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In Vitro Anticoagulant Effects of Aqueous Extract of *Allium Cepa* (Onion) on Albino Rat Blood

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Abstract

Blood samples collected from twenty one (21) albino rats were used to study the anticoagulant properties of aqueous extract of *Allium cepa*. The rats were divided into seven groups labeled A to G. 3 rats /group. Group A was a negative controlled group and was not treated with any agent. Group B to F were treated with 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL of the extract/1mL of blood respectively. Group G was a positive controlled group and was subdivided into G1 and G2 and were treated with standard anticoagulants. The highest anticoagulant effect was achieved in 1mL of the extract/1mL of albino rat blood. The results obtained were compared statistically with those obtained from two (2) standard anticoagulants (K3EDTA, fluoride oxalate). The results indicated that there was a highly significant differences ($P < 0.05$) in the value obtained. The study showed that, the aqueous extract of Allium cepa has anticoagulant property and may be used as a supplementary anticoagulant agent to improve and/or prevent cardiovascular diseases.

Keywords: *Allium cepa; Anticoagulant; Albino rat; K3EDTA; Fluoride oxalate*

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Introduction

Anticoagulants (blood thinners) are drugs that when added to blood have the ability to prevent clotting of such blood (Raffaele *et al.*, 2013). The drugs used as anticoagulants achieve their effects by suppressing the synthesis or function of various clotting factors that are normally present in the blood (Lisboa *et al*., 2015). The most widely used standard anticoagulants are heparin and warfarin, though they have different modes of action but they are able to achieve their effect readily by either inhibiting growth of existing blood clots or preventing the formation of new ones (Schattauer, 2013). Heparin is a mixture of mucopolysaccharides that promote the activity of antithrombin III, a blood plasma protein that inactivates thrombin which is an enzyme that promotes clotting (Schattauer, 2013).

Warfarin, a coumarin derivative, interferes with the metabolism of vitamin K. Since it is an important element in the synthesis of various clotting factors, interference with its metabolism gives rise to clotting factors that are defective and hence inactive in the blood (Lisboa *et al*., 2015). Other anticoagulants include ethylene diamine tetra acetic acid (EDTA) and sodium citrate. Drugs used as anticoagulant include; acecoumarol, delteparin, argatroban, danaparoid and hirudin (Schattauer, 2013). However, both heparin and warfarin have drawbacks that prompt the need to search for new anticoagulants. Of the most important drawback is their narrow window of effectiveness

before risk of bleeding and also their highly variable navture among individuals (Schattauer, 2013). Nevertheless, newer anticoagulants such as idraparinux, apizean and hondaprinux, are under study around the world. These will serve as substitutes with lower levels of the drawbacks of the other anticoagulants. Also one of such studies involves the use of plant and plant products such as Allium cepa (Onion) (Shikha *et al*., 2014).

Onion belongs to a family Amaryllidaceous. It is commonly called Albasa in Hausa. It has one or more leafless flower stalks that reach a height of 75–180 cm, terminating in a spherical cluster of small greenish white flowers. The concentric leaf bases of the developing plant swell to form an underground edible bulb (Hridaya and Simalchaur, 2007). Most commercially cultivated onions are grown from the plant's small black seeds, which are sown directly in the field, but it may also be grown from small bulbs or from transplants (Hridaya and Simalchaur, 2007). Onions are very hardy and can survive in a wide range of growing conditions. The bulbs vary in size, shape, colour and pungency, though warmer climate generally produces onion with milder sweeter flavour.

Onions have been used in many medicinal practices. Similar to garlic, they contain sulfur-compounds such as propyl disulphide that contribute to its pungent odour (Hridaya and Simalchaur, 2007). Medically, it has been shown to improve cardiovascular health. Current research has shown that the more pungent the onion, the stronger the anti-platelet activity (Hridaya and Simalchaur, 2007) and this is due to sulfur compounds, chromium and vitamin B6, all of which are known to decrease the homocysteine levels which is a known factor in heart attack, stroke and other heart diseases (Prakash, 2006). Similarly, atherosclerosis and other cardiovascular diseases such as heart attack and stroke are all associated with platelet aggregation and the clogging of arteries and veins that these compounds can help instigate (Hridaya and Simalchaur, 2007).

The importance of anticoagulants cannot be over emphasized. It is crucial in the determination of haematological parameters for diagnosis. Also some anticoagulants help in preventing heart related problems such as thromboembolism and heart attacks (Prakash, 2006). Many available anticoagulants have drawbacks such as haemorrhages and narrow therapeutic indices in the face of bleeding. As such, there is the need for research and development of alternative anticoagulants that will have less or no such drawbacks. Some of these substitutes are plants and plant products such as *Allium cepa* (Onion), as believed by traditionally practioners. This research was therefore conducted to scientifically verify this claim. The aim and objectives of this study is to scientifically validate the traditional belief that extract of *Allium cepa* has anticoagulant properties and also to investigate the suitability of *Allium cepa* extract as an anticoagulant on albino rat blood *in vitro*.

Materials and Methods

Collection of *Allium Cepa* Bulbs (Onions)

The bulbs of *Allium cepa* (onion) were purchased from Sokoto metropolis food market in Sokoto state, in the month of July and taken to the herbarium of Botany unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, for identification by Malam Umar Dange.

Laboratory Animals (Albino Rats)

Twenty -one albino rats of both sexes were purchased from the Department of Biological Sciences, Ahmadu Bello University Zaria and were aclimatised for two weeks and fed grower's mash and drinking water ad libitum.

Grouping of the Lab Animals (Albino Rats)

A total of 21 albino rats were divided into seven groups labeled A, to G and 3 rats/group. Five test tubes were used for each of the groups.

Preparation of the Extract

Fifty grams of the onion was weighed with a digital weighing balance. Its dried covers were removed with a knife, and then the fresh bulbs were washed with distilled water and sliced into pieces. The pieces of the sliced onion were blended with an electric blender for

10 minutes with 50 mL of distilled water. The blended onion was filtered with a wire mesh filter and the filtrate was centrifuged at 3000 rmp for 10 minutes, after which the supernatant was filtered with Whitman filter paper size 1 and the filtrate was collected into a beaker and stored in the refrigerator for further use.

Determination of the Physical Properties of the Extract

The physical properties of the extract were determined by visual examination and the pH was determined by pH meter.

Collection of Blood Samples

Blood samples were collected by cardiac puncture using a clean dried sterile syringe and needle. The blood was then dispensed into the test tubes containing the extract.

Determination of Minimum Volume of Extract for Anticoagulation

Fresh whole blood was collected and 1 mL of the blood was dropped into five different test tubes in each group. Five test tubes in same group were treated with same volume of the extract which is different from other groups. Group A was a negative control group and was not treated with any agent. Groups B, C, D, E, and F were treated with 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL of the extract/1mL of rat blood respectively. Whereas group G served as a positive control group and was subdivided into G1 and G2 and were treated with a standard anticoagulants (EDTA and Fluoride oxalate respectively). Time taken for blood clot formation in each test tube was taken (Odoula *et al*., 2005).

Determination of the Coagulation Time

Lee and White method was used to determine the coagulation time. 1 mL of fresh blood was dispensed into five different test tubes containing the quantity of the extract, and was kept in water bath set at 37^0C. Then, the tubes were tilted one by one in every 30 seconds. The clotting time was taken when the tubes were tilted without spilling of their content and the clotting time was calculated as the average of the five tubes used (Farlex, 2012).

Statistical Analysis

The mean ± standard error of mean was calculated for all the groups. The standard errors of mean were subjected to analysis of variance (ONE WAY ANOVA) in order to know if there were significant differences between the coagulation times obtained. $P < 0.05$ was considered statistically significant.

Results

The extract of *Allium cepa* was milky in colour, watery in consistency, pungent in odour, and with a pH of 7. Table 1 shows the result of the volumes of the extract that produced anticoagulation when the albino rat blood was added to each test tube containing different concentrations of the extract. With respect to table 4.1, the highest coagulation time was achieved in 1 mL of extract per 1 mL of blood sample. Although anticoagulation were achieved also at the concentration of 0.2 mL, 0.4 mL, 0.6 mL, 0.6 mL and 0.8 mL /mL of rat blood

Test tubes	Group A Untreated	Group B 0.2 mL extract	Group C 0.4 mL extract	Group D 0.6 mL extract	Group E 0.8 mL extract	Group F 1 mL extract	Group G1 10µL (K3EDTA) hrs	Group G2 10µL (Oxalate) hrs
1	0.5	3.5	4.5	8	10.5	17.5	> 24	> 24
2	0.5	4	4.5	8	10	16	> 24	> 24
3	0.5	4	5	8	10.5	16.5	> 24	> 24
4	0.5	4.5	5.5	8.5	10.5	17	> 24	> 24
5	0.5	4	5	7.5	11	18	> 24	> 24
X	0.5	4	5	8	10.5	17	24	24

	0.5	≥ 4	≥ 5	≥ 8	≥ 10.5	≥ 17	≥ 24	≥ 2
Key X= Average time Period before clotting (minutes)								

Table 1: *Coagulation Time (Minute) of Blood Treated with Different Volume of the Extract and Standard Anticoagulants.*

Comparism of the Coagulation Time in Blood Treated with Onion Extract and Two Standard Anticoagulants

Table 2 shows the comparison of the coagulation time between the onion extract and two standard anticoagulants (potassium EDTA and fluoride oxalate). The table showed significant differences between the coagulation time of the blood treated with onion extract and two standard anticoagulants with a P< 0.05

Volumes of the extract mL	Number of test tubes	X ± S.D of clotting time (minutes)
Negative	5	0.5 ± 0.00
0.2	5	4.00 ± 0.35
0.4	5	5.00 ± 0.50
0.6	5	8.00 ± 0.35
0.8	5	10.00 ± 0.22
1.0	5	17.00 ± 0.79
K3EDTA	5	1440.00 ± 0.00
Fluoride oxalate	5	1440.00 ± 0.00

A value of P less than 0.05 ($p < 0.05$) was considered statistically significant

Table 2: *Mean and standard error of mean of the coagulation time of all the albino rat blood treated with onion extract and standard anticoagulants.*

Discussion

The prevalence of atherosclerosis and coronary artery diseases have focused attention on the influence of diet on the cardiovascular system. Natural anticoagulant agents that influence platelet function and inhibit coagulation processes are of potential interest for primary prevention of cardiovascular diseases. Previous studies showed that nonsteroidal anti-inflammatory drugs (NSAIDs) in small doses for extended period of time inhibit platelet aggregation and thromboxane formation (Imadeldin., *et al*., 2013). This study demonstrates that aqueous extract of *Allium cepa* in different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mL/mL of blood sample) inhibits clot formation and increases coagulation time. It also shows that increasing the concentration of the aqueous extract of *Allium cepa* strongly inhibit the coagulation process and increase the coagulation time, and that aqueous extract of *Allium cepa* has anticoagulant properties through the prevention of clot formation for extended period of time (Table 1). The fresh aqueous extract of *Allium cepa* was found to have a pH of 7; However Mariae (2013) earlier reported that the extract has a pH of 5.5.

This variation in the pH could be as result of seasonal variation at the time of the harvest or collection of the onion, it could also be as a result of the location and soil quality of the place of the cultivation of the onion. For instance, if the fresh onion was collected during the dry season, then there is a possibility that the pH will be acidic due to low water content (F.A.O., 2000). In the present study, the onion extract was collected during the raining season when there was low environmental temperature, thus leading to decreased loss of water from the onions and likely contributed to the increase in pH (Encarta, 2008)

The coagulation time for untreated blood was 0.5 ± 0.00 minutes, Blood treated with 0.2, 0.4, 0.6, 0.8 and 1 mL of the extract had the coagulation time to be 4.00 ± 0.35, 5.00 ± 0.5, 8.00 ± 0.35, 10.00 ± 0.22 and 17.00 ± 0.00 minutes respectively whereas blood treated with standard anticoagulants had a coagulation time to be 1440 ± 0.00 minutes. The anticoagulant property of the fresh onion extract

was found in all concentrations tested on 1 mL of albino rat blood. But increase coagulation time occurs in dose dependent- manner. This is in agreement with Shikha *et al* (2013). Comparism of coagulation time of blood treated with aqueous extract of *Allium cepa* and standard anticoagulants (K3EDTA and fluoride oxalate) showed a significant difference at $P < 0.05$ which makes the coagulation time for the extract insignificant.

Conclusion

One of the criteria used for selecting a good anticoagulant is that, it should have a prolonged coagulation time and should not interfere by adding to or subtracting from the components of blood sample. Since the aqueous extract of *Allium cepa* does not exhibit a prolonged coagulation time required as a good anticoagulant, then it implies that aqueous extract of *Allium cepa* may not be suitable as an anticoagulant to be used on an albino rat blood for determination of haematological parameters. Nevertheless, observations made through this study, however confirmed that the aqueous extract of *Allium cepa* has anticoagulant property when used on albino rat blood. As such it may be used as a supplementary anticoagulant agent to improve and/or prevent cardiovascular diseases.

Recommendations

Further studies are recommended to evaluate this effect and to determine the mechanism of action. Testing concentrations greater than 1 mL of the extract/1 mL of blood are also recommended.

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