

Original Research Article

Anti-Thrombotic Effect of *Carthamus tinctorius* Linn Extracts in Rats

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Received: 13 August 2013

Revised accepted: 22 September 2014

Abstract

Purpose: To explore the effects of *Carthamus tinctorius* L. (CTL) extracts on thrombosis in rats.

Methods: CTL extract was obtained in hot water (60 °C), dried in a hot air oven and then freeze-dried. The rats were divided into 6 groups: normal group, control group, reference group (aspirin 5 mg/kg) as well as groups that received 20, 40 and 80 mg/kg doses of CTL, respectively. For each group, treatment was given orally once daily for 14 days. Common carotid artery FeCl₃-induced thrombus and inferior vena cava thrombosis occlusion time, as well as plasma concentrations of thromboxane B₂ (TXB₂) and 6-keto-prostaglandin F_{1α}(6-keto-PGF_{1α}) were measured in rats.

Results: Compared with the control group, all doses of CTL extracts significantly and dose-dependently prolonged thrombosis occlusion time, reduced the weight of thrombus and increased inhibition rate ($p < 0.01$). Plasma TXB₂ concentration of all CTL extracts groups decreased dose-dependently ($p < 0.05$) while that of 6-keto-PGF_{1α} was increased ($p < 0.05$). There was association between 6-keto-PGF_{1α}/TXB₂ and arterial or venous thrombus weight for all treatments, and also with occlusion time for CTL treatment but not for aspirin.

Conclusion: CTL has a significant effect on thrombosis in rats. However, further studies are required to determine its clinical potentials.

Keywords: *Carthamus tinctorius* L., Thrombosis, Thromboxane B₂, 6-Keto-prostaglandin F_{1α}, Aspirin, Occlusion time

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INTRODUCTION

Carthamus tinctorius L. or safflower, commonly called Honghua in Chinese, is an annual or biennial herbal plant in the family of Compositae. The red tubular flowers without ovary are usually picked in the summer when the color of flowers changes from yellow into red, and then dried in shady and well-ventilated places for the clinical usage [1]. With the increasingly extensiveness of studies on the chemical constituents of Chinese

Material Medica, phytochemical investigations have also been conducted on safflower. Currently, over 104 compounds from this plant have been isolated and identified, and they include quinochalones, flavonoids, alkaloids, polyacetylene, aromatic glucosides, organic acids, etc [2].

Modern pharmacological experiments have demonstrated that safflower with its active compounds possesses wide-reaching biological

activities, including dilating coronary artery, improving myocardial ischemia, modulating immune system, anticoagulation and antithrombosis, antioxidation, anti-aging, antihypoxia, antifatigue, antiinflammation, anti-hepatic fibrosis, antitumor, analgesia, etc [2-7]. Due to its traditional use of CTL in the prevention of cardiovascular disease [8], this study was therefore performed on models of thrombosis in rats and compared to the standard antiplatelet agent, aspirin [9].

EXPERIMENTAL

Material

The herbal samples of *Carthamus tinctorius* L. were collected from Bozhou City, Anhui Province in China in July 2013. Taxonomic identification of the plant was performed by Professor He-li Hu of Wenzhou Medical University in China. A voucher specimen of herbarium (No. CTL 201307013) was deposited in the College of Pharmacy, Wenzhou Medical University, China for future reference. The aqueous extract of CTL was obtained by steeping the dried *Carthamus tinctorius* L. in water at 60 °C three times, each for one hour before first drying in an oven and then freeze-drying the last extract thus obtained. The yield was 55.56 %.

Other drugs and reagents were aspirin (Sigma Co, USA), 6-keto-PGF 1 α and TXB2 RIA kits (Shenzhen Xin-Bo-Sheng Biological Technology Co Ltd, China).

Animals

SPF Male Wistar hypertensive rats weighing 300–350 g were provided by the Experimental Animal Center of Zhengjiang Province (Certificate no. SYXK 2004-0002). The 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 Wenzhou Medical University (approval ref no. 20131013) and was carried out in compliance with the Directive 2010/63/EU on the handling of animals used for scientific purposes [10].

Animal group

The rats were randomly divided into 6 groups of eight rats: normal group, control group, reference group (aspirin 5 mg/kg) as well as CTL extract groups, namely, 20, 40 and 80 mg/kg doses. Treatments were given orally once daily for 14 days, dissolved in water.

Common carotid artery thrombosis study

After the last administration, rats were anesthetized with 3 % barbital sodium (0.5 ml/100 g i.p.). 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 [11-14]. The left common carotid artery was isolated for 2 cm in length carefully and a plastic sheet (3 cm \times 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 \times 1 cm) saturated with 40 % FeCl₃ solution (normal saline in sham group) [15,16]. 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 (Ti) was computed as in Eq 1.

$$Ti (\%) = \{(A - A1)/A\}100 \dots\dots\dots (1)$$

where A and A1 are the wet weight of the thrombus in the control group and aspirin- or extract-treated groups.

Inferior vena cava thrombosis study

Under sterile conditions, the 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. 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. 4 h later the abdomen was reopened, the inferior vena cava was clamped about 2 cm below the ligature and other branches were ligated. The inferior vena cava vein was opened lengthwise, the thrombus was removed and placed on the filter paper to dry, then was weighed [17,18]. Thrombosis inhibition was calculated as in Eq 1 above.

Measurement of the plasma concentration of 6-keto-PGF1 α and TXB2

Under sterile conditions, 90 min after surgery, the abdominal aorta was isolated and punctured for collecting 3 ml blood. The plasma was separated and stored at -20 °C. The plasma concentrations of 6-keto-PGF1 α and TXB2 were measured by radioimmunoassay (RIA) [19,20].

Statistical analysis

Values are expressed as mean \pm SD. Significant differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by two-paired Student's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

FeCl₃-induced common carotid artery thrombosis

As shown in Table 1, compared with the control group, all doses of CTL extracts significantly and dose-dependently prolonged thrombosis

occlusion time, reduced the weight of thrombus and increased inhibition rate ($p < 0.01$). Aspirin (5 mg/kg) had the same effect as CTL (30 mg/kg) for inhibition of thrombus weight, but less effect on occlusion time.

Inferior vena cava thrombosis

As shown in Table 2, compared with the control group, CTL extracts significantly and dose-dependently reduced the weight of thrombus, increasing inhibitory rate ($p < 0.01$). The effects of aspirin were similar to that of the medium dose of CTL.

Table 1: Effect of CTL extracts on FeCl₃-induced common carotid artery thrombosis in rats (mean \pm SD, n = 8)

Group	N	Dose (mg/kg)	OT (min)	Weight of thrombus (mg)	Inhibition rate (%)
Control	8	—	8.56 \pm 1.3	14.33 \pm 0.37	
Aspirin	8	5	18.7 \pm 2.21 [*]	6.54 \pm 1.43 [*]	58.3
CTL-L	8	15	27.3 \pm 3.59 [*]	9.87 \pm 1.78 [*]	31.6 [*]
CTL-M	8	30	32.6 \pm 5.43 [*]	7.12 \pm 1.45 [*]	57.8 [*]
CTL-H	8	60	41.2 \pm 4.9 ^{*#}	5.28 \pm 1.32 ^{*#}	67.4 ^{*#}

OT = thrombosis occlusion time; ^{*} $p < 0.01$ vs. control group; [#] $p < 0.05$ vs. aspirin group

Table 2: Effect of CTL extract on inferior vena cava thrombosis in rats (mean \pm SD, n = 8)

Group	N	Dose (mg/kg)	Weight of thrombus (mg)	Inhibition rate (%)
Control	8	—	20.31 \pm 8.3	
Aspirin	8	5	6.87 \pm 1.58 [*]	65.4 [*]
CTL-L	8	15	11.2 \pm 5.1 [*]	39.7 [*]
CTL-M	8	30	7.64 \pm 2.6 [*]	63.5 [*]
CTL-H	8	60	5.24 \pm 1.13 ^{*#}	76.4 ^{*#}

^{*} $P < 0.01$ vs. control group; [#] $p < 0.05$ vs. aspirin group

Plasma TXB₂ and 6-keto-PGF₁ α

As shown in Table 3, compared with the normal group, arterial plasma 6-keto-PGF₁ α concentration was decreased ($p < 0.05$) and TXB₂ concentration was increased ($p < 0.05$) in the control group. Compared with the control group, the plasma TXB₂ concentration of all CTL extracts groups decreased dose-dependently ($p < 0.05$) while that of 6-keto-PGF₁ α increased dose-dependently ($p < 0.05$). Aspirin inhibited the secretion of both 6-keto-PGF₁ α and TXB₂ significantly ($p < 0.05$).

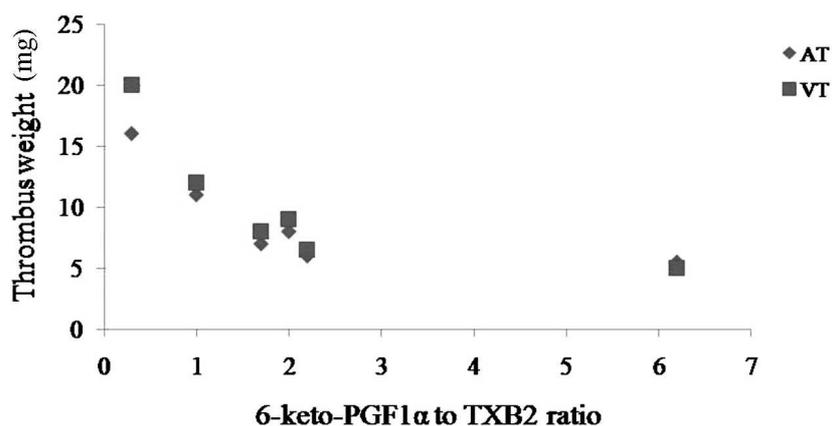
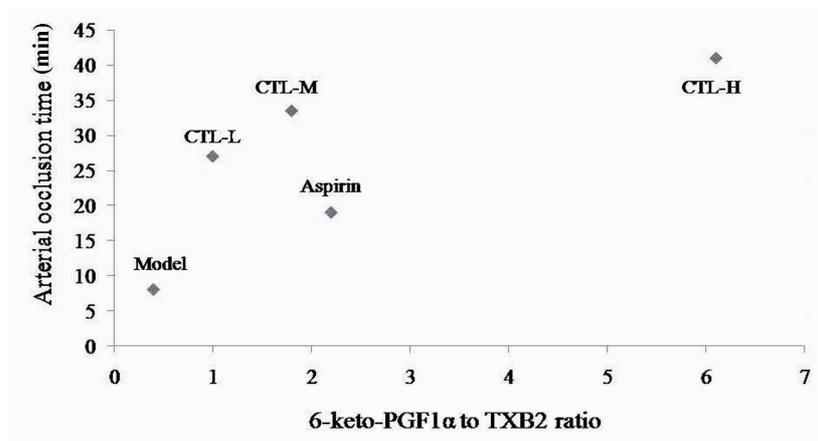
6-Keto-PGF₁ α to TXB₂ ratio went from 2.5 in normal control to 0.35 in control animals

indicating strong platelet activation. It went from 1.0 at the lowest dose to 1.92 at 30 mg, and 6.28 at the highest dose with increasing doses of CTL. And it was 2.24 with aspirin (Figs 1 and 2). There was an asymptotic relationship between this 6-keto-PGF₁ α to TXB₂ ratio and arterial or venous thrombus weight (Fig 1), 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 (Fig 2).

Table 3: Effect of CTL extract on the plasma concentrations of 6-keto-PGF1 α and TXB2 in rats (mean \pm SD, n = 8)

Group	N	Dose (mg/kg)	6-Keto-PGF1 α (pg/ml)	TXB2 (pg/ml)
Normal	8	—	586.37 \pm 321.54	238.45 \pm 108.76
Control	8	—	254.33 \pm 227.46	724.28 \pm 287.35
Aspirin	8	5	184.32 \pm 101.28*	83.14 \pm 37.19*
CTL-L	8	15	556.47 \pm 297.86*	425.15 \pm 217.38*
CTL-M	8	30	327.78 \pm 169.47*	201.75 \pm 144.27*
CTL-H	8	60	379.61 \pm 310.56*#	97.64 \pm 57.06*

* $P < 0.01$ vs. control group; # $p < 0.05$ vs. aspirin group

**Figure 1:** Relation between 6-keto-PGF1 α to TXB2 ratio and arterial (AT) or venous (VT) thrombus weight (mg, n = 8)**Figure 2:** Relationship between 6-keto-PGF1 α to TXB2 ratio and arterial occlusion time (n = 8)

DISCUSSION

CTL is traditionally used in Chinese medicine for cardiovascular prevention in China. The effects of the CTL extracts on hemostasis were confirmed in experimental thrombosis rats, both venous and arterial. Injury of blood vessel endothelium by FeCl₃ can induce platelet adhesion and aggregation, which leads to thrombosis [21]. Ligation of veins causes focal

blood stasis, injury of vascular endothelial cell and hypoxia [22]. In both cases, before activation of the intrinsic coagulation system, blood coagulation factors and thrombin are activated locally. Endothelial cell degeneration and necrosis then leads to exposure of sub-endothelial collagen, which activates the extrinsic coagulation system. The activated intrinsic and extrinsic coagulation systems activate thrombin and the blood coagulation factors. Due to injury

of vascular endothelial cell, synthesis of PGI₂ decreased and plasma TXA₂ increased [23], further promoting platelet adhesion and aggregation and imbalance of TXA₂/PGI₂, which leads to vasoconstriction, platelet aggregation, and thrombosis [24]. The biological half-life of serum TXA₂ is only 30 s, and TXA₂ is rapidly transformed to TXB₂. Therefore, TXB₂ was measured in this experiment.

Compared with the model group, CTL extracts dose-dependently prolonged OT, reduced the weight of arterial and venous thrombosis. The extracts also decreased the plasma TXB₂ concentrations and increased 6-keto-PGF₁α, thereby increasing the 6-keto-PGF₁α to TXB₂ ratio. The anti-thrombotic effect of CTL extracts was probably mediated 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, aspirin and CTL-H ($r^2 = 0.999$) but CTL-M and CTL-L appear not to lie on the same line (Fig 2). Furthermore, CTL perhaps had some additional effect that 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. It is uncertain whether this is related to a differential effect on platelets rather than to actual thrombosis, and therefore needs to be further investigated [25,26].

CONCLUSION

The result of this study demonstrates that CTL has a significant effect on thrombosis in rats. However, further studies are required to determine its clinical potentials.

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