples were collected via cardiac puncture according to the method of Kageyama *et al.* (2000). Blood samples were placed in Sodium Citrate tubes for the measurements of blood coagulation parameters according to the methods of Harrison *et al.* (1997) and Takano *et al.* (2001). A part of the blood samples was placed in EDTA tubes for platelets count. All the blood samples tubes were surrounded by small ice pieces and then sent within less than half an hour to a specialized medical laboratory (AL-Mamlaka Medical Laboratories, Jeddah) to avoid the formation of any small clots in the tubes. The blood samples were centrifuged at 3000 rpm for 5 min to separate the serum in order to measure the blood coagulation parameters and platelets count using a computerized coagulation analyzer.

Measurements of blood coagulation parameters: The haematological method applied to measure the blood coagulation parameters and platelets count in the serum samples was as that described by Chaloupecky *et al.* (2005) and Yang *et al.* (2006). The blood coagulation parameters were measured in the serum samples using a computerized (Dade Behring Coagulation Analyzer), manufactured by Dade Behring corporation, Marburg GmbH, Germany. Specific kits were used in the measurements, these kits were obtained from the same company. The following coagulation parameters were measured in the serum samples collected from the three animal groups:- Prothrombin time, INR (International Normalized Ratio), Fibrinogen level, Factor (IX), Factor (VIII), Fibrin Degraded Products (D-Diimer), Antithrombin (III), protein C and protein S. Meanwhile, the Platelets count was measured using computerized (CELL-DYN 1800), manufactured by Abbott Laboratories Inc. IL. USA.

Statistical analysis: Measurement data of the following coagulation parameters:- Prothrombin time, INR, APTT, Fibrinogen and Fibrin degraded products (D-Diimer) were expressed as Mean \pm SE. The significant differences between each coagulation parameter in the treated group and the control group was statistically determined by applying (t-test) using sigma state program (v.8) (Howell, 1987). On the other hand, the data of the following coagulation parameters: Factors (IX), Factor (VIII), Antithrombin (III), Proteins C and S were expressed in the form of percentage (%). The results of these

coagulation parameters were statistically analyzed by applying (Fisher Test) according to Howell (1987) using the same statistical computerized program.

RESULTS

In general, the present results indicated that the tested doses of the aqueous extract of Danshen roots (21 and 43 mg kg⁻¹, respectively) induced significant inhibitory effects on the blood coagulation process. This hypocoagulative effect of Danshen roots extract was found to be dose dependent.

Effect of low therapeutic dose: Table 1 clearly shows the low therapeutic dose (21 mg kg⁻¹) of Danshen extract induced limited physiological changes in the coagulation parameters compared to the control group. A significant increase was observed in the mean of Prothrombin time and partial thromboplastin time (APTT) (38±2.3 and 24.21±1.8 sec, respectively) compared to control group according to t-test (p≤0.05). On the other hand, significant decrease was recorded in the Fibrinogen level in the serum (2.4±0.35 g L⁻¹) compared to the control group according to t-test (p≤0.05). Meanwhile, significant decrease was found in the mean of the anti-thrombin (III) (62%) compared to control group according to Fisher test, (p≤0.05). Whereas, no significant changes were recorded in the means of factor (IX), factor (VIII), protein C and protein S (79, 87, 84 and 77%, respectively) according to Fisher test. Similarly, no significant differences were found in the means of platelets count and Fibrin degraded products (D-Diaper) (560±42×10⁹ plat. L⁻¹ and 0.92±0.07 mg L⁻¹) compared to the control group according to t-test.

Effect of high therapeutic dose: On the other hand, the results in Table 1 marked significant changes in most of the coagulation parameters induced by the high tested therapeutic dose (43 mg kg⁻¹). For instance, significant increases were recorded in the means of Prothrombin time (PT), INR value and partial thromboplastin time (APTT) (66.80±1.8 sec, 11 and 56.60±2.7 sec, respectively) compared to the control group according to t-test (p≤0.01). At the same time, significant decreases were recorded in the means of Fibrinogen level and Fibrin degraded products (D-Diaper) (1.65±0.12 g L⁻¹ and 0.63±0.05 mg L⁻¹, respectively) compared to the control group according to t-test (p≤0.05). Similarly, significant decreases were found in the means of the anti-thrombin (III), factor (IX), factor (VIII), protein C and protein S (57, 29, 46, 61 and 24%, respectively) compared to the control group according to Fisher test. Regarding

Table 1: Effect of Danshen roots extract doses on the coagulation parameters in the treated groups (G₂ and G₃) compared to the control group (G₁) in male Guinea pigs (n = 8)

Coagulation parameters	Groups		
	Control (G ₁)	Treated (G ₂)	Treated (G ₃)
Platelet count (×10 ⁹ L ⁻¹)	558±38	560±42	554±48
Prothrombin Time (PT)(sec)	27.0±1.6	38±2.3*	66.80±1.8**
INR	2.5	4	11**
Partial thromboplastin Time 9APTT) (sec)	15.0±0.7	24.21±0.35*	56.60±2.7**
Fibrinogen (g L ⁻¹)	3.20±0.5	2.4±0.35*	1.65±0.12*
Factor IX (%)	86	79	29 ²
Factor VIII (%)	94	87	46
Fibrin degraded Products (mg L ⁻¹)	0.89±0.05	0.92±0.07	0.63±0.05*
Anti-thrombin III (%)	77	62 ¹	57 ¹
Protein C (%)	87	84	61 ¹
Protein S (%)	81	77	24 ²

Number of animals in each group was eight (n = 8). Some data are expressed as Mean±SE, while other data expressed as (%). *Significant difference (p≤0.05) comparing to control group according to t-test. **Highly significant difference (p≤0.01) comparing to control group according to t-test. ¹Significant difference (p≤0.05) comparing to control group according to Fisher test. ²Highly significant difference (p≤0.025) comparing to control group according to Fisher test

platelets count, no significant change was observed in its count (554±48×10⁹ L⁻¹) according to t-test.

DISCUSSION

During last ten years, cardiologists in many countries noticed a marked increase in the rate of the cases of coronary and cerebral thrombosis (Kearon *et al.*, 2008). Vascular thrombosis, particularly coronary and cerebral thrombosis, are fatal cardiovascular diseases which threaten the lives of many patients around the world (Yarnell *et al.*, 2005). Therefore, this serious health problem attracted the attention of many investigators, some of them started to look for new natural thrombolytic phytochemicals that can be clinically used to remove these mortal thrombus (Argento *et al.*, 2000; Xie and Mao, 2001). In similar approach, the present study was designed to examine the anticoagulative effect of the Chinese Danshen roots extract and its physiological mode of action on certain blood coagulation parameters. The present results showed significant hypocoagulative effect of Danshen roots extract on the tested coagulation parameters specially under the influence of the second tested therapeutic dose (43 mg kg⁻¹). It showed also a significant increase in the INR value (more than 10) which proved that the extract induced marked decrease in the blood viscosity. The current results seem to support the previous findings of Chan (2001), who referred to the anticoagulation effect of the aqueous extract of Danshen roots in human patients. Similarly, the results also correspond with Izzo *et al.* (2005) study which pointed out

that Danshen roots have inhibitory effects on the blood coagulation process. Meanwhile, the results also seem to be matched with the Pan *et al.* (2005) findings which referred to the anti-coagulative effect of the aqueous extract of Danshen roots in rats. In contrary, the present results appeared to be not matched at all with Yu *et al.* (1997) study as they reported that Danshen roots extract induced over-coagulative effect in some human patients who treated through Chinese herbal medicine with this plant. However, in regard the mechanism of action which can help in explaining how the active compounds of Danshen roots produce this anticoagulant effect is still unknown. In spit of that, there are some hypotheses which attempted to discover this mechanism, for example, Rattner *et al.* (1990) suggested that this therapeutic anticoagulative effect of Danshen roots occurs through the direct effect of its active compounds on the platelet activating factor in particular. Whereas, Liu *et al.* (2000) hypothesized that the active compounds of Danshen roots inhibit the platelets adhesion required for the formation of the blood clots and at the same time these active compounds are believed to elevate the blood viscosity by increasing the levels of Nitric Oxide (NO) and Endothelin level in the blood. On the other hand, Hirsh *et al.* (2001) suggested that the active compounds of Danshen roots produce this anticoagulant effect by possessing fibrinolytic activities which helps in lysis of any abnormal blood clots in the circulation, at same time these active compounds are believed to have the ability to inhibit the platelets aggregation which is required for the blood clots formation. Zhang *et al.* (2003) study suggested that the active compounds of Danshen reduce the activity of the platelet membrane glycoproteins, preventing formation of any abnormal vascular thrombosis. The same investigators in second study reported that the active compounds inhibit the blood coagulation by blocking calcium ions inflow to the platelets (Zhang *et al.*, 2006). In conclusion, the present results may prove the therapeutic inhibitory effect (hypocoagulative effect) of Danshen roots active compounds on the blood coagulation. At end of this study, it is recommended that further pharmacological studies are needed to determine and separate the exact active compound which is responsible for this useful hypocoagulative effect of Danshen roots. Such compound can be then used as an alternative drug of Warfarin in treating the cases of thrombosis in human patients. Meanwhile, further toxicological studies are needed to examine the therapeutic safety of Danshen roots before we can recommend the therapeutic usage of this plant.

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REFERENCES

- Adams, J., R. Wang and E. Lien, 2006. Preclinical and clinical examination of Danshen and its tanshinones in ischemic condition. *J. Chinese Med.*, 1: 40-45.
- Ajarem, J.S., 1990. Effects of fresh Kola nut extract (*Cola nitida*) on the locomotor activities of male mice. *J. Acta Physiol. Pharmacol. (Bulg.)*, 16: 10-15.
- Al-Tayib, Y.A., 2004. Effects of melatonin on molybdenum hydroxylase activities of kidney and small of guinea pigs. *J. Egypt. Ger. Soc. Zool.*, 43A: 203-213.
- Argento, A., E. Tiraferri and M. Marzalani, 2000. Oral anticoagulants and medicinal plant. *Ann. Italian Int. J.*, 15: 139-143.
- Bristol, M., S. Somnad and C. Guerra, 2008. Uninformed complementary and alternative supplement use: A risky for cardiovascular patients. *J. Health Pract. Rev.*, 13: 100-109.
- Chaloupecky, V., R. Svobodova and J. Skovranek, 2005. Coagulation profile and liver functions in 102 Patients after pulmonary connection a t mid term follow up. *J. Heart*, 91: 73-79.
- Chan, T.T., 2001. Interaction between Warfarin and Danshen (*Salvia miltiorrhiza*). *Ann. J. Pharm. Therap.*, 35: 501-504.
- Chan, K., S. Chui and D. Wong, 2004. Protective effects of tanshinones from the aqueous extract of *Salvia miltiorrhiza* (Danshen) against homocysteine induced endothelial dysfunction. *Life Sci.*, 75: 3157-3171.
- Che, A., J. Zhang, Z. Hu and X. Chen, 2004. Separation and determination of active components in *Salvia miltiorrhiza* and its medicinal preparations by non-aqueous capillary electrophoresis. *J. Phytochem.*, 27: 569-575.
- Chen, J. and T. Chen, 2004. *Chinese Medicinal Herbology and Pharmacology*. 1st Edn., Art of Medicine Press, Pikin, China, pp: 1267.
- Cheng, T.K., 2000. Cardiovascular effects Danshen. *Int. J. Cardiol.*, 121: 9-22.
- Harrison, L., M. Johnston, K. Moffat and J. Hirsh, 1997. Comparison of 5 mg and 10 mg loading doses in initiation of (Warfarin) therapy. *Annl. J. Int. Med.*, 126: 133-136.

- Hirsh, J., J. Dalen, D. Anderson and D. Deykin, 2001. Oral anticoagulants: Mechanism of action, clinical effectiveness and optimal therapeutic range. *J. Am. Chest Phys.*, 119: 8S-21S.
- Howell, D.C., 1987. *Statistical Methods*. Duxbury Press, Boston, USA.
- Izzo, A., C. Diy, F. Borrelli and E. Ernst, 2005. Cardiovascular pharmacotherapy and herbal medicine: The risk of drug interactions. *Int. J. Cardiol.*, 98: 1-14.
- Kageyama, S., H. Yamamoto and R. Yoshimoto, 2000. Anti-Human (Von Willebrand) factor monoclonal antibody prevents thrombus deposition in guinea pigs. *J. Arteriosclerosis, Thrombosis Vas. Biol.*, 20: 2303-2303.
- Kearon, C., S. Kahn, S. Agnelli and A. Comerota, 2008. Anti-thrombotic therapy for venous thromboembolic disease. *Chest J.*, 133: 454S-545S.
- Lam, F., J. Yeung and P. Yao, 2006. Salvianolic acid (B), an aqueous component of Danshen (*Salvia miltiorrhiza*) relaxes rat coronary artery by inhibition of calcium channels. *Eur. J. Pharmacol.*, 553: 240-245.
- Lee, W., L. Chiu and J. Yeung, 2008. Cytotoxicity of major tanshinones isolated from (*Salvia miltiorrhiza*) on (HepG2 cells) in relation to glutathione perturbation. *J. Food Chem. Toxicol.*, 48: 328-338.
- Liu, J., X. Zhang, J. Fu and Y. Wu, 2000. Study on the changes of ET/NO ration during the therapeutic effect of Danshen in acute Pancreatitis. *J. Chinese Med.*, 20: 67-71.
- Ody, P.A., 2001. *Secrets of Chinese Herbal Medicine*. A Dorling Kindersley, London, UK., pp: 106-107.
- Pan, J., Y. Zhang, M. Wang and K. Jing, 2005. Therapeutic effect of Danshen solution on hemorrhagic shock combined with coagulopathy in rats. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*, 13: 456-459.
- Rattner, D., L. Napolitano, J. Corsetti and R. Warshaw, 1990. Hypocalcemia in experimental pancreatitis occurs independently of changes in non-esterified fatty acid levels. *Int. J. Pancreatol.*, 6: 249-262.
- Steinkamp, F., L. Bollinger, N. Voller and H. Li, 2007. Ursolic acid from the Chinese herb Danshen (*Salvia miltiorrhiza*) regulate NO and NO₄ in human endothelial cells. *J. Atherosclerosis*, 195: 104-111.
- Takano, S., J. Kimura and T. Ono, 2001. Inhibition of aggregation of rabbit and human platelets induced by adrenaline and 5-hydroxytryptamine by Na/Ca inhibitor. *Br. J. Pharmacol.*, 132: 1383-1388.
- Xie, F., X. Li and D. Mao, 2001. An experimental study on drugs for improving blood circulation and removing blood stasis in treating mild chronic hepatic damages. *J. Trad. Chin. Med.*, 21: 225-231.
- Yang, H., X. Jiang, G. Zhao and X. Guo, 2006. Thrombocytopenia in the leptospirosis of guinea pig is not related to disseminate intra-vascular coagulation. *J. BMC Infect. Dis.*, 6: 19-19.
- Yarnell, J., E. Mccrum, A. Rumley and A. Evans, 2005. Association of European population levels of thrombosis and inflammatory factors with risk of coronary heart disease. *Eur. Heart J.*, 26: 332-342.
- Yu, W., F. Shen and Z. Mau, 1997. Observation of the therapeutic effect of (*Salvia miltiorrhiza*) and cytosine diphosphate-choline injection on patients with hypertensive cerebral hemorrhage. *J. Zhongguo Zhang Xi*, 20: 94-98.
- Zhang, M., X. Li, G. Jiu and Y. Liu, 2003. Effect of Danshen extract injection on expression of platelet membrane glycoproteins in patients with type (II) diabetes mellitus. *J. Zhong Yao Cai*, 26: 738-740.
- Zhang, X., Z. Li and D. Liu, 2006. Progress in research into the mechanism of (*Salvia Miltiorrhize*) in treatment of acute pancreatitis. *J. Hepato-Biliary Pancreat. Dis.*, 5: 501-504.
- Zou, Z., L. Xu and J. Tian, 1993. Anti-thrombotic and anti-platelet effects of Rosmarinic acid, a water soluble isolated from (*Salvia Miltiorrhizae*) (Danshen). *J. Yao Xue Bao*, 28: 241-241.