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The Effect of Aqueous Extract of *Ginger Rhizomes* as in vitro Anticoagulant on the Prothrombin Time and Activated Partial Thromboplastin Time

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ABSTRACT

Background: Thrombotic disorders remain a major global health burden, and while synthetic anticoagulant therapies are effective, they are often associated with limitations such as bleeding risk, drug interactions, and the need for continuous monitoring. Ginger (*Zingiber officinale*), a widely used medicinal plant, is traditionally known for its anti-inflammatory and cardio protective effects, and bioactive compounds within its rhizomes. **Objectives:** The present experimental study aimed to evaluate the in vitro anticoagulant effect of aqueous ginger rhizome extract on coagulation. **Materials and methods**: The plasma prepared from 20 blood samples of healthy individuals and treated with varying concentrations (2.5%, 5%, and 10%) and volumes (25, 50, and 75 µl) of ginger extract. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using a semi-automated coagulometer. **Result:** Demonstrated a significant, dose-dependent prolongation of both PT and APTT compared with controls (both P values 0.00). The PT values showed for 2.5% concentration were (13.1 ± 0.2, 13.7 ± 0.4, and 14.4 ± 0.5 seconds) for 25µl, 50 µl, and 75 µl, respectively. For the 5% concentration, the prothrombin times were (14.2 ± 0.3, 16.8 ± 0.7, and 18.3 ± 0.5seconds) for the same volumes. At a 10% concentration, the prothrombin times were 17.1 ± 0.8, 19.5 ± 0.7 and 22.3 ± 1.1 seconds) for the same volumes. In addition, the APTT values showed for the 2.5% concentration, were 33.4 ± 1.3, 34.2 ± 1.1 and 35.0 ± 0.8 seconds) for 25 µl, 50 µl, and 75 µl, respectively. For the 5% concentration, the APTT values were 36.1 ± 1.1 seconds, 38.1 ± 0.9, and 40.8 ± 1.4 seconds) for 25 µl, 50 µl, and 75 µl, respectively. At a 10% concentration, the APTT values were 37.6 ± 1.2, 40.2 ± 1.5 and 44.8 ± 1.7 seconds) for the same volumes. **Conclusion**: The aqueous ginger extract exhibits significant anticoagulant activity, highlighting its potential as a natural source of anticoagulant compounds.

Keywords: Aqueous; Extract; Hemostasis; Anticoagulant Effect; Ginger (*Zingiber officinale*); Herbal Medicine; Medicinal Plant; Prothrombin Time; Activated Partial Thromboplastin; Thrombotic Disorders.

1. Introduction

Ginger (*Zingiber officinale*) is a widely recognized medicinal plant with diverse therapeutic applications, including anti-inflammatory, antioxidant, and anticoagulant properties [1]. The rhizomes, which represent the most commonly utilized part of the plant, contain numerous bioactive compounds such as *Gingerols* and *Shogaols* that exert significant effects on physiological processes and contribute to disease prevention and health promotion [2]. In hematology, coagulation tests such as prothrombin time (PT) and activated partial thromboplastin time (APTT) are fundamental tools for assessing clotting ability, with PT reflecting the extrinsic and common pathways and APTT assessing the intrinsic and common pathways [3]. Several investigations have shown that ginger extracts can prolong both PT and APTT, indicating their potential anticoagulant activity through inhibition of platelet aggregation and modulation of clotting factors [4]. These findings suggest that ginger may serve as a natural therapeutic option or adjunct to synthetic anticoagulants for managing thrombotic conditions [5].

Ginger is a perennial herb of the *Zingiberaceae* family, cultivated for centuries across Asia for both culinary and medicinal purposes. Its rhizomes are fleshy underground stems characterized by fibrous interiors and aromatic properties, containing high levels of *gingerols, shogaols,* paradols, and *zingerone. Gingerols,* abundant in fresh rhizomes, account for its pungency and therapeutic effects, while shogaols, produced during drying or cooking, enhance its medicinal potency. Paradols and zingerone contribute to antioxidant and anti-inflammatory actions, supporting ginger's pharmacological versatility [6,7]. The plant exists in several forms, including fresh, dried, preserved, pickled, and ground preparations, each with specific uses ranging from culinary applications to clinical practice. Fresh ginger is primarily used for flavoring, dried and powdered ginger is applied in traditional medicine, and pickled or preserved ginger is incorporated into various foods [8].

Clinically, ginger has been studied for its efficacy in alleviating gastrointestinal disorders, including nausea, vomiting, and indigestion. It is particularly effective in pregnancy-related morning sickness and chemotherapy-induced nausea [9]. Beyond digestive health, ginger exhibits strong anti-inflammatory activity, beneficial in conditions such as osteoarthritis and rheumatoid arthritis, through inhibition of pro-inflammatory cytokines and enzymes like COX-2. Its high antioxidant content further protects against oxidative stress-related diseases, including cardiovascular disorders and cancer [10]. Recently, research has also emphasized ginger's anticoagulant potential. Its active compounds have been shown to inhibit platelet aggregation and suppress thromboxane synthesis, critical mediators of clot formation. Both *gingerol* and *shogaol* have been implicated in prolonging coagulation times, positioning ginger as a promising natural alternative or complement to synthetic anticoagulants, which are often associated with adverse effects such as bleeding risks [11].

The coagulation system itself is complex, and laboratory tests such as PT and APTT remain essential for evaluating abnormalities. PT, first developed by Quick, was initially intended to measure prothrombin levels but is now recognized as being sensitive to deficiencies in factors VII, X, V, and prothrombin, as well as vitamin K deficiency and liver disease [12]. However, PT alone cannot fully reflect in vivo hemostasis, as patients with hemophilia may present with normal PT despite recurrent bleeding episodes. To address this limitation, the APTT assay was introduced, providing a more reliable measure of the intrinsic pathway. The use of partial thromboplastin and subsequent refinements with contact activators such as kaolin or ellagic acid have improved reproducibility, making APTT a cornerstone in coagulation testing [13,14].

Several experimental and clinical studies have investigated the anticoagulant properties of ginger. Hamedelniel et al. (2016) reported a dose-dependent prolongation of PT in blood samples treated with ginger extracts, with the highest concentration producing the most significant increase [15]. Jude Oliver et al. (2015) similarly found that adding different volumes of 5% aqueous ginger extract to normal blood samples significantly prolonged PT in healthy volunteers [16]. In contrast, Albara et al. (2022) examined ethanolic extracts of ginger and Curcuma longa and observed that ginger significantly prolonged PT in a dose-dependent manner, while its effect on APTT was insignificant; Curcuma showed no substantial anticoagulant activity [17]. Shadrack et al. (2021) confirmed that aqueous ginger extract significantly increased both PT and APTT in healthy Nigerian individuals, with values markedly elevated compared to controls [18]. More recently, Linconada et al. (2023) demonstrated that ginger aqueous extract prolonged both PT and APTT beyond normal limits in healthy participants, reinforcing the herb's potential as a source of novel anticoagulant compounds [19]. Taken together, these findings emphasize the therapeutic promise of ginger as a natural anticoagulant. Its widespread availability, established safety profile in culinary use, and multifaceted pharmacological activities highlight the importance of further investigating its role in clinical practice. Understanding its effects on PT and APTT may open avenues for integrating ginger into anticoagulant therapy, either as a preventive agent or as a supportive alternative to synthetic drugs.

1.1. General Objectives

- To measure Prothrombin Time in normal plasma as control groups using semi-automated technique.
- To measure Activated Partial Thromboplastin Time (APTT) in normal plasma as control groups using semi-automated.
- To measure Prothrombin Time in normal plasma treated with different concentrations and volumes of ginger Rhizomes.
- To measure Activated Partial Thromboplastin Time in normal plasma treated with different concentrations and volumes of ginger Rhizomes.
- To compare Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) in different concentrations and volumes of ginger Rhizomes with normal control groups.

2. Materials and Methods

This experimental in vitro study was conducted in the Department of Hematology, Faculty of Medical Laboratory Sciences, during the period from July to November 2024. The study aimed to evaluate the anticoagulant effect of aqueous extract of *Zingiber officinale* (ginger) rhizomes on normal human plasma through measurement of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT).

2.1. Preparation of Aqueous Ginger Rhizome Extract and procedures

Fresh ginger rhizomes were washed, peeled, sliced, and dried at room temperature for three weeks. The rhizomes were crushed in the laboratory using a pestle and mortar, after which it was grinded into fine powder. (2.5, 5 and 10) grams of ginger powder weighed separately by using a sensitive balance and then each was suspended in 100 mL of distilled water in a conical flask with shaking at intervals for twenty-four hours. The suspensions of the extract was filtered through Whatman No. 42 filter paper and the final aqueous extracts (2.5%, 5%, and 10%) then stored at 4°C until used for testing the anticoagulant activity.

2.2. Samples Preparation

Twenty blood samples were collected from healthy volunteers aged between 18 and 30 years. Participants had no history of bleeding disorders, chronic illnesses, or medication use affecting coagulation. Approximately five mL of venous blood samples were drawn aseptically from each individual and placed into 3.2% sodium citrate anticoagulated tubes in a ratio of 9:1 (blood to anticoagulant). The samples were centrifuged at 1006.2 X g for 10 minutes to obtain platelet-poor plasma, which was used for all subsequent analyses.

2.3. Testing for in vitro anticoagulant effect of ginger extract

The anticoagulant activity of ginger extract was evaluated by measuring PT and APTT for each individual five times. Aqueous extract of ginger was used at different concentrations (2.5%, 5%, and 10%) and the anticoagulant effect for each concentration was tested using different volumes (25, 50, 75 µL). The control samples were prepared by replacing the extract with an equal volume of distilled water to correct the dilution effects.

PT and APTT tests were performed using a semi-automated coagulometer (BioBas, BioSystem, Spain). For PT determination, 100 µL of plasma–extract mixture was incubated at 37°C, and 200 µL of pre-warmed thromboplastin reagent (BioSystem) was added to initiate clotting. The clotting time was recorded in seconds. For APTT determination, 100 µL of plasma–extract mixture was incubated with 100 µL of kaolin–cephalin reagent (BioSystem) for 3 minutes at 37°C. Subsequently, 100 µL of 0.025 M calcium chloride was added, and the time required for clot formation was recorded as the APTT value.

2.4. Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 29.0. The results were expressed as mean ± standard deviation (SD). Paired t-test was used to compare results of control and treated samples. A p-value ≤ 0.05 was considered statistically significant.

2.5. Ethical consideration

The study protocol was reviewed and approved by the Institutional Ethical Review Board of Alzaeim Alazhari University. Informed consent was obtained from all participants prior to blood collection. All procedures were performed according to the principles of the Declaration of Helsinki (2013) to ensure the safety, rights, and confidentiality of the participants

3. Results

This study examined the effects of aqueous ginger rhizome extract at different concentrations (2.5%, 5%, and 10%) and volumes (25, 50, and 75 µl) on prothrombin time (PT) and activated partial thromboplastin time (APTT) in plasma samples from 20 healthy individuals.

Table 3.1 shows the PT for different volumes (25µl l, 50 µl, and 75 µl) of 2.5%, 5%, and 10% concentrations of ginger extract.

For the 2.5% concentration, the prothrombin times were (13.1 ± 0.2, 13.7 ± 0.4, and 14.4 ± 0.5 seconds) for 25µl l, 50 µl, and 75 µl, respectively. For the 5% concentration, the prothrombin times were (14.2 ± 0.3, 16.8 ± 0.7, and 18.3 ± 0.5seconds) for the same volumes. At a 10% concentration, the prothrombin times were 17.1 ± 0.8, 19.5 ± 0.7 and 22.3 ± 1.1 seconds) for the same volumes. The PT increased significantly at the highest extract concentration (10%, 75 µl) with P value 0.000.

Table 3.2 displays the APTT for different volumes (25 µl, 50 µl, and 75 µl) of 2.5%, 5%, and 10% concentrations of ginger extract.

For the 2.5% concentration, the APTT values were 33.4 ± 1.3, 34.2 ± 1.1 and 35.0 ± 0.8 seconds) for 25 µl, 50 µl, and 75 µl, respectively. For the 5% concentration, the APTT values were 36.1 ± 1.1 seconds, 38.1 ± 0.9, and 40.8 ± 1.4 seconds) for 25 µl, 50 µl , and 75 µl , respectively. At a 10% concentration, the APTT values were 37.6 ± 1.2, 40.2 ± 1.5 and 44.8 ± 1.7 seconds) for the same volumes. On the other hand the APTT values increased from control (30.2 ± 1.1 s) to 44.8 ± 1.7 s at the highest concentration (10%, 75 µl) with P value 0.000. Both parameters showed a dose- and volume-dependent effect with statistically significant differences compared with control ($p < 0.05$).

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Table 3.1. The mean, SD, and P. value of PT test among different volumes and concentrations of aqueous extract of ginger Rhizomes compared with control

Volume of extract (2.5% concentration)	PT of normal plasma with aqueous extract (sec)	PT of Control (normal plasma with distilled water) (sec)	P value
25 µl	13.1 ± 0.2	12.3 ± 0.4	0.04
50 µl	13.7 ± 0.4	12.3 ± 0.4	0.04
75 µl	14.4 ± 0.5	12.3 ± 0.4	0.01
Volume of extract (5% concentration)			
25 µl	14.2 ± 0.3	12.3 ± 0.4	0.03
50 µl	16.8 ± 0.7	12.3 ± 0.4	0.00
75 µl	18.3 ± 0.5	12.3 ± 0.4	0.00
Volume of extract (10% concentration)			
25 µl	17.1 ± 0.8	12.3 ± 0.4	0.00
50 µl	19.5 ± 0.7	12.3 ± 0.4	0.00
75 µl	22.3 ± 1.1	12.3 ± 0.4	0.00

Table 3.2. The mean, SD, and P. value of APTT test among different volumes and concentrations of aqueous extract of ginger Rhizomes compared with control

Volume of extract (2.5% concentration)	APTT of normal plasma with aqueous extract (sec)	APTT of Control (normal plasma with distilled water) (sec)	P value
25 µl	33.4 ± 1.3	30.2 ± 1.1	0.01
50 µl	34.2 ± 1.1	30.2 ± 1.1	0.00
75 µl	35.0 ± 0.8	30.2 ± 1.1	0.00
Volume of extract (5% concentration)			
25 µl	36.1 ± 1.1	30.2 ± 1.1	0.00
50 µl	38.1 ± 0.9	30.2 ± 1.1	0.00
75 µl	40.8 ± 1.4	30.2 ± 1.1	0.00
Volume of extract (10% concentration)			
25 µl	37.6 ± 1.2	30.2 ± 1.1	0.00
50 µl	40.2 ± 1.5	30.2 ± 1.1	0.00
75 µl	44.8 ± 1.7	30.2 ± 1.1	0.00

4. Discussion

Ginger (*Zingiber officinale*) is a well-established medicinal herb with diverse therapeutic properties, including anticoagulant activity. Several studies have evaluated its effects on coagulation parameters, particularly prothrombin time (PT) and activated partial thromboplastin time (APTT), which are crucial indicators of hemostatic function. The findings of the present study demonstrate that aqueous ginger extract significantly prolongs both PT and APTT in a dose- and volume-dependent manner, providing further support for its anticoagulant potential.

The current results are consistent with those reported by Taj Eldin IM et al. [15] and Jude et al. [16], who also observed significant prolongation of PT with increasing concentrations of ginger extract. In this study, PT was markedly prolonged at higher concentrations (5% and 10%) with highly significant values ($p = 0.00$), comparable to their findings in which higher concentrations resulted in stronger anticoagulant effects. Similarly, Özdemir, E.Ş et al. [16] demonstrated a clear dose–dependent effect, reporting significant increases in PT ($p = 0.001$), which parallels the results obtained in this study.

Albara et al. [17] confirmed a significant prolongation of PT with ginger extract ($p = 0.001$) but did not find significant changes in APTT ($p = 0.139$). In contrast, findings of this study revealed significant prolongation of both PT and APTT, particularly at higher concentrations, suggesting that aqueous ginger extract may exert broader anticoagulant effects than the ethanolic extract used in their study. These differences may be attributed to variations in extraction methods, participant demographics, or other experimental factors. Furthermore, results of the current study are in agreement with Ahmed, S. H. H et al. [18], who reported significant increases in both PT and APTT following treatment with ginger extract. This concordance reinforces the hypothesis that ginger possesses multiple anticoagulant mechanisms, supporting its potential use as a natural alternative or adjunct to synthetic anticoagulant agents.

Taken together, the findings of this study strongly support the anticoagulant properties of ginger, demonstrating significant and dose-dependent prolongation of both PT and APTT. These results, in line with previous investigations, highlight the potential of ginger extract as a promising natural agent in the management or prevention of thrombotic conditions. However, discrepancies between studies, particularly in relation to APTT findings as noted by Albara et al. [17], emphasize the need for standardized extraction protocols and further research to elucidate the precise mechanisms underlying ginger's anticoagulant effects.

5. Conclusion

The current study confirms that aqueous ginger rhizome extract exerts a significant anticoagulant effect by prolonging both PT and APTT, with more pronounced activity observed at higher concentrations. These results provide additional evidence that ginger may be considered as a natural anticoagulant candidate, although its clinical application requires further validation. Future investigations should expand the scope of coagulation testing by including thrombin time (TT) and fibrinolytic assays, as well as assessing the effects of a broader range of concentrations. Moreover, advanced extraction methods, such as percolation, should be explored to optimize the yield of bioactive compounds. Comprehensive clinical trials are also warranted to establish both the efficacy and the safety profile of ginger extract in therapeutic contexts.

6. Future Suggestions

- Other tests should be included to confirm the coagulation activity like thrombin time (TT)

- More ginger extract concentrations should be included in further studies.
- Other studies should be conducted to explain the exact mechanism of ginger effect on coagulation.
- Other studies should be done to investigate the ginger effects on fibrinolysis activity.
- Other methods of ginger extraction like percolation method should be used for further studies to choose optimum methods for ginger extraction.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis, and manuscript writing equally.

Availability of data and materials

Supplementary information is available from the authors upon reasonable request.

Ethical Approval

The study protocol was reviewed and approved by the Institutional Ethical Review Board of Alzaeim Alazhari University.

Informed consent

Informed consent was obtained from all participants prior to blood collection. All procedures were performed according to the principles of the Declaration of Helsinki (2013) to ensure the safety, rights, and confidentiality of the participants

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