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ORIGINAL ARTICLE

Comparative Study of the Anticoagulant Activity of Zingiber Officinale and Curcuma longa Rhizomes Extracts in Blood Samples of Normal Individuals

ALBARA AHMAD[1], SAFAA JAFAR MOHAMMED[2], IKRAM MOHAMED ELTAYEB[3], YOUSIF MOHAMMED ELMOSAAD[4], HISHAM ALI WAGGIALLAH[5]

[1]*Department of Hematology, Medical Laboratory Program, Alfajr College for Sciences and Technology, Sudan*
[2]*Department of Hematology, Faculty of Medical Laboratory, Elrazi University, Sudan*
[3]*Department of Pharmacognosy, Pharmacy Program, Alfajr College for Sciences and Technology, Sudan.*
[4]*Department of Public Health, College of Applied Medical Sciences, King Faisal University, Saudi Arabia.*
[5]*Department of Medical Laboratory Sciences, College of Applied Medical Science, Prince Sattam Bin Abdulaziz Universiy, Alkharj, Saudi Arabia.*
Corresponding author: Hisham Ali Waggiallah, Email: hishamwagg30@hotmail.com

ABSTRACT

Objective: The current study compares the anticoagulant activity of curcumin and ginger rhizome extracts in vitro.
Background: Curcuma longa and Zingiber officinale (Ginger) (turmeric, curcuma, or curcumin) Rhizomes are widely used as a spice and in herbal medicine around the world.
Methods: To obtain the entire crude extract, Zingiber officinale and Curcuma longa rhizomes were pulverized and extracted in 70% ethanol. The phytochemical contents of the extracts were determined. Their anticoagulant impact was tested in vitro on blood samples from healthy Sudanese people by assessing prothrombin time (PT) and activated partial thromboplastin time (APTT).
Results: The ginger extract inhibited coagulation and significantly prolonged prothrombin time (PT) in a dose-dependent manner (25,50, 75 µl) with P. value 0.001, whereas APTT showed insignificant prolongation (P. value 0.139). PT and APTT of curcumin showed insignificant prolongation among (25, 50, 75µl) with P. value 0.55 and 0.146, respectively. **Conclusion:** The in vitro anticoagulant effect demonstrated that ginger and curcumin extracts had equal anticoagulant activity, but curcumin has a higher anticoagulant impact with considerable PT prolongation.
Keywords: Zingiber officinale, Curcuma longa, Coagulation, PT, APTT.

INTRODUCTION

The generation of thrombin, which is essential for the conversion of fibrinogen to fibrin, is the key to the blood clotting pathway. Thrombin is present in the cell in an inactive form known as prothrombin and is triggered by the coagulation cascade through the development of a complex known as the prothrombin activator complex. The intrinsic prothrombin activation pathway and the extrinsic prothrombin activation pathway both contribute to the synthesis of the prothrombin activator complex. Though the end goal of both paths is to produce the prothrombin activator complex, other methods are utilized, each producing a distinct form of the prothrombin activator. Prothrombin activator complex (FXa), tissue factor, activated factor VII, and the cofactor activated factor V comprise the extrinsic route[1]. This complex, particularly FXa, together with the cofactor FVa, transforms prothrombin to active thrombin. To stabilize clots, fibrin develops a network within the platelet aggregate. In the intrinsic pathway, the prothrombin activator complex is made up of FXa, FVa, activated factor VIII, and phopspholipid. The clotting time assay assesses the lag time of thrombin production, whereas the APTT is a performance indicator that assesses the efficacy of both the contact activation pathway and the common coagulation pathways. Furthermore, the prothrombin time (PT) is a measure of the extrinsic coagulation pathway. [1, 2]

Zingiber officinale and Curcuma longa are herbaceous perennials with rhizomes, fibrous roots, and aerial shots in the Zingiberaceae family. Their rhizomes have long been utilized as spices and medicinal herbs over the world.[3] Ginger's pungent odor is primarily attributable to its volatile oil. [4] Over 50 oil components have been identified, and the pungency is mostly attributed to gingerol, an oily liquid composed of homologous phenols generated in the plant from phenylalanine, malonate, and hexonate. [5]

Ar-turmerone and ar-curcumene are key ingredients of Curcuma longa essential oil. A- and b-pinene, sabinene, myrcene, a-terpinene, limonene, p-cymene, perillyl alcohol, turmerone, eugenol, iso-eugenol, eugenol methyl ether, and iso-eugenol methyl ether are among the other chemicals. Curcumin and similar chemicals have also been identified as important rhizome ingredients. A range of sesquiterpenes, including germacrane, bisabolane, and guainane skeletons, have recently been discovered from C. longa. [6] Curcumin contains alkaloids, saponin, anthocyanin, emodins, phytosterol, phlobatannin, phenol, anthroquinone, cardiac glycoside, and carbohydrates, and has been shown to be more effective. [7] Alkaloids, tannins, saponins, polysaccharides, sterols, reducing sugars, compound reducing sugars, terpenoids, and flavonoids were discovered in ginger. [8]

Ginger rhizome has been used in traditional medicine to cure many symptoms like fever and muscle pain. [9] C. longa rhizome is used to cure numerous ailments, including hypersensitivity and urinary tract infection, and its essential oil is used to treat carminative, stomachic, and tonic properties. [10] Sprains and swellings induced by damage for the therapy of liver diseases, rheumatoid arthritis, also it is used as an anti-inflammatry agent [11] and to treat flatulence, jaundice, and menstruation problems.[12] Turmeric's cardiovascular-protective actions comprise decreasing cholesterol and triglyceride levels, reducing the vulnerability of LDL to lipid peroxidation [13] and suppressing platelet aggregation and thromboxanesynthesis. [14] The effect of turmeric extract on cholesterol levels. [15] It has high antioxidant activity [16], and it reduces ischemia-induced alterations in the heart. [17] The goal of this reserch is to evaluate the anticoagulant activity of Zingiber officinale and Curcuma longa Rhizomes in normal blood samples in vitro.

MATERIALS AND METHODS

Study design: This experimental crossover investigation was carried out at the Al-Gofran Medical Complex in Khartoum, Sudan. This study's group consisted of healthy individuals who attended Al-Qofran Complex between the years of (Jan 2021to Apr 2021).
Inclusion Criteria: Sixty individuals in good health who show no signs of bleeding or thrombotic disease were involved in the study.
Exclusion Criteria: Persons with a history of bleeding, coagulation problems (hereditary or acquired), thrombotic diseases, pregnancy, and who have not recently used antithrombotic or thromboembolic medicines such as warfarin and aspirin were involved in the study.
Sampling: Trisodium citrate, an anticoagulant, was used to collect venous blood samples. To get platelet-poor plasma, a 15-minute centrifugation at 3000 rpm was performed.

Preparation of Platelet Poor Plasma (P.P.P): PPP was made by centrifuging it at 2000 g for 15 minutes (about 4000 rev/min on a standard bench). The sample was then counted by chamber and stored as a batch at -30 ºC for many weeks before being blended and centrifuged before testing.

Plant Material Collection and Preparation: The rhizomes of Zingiber officinale and Curcuma longa were acquired from the regional marketplace. For extraction, they were broken into smaller pieces, shade-dried, and ground into a fine powder. The plant materials were validated at the Medicinal and Aromatic Plants Research Institute (MAPRI), and a voucher specimen was placed in the herbarium.

Phytochemical Screening

A. Preparation of Extract: The extract was made by boiling the powdered plant material with 70% ethanol; the resulting macerate was then purified and used for phytochemical screening.

B. Detection of phytochemical constituents: The phytochemical constituents (secondary metabolites) of the extracts were determined using standard methods outlined in [18, 19].

In vitro Anticoagulant Test: The extracts' in vitro anticoagulant activity was determined by measuring prothrombin time and activated partial thromboplastin time; each individual's plasma sample was separated into five groups. Group 1 consists of the standard PT and APTT. Three volumes of curcumin and ginger extracts (25, 50, and 75µl) and distilled water (control) were separately added to the remaining four groups of plasma samples and the anticoagulant effect was assessed as reported by Taj et al [2].

Data analysis: The data was examined using the social science statistics software (SPSS). P value ≤0.05 was considered significant, and all data were reported as mean ± SD. Independent T test and regression test were used for data analysis.

Ethical consideration: Permission to conduct this research was granted by the research board of the faculty of medical laboratory science at Sudan's National University. Before collecting the specimen, the patient was told about the goal of the study, and signed agreement was obtained from each participant. During the sampling and data gathering processes, all ethical duties were followed.

RESULTS

Both Zingiber officinale and Curcuma longa included alkaloids, flavonoids, tannins, terpenoids, sterols, saponins, carbohydrates, reducing sugars, and compound reducing sugars, according to early phytochemical screening. The addition of curcumin extract (5%) in different volumes (25, 50, and 75µml) to the plasma samples of normal persons (Sudanese) resulted in curcumin and ginger have a highly significant effect on blood fluidity by increasing the added concentrations when independent T test has been used as (p=0.000) in both PT and APTT. Eta squared (²η) value between 0.50 and 1.0 (Table 2 & 3). When regression test has been used for correlation it was found that curcumin concentration of 50 µml is the most appropriate concentration when it causes an increase in blood fluidity when measured by PT, while ginger does not have a significant correlation with increasing the dose (Table 3). By adding the extract (5%) to the plasma samples of normal adults in varied volumes of 25, 50, and 75 µml, and regression test was conducted, it has been found that both curcumin and ginger have a significant effect by increasing the dose, especially at concentrations 25 µml and µml 50 for PT (p=0.003and 0.009) and for APTT (p=0.003 and 0.000) respectively as shown table 4

Table 1: Independent T test for PT of healthy individuals treated with different concentrations (25-75 µml) of curcumin and ginger 5%extracts.

Variable	concentrations	Mean ±SD	df	T-value	Sig	η²
Control		13.07±0.77				
Curcumin	25 µml	16.92±1.07	58	15.9	0.000**	0.81
	50 µml	19.49±1.48	58	21.04	0.000**	0.88
	75 µml	23.68±2.17	58	25.20	0.000**	0.92
Ginger	25 µml	17.35±1.25	58	15.9	0.000**	0.81
	50 µml	20.39±1.60	58	22.52	0.000**	0.91
	75 µml	24.75±2.40	58	25.32	0.000**	0.92

**P≤0.001is highly significant correlation

Table 2: Independent T test for APPT of healthy individuals treated with different concentrations (25-75 µml) of curcumin and ginger 5% extracts.

Variables	concentrations	Mean ±SD	df	T-value	Sig	η²
Control		30.69±2.38				
Curcumin	25 µml	35.52±2.04	58	8.43	0.000**	0.55
	50 µml	38.38±2.07	58	13.43	0.000**	0.76
	75 µml	42.34±2.42	58	18.78	0.000**	0.86
Ginger	25 µml	36.77±1.64	58	11.51	0.000**	0.70
	50 µml	40.30±2.48	58	15.30	0.000**	0.80
	75 µml	44.22±1.70	58	25.33	0.000**	0.92

**P≤0.001 highly significant correlation

Table 3: Regression analysis for the deviation of values of PT with curcumin and ginger extracts (5%).

Variables	Concentration	B	Beta	R	R^2	F	T-value	Sign.
Curcumin	C25	0.46	0.33	0.34	0.11	3.51	1.87	0.72
	C50	0.75	0.39	0.40	0.16	5.22	2.29	0.030*
	C75	0.81	0.30	0.29	0.09	2.59	1.61	0.119
Ginger	G25	-0.07	-0.05	0.02	0.34	0.06	0.24	0.809
	G50	0.46	0.23	0.22	0.05	1.45	1.20	0.238
	C75	0.64	0.20	0.21	0.04	1.27	1.13	0.270

*P≤0.05 significant correlation

Table 4: Regression analysis for the deviation of values of APTT with curcumin and ginger 5% extracts.

Variables	Concentrations	B	Beta	R	R^2	F	T-value	Sign.
Curcumin	C25	0.45	0.52	0.52	0.27	10.39	3.22	0.003*
	C50	0.41	0.47	0.47	0.22	7.80	2.79	0.009*
	C75	0.32	0.31	0.31	0.10	3.03	1.74	0.093
Ginger	G25	0.36	0.52	0.52	0.27	10.42	3.23	0.003*
	G50	0.64	0.61	0.61	0.37	16.93	4.12	0.000**
	C75	0.012	0.02	0.02	0.003	0.01	0.093	0.927

*P≤0.05 significant correlation, **P≤0.001 significant correlation

DISCUSSION

Coagulation and anticoagulation are remain medical mysteries with several application and use issues. Herbal medicine has gained popularity as a result of its low cost and wide availability. This study looked at the anticoagulant properties of curcumin and ginger in Sudanese people. In sixty healthy people, the anticoagulant impact was studied utilizing prothrombin time (PT) and activated partial thromboplastin time (APTT) assays. There were clear proportional relationships between the different amounts of Zingiber officinale and Curcuma longa extracts needed to decrease prothrombin time prolongation, activated partial thromboplastin time, and clot formation.

In this research, we discovered that increasing the added amounts of curcumin and ginger has a noticeable and extremely substantial influence on blood fluidity in the two coagulation pathways. These findings corroborate and are consistent with the prior study conducted by Dong-Chan et al., 2012. [1]

According to the findings, extracts of Zingiber officinale and Curcuma longa rhizomes inhibited prothrombin time and activated partial thromboplastin time in blood samples, indicating an anticoagulant effect of ginger and curcumin rhizomes, with Zingiber officinale having a stronger effect than Curcuma longa rhizomes. These effects are readily explained by the presence of several secondary metabolites (the above phytochemical screening results) that are well known for their anticoagulant properties, such as alkaloids [20-22], flavonoids [23-24], tannins [25], terpenoids [26], and sterols [27]. Furthermore, plants in the Zingiberaceae family have been shown to be high in essential oils such as monoterpenes, sesquiterpenes, and a few phenols [28], which are well-known for their potent anticoagulant properties. [29]

In this study, it was revealed that It has been discovered that a curcumin concentration of 50 µml is the most acceptable concentration for causing an increase in blood fluidity when measured by PT, although ginger has no significant association with increasing the dose. Also, when the APPT test was performed, it was revealed that increasing the amount of curcumin and ginger had a substantial effect, especially at concentrations of 25 µml and 50 µml which is inconsistent with the findings of Faeze Keihanian et al 2017 who concluded that curcumin has an effect on coagulation by prolonging PT and APTT. [30]

Another investigation on the anticoagulant effects of curcumin and its derivatives was conducted by monitoring activated partial thromboplastin time (aPTT) and prothrombin time (PT) data, which revealed that curcumin and BDMC considerably prolonged aPTT and PT and reduced thrombin and FXa activities. [1] In another study, the in vitro anticoagulant action of aqueous extract of ginger (Zingiber officinale) Rhizomes in blood samples of healthy people revealed that the aqueous extract of ginger inhibited clotting and considerably delayed prothrombin time according to the concentration. [2]

Furthermore, our findings agree with study by Dong-Chan et al., who conducted a study titled anticoagulant effects of curcumin and its derivatives. Curcumin and its derivatives' anticoagulant characteristics were assessed by measuring APTT, PT, and cell-based thrombin and activated factor X production activities. Curcumin and Bisdemethoxycurcumin (BDMC) were found to considerably lengthen APTT and PT while inhibiting thrombin and FXa activities. They prevented the formation of thrombin or FXa. Curcumin and BDMC demonstrated anticoagulant activity in vivo in agreement with these anticoagulant properties. Interestingly, curcumin's anticoagulant effects were superior to those of BDMC, demonstrating that the methoxy group in curcumin positively influenced curcumin's anticoagulant action. As a result of these findings, curcumin and BDMC have antithrombotic properties, and frequent ingestion of the curry spice turmeric may help maintain anticoagulant state. [1]

Taj Eldin et al. conducted a study titled an in vitro anticoagulant action of aqueous extract of ginger (Zingiber officinale) Rhizomes in normal blood samples, which is consistent with the current study. The anticoagulant properties of ginger were studied in vitro using blood samples from healthy people. The anticoagulant effect of ginger aqueous extract (5%) in different volumes (25, 50, 75, and 100 µml) was tested in vitro in blood samples from healthy people using prothrombin time (PT). According to the findings, ginger aqueous extract inhibited coagulation and considerably delayed PT according to concentration. [2]

CONCLUSION

The in vitro anticoagulant test demonstrated that ginger and curcumin extracts had equal powerful anticoagulant activity, while curcumin was found to have a greater anticoagulant impact with considerable PT prolongation in 50µml concentration.

Significance Statement: We discovered that curcumin and ginger has a very powerful effect on blood fluidity, and it can be used as a natural alternative to anti-thrombotic medicines, or at the very least as a prophylactic dose for patients with thrombosis

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