Indonesian Journal of Medical Laboratory Science and Technology

Open Access p-ISSN 2684-6748 | e-ISSN 2656-9825 IJMLST

Potential of Garlic Filtrate as An Alternative Anticoagulant for Whole Blood Samples

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Received: January 18, 2022
Revised: May 25, 2022
Accepted: September 13, 2022
Published: October 30, 2022

DOI: 10.33086/ijmlst.v4i2.2683

Abstract

Synthetic anticoagulants such as heparin, citric, and ethylenediaminetetraacetic acid (EDTA) are commonly used to prevent blood clots. In contrast, its widespread use in clinical laboratories is still constrained by price, its toxic nature, and its short shelf life. Therefore, an alternative material that is relatively cheap, non-toxic, and easy to obtain and process in a ready-to-use form is needed. Garlic contains allicin and ajoene, which are anti-platelet and anti-thrombogenic. This study's aim is to explore the potential of garlic filtrate as an alternative anticoagulant. Blood from 16 individuals was used and separated into four groups: non-anticoagulant, 50 µL/mL garlic filtrate, 100 µL/mL garlic filtrate, and 150 µL/mL heparin, for a total of 64 treatments. The Lee and White method showed that non-anticoagulated blood had normal clotting times (mean 8 minutes and 56 seconds), whereas heparin plasma and garlic filtrate plasma had longer clotting times (more than 20 minutes); and this is statistically different based on the Friedman test with a significance value (p) of 0.000 < 0.05. On spectrophotometric measurements, the levels of calcium ions in heparinized plasma and serum were 8.66 mg/dL and 8.52 mg/dL, respectively, while in garlic plasma filtrate of 50 µL/mL and 100 µL/mL were 4.13 mg/dL and 3.58 mg/dL, respectively; this is also statistically different based on the ANOVA test with a significance value of 0.000 < 0.05. The differences indicate that garlic filtrate can extend clotting time and reduce calcium ions therefore it is worth reviewing as an alternative anticoagulant.

Keywords

Anticoagulant, Calcium Ion Levels, Clotting Time, Garlic Filtrate.

INTRODUCTION

The hematological examination includes extensive laboratory measures and is essentials for diagnosing disease. Although modern technology has influenced many aspects of laboratory work, and examination procedures have become increasingly diverse, the basic principles of sample preparation remain the same. Knowledge of sample preparation relates to the technique or parameter to be performed, attempts to increase the validity of the results and, ultimately, to establish the diagnosis of the disease. Although almost anybody fluid can be a representative sample and provide information about a person's biological status, blood is the most widely used sample because it is easy to obtain also the analytes in it tend to be stable (1).

There are several causes of blood samples for hematological examination to be unusable, including hemolysis, insufficient volume, wrong container and undue clotting (2). The presence of clots, even if they are small, can affect the examination then the results will be inaccurate (3). When platelets, proteins, and blood cells adhere to one another, a blood clot form. Blood needs to be anticoagulated also known as blood thinners, to stop clots from forming. Several anticoagulants are used in the laboratory to prevent clotting or to obtain plasma. The three anticoagulants that are most often are heparin, ethylenediaminetetraacetic acid (EDTA), and citrate. Although novel anticoagulants like hirudin are available, they are not often utilized (4).

However, the use of anticoagulants does not always perform smoothly. Some laboratories that could not perform hematological examinations properly because they had run out of stock of anticoagulants. This condition worsens by its high price, dependence on distribution, short expiry date and its toxic or irritating nature. For this reason, the need to find alternative natural anticoagulants that address the challenges mentioned above, especially those from nature.

Natural products are usually made of several constituents, which have several targets. Therefore, providing a pleiotropic and synergistic effect to promote positive results. In addition, the constituents of natural products usually have minimal side effects, especially on the digestive system (5). Garlic (*Allium sativum*) is known to have various biological activities (6), including in-vitro anticoagulant effects (7).

Garlic has been used in many regions of the world since the Sumerians (2600–2100 BC); it is taken as food and medicine, particularly in the tuber; it contains antibacterial, antiviral, antifungal, and antiprotozoal properties; and it has favorable effects on the cardiovascular and immunological systems (8). Garlic's chemical composition changes depending on

the circumstances. Fresh and entire garlic bulbs contain γ-glutamyl cysteine, which can be oxidized and hydrolyzed to alliin when kept at low temperatures. When garlic is sliced, crushed, chewed, or dried, a vacuole enzyme called alliinase is released, causing alliin to be converted to allicin. Diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins, and ajoene are some of the chemicals that allicin decomposes into (9).

Garlic has the potential to be employed as an alternative source of anticoagulant replacements since allicin and ajoene exhibit antithrombin and antiplatelet action (10,11) in addition to antibacterial (12), antifungal (13), and anticancer (14–16) properties. Although there is a known relationship between garlic products with platelet aggregation (17) and calcium levels (18), the active element in garlic's capacity to inhibit blood coagulation in comparison to synthetic anticoagulants has not been thoroughly explored.

This study investigated the potential of garlic filtrate to be an alternative anticoagulant for hematology laboratory tests by analyzing blood clotting time and plasma calcium ion levels. The findings of this study are expected to pave the way for the use of garlic filtrate to replace synthetic anticoagulants like heparin and EDTA in emergencies, to avoid chemical side effects, or for efficiency reasons, particularly in laboratories with distance and operational cost issues.

MATERIALS AND METHODS

The Health Research Ethics Committee of the Health Polytechnic of Pontianak, Ministry of Health (177/KEPK-PK.PKP/VII/2020, July 8, 2020) approved this study, which was conducted in July–August 2020 at the Hematology Laboratory of the Department of Medical Laboratory Technology, Health Polytechnic of Pontianak, Ministry of Health. The blood of 16 volunteers who have stated their readiness and do not have blood coagulation issues were used in the study. This study used garlic filtrate because it is more accessible to collect than garlic extract. The research design was quasi-experimental, with each blood sample divided into four groups: without the addition of garlic filtrate (group A), with the addition of 50 µL/mL garlic filtrate (group B), with the addition of 100 µL/mL garlic filtrate (group C), and with the addition of 150 µL/mL heparin (group D) (19), for a total of 64 samples. The clotting time (11) and calcium level of each sample tested used spectrophotometry with the reagent kit from Biosystems (Barcelona, Spain).

The following methods were used to create garlic filtrate: (a) garlic bulbs were peeled, rinsed, and drained; (b) shredded; and (c) filtered through a funnel using Whatman paper and collected in an Erlenmeyer. The

garlic filtrate is the filtered and collected solution in the Erlenmeyer, while the residue is the solid left on the filter paper.

Measurement of clotting time is performed in the following ways: (a) the time measured with a stopwatch when blood enters the syringe; (b) 5 mL of blood collected and placed into the test tube according to the treatment group; (c) left the tube at room temperature (25°C); (d) all tubes tilted at a 45° angle after 5 minutes, and repeated every minute; and (e) the time recorded until the blood clots completely. If the blood did not clot for more than 20 minutes, the observation was over. Clotting periods are measured in minutes and categorized as follows: less than 5 minutes, 5–15 minutes, 15–20 minutes, and more than 20 minutes (20).

For examination of calcium ion levels, samples were obtained from venous blood. Fresh venous blood was centrifuged for 10 minutes at 1500 g, then left at room temperature to 2 hours; two layers formed and the top layer (supernatant) was serum. On the other hand, fresh blood was inserted into an anticoagulated tube and centrifuged for 15 minutes at 2000–3000 g to extract plasma (21). Heparin was employed as an anticoagulant, while 50 µL/mL and 100 µL/mL garlic filtrate were used as candidate anticoagulants in this investigation.

According to Biosystems (22), calcium measurement is as follows: (a) 1000 µL of reagent A and 1000 µL of reagent B were inserted into each blank, standard, and sample tube; (b) 20 µL of calcium standard solution was inserted into a standard tube; (c) 20 µL of serum/plasma was inserted into the sample tube; (d) homogenized and then incubated for 2 minutes at room temperature and (e) read the absorbance of the standard and sample against the blank at a wavelength of 610 nm and not more than 60 minutes.

Statistical studies of the variations in clotting time and calcium ion levels between the four treatment groups were carried out using the Friedman test and the ANOVA test, respectively, using SPSS (version 26).

RESULTS

This study compared the impact of adding garlic filtrate as an alternative to anticoagulants in extending clotting time in groups that received anticoagulants and those that received heparin. In non-anticoagulated samples, blood clots occurred between 5 and 15 minutes (mean time was 8 minutes 56 seconds), whereas in samples given 50 µL/mL garlic filtrate, 100 µL/mL garlic filtrate or heparin, no blood clots occurred for up to 20 minutes (Figure 1). The Friedman test revealed a significant difference in clotting time between non-anticoagulated samples and those given 50 µL/mL garlic filtrate, 100 µL/mL garlic filtrate, and heparin with a significance value of 0.000 < 0.05.

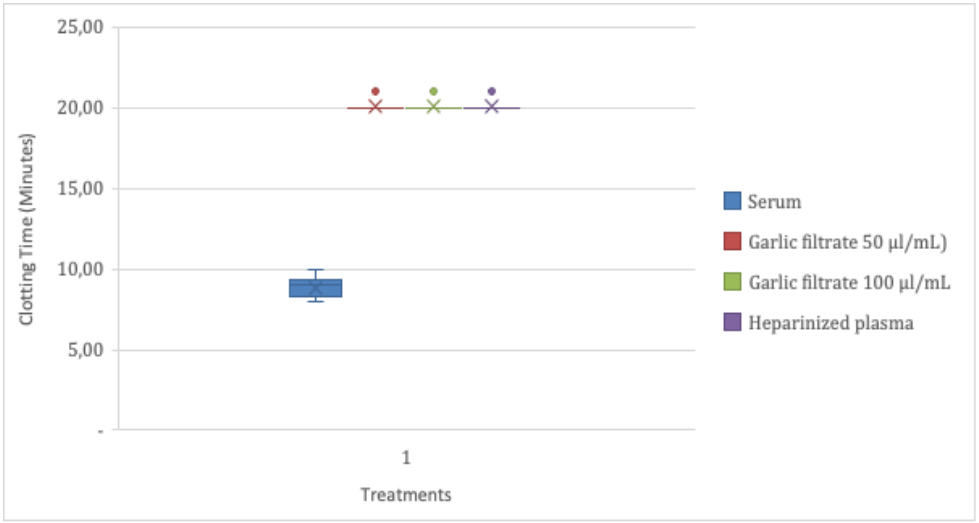

Figure 1. The Four Treatment Groups' Clotting Times

The calcium levels in heparinized plasma and serum were almost identical, at 8.66 mg/dL and 8.52 mg/dL, respectively, and did not differ statistically (significance value (p) 0.438). However, the calcium level in 50 µL/mL garlic filtrate plasma dropped dramatically to 4.13 mg/dL and was even lower in 100 µL/mL garlic filtrate plasma, at 3.58 mg/dL (Figure 2). There was a significant difference in serum calcium levels between 50 µL/mL garlic filtrate plasma and 100 µL/mL garlic filtrate plasma (p 0.000). A difference in calcium levels exists between plasma of 50 µL/mL garlic filtrate and heparinized plasma, as well as between plasma of 100 µL/mL garlic filtrate and heparinized plasma (p 0.000 and 0.004 respectively).

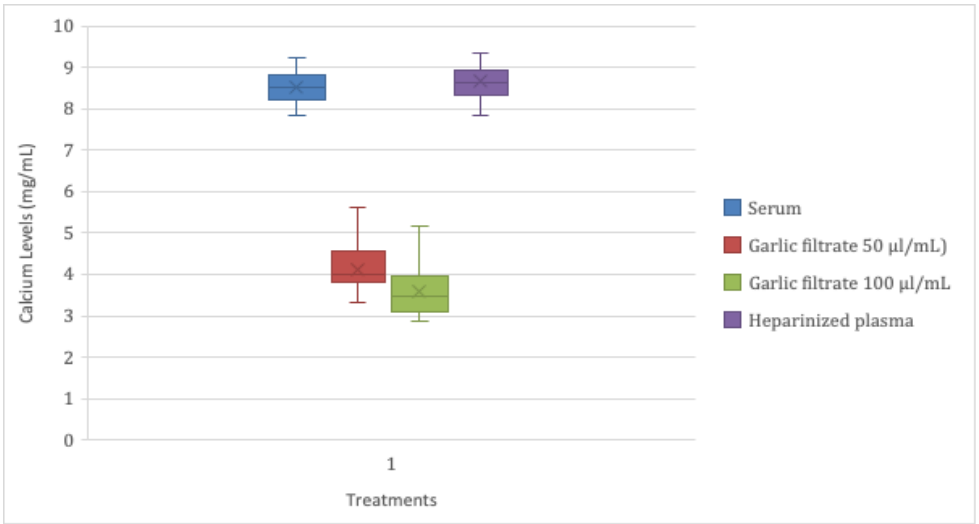

Figure 2. The Four Treatment Groups' Calcium Levels

DISCUSSION

Platelets get activated after vascular damage and produce thromboxane A2, adenosine diphosphate (ADP), serotonin, P-selectin, calcium ion, and fibrinogen, among other prothrombotic mediators. Platelets will stick to the subendothelial matrix receptors and produce aggregates (18).

The calcium ion (Ca^{2+}) is a coagulation factor IV that is essential for blood coagulation and homeostasis. After platelet activation, calcium binds to phospholipids, providing a surface for assembly with different coagulation components. Calcium ions and factor VIIa, in particular, stimulate alterations in the conversion of factor X to Xa (23,24).

Heparin binds to various proteins after administration, but it is binding to antithrombin, which is significant because it induces a surface change and inactivates thrombin. Antithrombin binds to clotting factors, but two are particularly important: thrombin (Factor IIa) and Factor Xa. It inhibits the development of clots and prolongs blood clotting time by inactivating thrombin and blocking the conversion of fibrinogen to fibrin (25).

Allicin (diallythiosulfinate) and propyl propane-thiosulfinate are two onion thiosulfinates that suppress human platelet aggregation by up to 90% (26). Allicin may easily pass across cellular membranes and bind to sulfhydryl (SH)-containing proteins, preventing them from forming free sulfhydryl residues. Garlic extract's antiplatelet action is attributable, at least in part, to the suppression of collagen binding to particular platelet receptors. Platelet adhesion and activation might be hindered as a result (27).

Ajoene, another active chemical, inhibits the binding of fibrinogen and von Willebrand factor (vWF) to the GPIIb/IIIa receptor (28). Because of its interaction with fibrinogen receptors, ajoene has anti-aggregatory and antithrombotic properties, and it also blocks arachidonic acid-stimulated platelet aggregation irreversibly (29).

In this study, similar calcium levels in serum and plasma heparin confirmed that heparin did not inhibit calcium ions. However, the decrease in calcium levels in the plasma of garlic filtrate gave evidence that the active ingredients in it bind calcium ions, prolonging clotting time, as shown by heparinized blood. It showed that garlic filtrate has the possibility as an anticoagulant replacement, especially under the conditions outlined above.

Garlic filtrate is considered to be superior to garlic extract. Apart from making the procedure accessible, the combination of components in garlic filtrate has an anticoagulant effect balanced by synthetic anticoagulants such as heparin. Because just one set of active chemicals is acquired when the extract is used, the anticoagulant's

potential is thought to be diminished. If the goal is efficiency, a dose of 50 µL/mL garlic filtrate is preferable to 100 µL/mL garlic filtrate due to their shared impact of lengthening clotting time.

This study, however, was unable to determine how long garlic filtrate may be stored before use, what parameters are safe or influenced when using garlic filtrate as an anticoagulant, or how long the effect lasts after being combined with blood.

CONCLUSIONS

Non-anticoagulated blood had an average clotting time of 8 minutes and 56 seconds, but heparinized and garlic filtrate plasma had a longer clotting time. This result might be attributed to the active components' performance, allicin and ajoene, which was statistically supported by the Friedman test findings, which revealed a significant difference between the four groups. Similarly, calcium ion levels in serum and heparinized plasma were somewhat similar, at 8.52 mg/dL and 8.66 mg/dL, respectively, but calcium ions were low in both treatments of garlic filtrate plasma (4.13 mg/dL and 3.58 mg/dL, respectively). This result suggests that garlic filtrate might be used as an anticoagulant alternative.

AUTHOR CONTRIBUTIONS

Ari Nuswantoro: concept and design, writing original and revising manuscript, analysis and interpretation of data, supervision and final approval of the version to be published. Jessica Ningtyas Berlianti: concept and design, methodology, laboratory analysis, administration, and research permission.

ACKNOWLEDGEMENT

The authors wish to acknowledge the contributions of participating volunteers on this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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