INTRODUCTION

Danshen, the dry root and rhizome of *Salvia miltiorrhiza* Bge. (Labiatae), is one of the popular herbs used in China and the neighboring countries. This herb is widely applied in traditional Chinese medicine for promotion of blood flow to overcome blood stasis and to resolve abscesses [1]. Moreover, the extracts from the roots of this plant effectively prevented development of bone loss and increase blood estrogen level in a rat model of osteoporosis [2]. Several preparations that contain its major bioactive ingredients are also effective for microcirculation and coronary vasodilation and prevent inflammatory factors, and are therefore used against hypertension and inflammatory diseases [3]. Furthermore, a purified extract of this plant containing its major constituents, cryptotanshinone, tanshinone I, and tanshinone IIA can protect against liver toxicity *in vivo* and *in vitro* [4]. It has been reported that cryptotanshinone, tanshinones I and IIA, and salvianolic acid B are the major bioactive hydrophilic constituents present in roots of *Salvia miltiorrhiza* Bge. collected from different areas of
China. In addition, *Salvia Miltiorrhiza Bge.* has been shown to possess unique efficacy in treating thromboangiitis obliterans [5].

Sequel to the use of *Salvia Miltiorrhiza Bge.* in the prevention of cardiovascular disease [6], this study was performed to study the effect of SMBE on models of thrombosis in rats [7].

**EXPERIMENTAL**

**Material**

Root of *Salvia miltiorrhiza Bge.* were collected from Shiyan City, Hubei Province in China in May 2015. Taxonomic identification of the plant was performed by Professor Ping He of College of Pharmacy of Southern Medical University in China. A voucher specimen of herbarium (no. SMBE 20150504) was deposited in the College of Pharmacy, Southern Medical University, China for future reference. SMBE was obtained by steeping the dried *Salvia miltiorrhiza Bge.* in water at 60 °C three times, each for 1 h in an oven and then it was freeze-dried. One gram powder was equivalent to about 1.5 g crude samples. The yield was 66.67 %.

Other drugs and reagents were aspirin (Sigma Co, USA), 6-keto-PGF 1α and TXB2 RIA kits (Nanjing Jiancheng Technology Co Ltd, China).

**Animals**

SD Wistar hypertensive rats weighing 200 - 250 g were provided by Experimental Animal Center of Guangdong Province (certificate no. SYXK 2002-0006). 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 Animal Care and Use Committee of Southern Medical University (approval ref no. 20150904) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [8]. The rats were randomly divided into 6 groups of ten rats: normal group, control group, reference group (aspirin 5 mg/kg) as well as SMBE groups, namely, 25, 50 and 100 mg/kg doses. Treatments were given orally once daily for 14 days.

**Studies on common carotid artery thrombosis**

After the last administration, rats were anesthetized with 3 % barbital sodium (0.5 ml/100 g i.p.). Under sterile conditions of class SPF animal room, 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 [9-12]. 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 % FeCl₃ solution (normal saline in sham group)[13,14]. 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.

\[
Ti(\%) = \left(\frac{A - A1}{A}\right)\times 100 ................. (1)
\]

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

**Studies on inferior vena cava thrombosis**

Under sterile conditions of class SPF animal room, the rats were fixed on anatomical planes in supine position, the hairs of the abdomen were scraped off 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 cava vein was opened lengthwise, the thrombus was removed and placed on the filter paper to dry, then was weighed [15,16]. Thrombosis inhibition was calculated as in Eq 1 above.

**Measurement of plasma concentration of 6-keto-PGF1α and TXB2**

One and half hours after surgery, the abdominal aorta was isolated and punctured for collecting 3 mL blood. Plasma was separated and stored at -20 °C. The plasma concentrations of 6-keto-PGF1α and TXB2 were measured by radioimmunoassay (RIA) [17,18].

**Statistical analysis**

Data are expressed as mean ± standard deviation (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

Compared with the control group, SMBE 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 SMBE (50 mg/kg) for inhibition of thrombus weight, but less effect on occlusion time (Table 1).

Inferior vena cava thrombosis

Compared with the control group, SMBE 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 SMBE (Table 2).

Plasma TXB2 and 6-keto-PGF1α

Compared with the normal group, arterial plasma 6-keto-PGF1α 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 SMBE groups was decreased dose-dependently (p < 0.05) while that of 6-keto-PGF1α was increased but with a reversed dose-dependence (p < 0.05).

Aspirin inhibited the secretion of both 6-keto-PGF1α and TXB2 significantly.

<p>| Table 1: Effect of SMBE on FeCl3-induced common carotid artery thrombosis in rats |
|-----------------|---|-----------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Dose (mg/kg)</th>
<th>OT (min)</th>
<th>Weight of thrombus (mg)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>—</td>
<td>8.5±1.4</td>
<td>16.8±0.6</td>
<td>52.8</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10</td>
<td>5</td>
<td>20.5±2.1</td>
<td>6.6±1.3</td>
<td>34.6</td>
</tr>
<tr>
<td>SMBE-L</td>
<td>10</td>
<td>25</td>
<td>27.2±3.2</td>
<td>9.8±1.4</td>
<td>55.3</td>
</tr>
<tr>
<td>SMBE-M</td>
<td>10</td>
<td>50</td>
<td>34.1±5.2</td>
<td>7.5±1.4</td>
<td>58.2*</td>
</tr>
<tr>
<td>SMBE-H</td>
<td>10</td>
<td>100</td>
<td>45.3±4.5</td>
<td>5.7±1.3</td>
<td>86.7*</td>
</tr>
</tbody>
</table>

OT = thrombosis occlusion time; SMBE-L: low-dose of SMBE; SMBE-M: middle-dose of SMBE; SMBE-H: high-dose of SMBE (mean ± SD, n = 10). *p < 0.01 vs. control group; #p < 0.05 vs. aspirin group

<p>| Table 2: Effect of SMBE on inferior vena cava thrombosis in rats |
|-----------------|---|-----------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Dose (mg/kg)</th>
<th>Weight of thrombus (mg)</th>
<th>Inhibition rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>—</td>
<td>24.6±7.8</td>
<td>66.9*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10</td>
<td>5</td>
<td>6.3±1.7</td>
<td>39.2*</td>
</tr>
<tr>
<td>SMBE-L</td>
<td>10</td>
<td>25</td>
<td>13.7±4.9</td>
<td>63.5*</td>
</tr>
<tr>
<td>SMBE-M</td>
<td>10</td>
<td>50</td>
<td>7.6±2.2</td>
<td>64.5*</td>
</tr>
<tr>
<td>SMBE-H</td>
<td>10</td>
<td>100</td>
<td>5.8±1.5</td>
<td>67.4*</td>
</tr>
</tbody>
</table>

SMBE-L: low-dose of SMBE; SMBE-M: middle-dose of SMBE; SMBE-H: high-dose of SMBE. *p < 0.01 vs. control group; #p < 0.05 vs. (mean ± SD, n = 10) aspirin group

<p>| Table 3: Effect of SMBE on the plasma concentrations of 6-keto-PGF1α and TXB2 in rats |
|-----------------|---|-----------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Dose (mg/kg)</th>
<th>6-Keto-PGF1α (pg/ml)</th>
<th>TXB2 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>—</td>
<td>568.3±305.12</td>
<td>238.17±103.43</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10</td>
<td>—</td>
<td>247.26±214.22</td>
<td>719.24±278.36</td>
</tr>
<tr>
<td>SMBE-L</td>
<td>10</td>
<td>25</td>
<td>186.37±102.25</td>
<td>91.24±35.17</td>
</tr>
<tr>
<td>SMBE-M</td>
<td>10</td>
<td>50</td>
<td>533.56±276.33</td>
<td>431.18±218.32</td>
</tr>
<tr>
<td>SMBE-H</td>
<td>10</td>
<td>100</td>
<td>318.24±149.28</td>
<td>211.53±128.26</td>
</tr>
</tbody>
</table>

SMBE-L: low-dose of SMBE; SMBE-M: middle-dose of SMBE; SMBE-H: high-dose of SMBE (mean ± SD, n = 10). *p < 0.01 vs. control group; #p < 0.05 vs. aspirin group

DISCUSSION

Many clinical studies showed that Danshen and its preparations are effective for the treatment of coronary artery diseases, angina pectoris, myocardial infarction, cerebrovascular diseases, various types of hepatitis and chronic renal failure [19]. Ligation of veins causes focal blood stasis, injury of vascular endothelial cell and hypoxia [20]. Danshen and its medicinal products

are widely used in Asian area for supporting cardiovascular function; evaluation of the active constituents in this herb is essential to ensure the efficiency of medication. Studies showed that this herb contains several pharmacologically active compounds, especially the diterpene diketones known as tanshinones. Due to injury of vascular endothelial cell, synthesis of PG12 decrease and plasma TXA2 increased [21], further promoting platelet adhesion and aggregation and imbalance of TXA2/PGI2, which leads to vasoconstriction, platelet aggregation, and thrombosis [22]. The biological half-life of serum TXA2 is only 30 seconds, and TXA2 transformed to TXB2 quickly. Therefore, we measured TXB2 in our experiment.

Compared with the model group, SMBE dose-dependently prolonged OT, reduced the weight of arterial and venous thrombosis. The extracts also decrease plasma TXB2 concentrations and increased 6-keto-PGF1α, thereby increasing the 6-keto-PGF1α to TXB2 ratio. The anti-thrombotic effect of SMBE was probably mediated by regulating the prostacyclin/thromboxane balance and the ratio, resulting in a ratio that was dependent on thrombus weight. Arterial occlusion time was linearly related to the ratio for control, aspirin and SMBE -H ($r^2 = 0.999$) but SMBE -M and SMBE -L appear not to lie on the same line. Furthermore, SMBE perhaps had some additional effect that prolongs occlusion time beyond what would be expected from the effect on cyclooxygenase, or more generally on the synthesis of thromboxane and PGI2, especially for the lower SMBE 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 [23,24].

CONCLUSION

The findings of this study indicate the anti-thrombosis effect of SMBE in Further studies are, however, required to ascertain its therapeutic significance in the treatment of thrombosis in humans.

DECLARATIONS

Acknowledgement

None declared.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/ra), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES


