Evidence based Scientific Narrative Review on the Anticoagulant Mechanisms of *Allium sativum* (Garlic)

Hareera M.N.F.Z.1, Wijesekara G.U.S.1*, and Bandara E.M.S.1

1Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Sri Jayewardenepura, Sri Lanka

Abstract

Introduction: Garlic (*Allium sativum*, Family: Amaryllidaceae) is a seasoning plant cultivated all over the world. Garlic contains a lot of bioactive compounds including organosulfur compounds, saponins, phenolic compounds, and polysaccharides. Anticoagulant activity is one of the pharmacological properties of Garlic. The aim of this study was to review the mechanism of the anticoagulant activity of garlic and summarise the potential evidence about the anticoagulant activity of garlic from past studies. Method: Literature searches were conducted on PubMed and Google Scholar databases. 44 full articles were reviewed in detail in this study. The findings were reviewed and potential evidence for the anticoagulant mechanisms of garlic was summarised. Results: Organosulfur compounds in garlic play a major role in the anticoagulant property of garlic. Garlic exhibits anticoagulant activity mainly through three mechanisms. Those are inhibiting platelet aggregation, retarding thrombin formation, and promoting fibrinolysis. The anticoagulant activity of garlic is proven by several human, animal and *in vitro* studies. Garlic significantly inhibits adenosine diphosphate and epinephrine induced platelet aggregation in healthy subjects. Garlic administration inhibit the thrombus formation in rats. Aqueous and methanolic extracts of garlic prolong prothrombin time. Extension in clotting time is reported with increasing concentrations of garlic extract. Conclusions: Garlic has been reported as a medicinal plant showing anticoagulant activity by resisting platelet aggregation, inhibiting thrombin formation and enhancing fibrinolysis and this study suggests that garlic reduces the risk of thrombosis therefore cardiovascular complications.

Keywords: Anticoagulant Activity, Garlic, Thrombosis

Introduction

Garlic (*Allium sativum*, Family: Amaryllidaceae) is a seasoning plant cultivated all over the world. It produces bulbs, with five to ten cloves (Figure 1). It is utilized as a spice for flavouring food during the cooking process [1]. Several parts of garlic are used in traditional folk medicine. Leaves and cloves are mostly used for medicinal purposes [2]. Since ancient times, people use garlic to treat cardiac diseases, hypertension, arthritis, pulmonary complaints, respiratory infection, abdominal growth, cold, diarrhea, headache, bites, skin diseases, wounds, worms, influenza, ulcers and tumours [1,3,4]. Garlic contains many bioactive compounds including organosulfur compounds, saponins, phenolic compounds, and polysaccharides [5].

*Corresponding author: udaya_wije@sjp.ac.lk
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Among them, organosulfur compounds are the major compounds found in garlic [2]. The four major organosulfur compounds found in garlic are alliin (S-allyl cysteine sulfoxide), S-allylcysteine (SAC), S-methylcysteine (SMC), and S-ethylcysteine (SEC) [5]. Allicin (Diallyl thiosulfinate) is the principal bioactive compound responsible for most activities of garlic, released by crushing or chopping garlic. Crushing of garlic releases an alliinase enzyme that catalyzes formation of allicin from alliin [6,7,8]. Allicin is a very unstable molecule. Allyl methyl disulfide (MADS), diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), E/Z-ajoene, and dithiins found in garlic are produced due to breakdown of allicin [7,8]. The characteristic odor of garlic is due to the presence of allicin and other oil soluble sulfur compounds [6,8].

Compounds in garlic have several pharmacological properties that are very useful in herbal medicine such as anticoagulant, antimicrobial, anti-inflammatory, anticancer, antioxidant, antidiabetic, anti-obesity, antihypertensive, hypolipidemic, hypoglycemic, and fibrinolytic activities [9-16].

The aim of this study is to find out the mechanisms of anticoagulant activity and evidence for the anticoagulant activity of garlic from existing literature.

**Methods**

To gather the needed information, literature searches were conducted using keywords on PubMed and Google Scholar databases. A total of 104 articles published between 1963 and 2020 were selected for this study. Duplicate articles, articles that have no specific information, and articles that cannot be accessed to full articles (abstract and conference presentations) were excluded. Finally, 44 full articles were included to review in detail in this study. The full text of each article was read by the authors and information related to anticoagulant mechanisms of garlic was extracted. Finally extracted information was analysed qualitatively and potential evidence for the anticoagulant mechanism of garlic was summarised.

**Results and discussion**

Evidence for anticoagulant mechanisms of garlic, extracted from *in vitro*, animal, and human studies are given below.

**Anticoagulant activity of garlic**

Garlic shows anticoagulant activity by several mechanisms. Garlic enhances the anticoagulant activity of non-steroidal anti-inflammatory drugs and blood thinning medications. It recommends that a dosage of 4 g or 1-2 cloves of garlic per day is health beneficial [17].

**Compounds in garlic responsible for its anticoagulant activity**

There are several compounds that are responsible for anticoagulant activity in garlic. Organosulfur compounds in garlic play a major role in its anticoagulant property. Allicin, other thiosulfinates, allicin derivatives such as ajoene, diallyl trisulfide (DATS), diallyl disulfide (DADS), methyl allyl trisulfide (MATS), dithiins and adenosine in garlic are responsible for the antithrombotic effect of garlic. The most powerful antiaggregatory compound in raw garlic is allicin, but it cannot be identified in garlic extracts [18-
Steroid saponins such as proto-isoeruboside-B and isoeruboside-B separated from garlic also affect blood coagulation therefore anticoagulant activity [23]. Saponin is a major non sulfur compound in garlic. It is not affected during the cooking process. It has the ability to inhibit the formation of clots (thrombus) [24]. Galactolipid and a phytosterol from garlic can affect adenosine diphosphate (ADP) induced platelet aggregation [25]. In addition to the above compounds, L-methionine, L-arginine and L-cysteine in aged garlic extract have been found to have antiaggregatory effects [26].

**Mechanism of the anticoagulant activity of garlic**

Garlic exhibits anticoagulant activity mainly through three mechanisms. Those are inhibiting platelet aggregation, retarding thrombin formation, and promoting fibrinolysis (Figure 2) [6]. Platelet aggregation and adhesion are significantly reduced by garlic [27]. Garlic shows anticoagulant effect by inhibiting collagen, adenosine diphosphate (ADP) and epinephrine induced platelet aggregation [28].

Platelet aggregation is a complex process that includes activation of cytosolic Ca$^{2+}$, thromboxane production, fibrinogen receptors, glycoprotein IIb/IIIa (GPIIb/IIIa) and other modulators like cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), lipoxygenase, protein kinase enzyme, and nitric oxide (NO). The rise in platelet aggregation can result in pathological thrombus formation. Some of the mechanisms said above are affected by garlic thus helping to reduce the risk of thrombosis, therefore, cardio associated death [7,29,30]. Platelet aggregation is inhibited by several mechanisms such as inhibition of cyclooxygenase enzyme, retardation of movement of calcium into platelets, and elevation of cAMP, and cGMP [31]. Diallyl trisulfide (DATS), a garlic component inhibits platelet aggregation induced by thrombin and collagen related peptides, and inhibition is increased with increasing concentration. DATS inhibits platelet aggregation without interfering the formation of cAMP, and cGMP [32].

Thromboxane production is retarded by garlic by the following mechanisms. DATS in garlic reduces the synthesis of thromboxane B$_2$ by repressing the signal transduction pathway, but not by retarding the cyclooxygenase [32]. Platelet lipoxygenase and cyclooxygenase enzymes are inhibited by allylpropyl disulfide, diallyl disulfide, and other sulfur compounds in garlic oil in order to prevent the synthesis of thromboxane B$_2$ (TXB$_2$) [19].

Platelet aggregation is inhibited through several mechanisms. Aged garlic, prepared by soaking raw garlic in 15-20% ethanol for 20 months [6] inhibits platelet aggregation through several mechanisms. It prevents the inflow of calcium ions by chelating calcium within cytosol or interfering with intracellular secondary messengers within the platelets [26]. Platelet aggregation is also retarded by blocking the synthesis of thromboxane A$_2$, changing the membrane fluidity and increasing the production of nitric oxide and cAMP [26,33]. It also induces disaggregation of platelets by resisting the fibrinogen to bind with glycoprotein IIb/IIIa receptor, increasing the bioavailability of nitric oxide, and decreasing the thromboxane production [34].

Blood coagulation is suppressed by consumption of garlic which lowers fibrinogen level and promotes fibrinolysis [19]. In normal conditions, tissue type plasminogen activator (t-PA) produces plasmin from plasma precursor plasminogen and promotes fibrinolysis. Plasmin is a proteolytic enzyme which digests the stable fibrin clot to fibrinogen degradation products (FDP). Fibrinolysis is regulated by plasminogen activator inhibitor-1 and 2 (PAI-1 and PAI-2) [28]. Fibrinolysis is promoted by garlic. The
mechanism of improvement in fibrinolysis by garlic is due to enhancement in t-PA mediated plasminogen activation. Components in garlic function as a cofactor and increase the affinity of t-PA for plasminogen. Therefore, plasminogen is highly activated and produces a large amount of plasmin [14]. This enhancement depends on the concentration and duration of intake. It has also been found that the consumption of garlic with low concentration for a long time has the best effect on fibrinolysis [35]. There is no effect on platelet aggregation by steroid saponins, but they enhance fibrinolysis and inhibit blood clotting [23].

Anticoagulant activity is exhibited by DATS rich garlic oil by enhancing the activity of antithrombin III and protein C. Antithrombin III activates proteases which suppress the production and activity of thrombin. Protein C also inhibits the formation of thrombin [36]. The adenosine diphosphate (ADP) pathway is also suppressed by alcoholic garlic extract [37]. The description of the anticoagulant mechanisms of garlic is explained in Figure 2.

![Anticoagulant Activity of Garlic](image)

**Figure 2:** Description of the anticoagulant mechanisms of garlic
Evidence from in vitro studies

An in vitro study has been conducted by Reddy et al in 2017 to see the effect of raw garlic extract on blood coagulation and fibrinolysis. In this study, clotting time had been measured by capillary method before and after mixing blood with different volumes of garlic extract. It could be observed in this study that clotting time is increased with an increasing volume of extract. Fibrinolytic activity has been analysed by adding garlic extract after formation of a clot in tubes. The clot had partly dissolved with the addition of garlic extract [11].

Another study by Vaijayanthimala et al in 2017 has been conducted to see the in vitro anticoagulant activity of aqueous and methanolic A. sativum plant extracts using plasma of normal healthy individuals. This study had found that both aqueous and methanolic extracts of garlic prolong prothrombin time. Extension in clotting time had been observed with increasing concentration of extract [38].

The research by Song et al in 1993 had isolated an anticoagulant substance from garlic. In this study, prothrombin time and blood clotting time of garlic extract added plasma and potassium oxalate added plasma had been compared to see the antithrombotic, fibrinolytic activities. Prolongation of clotting time observed with garlic extract added plasma was higher than potassium oxalate added plasma [39].

Enhancement in fibrinolytic activity by odorless garlic has been proven in a study by Fukao et al in 2007, using fibrin film assay and chromogenic assay. It was found that, according to the fibrin film assay, t-PA activity had raised by 180% in the presence of garlic compared to the absence of garlic. In chromogenic assay plasmin had been used as a substrate and enzymatic activity had been measured calorimetrically. The results from the Lineweaver–Burk plot had reported that there is no effect in maximal reaction rate (V_max) by garlic, but Michaelis constant (Km) is reduced by garlic showing a high affinity of t-PA to plasminogen [14].

Another in vitro study by Hiyasat et al in 2009 has been conducted to see the antiplatelet activity of A. sativum and A. ursinum. In this study, in vitro platelet aggregation induced by ADP, collagen, A23187, epinephrine and arachidonic acid have been analysed in a four-channel aggregometer by measuring the amount of turbidity. Plasma pre-incubated with an alcoholic extract of A. sativum had shown a decrease in platelet aggregation as compared to normal (baseline) conditions. Furthermore, it was evident that alcoholic garlic extracts significantly reduce ADP induced platelet aggregation rather than other agonists [37].

A study on fibrinolytic activity of the blood clot in vitro has been carried out by Ansari et al in 2011. This study had been done by adding various volumes of ethanolic garlic extract into plasma followed by addition of fluorescent labeled clot into that mixture. Fluorescent intensity had been measured after adding labelled clots. A significant increase in fibrinolytic activity had been observed with garlic extracts than with control [35].

Evidence from animal studies

In an experimental study conducted on a group of albino rabbits by Singh et al in 2015, an alcoholic extract of garlic had been fed to albino rabbits for two weeks to analyse the anticoagulant and fibrinolytic activity of garlic. It has been reported in the study that blood coagulation time significantly increases by 61.9%, and prothrombin time by 15.2% after a week of administration of alcoholic garlic extract [40].

Another study by Fukao et al in 2007 has been performed to see the antithrombotic effect of odorless garlic in rat models. Thrombosis had been induced in rats by in situ loop operation (control).
Another group of rats had been administered odourless garlic powder with diet and also in situ loop operation had been done (test). At the end of the experiment, blood had been collected from the vena cava of anesthetized rats. According to the results, prothrombin time (PT), activated partial thrombin time (APTT), thrombin time (TT) and fibrinogen degradation products (FDP) had been analysed. PT, APTT, TT, and FDP had decreased in test rats as compared to control as coagulation factors were consumed in control group rather than the test group. Garlic administration had inhibited the thrombus formation in test rats [14].

According to an animal study done by Miao et al in 2020 to find the anti-atherosclerotic property of total saponins of garlic in rats, administration of saponins extracted from garlic to rat models reduces the thromboxane B$_2$ (TXB$_2$) level and increases the 6-keto-prostaglandin F$_1\alpha$ (6-keto-PGF$_1\alpha$). Both have the opposite effect on formation of atherosclerosis. TXB$_2$ enhances vasoconstriction and platelet aggregation while 6-keto-PGF$_1\alpha$ relaxes blood vessels and inhibits platelet aggregation [24].

**Evidence from human studies**

A study has been done by Ali et al in 1995 to evaluate the effect of minimal intake of garlic on thromboxane synthesis. In this study, eight healthy male volunteers had been fed with one clove of fresh garlic daily for 16 weeks. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) had not been taken by subjects during the study period. It was found in this study that the intake of one clove of fresh garlic regularly for a long period decreases the level of TXB$_2$[41].

Another study has been conducted by Bordia et al in 1996 to evaluate the effect of garlic on platelet aggregation in humans by administrating different numbers of capsules containing oil equivalent to 1g of raw garlic to healthy subjects and patients with coronary artery diseases (CAD). The study consisted of two parts to check the acute and long-term effects of garlic. It has been shown in this study that acute effect of garlic is dose related and epinephrine induced platelet aggregation is only inhibited by garlic. It has been found in long term effect studies that garlic significantly inhibits ADP, epinephrine induced platelet aggregation in healthy subjects and epinephrine induced platelet aggregation in patients with CAD. According to the results, there is no significant inhibition of ADP, collagen induced platelet aggregation by garlic in patients with CAD. The metabolism of arachidonic acid in washed platelets had also been studied in this study. It has been found that garlic oil affects the metabolism of arachidonic acid thus preventing the formation of thromboxane [42].

Another study by Bordia et al in 1998 has been conducted to see the effects of garlic on various factors. It had been done by administrating garlic oil to myocardial patients. It has been found that garlic oil significantly enhances fibrinolytic activity by 39.4% and 55.1% at 1.5 months and 3 months respectively, but there is no effect on fibrinogen level. Platelet aggregation induced by arachidonic acid, collagen, adrenaline, and calcium ionophore was also affected by garlic oil due to presence of DADS and DATS which inhibit thromboxane B$_2$ production [43].

A double-blind placebo controlled crossover study by Wojcikowski et al in 2007 has been conducted to see the effect of garlic oil on platelet aggregation. It has been proven in this study that adrenaline induced platelet aggregation is significantly reduced by garlic oil but, there is no significant effect on collagen and ADP induced platelet aggregation [44].

The summary of the evidence for anticoagulant mechanisms of garlic, extracted from in vitro, animal, and human studies are stated below in Table 1.
Conclusions
Garlic has been reported as a medicinal plant showing anticoagulant activity. It shows anticoagulant activity by resisting platelet aggregation, inhibiting thrombin formation and enhancing fibrinolysis. Several compounds in garlic mainly organosulfur compounds are responsible for its anticoagulant activity. Anticoagulant activity of garlic is proven by several in vitro, animal and human studies. So, this study suggests the garlic to reduce the risk of thrombosis therefore cardiovascular complications.

Table 1: Evidence of anticoagulant mechanisms of garlic extracted from previous studies

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Reference</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro study</td>
<td>Vaijayanthimala et al. [38] 2017</td>
<td>Clotting time is prolonged with the addition of garlic extract and the prolongation is increased with increasing concentration of garlic extract.</td>
</tr>
<tr>
<td>In vitro study</td>
<td>Song et al. [39] 1993</td>
<td>Prothrombin time and clotting time are prolonged with garlic extract added plasma than control.</td>
</tr>
<tr>
<td>In vitro study</td>
<td>Fukao et al. [14] 2007</td>
<td>The affinity of tissue-type plasminogen activator (t-PA) to plasminogen is increased by garlic extract.</td>
</tr>
<tr>
<td>In vitro study</td>
<td>Hiyasat et al. [37] 2009</td>
<td>Garlic extracts significantly reduce adenosine diphosphate (ADP) induced platelet aggregation rather than other agonists.</td>
</tr>
<tr>
<td>In vitro study</td>
<td>Ansari et al. [35] 2011</td>
<td>A significant increase in fibrinolytic activity exists with garlic extracts.</td>
</tr>
<tr>
<td>Animal study</td>
<td>Singh et al. [40] 2015</td>
<td>Blood coagulation time and prothrombin time significantly increase after an administration of alcoholic garlic extract.</td>
</tr>
<tr>
<td>Animal study</td>
<td>Miao et al. [24] 2020</td>
<td>Administration of saponins extracted from garlic to rat models reduces the thromboxane B2 (TXB2) level and increases the 6-keto-prostaglandin.</td>
</tr>
<tr>
<td>Human study</td>
<td>Ali et al. [41] 1995</td>
<td>Intake of one clove of fresh garlic regularly for a long period decreases the level of TXB2.</td>
</tr>
<tr>
<td>Human study</td>
<td>Bordia et al. [42] 1996</td>
<td>Garlic significantly inhibits ADP, epinephrine induced platelet aggregation.</td>
</tr>
<tr>
<td>Human study</td>
<td>Bordia et al. [43] 1998</td>
<td>Garlic oil significantly enhances fibrinolytic activity and platelet aggregation induced by arachidonic acid, collagen, adrenaline, and calcium ionophore is also affected by garlic oil.</td>
</tr>
<tr>
<td>Human study</td>
<td>Wojcikowski et al. [44] 2007</td>
<td>Adrenaline induced platelet aggregation is significantly reduced by garlic oil.</td>
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References


