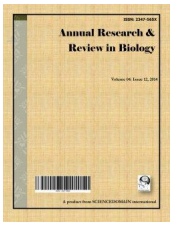

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# *In vivo* Antithrombotic Activity of Ethanolic Extract from *Ocimum basilicum L.*

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author AK designed the study, performed the statistical analysis and wrote the protocol. Authors AK and MN wrote the first draft of the manuscript. Authors YQA and RAS managed the analyses of the study. Authors SAA and HAAT managed the literature searches. Authors SSM and KOA reviewed the study. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** In this study, the antithrombotic activity of ethanolic extract from *Ocimum basilicum L.* was investigated on mice. The active component of *Ocimum basilicum L.* was extracted in 40% ethanol by Soxhlet extractor.

**Place and Duration of Study:** Department of Pharmacology, Faculty of Pharmacy, Xinjiang Medical University, Urumqi, Xinjiang, China, between February, 2009 and July, 2009.

**Methodology:** The crude extract was gavaged to mice in different concentration (100mg/ml, 200 mg/ml, and 400 mg/ml), for 21 days, Salvia Tablets were used as a positive control. On the final day, 50 mg/kg carrageenan was injected intraperitoneally to induce blood coagulation. Fibrin degradation products (FDPs) were assayed.

**Results:** Results showed that the content of mice FDPs significantly（P<0. 05）increased in the 200 mg/ml dose group and 400mg/ml dose group compared to the control group. This was dose-dependent.

**Conclusion:** The ethanolic extract of *Ocimum basilicum L* can activate the fibrinolytic system so as to stimulate Plasminogen enzyme into fibrinolytic enzyme to show antithrombotic activity.


*Keywords: Ocimum basilicum L; Plasminogen; fibrinolytic enzyme; FDPs.*

## 1. INTRODUCTION

The use of synthetic fibrinolytic medicines in treating vascular blockage, atherothrombotic diseases like myocardial or cerebral infarction is associated with a hyper risk of haemorrhage, severe anaphylactic reaction and lack of specificity [1]. The hemostatic system involves equilibrium between fibrin formation (coagulation) and fibrin dissolution (fibrinolysis) to prevent blood loss and seal the sites of injury and ensure perfusion through tissues [2]. This system is to protect against the threat of fatal hemorrhage [3], [4]. The interaction between platelets and clotting factors results in the generation of a protective hemostatic plug and run the flow of blood at the site of vascular injury. The key enzyme responsible for this is thrombin [3]. Under the physiological conditions, it transforms fibrinogen into fibrin, while plasmin is the main enzyme capable of degrading both fibrinogen and fibrin. Any defect of this equilibrium may cause thrombosis or hemorrhage. As well as, local or systemic higher fibrinolytic activity may evolve into heavy bleeding or clotting incidents [5]. These coagulation and fibrinolysis disorders cause different medical problems. Sequentially, isolation and sortation of anti- and pro-coagulant compounds as fibrinolytic and antifibrinolytic components from various sources such as caterpillars, snakes, and plants have been practiced [6]. Plant remedies are the most prevalent treatments in most of the developing countries. Recipes of these remedies have been handed down from one generation to next generation, and every culture has used decoctions or extracts of a different part of plants like leaves, flowers, barks or roots to treat various health problems [7]. There are different plant components like flavonoids, coumarins, proteases and phenolic compounds have been isolated, and they showed an effect on the hemostasis.

*Ocimum basilicum L.,* also known as sweet basil belongs to the Lamiaceae family. It is an annual herb growing in several regions around the world [8,9]. Traditionally, it has been used as a medicinal plant for the treatment of headaches, coughs, diarrhea, constipation, worms, heart problems and kidney malfunction [10]. It is also used as a flavoring agent in sausages, meats, on pizzas and in salads [11]. However, Scientists have studied different biological activities of *O. basilicum L.* such as antiviral, antispasmodic, carminative, analgesic, antiseptic, respiratory tract and anti-inflammatory activities [11-13]. In the present study, due to the rare actual scientific evidence and interest in the haemostatic activity compounds with the great medical potential present in *O. basilicum L.*, an in vivo investigation was carried on for the antithrombotic activity of ethanolic extract of this plant.

## 2. MATERIALS

### 2.1 Plant Material

*O. basilicum L.* shoots were collected from Toksun, Xinjiang, China. April 2009. The plant material was identified by an expert, from the Department of Pharmacognosy, Faculty of Pharmacy, Xinjiang Medical University, China.

## 2.2 Chemicals

Carrageenan, ethanol, sodium citrate, dimethyl sulfoxide were purchased from Sigma (St. Louis, USA). Fibrinogen Degradation Products (FDPs) ELISA Kit was purchased from Shanghai Sun Biology Technology Co., Ltd.

## 2.3 Animal

Swiss albino mice (35male, 35female, 18–22 g) were obtained from Xinxiang Medical University Experimental Animals Research Centre, China. Mice were kept in the animal laboratory with controlled temperature (25±2°C) for 12 h light/12 h dark cycle with free access to food and water. Mice care and research protocol were based on the principles and guidelines adopted by the Guide for the Care and Use of Laboratory Animals and approved by the Xinxiang Medical University Experimental Animals Ethics Committee.

## 3. METHODS

## 3.1 Preparation of the Ethanol Extract of *O. basilicum L.* Shoots

*O. basilicum L.* shoots (1000 g) were air-dried at room temperature and under shade and then cut into small pieces. Plant material was extracted in 40% ethanol [14,15] using a Soxhlet apparatus for 10 h. The ethanol was removed by Rotary evaporator, and water was removed by lyophilization. The extract was kept in -20℃.

## 3.2 Animal Experiment

A total of 70 male and female mice were randomly divided into 5 groups (n=14), and mouse gender was equally distributed throughout groups. Group 1 served as the control with saline; Groups 2, 3, 4 were, respectively gavaged with 100, 200 and 400 mg/kg extract [15]; Group 5 was given Salvia Tablets, a well-known antithrombotic chinese medicine approved by Chinese food and drug administration, as the positive control. The experiment was continued for 21 days and on the final day, 50 mg/kg carrageenan prepared in saline was administered intraperitoneally to induce blood coagulation after one hour of the test samples administration.

## 3.3 FDPs Determination

One hour after carrageenan injection, blood samples were collected in the tube with 3.8% sodium citrate by retro-orbital blood collection method. Platelet was removed by centrifugation (3000 r/min, 10 min). FDPs were measured immediately according to the kit manual [16].

## 3.4 Statistical Analysis

GraphPad Prism7 software was used for Statistical analysis, differences between the control group and remaining groups were analyzed by using Student's t-test. $P<0.05$ was considered statistically significant. All the data are expressed as the means ± standard deviation.

## 4. RESULTS

Fibrinolytic enzyme activation leads the generation of FDPs and inhibits thrombosis process. It can be seen from Table 1 that FDPs level in 100 mg/ml extract group remained almost same with control group while FDPs

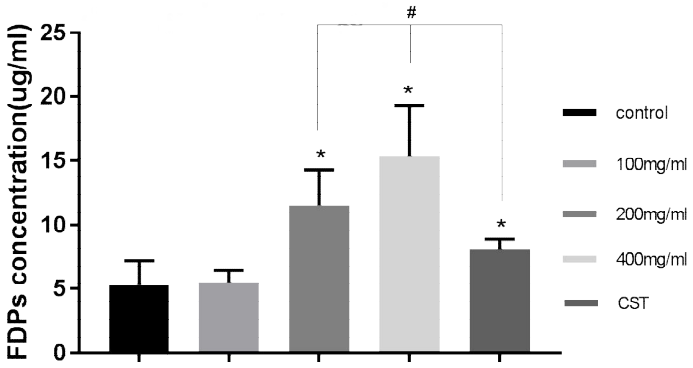


**Fig. 1. FDPs assay for blood samples of carrageenan induced thrombosis mice model**

*p<0.05 for comparison with control group, # for comparison with CST. Mean ± S.E.M = Mean values ± Standard error of means of n=70*

concentration in 200 mg/ml, 400 mg/ml, and Salvia Tablets groups significantly (P<0.05) increased compared to the control group. Noticeably, the highest concentration of FDPs was detected in 400 mg/ml, and 200 mg/ml was next, and the differences were significant to the positive control group, Salvia Tablets group (Fig. 1).

**Table 1. FDPs concentration in blood (ug/ml)**

| Group | n | FDPs (ug/ml) |
|---|---|---|
| Control | 9 | 5.277±1.954 |
| 100 mg/ml | 9 | 5.500±0.956 |
| 200 mg/ml | 13 | 11.538±2.755*# |
| 400 mg/ml | 12 | 15.416±3.822*# |
| *Salvia Tablets* | 11 | 8.124±0.810* |

**represents P<0. 05 while compared to the control group; #represents P<0.05 while compared to the Salvia Tablets group*

## 5. DISCUSSION

Fibrinolysis is very important mechanism of human body that prevents problematic blood clots formation and growing. This is a normal body process and it can be stimulated by medicine to breakdown of clots resulted from a medical disorder, or some other cause [17]. The plasmin is the main enzyme in this process which cuts the fibrin clot, the product of coagulation, in to small pieces. These are called FDPs. FDPs compete with thrombin, and thus slow down clot formation by preventing the conversion of fibrinogen to fibrin [18]. Carrageenans belong to polysaccharide polymers family. They are prepared from red seaweeds and used as gelling and thickening agents in the food and pharmaceutical industry [19]. It has been proved to be most potent thrombogens and frequently used to induce thrombotic animal model for evaluation of antithrombotic activity of different compounds [20,21]. R. Arslan et al. [22] investigated the antithrombotic effect of ethanol extracts from *Crataegus* species on carrageenan induced tail thrombosis mice model. They reported that the flavonoids of the extract might contribute to the therapeutic effect, this finding is match with many other reports. Flavonoids bind to cell receptors and alter the signaling pathways for example; blocking the platelet glycoprotein Ibα binding receptors (adenosine receptors and von Willebrand factor) [23,24]. *Ocimum basilicum* L. is one of the commonly used plants which is a rich source of flavonoids, phenolics, and anthocyanins. It has been reported to exhibit anti-oxidant, anti-inflammatory, anti-hypertension, and even anti-cancer activity in invitro and invivo studies [25,26]. In this study, the antithrombotic activity of ethanol extract of *Ocimum basilicum* L. was investigated in carrageenan induced thrombosis mice model. Three different concentration (100, 200, 400 mg/ml) were used. The increased amount of FDPs were investigated in blood samples. The lowest concentration did not show antithrombotic effect, but the middle and high dose of extract exhibited promising antithrombotic activity compared to the positive control group, this result indicated the dose dependent antithrombotic ability of this plant. However, more studies are needed to identify the antithrombotic component of this plant and the possible mechanism of it.

## 6. CONCLUSION

Herbal medicines are still widely practiced worldwide today, as the practice of herbalism is not strictly based on evidence, experimental investigations are needed to prove their efficacy. In this study, the bioactive components of *Ocimum basilicum* L. were extracted, and the antithrombotic property was investigated invivo. Our findings provide solid evidence to use *Ocimum basilicum* L. in treading cardiovascular diseases.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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