

An *in vitro* Anticoagulant Effect of Aqueous Extract of Ginger (*Zingiber officinale*) Rhizomes in Blood Samples of Normal Individuals

Taj Eldin IM^{1*}, MA Elmutalib², Hiba A.³, Hiba F.³, and Thowiba S.³, Elnazeer I. Hamedelnie⁴

¹Department of Pharmacology, Faculty of Pharmacy, University of Gezira, Sudan

²Islamic Medical Association laboratory, Wad Medani, Sudan

³Pharmacy graduates, batch 30, Faculty of Pharmacy, University of Gezira, Sudan

⁴Department of Pharmaceutics, Faculty of Pharmacy, Omdurman Islamic University, Sudan

Corresponding E-mail: omdataj64@gmail.com, nizophar@hotmail.com

Abstract

Background: Haemostasis is the process that arrests bleeding from an injured blood vessel required the combined activity of vascular, platelets and plasma factors. Ginger is widely used around the world throughout history as both medicine and spice.

Objective: To investigate the anticoagulant effects of ginger *in vitro*, using blood samples of normal individuals.

Methods: the anticoagulant effect of ginger aqueous extract (5%) in different volumes (25, 50, 75 and 100 μ L) was examined *in vitro* in blood samples of normal individuals through measuring of prothrombin time (PT).

Results: The aqueous extract of ginger inhibited coagulation process and significantly prolonged prothrombin time in a dose-dependent manner.

Conclusion: ginger aqueous extract in different concentrations inhibited clot formation and increased prothrombin time. Ginger can be used as a supplementary anticoagulant agent to improve and/or prevent cardiovascular disorders.

Key words: Haemostasis; Anticoagulant; Ginger; *Zingiber officinale*; Prothrombin time; Medicinal plants.

{**Citation:** Taj Eldin IM, MA Elmutalib, Hiba A., Hiba F., Thowiba S., Elnazeer I. Hamedelniei. An *in vitro* anticoagulant effect of aqueous extract of ginger (*Zingiber officinale*) rhizomes in blood samples of normal individuals. American Journal of Research Communication, 2016, 4(1): 113-121} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Haemostasis is the process that arrests bleeding from an injured blood vessel, required the combined activity of vascular, platelets and plasma factors. During hemostasis three steps occur in a rapid sequence. Vascular spasm is the first response as the blood vessels constrict to allow less blood to be lost. In the second step, platelet plugs formation, platelets stick together to form a temporary seal to cover the break in the vessel wall. The third and last step is called coagulation or blood clotting. Coagulation reinforces the platelet plug with fibrin threads that act as a “molecular glue”.^[1] Platelets are a large factor in the hemostatic process. They allow for the creation of the “platelet plug” that forms almost directly after a blood vessel has been ruptured. Within seconds of a blood vessel’s epithelial wall being disrupted platelets begin to adhere to the sub-endothelium surface. It takes approximately sixty seconds until the first fibrin strands begin to intersperse among the wound. After several minutes the platelet plug is completely formed by fibrin.^[2]

Prothrombin time gives an indication of the concentration of prothrombin in the blood. The time required for coagulation to take place is known as the prothrombin time. The shortness of the time is determined mainly by prothrombin concentration. The normal prothrombin time is about 11-15 seconds^[3].

Ginger is widely used around the world in foods as spice. Native to tropical Asia, ginger is a perennial cultivated in the tropical climates of Australia, Brazil, China, India, Jamaica, West Africa, and parts of the United States ^[4]. Ginger rhizome has a long history of use in Chinese and Ayurvedic medicine as an antiemetic, antipyretic, and anti-inflammatory agent ^[5].

The therapeutically useful portions of ginger are the rhizome (root). Active constituents are called gingerols found in ginger's oleo-resin. These compounds have the following properties: antipyretic, analgesic, antitussive, cardiac inotropic and sedative. Dehydration products of gingerols are called shogaol homologues. One of these products, 6-shogaol and galanolactone are thought to act on serotonin (5-HT) receptors. Active constituents for topical applications are in ginger's volatile oils. Major constituents are beta-bisabolene and zingiberene. Other compounds in the oils include zingiberol, zingiberenol, ar-curcumene, beta-sesquiphellandrene, beta-sesquiphellandrol (cis and trans), and numerous monoterpene hydrocarbons, alcohols and aldehydes ^[6; 7].

The primary pungent agents (phenylalkylketones or vanillyl ketones) of ginger are gingerol, with other gingerol analogues such as the shogaols, paradol and zingerone also found in high levels in rhizome extracts. The major pharmacological activity of ginger appears to be due to gingerol and shogaol. Phenylalkylketones or vanillyl ketones of ginger include 6-gingerol 8-gingerol and 10-gingerol, 6-shogaol, 8-shogaol, 10-shogaol and zingerone. 6-paradol, 6- and 10-hydrogingerdione and 6- and 10-gingerdione have also been identified ^[8].

The present study was carried out to examine the possible anticoagulant effect of ginger in blood samples of normal individuals.

Materials and Methodology

Preparation of *Zingiber officinale* extract

Dried Ginger (*Zingiber officinale*) rhizomes were purchased from the local vegetable market in Wad Medani City; Central Sudan. The dry rhizomes ground into a fine powder and five grams of the powder were weighed using sensitive balance and then suspended in 100 ml of distilled water in a conical flask with continues shaking for twenty four hours. The supernatant of *Zingiber officinale* extract filtrated using filter paper size 42 mm. The final aqueous extract (5%) of *Zingiber officinale* was used for an *in vitro* testing of its possible anticoagulant activity in blood samples of normal individuals using the principles of prothrombin time test^[9].

Study population

Blood samples obtained from thirty normal individual volunteers attending to the Pharmacology Laboratory in Faculty of Pharmacy, University of Gezira and Islamic Medical Association laboratory, Wad Medani City, Central Sudan. Participant of both sexes, females (n=15) and males (n=15) were used to assess the anticoagulant effects of aqueous extract of *Zingiber officinale*^[9]. The age of the participants ranged between 15-30 years old. Consent forms were signed by participants, being interested in joining the study completely voluntary.

Criteria for choosing the participants for the study

Participants were selected according to the following criteria: having normal prothrombin time (11-15 seconds), not suffering from any cardiovascular diseases (hypertension, congestive heart failure, coagulation disorders such as, Hemophilia A or B) or diabetes, not recently using nonsteroidal anti-inflammatory drugs (NSAIDs), not obese or smokers and free from dyslipidemic disorders^[9].

Collection of blood samples

Venous blood samples were obtained from the right arm using sterile syringes, and placed separately in containers containing trisodium citrate to prevent the clotting process. Centrifugation was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma for PT test. Each plasma sample was separately poured in plane containers using automatic pipette and stored at room temperature ^[10].

In vitro anticoagulant test of *Zingiber officinale* extract

For determination of the prothrombin time, the plasma sample of each individual was divided into five groups each of 50 μ L. Group 1 (n=30) was tested first to determine the normal PT (positive control group) using the stable, liquid, combined calcium/thromboplastin rabbit brain (DiaMed LTD, UK) as a gold standard. Four volumes of ginger aqueous extract (25, 50, 75 and 100 μ L) were added separately to the remaining four groups of plasma samples in a water bath with gentle shaking. Then thromboplastin reagent (100 μ L) was added separately to the mixture of each plasma sample using pipetador volume adjustment. Stop watch was used for measuring the time of the clot formation, the PT ^[11; 12]. Thromboplastin reagent was added to the plasma in order to counteract the sodium citrate and allow clotting to proceed ^[13].

Statistical Methods

All the data were expressed as means \pm standard error of means (SEM) and analyzed by analysis of variance (ANOVA). Comparisons with the control group were made using two-way ANOVA. Differences were considered significant if $P < 0.05$.

Results and Discussion

In this study the effects of the aqueous extract of ginger (*Zingiber officinale*) as an anticoagulant agent had been investigated, using the principles of prothrombin time test in thirty normal individuals. The prothrombin time for all of them was found to be within the normal range (14.23 ± 1.073 seconds). When aqueous extract of *Zingiber officinale* (5%) was added in different volumes (25, 50, 75, and 100 μL) to plasma samples of normal individuals, it significantly ($P = 0.001$) showed prolongation in the prothrombin time in a dose dependent manner as 15.8 ± 0.12 , 18.5 ± 0.2 , 20.20 ± 0.22 and 21 ± 0.27 seconds respectively (Table 1; Figure 1).

Table 1: Prolongation of prothrombin time using different volumes of aqueous extract of ginger (5%)

Volume of ginger (μml)	25 μml	50 μml	75 μml	100 μml
Prothrombin time (seconds)	15.80 ± 0.12	18.53 ± 0.2	20.20 ± 0.22	21.00 ± 0.27

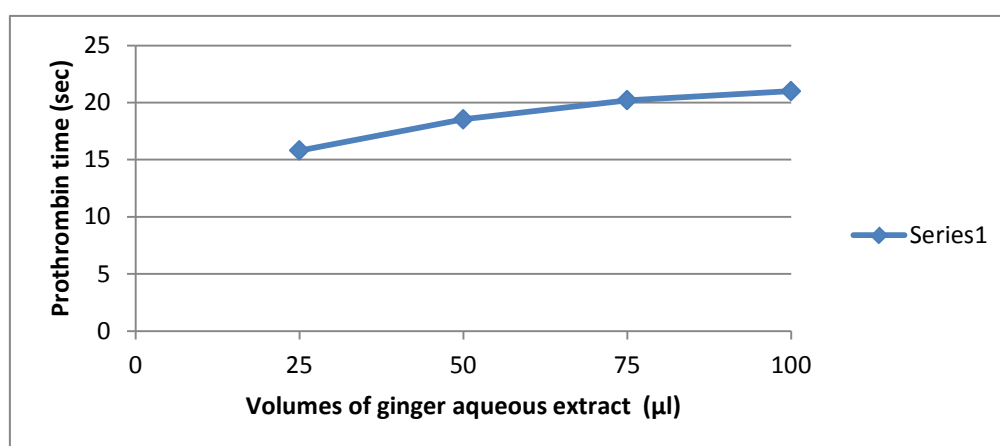


Figure 1: Prolongation of prothrombin time using different volumes of aqueous extract of ginger (5%).

It was evident that there were proportional correlations between the different concentrations of *Zingiber officinale* aqueous extract required to inhibit clot formation and prolongation of prothrombin time. Accordingly, increasing concentrations of ginger aqueous extract significantly ($P = 0.001$) inhibited the blood coagulation process and increased the prothrombin time. These findings demonstrated that, the aqueous extract of *Zingiber officinale* possesses anticoagulant properties through prevention of coagulation process and clot formation.

The obtained results may indicate that, the use ginger may intensify the properties of prescribed anticoagulants. Thus the concomitant use of excessive amounts of ginger and anticoagulants and/or antiplatelet drugs could increase the risk of bleeding, due to an interaction between ginger and oral anticoagulant ^[6; 14-16].

It is therefore essential to investigate the physiological role of active constituents of ginger and their potential effects on blood coagulation.

References

1. Marieb, Elaine Nicpon; Hoehn, Katja (2010). *Human Anatomy & Physiology* (8th ed.). San Francisco: Benjamin Cummings. pp. 649–50.
2. Clemetson, Kenneth J. "Platelets and Primary Haemostasis." *Thrombosis Research* 129.3 (2012): 220-224.
3. Guyton and Hall. Hemostasis and Blood Coagulation. *Text book of Medical Physiology*. 11th ed., Jaypee Brothers Medical Publishers (2006); 36: 457-468.

4. Langner E, Greifenberg S and Gruenwald J. (1998), Ginger: history and use. *Advance Thera.*; 15;25-44.
5. Leung AY. Chinese Herbal Remedies, Universe Books, New York, 124.
6. Jellin J, Gregor PJ, Batz F, Hitchens K *et al.* (2002) Pharmacist's letter/prescriber's letter. *Natural Medicines Comprehensive Database*. 4th ed. Stockton, CA: Therapeutic Research Faculty;; p.584-586.
7. Newall CA, Anderson LA, Philpson JD. (1996) Herbal Medicine: A Guide for Health Care professionals. London, UK: *The Pharmaceutical Press*; p. 135-137.
8. Chrubasik J, Roufogalis B and Chrubasik D. (2007) Evidence of effectiveness of herbal anti-inflammatory drugs in the treatment of painful osteoarthritis and chronic low back pain. *Phytother Res.*; 21:675-683.
9. Taj Eldin IM, Abdalmutalab MM, Izzaldeen HM, Abdalkareem MA, Abdalraheen MA, Alhassan MB. Evidence for an in vitro Anticoagulant Activity of Red Onion (*Allium cepa* L.). *Sudan Journal of Medical Sciences (JMS)* (2011); Vol.6. No.2: 85 - 88.
10. Sun WY, Ruiz-Saez A, Burkart MC, Bosch N, Degen SJ. Prothrombin carora: hypoprothrombinaemia caused by substitution of Tyr-44 by Cys. *Br J Haematol.* Jun 1999; 105(3):670 - 2.
11. Narayanan S. Multifunctional roles of thrombin. *Ann Clin Lab Sci.* Oct-Dec 1999; 29 (4): 275-80.
12. Manicam C, Abdullah JO, Rahayu E, Tohit M, Seman Z, Seieo CC, Hamid M. In vitro anticoagulant activities of *Melastoma malabathricum* Linn. Aqueous leaf extract: A preliminary novel finding. *Journal of Medicinal Plants Research*; 08/2010; 4:1464-1472.

13. Chernecky, Cynthia C. and Barbara Berger. Lab Tests and Diagnostic Processes. 3rd ed. Philadelphia: W. B. Saunders Company, 2001.
14. Newall CA, Anderson LA, Philpson JD. (1996) Herbal Medicine: A Guide for Health Care professionals. London, UK: *The Pharmaceutical Press*; p. 135-137.
15. Fetrow CW, PharmD; Avila JR, PharmD, (1999). *Professionals' Handbook of Complementary and Alternative Medicines*. Pennsylvania: Springhouse Co.; p. 275-277.
16. Vaes LPJ, Chyka PA, (2000), Interactions of warfarin with garlic, ginger, ginkgo, or ginseng: nature of evidence. *Ann of Pharmacother*. 34: 1478-82.