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ETHANOL EXTRACT OF *CARTHAMUS TINCTORIUS* L. SHOWS ANTI-THROMBOSIS ACTIVITY IN RATS.

Tong-bin Ding, Li-guoJian, Jiang-tao Zhao, Shi-chao Liu, Jin-ying Zhang* Sheng hao Wu

The First Affiliated Hospital of ZhengzhouUniversity, Zhengzhou, Henan, 450000, China.
*Corresponding author, **E-mail:** tongbinding133494@163.com

Abstract

Background: The study was designed to study the anti-thrombosis activity of *Carthamustinctorius* L. (CTL) ethanol extraction rats.

Material and Methods: Common carotid artery $FeCl_3$-induced thrombus and inferior vena cava thrombosis occlusion time, plasma concentrations of thromboxane B2(TXB2) and 6-keto-prostaglandin F1α(6-keto-PGF1α) were measured in rats.

Results: Venous occlusion time was prolonged in rats. Arterial and venous thrombus weights were dose-dependently reduced in CTL groups.TXB2 decreased and 6-keto-PGF1α increased with CTL and aspirin, with an association between 6-keto-PGF1α/TXB2 and arterial or venous thrombus weight for all products, and for occlusion time with CTL but not for aspirin.

Conclusion: The experimental effects of CTL on thrombosis in rats were confirmed. Again, further explorations of putative clinical effects appear justified.

Keywords: *Carthamus tinctorius* L.; thrombosis; TXB2; 6-keto-PGF1α.

Introduction

*Carthamustinctorius*L., a member of Asteraceae family, is a traditionally medicinal and edible plant in use over the years. The red tubular flowers without ovary are usually picked in summer when the color of flowerschanges from yellow to red, and dried in shady but well-ventilated places for clinical usage (Committee for the Pharmacopoeia of PR China, 2010). With increase in extensive study on chemical constituents of Chinese Material, Medica, investigations related to phytochemistry have been conducted on safflower. Currently, over 104 compounds such as quinochalones, flavonoids, alkaloids, polyacetylene, aromatic glucosides, organic acids from this plant have been isolated and identified (He, J.,2011).

Many scientists have demonstrated that safflower promotes blood circulation by removing blood stasis and pain alleviation. In China, safflower has been used clinically for cerebrovascular disease, coronary heart disease, improving myocardial ischemia, modulating immune system, anticoagulation and antithrombosis, antioxidation, anti-aging, antihypoxia, antifatigue, antiinflammation, anti-hepatic fibrosis,antitumor, analgesia, etc.(He J.,2011). Due to its traditional use in the prevention of cardiovascular disease, it was tested on models of thrombosis in rats, compared to the standard antiplatelet agent aspirin, to continue from *in vitro* test to *in vivo* (animal test).

Materials and methods

Material

The herbal samples of *Carthamustinctorius L.* were collected from Zhengzhou City, Henan Province in China in May 2014. Taxonomic identification of the plant was performed by Prof. XianWang of Zhengzhou University in China. A voucher specimen (NO.CTL 201405024) was deposited in the college of Pharmacy, Zhengzhou University, China for future reference. Ethanol extract of CTLwas obtained by steeping the dried *Carthamustinctorius L.* in 90% ethanol at 70 ℃ three times, eachfor 45 minutes before first drying and thenfreeze-drying the extract thus obtained. One gram powder was equivalent to about 0.8 g crude samples. The yield was 80.0%.

Aspirin was purchased from Sigma Co, USA 6-keto-PGF 1αand TXB2 ELISA kits were purchased from NanjingJiancheng Biological Technology Co., Ltd., China.

Animals and group

Male SD rats (280–320 g) were provided by the Experimental Animal Center of Henan Province (certificate number SYXK2005-0004).The

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animals had free access to food and water, and were allowed to acclimatize for at least one week before use.The rat experiment was approved by the Animal Care and Use Committee of Zhengzhou University (Approval reference NO. 20111024) and were carried in compliance with the Animal Welfare Act and the NIH guidelines (NIH publication No. 80-23, revised 1996).

All rats were randomly divided into 6 groups of ten rats: sham group, model group, aspirin 5 mg/kg,low extract of CTL10 mg/kg, medium extract of CTL 20 mg/kg and high extract of CTL 40 mg/kg dose. Treatments were given orally once daily for 10 days, dissolved in water.

Inferior vena cava thrombosis

Rats were fixed on anatomical planes in supine position, the hairs on the abdomen were sheared and the skin was disinfected with iodine and draped under sterile conditions. An abdominal incision was made along the medio-ventral line. Inferior vena cava was isolated and ligated with silk thread below the left renal vein branch. The abdominal walls were subsequently closed. The abdomen was reopened, the inferior vena cava was clamped about 2 cm below the ligature and other branches were ligated after 4 hr. The inferior cava vein was opened lengthwise, the thrombus was removed and placed on the filter paper to dry, then was weighed (Rhodes et al., 2000; Johnstone et al., 2001). Thrombosis inhibition was calculated with the same equation described in “Common carotid artery thrombosis”.

Common carotid artery thrombosis

Rats were anesthetized with 3% barbital sodium after the last administration,. Under sterile conditions, the rats were fixed on anatomical plane in supine position, the hairs on the throat were sheared and the skin was disinfected with iodine. An incision was made of about 3 cm in the midline on the throat (Liu and Xu, 1995; Zhang et al., 1998; Dieude et al., 2009; Eckly et al., 2011).The left common carotid artery was isolated for 2 cm in length carefully and a plastic sheet (3 cm × 1.5 cm) was placed under the vessel to separate it from the surrounding tissue. The surface of carotid artery was covered with a piece of filter paper(1 cm × 1 cm) saturated with 40% $FeCl_3$ solution (normal saline in sham group) (Nakata et al., 2008; Zhang et al., 2004).The temperature of the distal arterial surface was monitored by a thermometer. The time from when the filter paper was placed to a sudden drop in the temperature was recorded as thrombosis occlusion time (OT). An injured carotid artery segment (0.6 cm) was then cut off and placed on the filter paper to dry and was then weighed. The rate of thrombosis inhibition was calculated as: thrombosis inhibition (%) = (A - A1) / A × 100%, where A was the wet weight of the thrombus in the model group and A1 was that in agents-treated groups.

Serum concentration assay of 6-keto-PGF1α and TXB_2

The abdominal aorta was isolated and punctured for collecting 2 ml blood under sterile conditions 90 min after surgery. The serum was separated and serumconcentrations of 6-keto-PGF1α and TXB_2 were measured by ELISA kits.

Statistical analysis

Values were expressed as the means±SD. Significant differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by a two pairs Student’s t-test. $P<0.05$ was considered statistically significant.

Results

Inferior vena cava thrombosis

Compared with the model group, CTL extracts significantly and dose-dependently reduced the weight of thrombus, increasing inhibitory rate ($P< 0.01$). The effects of aspirin were the same as that of the medium dose of CTL (Table 1).

$FeCl_3$-induced common carotid artery thrombosis

Compared with the model group, all doses of CTL extracts significantly and dose-dependently prolonged thrombosis occlusion time,

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reduced the weight of thrombus and increased the inhibitory rate(P<0.01). 5 mg/kg aspirin had the same effect as the medium doses of CTL (20 mg/kg) for inhibition of thrombus weight (Table 2).

Table 1: Effect of CTL extracts on inferior vena cava thrombosis (mean±SD, n = 10 per group).

Group	N	Dose(mg/kg)	Weight of thrombus(mg)	Inhibitory rate(%)
Sham	10	—	22.45±7.5	
Aspirin	10	5	6.93±1.49*	66.7*
CTL-L	10	10	12.34±4.97*	41.3*
CTL-M	10	20	6.83±3.12*	65.7*
CTL-H	10	40	5.43±1.36*#	78.3*#

*P < 0.01 vs. model group. #P < 0.05 vs. aspirin group.

Table 2: Effect of CTL extracts on FeCl3-induced common carotid artery thrombosis in rats (mean±SD, n=10per group).

Group	N	Dose(mg/kg)	OT(min)	Weight of thrombus(mg)	Inhibitory rate(%)
Sham	10	—	7.92±1.29	13.45±0.58	
Aspirin	10	5	19.1±2.13*	6.49±1.35*	56.8
CTL-L	10	10	26.7±3.32*	8.27±1.63*	32.4*
CTL-M	10	20	29.1±4.89*	7.47±1.39*	58.2*
CTL-H	10	40	38.6±4.71*#	5.37±1.15*#	68.1*#

OT: thrombosis Occlusion Time.

*P<0.01 vs. model group. #P<0.05 vs. aspirin group.

Serum TXB2 and 6-keto-PGF1α concentrations

Compared with the normal group, the arterial serumconcentration of 6-keto-PGF1α was significantly decreased (P<0.05) and TXB2 was significantly increased (P<0.05) in the model group. Compared with the model group, the serumconcentration of TXB2 of all CTL extracts groups wasdecreased significantly and dose-dependently (P<0.05) and that of 6-keto-PGF1α was increased significantly but with an inversed dose-dependence (P<0.05).The secretion of 6-keto-PGF1α and TXB2 were both inhibited by aspirin significantly (Table 3).

The 6-keto-PGF1α to TXB2 ratio went from 2.4 in normal controls to 0.3 in model animals indicating strong platelet activation. It went from 1.1at the lowest dose to 1.86 at 20 mg, and 6.30 at the highest dose with increasing doses of CTL. And it was 2.2 with aspirin (Fig. 1 and 2).

Table 3: Effect of CTL extracts on serum concentrations of 6-keto-PGF1α and TXB2 (mean±SD, n=10 per group).

Group	N	Dose(mg/kg)	6-keto-PGF1α (pg/ml)	TXB2(pg/ml)
Normal	10	—	554.68±309.52	197.84±94.15
Model	10	—	248.54±231.17	687.16±237.47
Aspirin	10	5	176.43±98.73*	107.25±42.26*
CTL-L	10	10	537.16±273.25*	467.51±199.24*
CTL-M	10	20	348.36±158.86*	217.68±128.33*
CTL-H	10	40	385.54±287.72*#	102.53±62.15*

*P<0.01 vs. model group. #P<0.05 vs. aspirin group.

Discussion

With a wide spectrum of biological and pharmacological effects, safflower has been traditionally used in China for many years with the dried tubular flower as the effective agent. The anti-hemostasis activity of CTL extracts in both venous and arterial rats were studied and confirmed. Injury of blood vessel endothelium by $FeCl_3$ can induce platelet adhesion and aggregation, which leads to thrombosis (Du et al., 2007).

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Ligation of vein causes focal blood stasis, injury of vascular endothelial cell and hypoxia (Eckly et al., 2011). According to the literature of Science of Chinese Material Medica, safflower is pungent in flavor, warm in nature and attributive to the heart and liver meridians. Safflower has the power to promote blood circulation to reduce blood stasis, promote menstruation and alleviate pain. In the aspect of clinical practice, safflower is mainly applied for blood-stasis syndrome with dysmenorrheal, amenorrhea, postpartum abdominal pain and mass, trauma and pain of joints, etc. The activated intrinsic and extrinsic coagulation systems activate thrombin and the blood coagulation factors. Due to injury of vascular endothelial cell, synthesis of PGI_2 decreased and plasma TXA_2 increased (Bult et al., 1988), further promoting platelet adhesion and aggregation and imbalance of TXA_2/PGI_2, which leads to vasoconstriction, platelet aggregation, and thrombosis (Fuchs et al., 2010).The biological half life of serum TXA2 is only 30 seconds, and TXA2 transformed to TXB2 quickly. Therefore, the TXB2 concentration was measured and confirmed in this experiment.

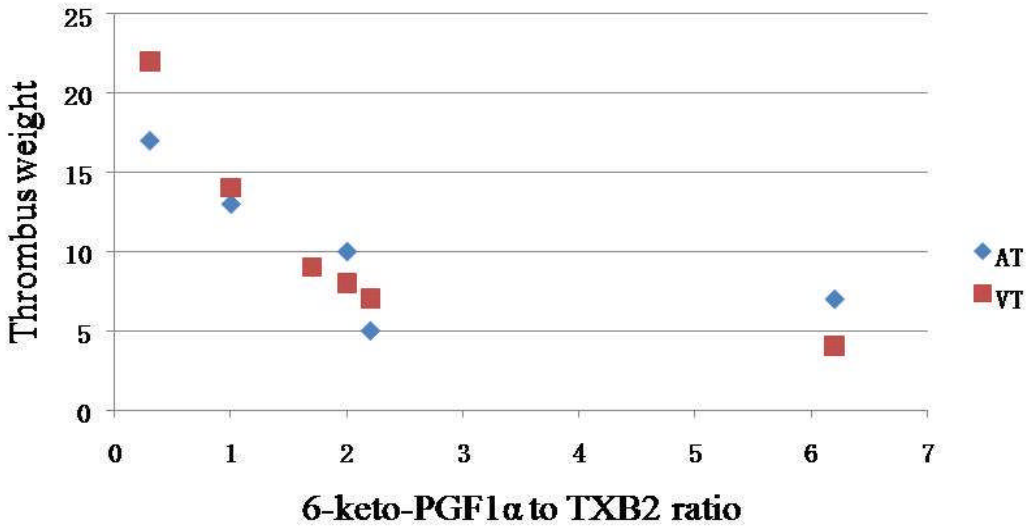

Figure 1: Relation between the 6-keto-PGF1α to TXB2 ratio and the arterial (AT) or venous (VT) weight (g).

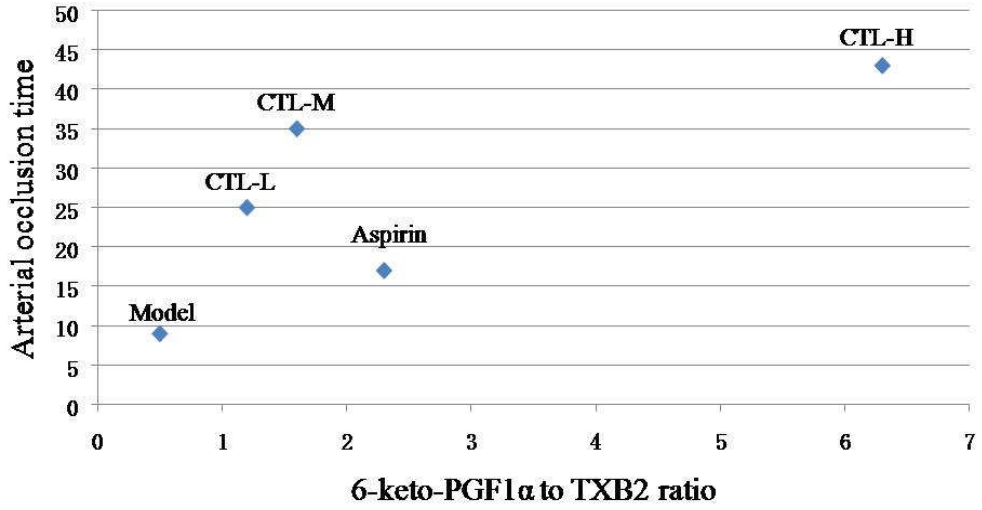

Figure 2: Relationship between the 6-keto-PGF1α to TXB2 ratio and the arterialocclusion time.

CTL could dose-dependently improve the neurological deficit scores and reduced the cerebral infarct area, which might be involved in its inhibitory effects on thrombosis formation and platelet aggregation as well as its beneficial action on regulation of PGI_2/TXA_2 ratio and blood rheological changes.Compared with the model group, CTL extracts dose-dependently reduced the weight of arterial and venous thrombosis,and prolonged OT.They decreased the serum concentration of TXB2 and increased 6-keto-PGF1 α, thereby increasing the 6-keto-PGF1α to TXB2

ratio.There was an asymptotic relationship between this 6-keto-PGF1 α to TXB2 ratio and arterial or venous thrombus weight, and a slightly more complex relationship between the 6-keto-PGF1 α to TXB2 ratio and arterial occlusion time: though the value for the highest dose of CTL was in line with control and aspirin values, the values for low and medium doses of CTL were clearly above that line.The anti-thrombotic activity of CTL extracts was probablymediated by acting on the prostacyclin/thromboxane balance, acting on both sides of the ratio, resulting in a ratio that was dependently related to thrombus weight (Fig. 1). Arterial occlusion time was linearly related to the ratio for control, aspirinand high extract of CTL (r^2=0.998) but medium extract of CTL and low extract of CTL appear not to lie on the same line (Figure 2). And more, CTL perhaps had some additional effectthat prolongs occlusion time beyond what would be expected from the effect on cyclo-oxygenase, or more generally on the synthesis of thromboxane and PGI2, especially for the lower CTL doses. Our study revealed that the effective dose of CTL was between 10 mg/kg and 40 mg/kg. It was not clear whether this was related to a differential effect on platelets rather than to actual thrombosis, and needs further study (Baxi et al., 2008; Kaptanoglu et al.,2008). Our experimental result demonstrated that *Carthamustinctorius*L., ethanol extract showed significant anti-thrombotic activity, which could be used for further prevention of vascular disease.

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