The Attenuation Effects of Apigenin, Vitexin, Hyperoside and Quercetin on Carrageenan-Induced Mice Tail Thrombosis Model

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ABSTRACT

Flavonoids exert different pharmacological actions, such as analgesic, anti-inflammatory and cardioprotective activities. Apigenin, vitexin, quercetin and hyperoside are flavonoids found within medicinal plants. We aimed to study selected flavonoids’ antithrombotic effects using a carrageenan-induced tail thrombosis model in mice. To evaluate the antithrombotic effects of selected flavonoids, a carrageenan-induced tail thrombus mouse model was used. Flavonoids were administered intraperitoneally in doses of 50, 100 and 200 mg/kg. Forty μl (1%) carrageenan (type I) injected in the mouse’s hind paw 45 min after drug administration. The wine-colored region at the mouse tail was then measured at 24, 48 and 72 h. The findings showed that all flavonoids tested had antithrombotic effects. A dose of 200 mg/kg of quercetin and hyperoside decreased the length of tail thrombosis significantly (P<0.001) at day three. Apigenin and vitexin were more effective at 50 and 100 mg/kg doses than at the 200 mg/kg dose. The results obtained indicate that selected flavonoids exert preventive anticoagulant effects. Apigenin and vitexin showed antithrombotic effects at all tested doses, while quercetin and hyperoside were effective only at the highest doses. We believe that these flavonoids are promising new antithrombotic agents or could be used as complementary therapy with other anticoagulant drugs.

Keywords: Apigenin, vitexin, quercetin, hyperoside, antithrombotic effects, thrombosis

INTRODUCTION

Hemostasis is a necessary mechanism to stop bleeding, whereas thrombosis can be either a pathological or a physiological event, and therefore may require treatment. Thrombus formation is one of the main causes of many cardiovascular diseases, such as myocardial infarction, ischemia, deep vein thrombosis and angina [1,2]. Thrombus formation is a highly complicated physiological process involving many factors, such as platelets and coagulation factors. Intravascular arterial thrombosis causes multiple types of cardiovascular damage and arises primarily from platelet activation. Venous thrombosis, which can cause deep vein thrombosis and pulmonary embolism, arises mostly from activation of coagulation factors [1-3].

Flavonoids are large polyphenolic compounds derived from medicinal plants, and their health benefits have been reported in various epidemiological studies. These compounds are used to obtain different pharmacological effects, such as analgesic, anti-inflammatory, antihepatotoxic, anti-ulcer, anticancer, antimutagenic, antispasmodic, anti-allergic, antiviral and cardioprotective activities [4-7]. Flavonoids are also part of a therapeutic diet to help prevent mortality due to coronary artery disease. It is believed that their inhibitory effects on low-density lipoprotein oxidation and platelet aggregation are responsible for these beneficial effects [8-11]. Additionally, it has been reported that flavonoids exhibit their antithrombotic effects by inhibiting arachidonic acid metabolism and modulating endothelial nitric oxide and prostacyclin levels [12, 13].

Traditional medicine, such as hawthorn plant products, is utilized in the management of thromboembolic and multiple cardiovascular diseases [14, 15]. It was previously demonstrated by us [16, 17] that the flavonoid-rich plant hawthorn (genus Crataegus), commonly used to treat angina, hypertension, arrhythmias, and congestive heart failure [14,15], possesses an in vivo antithrombotic effect in a carrageenan-induced
Intraperitoneal heparin (100 IU) inhibited platelet aggregation. Flavonoids were dissolved in a vehicle (0.3 ml saline) and injected into the mouse hind paw 45 min before carrageenan injection. Intraperitoneal heparin (100 IU) was used as a reference drug in the positive control group. The length of tail thrombosis was measured using a ruler and was photographed at 24- to 72-h intervals.

**RESULTS**

The subplantar injection of carrageenan resulted in thrombosis tail infarction becoming visible in the control group. The lengths of tail thrombosis at 24, 48 and 72 h are shown in Figure 1. The inhibition activities of flavonoids tested in carrageenan-induced tail thrombosis in mice are shown in Table 1. Heparin (100 IU) prevented the tail vein thrombosis from developing significantly (P<0.01) compared with the control group’s treatment. Quercetin and hyperoside decreased the length of tail thrombosis significantly at a dose of 200 mg/kg (P<0.001) at day three relative to the doses of 50 and 100 mg/kg and compared with the control group. At day three, the inhibition of thrombosis (%) for 200 mg/kg quercetin was 75% on average and for 200 mg/kg hyperoside was 73% on average. The length of tail thrombosis significantly decreased with doses of 50, 100 and 200 mg/kg apigenin (P<0.001, P<0.001 and P<0.05, respectively) in the treatment groups at day three. However, apigenin appeared most effective at the 100 mg/kg dose. Table 1 shows that, at all days, apigenin decreased thrombosis by on average 76% and 89% at doses of 50 and 100 mg/kg. The administration of vitexin also significantly prevented the developing of tail vein thrombosis (P<0.001, P<0.001 and P<0.05, respectively) at 24- and 48-h time points. Although the effectiveness of 200 mg/kg vitexin faded out, 50 and 100 mg/kg vitexin injected into the treated mice remained effective (P<0.001) at 72 h. At day three, the 50 mg/kg dose of vitexin inhibited thrombosis by 84% on average (Table 1).
Photographs of tail thrombosis for each groups at 48 h are shown in Figure 2-3 (photographs at 24 and 72 h not shown). The findings obtained in the present study indicate that apigenin and vitexin are highly potent against carrageenan-induced tail thrombosis in mice.

**DISCUSSION**

In this study, the preventive anticoagulant effects of apigenin, vitexin, quercetin and hyperoside were examined using a carrageenan-induced tail thrombosis model in mice. Apigenin and vitexin showed antithrombotic effects at all tested doses, while quercetin and hyperoside were effective only at the highest doses.

Physiologically, thrombosis is a similar event to hemostasis, but thrombosis can be a pathological process, whereas hemostasis is a physiological reaction of metabolism. In thrombosis, a thrombus forms in a vessel and obstructs blood flow through the circulatory system. This blockage causes different cardiovascular health problems [22,23]. Thus, the management of thrombosis with anticoagulant drugs is vital in treating cardiovascular diseases [24,25]. Many recent studies have focused on the investigation of new agents that are effective for treating thrombosis, because available antithrombotic drugs have many side effects owing to their narrow therapeutic index [26,27].

The carrageenan-induced tail vein thrombosis model used in this study employed carrageenan, a polysaccharide polymer extracted from various red seaweeds (Rhodophyceae). Carrageenan is used for gelling, thickening, and emulsifying food during preparation and in experimental pharmacological studies of anti-inflammatory and antithrombotic and other effects. Various types of carrageenan have been used, such as λ, κ, τ, ε and μ. It is known that the κ type of carrageenan induces tail thrombus in mice and enables researchers to observe the progression of thrombosis in a time-dependent manner [28,29]. In this study, type I carrageenan was chosen because it contains large amounts of κ type carrageenan. Tail thrombosis was successfully induced in mice and became visible within 24 h. At a later time, tail necrosis due to thrombosis was observed. It is known that the formation of a carrageenan-induced thrombus causes the release of inflammatory factors [30].

*Crlataegus* plant species, which are known to contain various flavonoids, have been used to treat cardiovascular disease [31]. The antithrombotic effects of *Crataegus oxyacantha* (a common hawthorn) and *Crataegus aronia* syn. *Azarolus* (L) were demonstrated in *in vitro* studies, and it has been suggested that *Crataegus* species inhibit platelet aggregation [32,33]. *Crataegus* species contain various types of flavonoids, such as hyperoside, apigenin, apigenin 7-glucoside, vitexin and quercetin [34,31]. Thus, the *in vivo* effects of hyperoside, apigenin, vitexin and quercetin on thrombosis were investigated and it was found that these flavonoids are effective at different doses in mice.

Many different mechanisms may play a role in the antithrombotic activity of flavonoids, such as inhibiting TXA2 release, decreasing the level of Ca2+ in platelets or blocking glycoprotein IIb/IIIa receptors [35]. It has been demonstrated that apigenin and quercetin inhibit collagen-induced aggregation in platelet-rich plasma *in vitro* [36]. Apigenin, genistein, luteolin and quercetin block the TXA2 receptors (TP), similarly to the TP receptor antagonist SQ29548. Aspirin, which suppresses the TXA2 pathway, shows more potent antiplatelet effects when combined with apigenin [37,38]. In this study, thrombosis was induced by carrageenan. It has been reported that the thrombotic activity of carrageenan results from the activation of Hageman factor, also known as factor XII, which is followed by intravasal coagulation [39]. Other proposed mechanisms for carrageenan-induced thrombosis include local blood vessel inflammation causing the release of interleukin-1 (IL-1) and tumor necrosis factor (TNF), which may destroy the functioning of normal endothelial cells that maintain the balance between hemagglutination and fibrinolysis, and endothelial cell damage due to promoting thrombus formation by reducing relaxing factors and increasing constrictor factors [40,41]. It is thought that hyperoside, apigenin, vitexin and quercetin thereby prevent thrombosis by inhibiting platelet aggregation through antagonizing TXA2 or Hageman factor which initiate the thrombosis formation, or reducing the effectiveness or levels of TNF and IL-1, which were released via local blood vessel inflammation following carrageenan-induced thrombosis in this study. Therefore, reducing the effects of thrombus formation induced by carrageenan could be accomplished using the tested substances through any of the mechanism proposed here. We contemplate that the antithrombotic activity of these flavonoids could be used as monotherapy or as a complementary or combination treatment for thrombosis.

The present *in vivo* study of the antithrombotic activity of flavonoids supported the results of previous *in vitro* studies. All tested flavonoids possessed antithrombotic activity. It can be seen that these flavonoids are potential prophylactic
compounds for the treatment of thrombosis or could be used as complementary drugs with other anticoagulant medicines. Our findings suggest that these flavonoids could be used to develop antiplatelet and/or anticoagulation agents and that they should be further assessed in clinical studies.

Acknowledgments

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

Table 1: The inhibition activity of COE against carrageenan-induced tail-thrombosis in mice.

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>Doses (mg/kg)</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
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<tr>
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<td>37.20366</td>
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Figure 1. The antithrombotic effects of quercetin, vitexin, apigenin, hyperoside and heparin in carrageenan-induced mouse tail thrombosis at 24, 48 and 72 h. Values are presented as the mean ± S.E.M; *P<0.05, **P<0.01 and ***P<0.001 were significant differences from controls (n= 7–8).
Figure 2. The effects of hyperoside, quercetin, control (0.5% CMC-SF) and heparin on carrageenan-induced mouse tail thrombus length (48 h after carrageenan injection). a: Control [0.5 % CMC – saline (3:7)]; b: 100 IU Heparin; c: quercetin 50 mg/kg; d: quercetin 100 mg/kg; e: quercetin 200 mg/kg; f: hyperoside 50 mg/kg; g: hyperoside 100 mg/kg; h: hyperoside 200 mg/kg. Data represent two animals for each group.

Figure 3. The effects of vitexin and apigenin on carrageenan-induced mouse tail thrombus length (48 h after carrageenan injection). Control and 100 IU Heparin group not given again). a: apigenin 50 mg/kg; b: apigenin 100 mg/kg; c: apigenin 200 mg/kg; d: vitexin 50 mg/kg; e: vitexin 100 mg/kg; f: vitexin 200 mg/kg. Data represent two animals for each group.

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