

Evidence for an *in vitro* Anticoagulant Activity of Red Onion (*Allium cepa* L.)

Taj Eldin IM^{1*}, Majed M. Abdalmutalab² Hajir M. Izzaldeen³,
Madina A. Abdalkareem³, Marwa A. Abdalraheem³ and Marwa B. Alhassan³

Abstract

Background: Haemostasis is the process that retains the blood within the vascular system during periods of injury, localizes the reactions involved to the site of injury, repairs and re-establishes blood flow through the injured vessel. Onion (*Allium cepa*) is a largely universal staple herb popular throughout history as both food and medicine and it has been consumed for prevention of cardiovascular disorders.

Objectives: To study the possible anticoagulant effects of red onion *in vitro*, by using blood samples of normal individuals.

Methods: *In vitro* anticoagulant effects of an aqueous extract (5%) of red onion in different volumes (25, 50 and 75 µL) were examined on the blood samples of normal individuals by measuring prothrombin time (PT).

Results: The aqueous extract of red onion was found to inhibit coagulation process *in vitro* and significantly prolonged prothrombin time in a dose-dependent manner.

Conclusion: This study showed that red onion aqueous extract in different concentrations inhibits clot formation and increases prothrombin time. Red onion can be used as a supplementary anticoagulant agent to improve and/or prevent cardiovascular diseases.

Key words: Haemostasis, prothrombin time, staple herb.

Haemostasis is the process that retains the blood within the vascular system during periods of injury. The coagulation mechanism may be thought of as a complex series of cascading reactions involving development of enzymes from their precursor (zymogens, procoagulants proenzymes). Most of the substances necessary for coagulation are present in an inert form and must be converted to an activated state. As one enzyme is formed it then becomes available to convert the next zymogen to its activated enzyme (serine protease). This process continues until a fibrin meshwork clot has formed. In addition to the zymogens, protein cofactors and membrane phospholipids surfaces, calcium ions play an active role in the final development of the fibrin clot¹.

Most adult cardiovascular disorders involving hypertension, cerebral hemorrhage, coronary thrombosis, arteriosclerosis and congestive heart failure are caused by problems in the blood circulatory system as blood clotting disorders which constitute a serious medical problem. A number of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin have been used as antithrombotic agents. These drugs *in vitro* and *in vivo* cause inhibition of platelet aggregation and thromboxane formation²⁻⁵.

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

1. Department of Pharmacology, Faculty of Pharmacy, University of Gezira, Sudan
2. Islamic Medical Association laboratory, Wad Medani, Sudan
3. Pharmacy graduates (2010). Batch 28, Faculty of Pharmacy University of Gezira
* Corresponding author. omdatj64@gmail.com

II, V, VII and X. Thus prolongation indicates a deficiency in one or more of these factors^{6,7}. The normal prothrombin time is 11-15 seconds. Each prothrombin time within this range indicates that the person has normal amounts of clotting factors VII and X while prolongation in prothrombin time is considered abnormal⁸.

Onion, garlic, clove, fenugreek, mugwort, sage and marine algae as food materials have been reported to have anticoagulative activity⁹⁻¹¹. In recent years, extensive research has been focused on the beneficial and medicinal properties of the genus *Allium*. *Allium* species, especially onion and garlic contain biologically active substances affecting blood clotting. Onions contain a number of sulphur and sulfides compounds similar to those found in garlic which may lower blood lipids, glucose and blood pressure. The anticlotting effect of onions closely correlates with their content of organosulfur compounds¹²⁻¹⁴. The purpose of this study is to investigate the *in vitro* anticoagulant activity of red onion (*Allium cepa*) in blood samples of normal individuals.

Materials and Methods

Preparation of *Allium cepa* extract

Fresh and recently cropped red onions (*Allium cepa*) were purchased from the local vegetable market in Wad Madani city, Central Sudan. Fifty grams of the bulb were cut into small slices and dried at room temperature. After complete drying the slices were grinded into a fine powder. Five grams of the dried 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 *Allium cepa* extract was filtrated using sucking pump. The final clear solution of *Allium cepa* aqueous extract was used for *in vitro* testing of anticoagulant activity in blood samples of normal individuals using the principles of prothrombin time test.

Study population

Blood samples obtained from thirty normal individual volunteers attended to the Islamic Medical Association Laboratory in Wad Madani, Central Sudan, of both sexes

(females (53.3%) and males (46.7%)), were used to assess the anticoagulant effects of *Allium cepa*. Participants were 15-30 years old. They had been chosen for this study according to the following criteria: having normal prothrombin time, 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, not obese or smokers and free from dyslipidemic disorders.

Collection of blood samples

The blood samples were obtained from normal individuals by using sterile syringes, withdrawn from vein of right arm of each individual 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 (ppp) for prothrombin time test. The obtained plasma sample of each individual were poured separately in plane containers using automatic pipette and stored at room temperature^{15,16}.

***In vitro* anticoagulant test of *Allium cepa* extract**

For determination of the prothrombin time, the plasma sample of each individual was divided into four groups each of 50 μ L. Group 1 (n=30) was tested first to determine the normal prothrombin time (positive control group) using the stable, liquid, combined calcium/thromboplastin rabbit brain (DiaMed LTD, UK) as a gold standard. Three volumes of *Allium cepa* extract (25, 50 and 75 μ L) were added separately to the 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 thereafter used for measuring the time of the clot formation. This time is called the prothrombin time^{17,18}. Thromboplastin reagent was added to the plasma in order to counteract the sodium citrate and allow clotting to proceed.

Ethical approval

The ethical approval for this study was obtained from the Ethical Committee/

University of Gezira/ Gezira State, Ministry of Health, Wad Medani-Sudan. Consent forms were signed by participants, being interested in joining the study completely voluntary.

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 One-way ANOVA. Differences were considered significant if $P < 0.05$.

Results and Discussion

Red onion (*Allium cepa*) is a vegetable plant that has been postulated to have similar activities as garlic (*Allium sativum*) however; it has not been extensively assessed scientifically in terms of its biological activities. Only a few publications have been reported on the biological activities of *Allium cepa* as compared to garlic.

In this study the effects of the aqueous extract of *Allium cepa* (5%) 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 normal (13 ± 0.16 seconds). When aqueous extract of *Allium cepa* was added in different volumes (25, 50 and 75 μ L) to plasma samples of normal individuals, it significantly ($P = 0.001$) showed prolongation in the prothrombin time (Figure 1) from 13 ± 0.16 to 14.8 ± 0.23 , 17.1 ± 0.33 and 19.5 ± 0.36 seconds respectively.

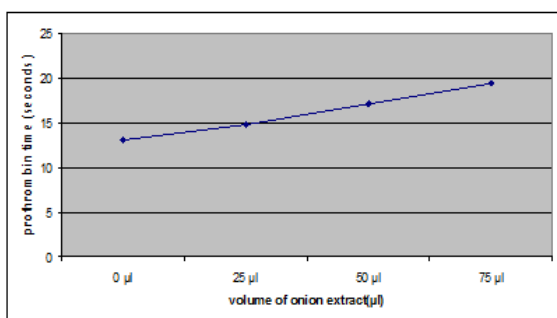


Figure 1: The effect of different volumes (25, 50 and 75 μ L) of aqueous extract of *Allium cepa* (5%) on prothrombin time values of plasma samples of normal individuals.

It was noticed that there were proportional correlations between the concentration of *Allium cepa* aqueous extract needed to inhibit clot formation and prolong of prothrombin time. That is, an increasing concentration of red onion extract strongly inhibited the coagulation process and increased the prothrombin time. These findings clearly showed that, aqueous extract of *Allium cepa* may have anticoagulant properties through prevention of coagulation process and clot formation.

It was reported that members of *Allium* family, especially garlic and onion, have been used as traditional medicines to treat a variety of diseases, including common cold, arthritis, headache, diabetes, and heart diseases. These beneficial properties have been attributed to their phytoconstituents which have hypocholesterolemic and hypoglycemic effects as well as their ability to inhibit platelet aggregation and thromboxane formation¹⁹⁻²¹. Jia-Huey, *et al*²², mentioned that the consumption of raw Welsh onion juice, but not boiled juice, has blood lowering and antithrombotic effects in rats²². The present study demonstrated that the use of aqueous extract of *Allium cepa* especially red onion can affect *in vitro* platelet functions and this may be due to inhibition of platelet adhesion, aggregation or thromboxane release.

Although the mechanism of onion-induced anticoagulative activity is not clearly understood, onion is a promising anticoagulant agent. It is therefore important to investigate the physiological role of its potential effects on blood coagulation.

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