Original Article

An in vitro anticoagulant effect of Fenugreek (Trigonella foenum-graecum) in blood samples of normal Sudanese individuals

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Abstract

Haemostasis is the process of forming clots in the walls of damaged blood vessels to prevent abnormal bleeding and to maintain intravascular blood in a fluid state. Fenugreek is largely universal staple herb, popular throughout history and it has been consumed for treatment of different disorders. We aimed to study the possible anticoagulant effect of Fenugreek aqueous extract in vitro by using blood samples of normal individuals. In vitro anticoagulant effects of Fenugreek aqueous extract (5%) in different volumes (25, 50 and 75 µL) were examined in the blood samples of normal individuals by measuring prothrombin time (PT). The aqueous extract of Fenugreek was found to inhibit coagulation process in vitro and significantly prolonged prothrombin time in a dose-dependent manner. Fenugreek aqueous extract in different concentrations inhibits clot formation and increases prothrombin time. Subject to further studies on efficacy and safety, It can well be used, in the future, as a supplementary anticoagulant agent in cardiovascular diseases and to prevent hypercoagulable states.

Key words

Haemostasis; Anticoagulant; Fenugreek; Prothrombin time; Medicinal plants; Sudan

Introduction

Haemostasis is an interaction process between coagulation and anticoagulants comprises a complex interrelated systemic mechanism that retains the blood within the injured vascular system [1]. The normal haemostasis is affected by local factors in various organs and depends on interacted haemostatic response to vascular damage between the blood vessel wall, the circulating platelets, the coagulation factors, the coagulation inhibitors and the fibrinolytic agents to arrest blood loss from damaged blood vessels which is essential for life [2]. The relative importance of haemostatic cascade depends on the type of vessel (arterial, venous or capillary) that has been injured [3].

The prothrombin time test (PT test) is a useful screening procedure for the extrinsic coagulation mechanism including the common pathway. It detects deficiencies

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in factor II, V, VII, and X. The prothrombin time test is frequently used to follow oral anticoagulant therapy that inhibit factors II, VII, IX and X. Thromboplastin activates the extrinsic coagulation system in plasma in the presence of calcium ions. The subsequent clotting time is dependent on the concentration of factors II, V, VII and X. Thus prolongation indicates a deficiency in one or more of these factors. The new oral anticoagulants have advantages over old ones such warfarin which include no need for laboratory monitoring, less drug-drug interactions and less food-drug interactions [4,5]. The normal PT is 11-15 seconds and normal amounts of clotting factors VII and X while prolongation in prothrombin time is considered abnormal [6].

Clotting factor II, or prothrombin, is a vitamin K–dependent proenzyme that functions in the blood coagulation cascade. Factor II deficiency is a rare, inherited or acquired bleeding disorder. Several specific missense mutations of the prothrombin gene have been documented [7]. These single amino acid substitutions can cause hypoprothrombinemia and/or dysprothrombinemia [8 - 12].

Several drugs are used in haemostatic disorders including anticoagulants, fibrinolytics (thrombolytics) and antiplatelets. Warfarin and heparin are the most commonly used anticoagulant agents and haemorrhage is their principal side effect. Also non-steroidal anti-inflammatory drugs (NSAIDs) particularly Aspirin, have the potential of anti platelets activity [13]. Some medicinal plants such as Ginger, Garlic, Onion and Fenugreek inhibit human platelet aggregation in vitro and/or in vivo and might thus enhance the risk of bleeding [14 -16].

Fenugreek is available as dried, ripe seed and its extracts are used as an artificial flavour for maple syrup. The seeds contain from 0.1 to 0.9% diosgenin and several coumarin compounds have been noted in the seed as well as a number of alkaloids such as trigonelline, gentianin, and carpaine. The seeds also contain approximately 8% of foul-smelling oil. Fenugreek has been noted to reduce plasma cholesterol in animals when 50% of their diet contained fenugreek seeds this may be attributed to its high fiber content, although it may be due to the steroid saponins. A hypoglycemic effect of Fenugreek has been also reported.

Fenugreek has limited toxicity when dosed in moderation a maple syrup odor via urine and sweat is commonly observed but the unpleasant effects of Fenugreek have been rarely reported. It is generally regarded and listed as safe herb in the USA [17].

This study is a preliminary attempt to evaluate the in vitro anticoagulant effect of an aqueous extract of Fenugreek in the blood samples of normal individuals by measuring PT.

MATERIALS AND METHODS

Preparation of Fenugreek extract

Fresh, dried, ripe and recently cropped Fenugreek seeds (Trigonella foenum-graecum) were purchased from the local market in Wad Medani city, Central Sudan. Fifty grams of the seeds were grinded 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 continuous shaking for three hours. The supernatant of Fenugreek extract was filtrated using sucking pump. The final clear solution of Fenugreek aqueous extract was used for in vitro testing of anticoagulant activity in blood samples of normal individuals using the principles of PT test.

Study population

Blood samples were obtained from thirty normal volunteers who attended the Islamic Medical Association Laboratory in Wad Medani city, Central Sudan. Participants of both sexes were recruited to assess the in vitro anticoagulant effects of Fenugreek

aqueous extract. The participants had been chosen according to the following criteria: having normal PT, not suffering from any cardiovascular diseases (hypertension, congestive heart failure or coagulation disorders such as; Hemophilia A or B) or diabetes, not recently using NSAIDs and not obese, alcoholics or smokers and also free from dyslipidemic disorders.

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 [18].

In vitro anticoagulant tests of Fenugreek aqueous extract (5%)

For determination of the prothrombin time, the plasma sample of each individual was divided into four groups each of 50 µL. 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. Three volumes of Fenugreek aqueous extract (25, 50 and 75 µL) were added separately to the remaining three groups of plasma samples in a water bath with gentle shaking. Then thromboplastin reagent (100 µL) 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 [19,20]. Thromboplastin reagent was added to the plasma in order to counteract the sodium citrate and allow clotting to proceed [21].

Statistical Methods

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

RESULTS

This study was carried out to evaluate the effect of Fenugreek (Trigonella foenum graecum) as an anticoagulant in blood samples of normal individuals by using principles of prothrombin time test. Thirty normal human individuals with normal prothrombin time (14.6±0.7 seconds) were randomly selected to participate in this study. The participants were 14 (46.7%) males and 16 (53.3%) females. Their average age is 16 ±2SD year. Different volumes (25, 50, 75µL) of Fenugreek aqueous extract (5%) were tested in vitro using blood samples from normal individuals. The addition of the different volumes (25, 50, 75µL) of Fenugreek aqueous extract significantly ($P = 0.001$) showed prolongation in the prothrombin time, from14.6±0.7 to 18.4±1.14, 21.16±1.2 and 23.6±1.23 seconds respectively (Figure 1).

Proportional correlations were noticed between the concentrations of Fenugreek aqueous extract needed to inhibit clot formation and prolongation of PT. The present study elucidated that the aqueous extract of Fenugreek prolonged prothrombin time in a dose dependent manner when tested in vitro for anticoagulant activity.

Figure 1- The effect of different volumes (25, 50 and 75 µL) of aqueous extract of Fenugreek (5%) on prothrombin time of plasma samples of normal individuals.

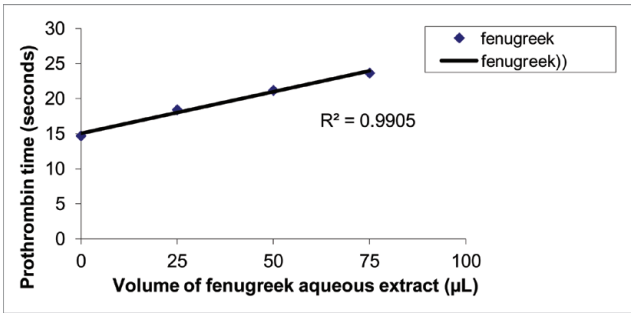

Table 1- The effect of Fenugreek aqueous extract on prothrombin time

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1089.825	03	363.275	1.189	0.001
Within Groups	137.967	116	1.189	305.435	
Total	1227.792	119			

DISCUSSION

The prevalence of atherosclerosis and coronary artery diseases has focused attention on the influence of diet on the cardiovascular system. Natural anticoagulant agents that influence platelet function and inhibit coagulation process are of potential interest for primary prevention of cardiovascular diseases.

Previous study showed that NSAIDs in small doses for an extended period of time inhibit platelet aggregation and thromboxane formation [20]. This study demonstrates that Fenugreek aqueous extract in different concentrations (25, 50, 75µL) inhibits clot formation and increases PT. It also shows that increasing concentrations of Fenugreek extract strongly inhibits the coagulation process and increases PT, and that aqueous extract of Fenugreek have anticoagulant properties through the prevention of clot formation. This may be attributed to several coumarin compounds that have been noted in the seed [19].

These results confirm the previously reported observation that, Fenugreek has been noticed to increase the anticoagulant effect of warfarin. It was also mentioned that there is one case of gastrointestinal bleeding in a premature infant (30 weeks) following introduction of Fenugreek to the mother [22].

In conclusion, fenugreek can be used as a supplementary anticoagulant agent to improve and/or prevent cardiovascular diseases and prevention of prolonged bleeding disorders of the extrinsic system. It is more beneficial if administered over a longer period of time. Further large studies are recommended to evaluate this effect and to determine the mode of action.

References

1. Sirridge MS, Shannon R. Hematology Principles and Procedures, 6Th ed. Philadelphia: Lea & Febiger; (1993); 5: 202-278.
2. Guyton and Hall. Hemostasis and Blood Coagulation. Text book of Medical Physiology. 11th ed., Jaypee Brothers Medical Publishers (2006); 36: 457-468
3. Rang H, Dale M, Ritter J, Flower R. Rang and Dale's Pharmacology, 6th ed. London: Churchill Livingstone; (2007); 21: P. 331 - 344.
4. Kaatz S, Kouides PA, Garcia DA, Spyropolous AC, Crowther M, Douketis JD, et al. Guidance on the emergent reversal of oral thrombin and factor Xa inhibitors. Am J Hematol. 2012 May;87 Suppl 1:S141-5.
5. Hoffbrand AV, Moss PAH. Essential Haematology. Willey-Blackwell Publisher, 6 Edit. (2008); 314-380.
6. Saxena R, Kannan M, Choudhry VP. Laboratory Studies in Coagulation Disorders. Indian Journal of Pediatrics; (2007); 74 (7): 649-655.

7. Akhavan S, Mannucci PM, Lak M, Mancuso G, Mazzucconi MG, Rocino A, et al. Identification and three-dimensional structural analysis of nine novel mutations in patients with prothrombin deficiency. Thromb Haemost. Dec 2000;84(6):989-97.
8. Aranson J. Herbal Medicine, 1st edition .USA: Elsevier.2009; 12.
9. Abebe W. Herbal medication potential for adverse interaction with analgesic drugs. Journal of clinical pharmacy and therapeutic. (2002); 27:391-401.
10. Naham G., Lee M, Park I. Acid and Heat Stability of Anticoagulative Activity of Onion Extract. Bioscience Biotechnology and Biochemistry. 2002; 66: 859 - 565.
11. Bordia T, Mohammed N, Thomson M, Ali M. An evaluation of garlic and onion as antithrombotic agents. Prostaglandins, Leukotrienes and essential fatty acids (PLEFA). (1996); 54. 183186-.
12. Taj Eldin IM, Abdalmutalab MM, Izzalddeen 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.
13. Hoffbrand AV, Moss PAH, Pettit JE. The essential haematology. 5th edition .USA, Australia, UK: Blackwell. (2006)
14. Review of Natural Products Facts and Comparisons (1996), St Louis, MO.
15. Sauvaire Y, Baccou JC. Extraction of diosgenin, (25R)-spirost-5-ene-3beta-ol; problems of the hydrolysis of the saponins. Lloydia; (1978); 41:247-251.
16. Valette G, Sauvaire Y, Baccou JC, Ribes G. Hypocholesterolaemic effect of fenugreek seeds in dogs. Atherosclerosis; (1984); 50 (1):105-111.
17. Lambert JP, Cormier A. Potential interaction between warfarin and boldo-fenugreek. Pharmacotherapy; (2001); 21(4): 509-512.
18. 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.
19. Narayanan S. Multifunctional roles of thrombin. Ann Clin Lab Sci. Oct-Dec 1999; 29 (4): 275-80.
20. 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.
21. Chernecky, Cynthia C. and Barbara Berger. Lab Tests and Diagnostic Processes. 3rd ed. Philadelphia: W. B. Saunders Company, 2001.
22. Swafford S, Berens P. Effect of fenugreek on breast milk production. Abstract. ABM News and Views; (2000); 6 (3): 20.