RESEARCH ARTICLE

Effect of chronic ingestion of *Allium cepa* (white onion bulb) on platelet aggregation in an aged female wistar rat

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ABSTRACT

Aging is associated with an increased incidence of cardiovascular disease and thrombosis. Several lines of evidence support that platelets from older subjects differ in their function and structure, making platelets more prone to activation and less sensitive to inhibition. These age-related changes could lead to platelet hyperactivity and to the development of a prothrombotic state in advanced age. The effect of aging on platelet may be related to reduce hematopoietic stem cell reverse in aging individuals. As such, discovery of natural products or supplements that will provide anti-thrombotic effect on the platelets of aged female wistar rat is sorely needed. The aim of this study is to investigate the effect of chronic ingestion of *Allium cepa* on platelet aggregation and some coagulation parameters in aged female wistar rats. A total of 30 aged female wistar rats were used in this study comprising of three groups. The three groups were Group A; negative control, Group B; positive control, and Group C as the experimental group. Hematological parameters such as PCV, WBC and platelet count as well as PT/ PTTK and platelet aggregation tests were analyzed and compared across all the study groups. There was no significant difference in the mean value of RBC, WBC, and platelet count between the study groups ($p>0.05$). However, there was a significant difference in the mean value of PCV when compared to control group ($p>0.05$). There was no significant difference in the mean value of platelet aggregation ratio. However, there was a significant difference in PT and PTTK between the study groups ($p<0.05$). The study shows enhanced coagulation effect of white onion in aged rats, though not statistically significant.

Key Words: *Platelet; Thrombosis; Chemopreventive*

INTRODUCTION

Aging is associated with an increased incidence of cardiovascular disease and thrombosis. Platelets play a major role in maintaining hemostasis and in thrombus formation, making them a key player in thrombotic disorders. Whereas it is well-known that platelet aggregability is increased in vascular diseases, the contribution of age-related changes in platelet biology to cardiovascular risk is not well-understood. Several lines of evidence support that platelets from older subjects differ in their function and structure, making platelets more prone to activation and less sensitive to inhibition. These age-related changes could lead to platelet hyperactivity and to the development of a prothrombotic state in advanced age. Platelet count is inversely associated with age. A large study based on the Third National Health and Nutrition Examination Survey including 12,142 American subjects showed a significant decrease of 10×10^3 platelets/μL in individuals in the 60 years–69 years age group as compared with those between the ages of 20 years–59 years, and of 20×10^3 platelets/μL in patients aged over 69 years of age, after adjusting for many covariates such as nutritional deficiencies, medication, inflammatory conditions, autoimmune or viral illnesses and consumption of alcohol and tobacco [1]. One of the most documented changes in platelet function during aging is platelet hyperactivity. Bleeding time decreases significantly in aging, denoting a faster clot formation and indirectly an enhanced platelet activity in the elderly [2,3].

When normal blood vessels are damaged, platelets are activated by stimuli which are present in the walls of blood vessels, and induce aggregation. Platelets are activated by agonists such as collagen, thrombin and Adenosine Diphosphate (ADP), and it induces the signals by activating multiple G protein-mediated pathways to activate platelet-shape change, degranulation and aggregation [4,5]. But in many cardiovascular diseases such as acute coronary syndromes, atherosclerosis, stroke and peripheral vascular diseases, excessive platelet activation is regarded as the cause of thrombosis [6-8]. Abnormal platelet aggregation leads to excessive Thromboxane A2 (TXA2) formation interacts with other platelets, inducing thrombotic disorders [9]. Therefore, the development of an anti-platelet agent could be a fundamental therapeutic approach to cardiovascular diseases [10,11].

The traditional system of medicine has become a topic of global importance during the past decade. Current estimates suggest that in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system to treat or prevent many diseases [12].

Onion (*Allium cepa*) has been reported to have beneficial effects, including preventing stroke, coronary thrombosis, atherosclerosis, hyperlipidemia, and hypertension [13,14]. Especially, it has been reported to inhibit platelet aggregation induced by various agonists *in vitro* and *in vivo* [15,16].

Onions (*Allium cepa*) are perennials that are cultivated for food worldwide. Onion which derived its name from the Latin Onion and French Oignon, has been described as the dynamite of all natural foods. It is a tunicated bulb, compressed, round, or oblong in Figure 1, invested with a shining, thin, dry membrane, of a reddish or white color [17]. Onion (*Allium cepa*) is the best widely cultivated species of the genus *Allium* and it is widely distributed in the temperate regions [18].

Onion has been used as food additive or supplement for many centuries.

Figure 1) *Allium cepa (Sokoto breed onion)*

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TABLE 1

Scientific classification of oni

Kingdom	Plantae
Division	*Magnoliophyta*
Class	*Liliopsida*
Order	*Asparagales*
Family	*Alliaceae*
Genus	*Allium*

Edible Parts: Flowers, Leaves, Root, Seed

The onion plant is a bulbulous vegetable 1.2 m in height, with 4 to 6 hollow, cylindrical leaves. It is botanically referred to as *Allium cepa*. Its underground bulb, comprises of fleshy leaf sheaths forming a thin-skinned capsule, and varies greatly in size (2 cm to 20 cm). Onion is also a popular folk remedy and is a staple food with a high content of flavonoids [19]. It contains 89% water, 1.5% protein, and vitamins B1, B2, C, K, and E, along with calcium, potassium, magnesium, iron, phosphorus, zinc, sodium, selenium [20], rich in flavonoids such as quercetin and sulfur compounds, such as allyl propyl disulphide that have perceived benefits to human health [21]. In addition, onions are rich in sulfur containing compounds mainly in the form of cysteine derivatives, viz. S-alkyl cysteine sulfoxides which are decomposed by the enzyme allinase into a variety of volatile compounds such as thiosulfinates and polysulfides during extraction. These compounds possess anti-diabetic, antibiotic, hypocholesterolaemic, fibrinolytic, and various other biological effects. In addition to volatile substances in *Alliums*, there are non-volatile sulfur-containing peptides and proteins which have been shown to have potential health benefits [22]. *Allium* containing substances have antibiotic effects and antibiotics should enable the proliferation of circulating white blood cells considering that white blood cells function to protect the body from teratogens. *Allium* species have been implicated in the induction of hemolytic anemia. They contain toxic components that may damage red blood cells and provoke hemolytic anemia accompanied by Heinz bodies in erythrocytes of animals such as cattle, water buffalos, sheep, horses, dogs and cats [23].

Allium cepa juice has been found to exhibit antioxidant effect in alloxan induced diabetic rats; it also repaired hepatic and renal damage caused by the administered alloxan [24]. Epidemiological, clinical and laboratory studies have demonstrated the role of onion in cancer prevention especially in relation to digestive tract cancers, including esophageal and stomach cancers [25,26]. In the case of GI-related cancers, proposed mechanisms of action for the *Allium* species include an inhibition of *Helicobacter pylori* and other bacterial activity, as well as a general decrease in the endogenous production of carcinogenic N-nitroso compounds. The chemopreventive activity has been attributed to the ability to modulate the activity of several metabolising enzymes that activate (cytochrome P450s) or detoxify (glutathione S-transferases) carcinogens and inhibit the formation of DNA adducts in several target tissues [27]. The fact that *Allium cepa* (onion) being widely used as food additive, due to its rich active constituents and antioxidant properties necessitated the need to investigate its effect on platelet count and prothrombin time (Table 1).

STATEMENT OF PROBLEM

Onion (*Allium cepa*) has been reported to have beneficial effects, including preventing stroke, coronary thrombosis, and hypertension [28]. Also, it has been reported to inhibit platelet aggregation induced by various agonists *in vitro* and *in vivo*.

Therefore, this study is designed to find out if the anti-thrombotic constituent of *Allium cepa* (quercetin) through consumption of onions will have effect on PT, APPT and platelet counts of aged female wistar rats.

RATIONLAE OF STUDY

The effect of aging on platelet may be related to reduce hematopoietic stem cell reverse in aging individuals. Physiological aging is associated with increased plasma levels of many protein of blood coagulation with fibrinolysis impairment [28,29]. As such, discovery of natural products or supplements that will provide anti-thrombotic effect on the platelets of aged female wistar rat is sorely needed. *Allium cepa* may serve as a potential candidate for inhibiting platelets aggregation leading to stroke in aged people, hence this study. This study is therefore designed to find out if the anti-thrombotic constituent of *Allium cepa* through consumption of onions will have effect on PT, APPT and platelet counts of aged female wistar rats.

AIM OF THE STUDY

The aim of this study is to investigate the effect of chronic ingestion of *Allium cepa* (white onion bulb) on platelet aggregation and some coagulation parameters in aged female wistar rats.

HYPOTHESIS

Alternate hypothesis

Allium cepa (white onion bulb) supplementation inhibits platelet aggregation and enhances hemostasis in aged female wistar rats.

Null hypothesis

Allium cepa (white onion bulb) supplementation does not inhibit platelet aggregation and enhances hemostasis in aged female wistar rats.

OBJECTIVES OF THE STUDY

The objectives of the study are to investigate the effects of:

- Determination of the effect of *Allium cepa* (white onion bulb) extract on hematological parameters such as Prothrombin Time (PT), activated partial thromboplastin time (APTT) and Platelet counts of aged female wistar rats.
- Assessment of the effect of *Allium cepa* (white onion bulb) extract on platelet aggregation in aged female wistar rats.

LITERATURE REVIEW

Aging is accompanied by many biological changes at molecular and cellular levels. Senescence, the most striking cellular alteration in aging, is a hypo replicative state implicated in age-related diseases [30]. Senescent cells adopt a specific secretion signature rich in pro-inflammatory cytokines called the Senescence-Associated Secretory Phenotype (SASP). It has been suggested that SASP induces changes in the functions of neighboring cells [31,32], and some authors hypothesize that it might potentially contribute to increased susceptibility to thrombosis in elders [33].

Aging is associated with an increased incidence of cardiovascular disease and thrombosis. Platelets play a major role in maintaining hemostasis and in thrombus formation, making them a key player in thrombotic disorders. Whereas it is well-known that platelet aggregability is increased in vascular diseases, the contribution of age-related changes in platelet biology to cardiovascular risk is not well-understood. Several lines of evidence support that platelets from older subjects differ in their function and structure, making platelets more prone to activation and less sensitive to inhibition. These age-related changes could lead to platelet hyperactivity and to the development of a prothrombotic state in advanced age. Several lines of evidence suggest that platelet hyperactivity observed in advanced age could be responsible for vascular and thrombotic disease development [34]. However, molecular mechanisms of platelet hyperactivity in aging are only partially understood.

Platelet physiology

Platelets are small anucleated cells packed with complex signalling machinery that enables them to react rapidly and specifically to a variety of stimuli, most notably at sites of tissue injury. At sites of vascular damage, platelets adhere to the sub-endothelial matrix via interactions between Von Willebrand Factor (VWF) and the platelet receptor complex GPIb-V-IX [35-37]. The first adherent platelets are stabilized and activated by the binding of GPVI and integrin α β to exposed collagens [38]. Following this initial deposition, subsequent platelets are recruited to the forming thrombus via integrin α β anchored tethers [39]. Once tethered, platelets encounter a host of agonists which are either generated or secreted by activated platelets (e.g. thromboxane A) or released upon platelet degranulation (e.g. ADP) or synthesized on the platelet surface or at the site of thrombus formation (e.g. thrombin) [40-43]. Intracellular signalling cascades initiated upon platelet activation lead to calcium mobilization from both internal stores and the extracellular space into the cytoplasm, platelet shape change, degranulation and a change in the affinity of integrin αβ for VWF and fibrinogen [44-48]. The binding of fibrinogen to integrin α β on different platelets supports aggregation and thrombus formation [49,50], and further stimulates platelets by the activation of integrins [51-53]. Unfettered or inappropriate platelet activation in kept in check by a series of inhibitory 2 1 IIb 3 2 IIb 3 IIb 3 signalling pathways, the most powerful of which are Nitric Oxide (NO) and prostacyclin (PGI2) released into the blood the healthy arterial endothelium [54].

This coordinated response enables platelets to respond rapidly to vascular damage and, in most circumstances, allows for the formation of

stable thrombi that prevent excess bleeding without blocking the flow of blood past the site of damage. Changes in platelet count or responsiveness alter the dynamics of this highly regulated response, with the consequent increase in the risk of bleeding or vessel occlusion and thrombotic disease. One of the leading causes of change in platelet physiology is an individual's age. This review focus on the changes in platelets associated with old age because of their particular relevance to the development of thrombotic disease (the majority of which occurs in people over 75 years of age) and its treatment [55-58].

Age-associated decrease in platelet count

Platelet count is inversely associated with age. A large study based on the Third National Health and Nutrition Examination Survey including 12,142 American subjects showed a significant decrease of 10×10^3 platelets/μL in individuals in the 60 years–69 years age group as compared with those between the ages of 20 years–59 years, and of 20×10^3 platelets/μL in patients aged over 69 years of age, after adjusting for many covariates such as nutritional deficiencies, medication, inflammatory conditions, autoimmune or viral illnesses and consumption of alcohol and tobacco. This suggests that the drop in platelet count with age is part of the biological aging process per se and not only due to environmental factors. Another large study on 33,258 subjects in France examined full blood count normal references values by age and sex. It showed the same tendency of platelet count being lower in older adults vs. their younger counterparts [59].

Despite evidence of an inverse correlation between aging and platelet count, the cause of the decline in platelet count and the physiological consequences of this phenomenon in older subjects remain to be elucidated. Two hypotheses have been put forward in an attempt to rationalize this observation:

- older individuals have a lower stem cell reserve compared to younger subjects; and
- a reduced platelet count confers a biological advantage, so the individual with this characteristic may have a better chance to reach an older age.

Enhanced platelet activity in the elderly

One of the most documented changes in platelet function during aging is platelet hyperactivity. Bleeding time decreases significantly in aging, denoting a faster clot formation and indirectly an enhanced platelet activity in the elderly. Furthermore, platelets from older men and women have a greater sensitivity to aggregation induced by classical agonists. Platelets aggregation of older subjects occurs at a lower concentration threshold of ADP, epinephrine, collagen and arachidonic acid than platelets from younger subjects. Meade et al. reported an increase in platelet aggregability of ~8% per decade of age, calculated by the EC50 of ADP in a cohort of 958 participants of all ages [60]. At all ages, aggregability was found to be more pronounced in women than in men [61]. A recent study suggests that age-related changes in platelet behavior on von Willebrand factor are more pronounced in women than in men [62], also supporting the concept that the aging process could affct platelet function diffrently in both sexes. Furthermore, β-thromboglobulin and Platelet Factor 4 (PF4), two proteins secreted from platelets α-granules, are both found at a significantly higher level in plasma of older compared with younger subjects [63,64]. This is consistent with the hyperaggregability observed in elderly individuals since platelets release their granule content during activation. Interestingly, PF4 has also been found to have a procoagulant effect [65], showing that the age-related prothrombotic state is probably due to a number of biological changes in the thrombotic pathway, not only occurring in platelets themselves. The mechanisms of this age-related platelet hyperactivity remain unclear. Bastyr have tested the hypothesis that modifications in phosphoinositide turnover, an important signaling mechanism of platelet activation, may be responsible for platelet hyperactivity in aging. They have found that platelet phosphoinositide turnover is enhanced in aging and correlates positively with platelet aggregation and plasma β- thromboglobulin levels. It has also been suggested that there could be a functional or expressional change in platelet α and β-adrenoreceptors, however the reported literature is conflcting [66]. These receptors, respectively, enhanced or inhibited platelet aggregation induced by epinephrine and noradrenaline by decreasing or increasing platelet cAMP levels. Yokoyama et al. observed an increase in α-adrenoreceptor binding capacity in platelets of elderly people without change in binding affity [67]. However, two other groups have observed the opposite, i.e., a decrease in α-adrenoreceptor binding capacity [68,69]. Finally, another study suggested a decrease of β-adrenoreceptor affity but an unchanged binding capacity in older subjects.

***Allium cepa* (Onion)**

Onion has been used as food additive or supplement for many centuries. The onion plant is a bulbulous vegetable 1.2m in height, with 4 to 6 hollow, cylindrical leaves. It is botanically referred to as *Allium cepa*. Its underground bulb, comprises of fleshy leaf sheaths forming a thin-skinned capsule, and varies greatly in size (2 cm to 20 cm). Onion is also a popular folk remedy and is a staple food with a high content of flavonoids. It contains 89% water, 1.5% protein, and vitamins B1, B2, C, K, and E, along with calcium, potassium, magnesium, iron, phosphorus, zinc, sodium, selenium, rich in flavonoids such as quercetin and sulfur compounds, such as allyl propyl disulphide that have perceived benefits to human health.

In addition, onions are rich in sulfur containing compounds mainly in the form of cysteine derivatives, viz. S-alkyl cysteine sulfoxides which are decomposed by the enzyme allinase into a variety of volatile compounds such as thiosulfinates and polysulfides during extraction. These compounds possess anti-diabetic, antibiotic, hypocholesterolaemic, fibrinolytic, and various other biological effects. In addition to volatile substances in alliums, there are non-volatile sulfur-containing peptides and proteins which have been shown to have potential health benefits. *Allium* containing substances have antibiotic effects and antibiotics should enable the proliferation of circulating white blood cells considering that white blood cells function to protect the body from teratogens.

Chemical composition

Active ingredients in onions include phenolic compounds (flavonoids, antocyanins, phenolic acids and flavonols), organosulphur compounds, vitamins and some minerals. Researches also shown that onion contains exogenous and endogenous antioxidants such as selenium, glutathione, vitamins A, B, and C, and flavonoids such as quercetin and isorhamnetin.

Action and medical uses

The onion (*Allium cepa*) has long been used in traditional medicine, is one of the important *Allium* species commonly used in our daily diet, and has recently been the source of much interest because of its antithrombotic, hypolipidaemic, hypotensive, diaphoretic, antibiotic, antidiabetic, antiatherogenic, and anticancer medicinal properties [70,71]. The pharmacological evidence for the use of onions as an anti-asthmatic, anti-hypertensive, anti-hyperglycemic, anti-hyperlipidemic and anti-tumor agent has been reported.

Quercetin is one of the well-studied flavonoids and is particularly high in onions. Quercetin is thought to be protective against coronary heart disease, stroke, and certain cancers. The organosulfur compounds in onions are believed to possess anti-inflammatory, anti-allergic, anti-microbial, and anti-thrombotic activity by inhibition of cyclooxygenase and lipoxygenase enzymes [72].

Anti-bacterial activity

Onions have been shown to possess antibacterial and antifungal properties [73]. Onion oil has been shown to be highly effective against gram positive bacteria [74]. Organosulfur compounds were cited as protective agents by researchers finding antibacterial effects of onion extract against oral pathogenic bacteria [75].

Anti-fungal activity

Onion oil has been shown to be highly effective against dermatophytic fungi growth and aflatoxin production of *Aspergillus* fungi genera. Welsh onion extracts have been proven to be more inhibitory toward aflatoxin production than the preservatives sorbate and propionate at values near 6.5 [76].

Anti-oxidant activity

Onions are good sources of dietary phytochemicals with proven antioxidant properties and ability to modulate the detoxification systems. Phenolic acids

in onions, such as caffeic, chlorogenic, ferulic, sinapic, p-coumaric acids, vanillic, syringic and p-hydroxybenzoic appear to be active antioxidants. Its vitamins, especially vitamin C have a protective function against oxidative damage and a powerful quencher of singlet oxygen, hydroxyl and peroxyl radicals.

Flavonoids have been widely studied for their antioxidative effects [77,78]. Onions are known to contain the flavonoids quercetin and Kaempferol [79,80]. The mechanisms of action quercetin include free radical scavenging, chelation of transition metal ions, and inhibition of oxidases such as lipoxygenase [81-83]. The antioxidative effects of consumption of onions have been associated with a reduced risk of neurodegenerative disorders [84], many forms of cancer, cataract formation [85,86], ulcer Development, and prevention of vascular and heart disease by inhibition Of lipid peroxidation and lowering of Low Density Lipoprotein (LDL) cholesterol levels [87-89].

MATERIALS AND REAGENTS

The materials involved in this study includes hand gloves, wistar rats, wooden cages, oral cannula, EDTA bottles, plain bottles, tri sodium citrate bottles, needles and syringes, *Allium cepa*, weighing balance, cotton wool, scissors and blades, methylated spirit, commercial feeds, water, auto analyzer, microscope, measuring cylinder, Lieshman stain, aspirin, universal bottle, distilled water, hypochlorite, collagen, water bath, mortar and pestle, PT/PTTK reagent, glass slides, cover slips, immersion oil, EDTA/formalin solution, EDTA/ buffer solution, stop watch, cryo vial, improved neubauer counting chamber, Turk's solution, blender, muslin bag, automatic pipette, Pasteur pipette.

STUDY DESIGN

This study was an experimental study.

STUDY SITE

The study was carried out at the animal house, Department of Medical Laboratory Science, College of Health Sciences, Ladoke Akintola University of Technology, Mercy land, Osogbo, Osun State.

ETHICAL CLEARANCE

An approval from the College of Health Sciences Ethical Committee of Ladoke Akintola University of Technology was collected.

PROCUREMENT OF *ALLIUM CEPA*

Allium cepa (white onion bulb) was purchased from Oja Oba Market, Osogbo, Osun state.

EXPERIMENTAL ANIMALS

Thirty (30) female wistar rats weighing between 180 g-200 g and about 2 years old were purchased from the animal house of Ladoke Akintola University of Technology (LAUTECH), Mercyland, Osogbo, Osun state. The rats were randomly divided into three groups of 10 rats per group. They were made to acclimatize to animal house condition for a week before the commencement of the research and were fed with a standard commercial pelleted rat feed and clean water. The room temperature in the animal house was maintained at 28°C ± 2°C and 12 hours light and dark cycle was employed. The weight of the animals was measured using an electronic analytical and precision balance before the commencement of the study. Experimental procedures involving the animals and their care were conducted in conformity with international, national and institutional guidelines for the care and use of laboratory animals in biomedical research promulgated by the Canadian Council of Animal Care (Table 2).

TABLE 2
Experimental protocol

Group	Treatment	Inference
A	Rats fed with commercial feed and water only Control	(Normal feed)
B	Rats fed with commercial feed and water + aspirin Control	(Standard drug)
C	Rats fed with commercial feed and water + *Allium cepa* (white onion bulb)	Experimental group

EXTRACTION AND ADMINISTRATION OF *ALLIUM CEPA*

Allium cepa (white onion bulb) was purchased from Oja Oba market, Osogbo, Osun state. The *Allium cepa* were weighed and blended severally using an electronic blender. The blender and cup was first weighed using micro weighing balance to know the weight of the blender and cup. The *Allium cepa* (white onion bulb) blended was then weighed with the blender to know the weight of the blended onion and blender. This was ingested orally (0.2 ml) morning and night by gavage using oral cannula into the test rats according to the experimental design. The volume ingested (0.2 ml) was calculated pharmacologically. The rats were observed after 1 hour of administration and the administration process was repeated after 12 hours for 2 months (60 days).

ADMINISTRATION OF ASPIRIN (ACETYLSALICYLIC ACID)

Aspirin (30 mg/ml) was gotten from the pharmacological department, Ladoke Akintola University of Technology (LAUTECH) Osogbo, Osun state [90]. The administered volume was deducted using the formula below:

Normal dose 3 mg/kg

Aspirin concentration = 30 mg/ml

Average weight of animal =162 g

Therefore: $$Administered\ volume = \frac{normal\ dose / 1000g\ X\ average\ weight /\ concentration}{3mg / 1000g\ X\ 162g / 3mg / ml}$$

Administered volume = 0.2 ml

0.2 ml of aspirin was ingested to the standard group every morning and night for 2 months (60 days).

ANIMAL SACRIFICE AND BLOOD SAMPLE COLLECTION

At the end of the experiment, the animals were sacrificed such that enough bloods can be collected into EDTA anticoagulant bottles, and tri sodium citrate bottles. The blood samples were analyzed for the various hematological parameters which include platelets counts by automated machine (Sysmex-KX-21N), platelets aggregation test using manual counting, coagulation test (PT/PTTK) and thin film for blood pictures.

HAEMATOLOGICAL ANALYSIS USING AUTOMATED MACHINE

Automation method-(Sysmex-KX-21N) plus is a quantitative automated hematology analyzer for invitro diagnostic use which can determine nineteen (19) parameter. It directly measures hematocrit, total WBC counts, RBC counts, Hemoglobin (Hb), MCV, MCH, MCHC, RDW, absolute lymphocytes counts and absolute mixed counts, platelet aggregation while platelets counts was calculated.

PLATELET AGGREGATION TEST (WU AND HOAK, 1974)

Principle

The method is based on the fact that in an EDTA/FORMALIN solution; EDTA acts as anticoagulant by chelating calcium while formalin will fixed the platelet aggregate in the blood and centrifuge down in a platelet rich plasma of EDTA/FORMALIN. Platelet aggregates are fixed thereby reducing platelet counts.

Moreover platelet aggregates flow freely in an EDTA/BUFFER solution without hindering the platelet counts of the platelet rich plasma.

The ratio of EDTA/FORMALIN to that of EDTA/BUFFER therefore, serves as a measure of platelet aggregation in the sample. This value approaches one (1) when there is no aggregation or when aggregation is low but less than one (1) when there is aggregation.

Methodology

Platelet counts ratio method was used for the quantitative determination of circulating platelet aggregates.

Various blood was drawn into two separate containers, 0.25 ml of blood was delivered into each. One of the container has 1 ml ratio of EDTA/ FORMALIN solution and the other has 1ml of EDTA/BUFFER solution.

The sample were kept at 22°C for 15 minutes and then centrifuged at 150 g for 8 minutes to obtain their Platelet Rich Plasma (PRP).

Platelet counts on both PRP samples were determined using the improved

neubauer counting chamber under x40 objective of the microscope lens.

The results were expressed as follows:

$$Platelet\ aggregation\ ratio = \frac{platelet\ counts\ in\ EDTA/FORMALIN}{Platelet\ counts\ in\ EDTA/BUFFER}$$

To validate this hypothesis, an invitro experiment was conducted as follows: collagen was added to citrated blood obtained from a healthy rats.

After stirring for 5 minutes, 0.25 ml aliquots of this blood sample was put into container:

- containing buffered EDTA/FORMALIN solution and EDTA/BUFFER solution in container
- . and then processed as described above to separate PRP from the sediment in order to out platelet count. The tests samples were all treated in this way.

DETECTION OF *IN-VITRO* INDUCED PLATELET AGGREGATES

A 0.130 ml (130 µl) collagen solution was added to 0.630 ml (630 µl) citrated blood samples obtained from pooled healthy rats. There were stirred at room temperature for 5 minutes and the platelet aggregate ratio was calculated.

BLOOD COAGULATION STUDY

Prothrombin Time (PT) test

Principle

The calcium in the whole blood bound by sodium citrate, thus preventing coagulation. Tissue Thromboplastin, to which calcium has been added, is mixed with the plasma, and the clotting is noted.

Methodology

The anticoagulated blood was centrifuge at 2,500 rpm for 10 minutes as soon as possible after collection. Two hundred microliter (200 µl) of thromboplastin –calcium mixture was added to a clean glass test tubes. The test tubes were pre-warmed in water bath at 37^{0}C for 1 minutes. The plasma sample was incubated for 3 minutes in water bath at 37^{0}C. One hundred microliter (100 µl) of plasma was added into the 200 µl of thromboplastin-calcium mixture in the water bath and simultaneously started the stop watch immediately.

The tube was removed from the water bath and gently tilted back and forth until a clot is formed, the timing is stopped immediately.

Activated Partial Thromboplastin Time (APTT) test

Principle

The calcium in a whole blood sample is bound by sodium citrate, thus preventing coagulation. The plasma, after centrifugation, contains all intrinsic coagulation factors except calcium and platelets. In the APTT test, partial thromboplastin (a phospholipid substitute) and an activator (to ensure maximum activation) are added to the plasma allowing the coagulation cascade to begin. During incubation, factors XII, PK, and XI are activated, building up the level of XIa in the reaction tube. Once $CaCl_2$ is added, the rest of the coagulation cascade is allow to continue and timing of the event is obtained. The time required for the plasma to clot is the activated partial thromboplastin time [91].

Methodology

The anticoagulated blood was centrifuge at 2,500 rpm for 10 minutes as soon as possible after collection. A sufficient amount of 0.025 M $CaCl_2$ was incubated at 37^{0}C. One hundred microlitre (100 µl) of plasma was added into a clean glass tube. One hundred microlitre (100µl) partial thromboplastin was pippeted into the test tubes containing the test plasma. The contents was mixed and incubated at 37^{0}C for 5 minutes. After 5 minutes, 100 µl of prewarmed $CaCl_2$ was added and the stop watch was started immediately. The tube was mixed and gently tilted every 5 seconds.

At the end of 20 seconds, the tube was removed and held such that the content of the tube can be monitored for formation of the clot. The tube was gently tilted back and forth until a clot was formed, at which point the timing was stopped immediately.

Thin blood film and staining

Principle

Lieshman stain is a romanowsky stain which contain eosin Y which is an acidic anionic dye and azure B and other thiazine dyes (derived from the oxidation, or polychroming of methylene blue) which are basic cationic dyes. When diluted in buffered water ionization occurs. Eosin stains the basic components of blood cells, e.g. hemoglobin stains pink-red and the granules of eosinophil stains orange-red. Azure B and other methylene-blue derived dyes, stains the acidic components of the cell. Nucleic acids and nucleoproteins, stain various shades of mauves-purple and violet, the granules of basophils stain dark blue-violet, and the cytoplasm of monocytes and lymphocytes stains blue or blue-grey [92].

Methodology

A thin film was made having head, body and tail. It was allowed to air dry. The film was flooded with Lieshman stain for 10 minutes. The film was diluted with buffered water to allow staining for 15 minutes.

The film was washed of gently with distilled water and it was allow to stand on a draining rack for the smear to dry. It was observed microscopically using X100 objective (oil immersion).

STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) software package was used for statistical analysis. The values obtained were expressed as mean ± standard deviation and compared using one way Analysis of Variance (ANOVA) and

TABLE 3

Mean ± SD of some hematological among study groups

	Group A	Group B	Group C	p-value	Remark
PCV	39.62 ± 2.52	40.81 ± 2.75	45.39 ± 3.70	0.010*	Significant
RBC	6.65 ± 0.47	6.87 ± 0.50	7.20 ± 0.84	0.372	Insignificant
WBC	13.06 ± 6.23	11.26 ± 1.02	9.22 ± 2.07	0.372	Insignificant
Platelet	828.25 ± 222.04	695.4 ± 231.79	808.85 ± 91.28	0.394	Insignificant

Data presented as mean ± standard deviation
**Significance at p-value<0.05*
Legend: PCV, Packed cell volume; WBC, white blood cells;

TABLE 4

Mean ± SD of platelet aggregation indices among study groups

	Group A	Group B	Group C	p-value	Remark
Platelet aggregation ratio	0.87 ± 0.09	1.27±0.66	1.27±0.66	0.466	Insignificant
PT	154.5 ± 19.09	153.00±22.62	78.33±53.67	0.043*	Significant
PTTK	159.5 ± 14.8	184.10 ± 10.32	148.33±13.61	0.012*	Significant

Data presented as mean ± standard deviation

**Significance at p-value <0.05*

Legend: PT, prothrombin time; PTTK, partial thromboplastin time with kaolin.

TABLE 5

Mean ± SD multiple comparison of platelet aggregation indices of group A compared to B and C

		Group A	Group B	Group C
Platelet aggregation ratio		0.87± 0.09	1.27 ± 0.66	1.27 ± 0.66
	p-value		0.656	0.774
PT		154.5 ±19.09	153.00 ± 22.62	78.33 ± 53.67
	p-value		0.999	0.116
PTTK		159.5±14.8	184.10 ± 10.32	148.33 ± 13.61
	p-value		0.115	0.619

Data presented as mean ± standard deviation
**Significance at p-value <0.05*

Legend: PT, prothrombin time; PTTK, partial thromboplastin time with kaolin

TABLE 6

Mean ± SD multiple comparison of platelet aggregation indices between group B and C

	Group B	Group C	p-value
Platelet aggregation ratio	1.27 ± 0.66	1.27 ± 0.66	0.98
PT	153.00 ± 22.62	78.33 ± 53.67	0.05*
PTTK	184.10 ± 10.32	148.33 ± 13.61	0.014*

Data presented as mean ± standard deviation

**Significance at p-value<0.05*

Legend: PT, prothrombin time; PTTK, partial thromboplastin time with kaolin

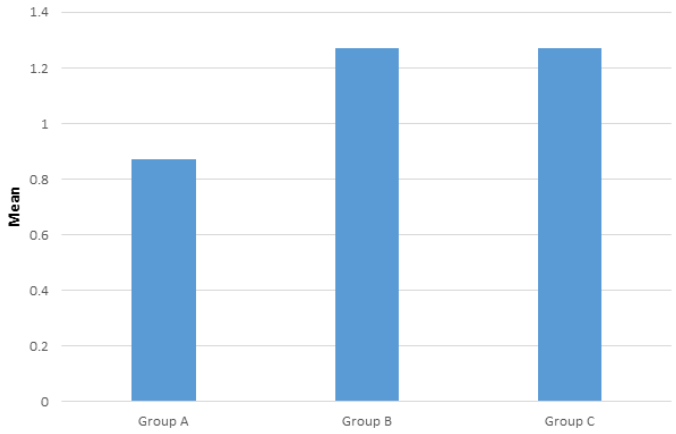

Figure 2) *Mean distribution of platelet aggregation ratio among study groups.*

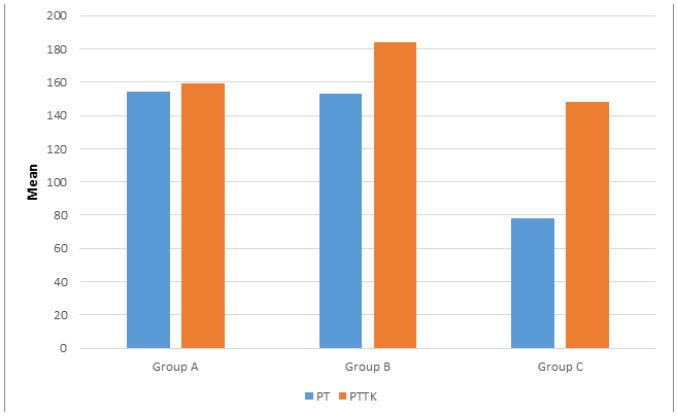

Figure 3) *Mean distribution of clotting profile parameter among study groups.*

the Significance was measured at p<0.05.

RESULTS

A total of 30 aged female wistar rats were used in this study comprising of three groups. The three groups were Group A; negative control, Group B; positive control, and Group C as the experimental group. Hematological parameters such as PCV, WBC and platelet count as well as PT and PTTK were analyzed and compared across all the study groups as shown in Table 3. There was no significant difference in the mean value of RBC, WBC, and platelet count between the study groups (p>0.05). However, there was a significant difference in the mean value of PCV when compared to control group (p>0.05).

As shown in Table 4 and Figure 2 there was no significant difference in the mean value of platelet aggregation ratio. However, there was a significant difference in PT and PTTK between the study groups (p<0.05).

In Tables 5 and 6, and Figure 3 the post hoc analysis of variance in multiple comparison of platelet aggregation indices parameter among study groups when compared to group A. There was a significant difference in the mean value of PT, PTTK, and platelet aggregation ratio among study group. However, there was a significant difference in the mean value of PT and PTTK of group B when compared to C (p<0.05).

DISCUSSION

Aging is associated with an increased incidence of cardiovascular disease and thrombosis. Platelets play a major role in maintaining hemostasis and in thrombus formation, making them a key player in thrombotic disorders. Several lines of evidence support that platelets from older subjects differ in their function and structure, making platelets more prone to activation and less sensitive to inhibition. These age-related changes could lead to platelet hyperactivity and to the development of a prothrombotic state in advanced age. Platelet count is inversely associated with age.

Onions contains 89% water, 1.5% protein, and vitamins B1, B2, C, K, and E, along with calcium, potassium, magnesium, iron, phosphorus, zinc, sodium, selenium, rich in flavonoids such as quercetin and sulfur compounds, such as allyl propyl disulphide that have perceived benefits to human health. In addition, onions are rich in sulfur containing compounds mainly in the form of cysteine derivatives, viz. S-alkyl cysteine sulfoxides which are decomposed by the enzyme allinase into a variety of volatile compounds such as thiosulfinates and polysulfides during extraction. These compounds possess anti-diabetic, antibiotic, hypocholesterolaemic, fibrinolytic, and various other biological effects. *Allium* containing substances have antibiotic effects and antibiotics should enable the proliferation of circulating white blood cells considering that white blood cells function to protect the body from teratogens.

The mechanisms of action under-pining these diverse effects of plants extracts on hematological parameters remain disjointed and relatively poorly understood. Nevertheless, postulated mechanisms of action supposedly revolve around their stimulatory effects on some cytokines, their role in iron bio- availability, and presence of vitamins, amino acids and phytochemicals.

Onion compounds seem to have a stimulatory effect along particular pathways on some hematopoietic growth factors (cytokines) which interacts with specific receptors on the surface of hematopoietic cells, regulating the proliferation and differentiation of progenitor cells and maturation and functioning of mature cells [93].

The outcome of this study showed that in the hematological parameters analyzed, there was a significant difference in the mean value of PCV of the subjects when compared to the control group. The increase in PCV level of group C compared to the control groups is in accordance with the work done by Salah, Enitan and Meraiyebu but however, contradicts the work done by Barnerjee and Maulik and Adebolu, who reported a significant decrease in PCV in the treated rats compared to control [94-96].

There is a non-significant increase in mean red blood cell count of the test group compared to the control groups. This is in concordance with the work done by Enitan and Meraiyebu, where an increase in red blood cell count was observed in the group administered with onion than those not administered with it, this may be due to onion having a stimulatory effect on some cytokines, their role in bio-availability and presence of some vitamins, essential amino acids and phytochemicals. This is also in discord with the outcome of the work done by Ugwu and Omale who reported a non-significant decrease in RBC count [97].

The mean total white blood cell count of the group administered with onion decreases compared to the control group (Normal feed). This is in line with the work done by Micheal, Ugwu and Omale and Meraiyebu, who all reported a decrease in the total white blood cell count of the test group compared to the control group but this is in contrast with the outcome of the work done by Enitan [98].

CONCLUSION

In conclusion, the results obtained from this study showed that oral administration of *Allium cepa* (White onion bulb) on collagen-induced platelet aggregation in aged-female wistar rats resulted in an inhibition of platelet aggregation. It also shows a significant increase in their PCV which may suggest that *Allium cepa* (White onion bulb) possess erythropoietin stimulating activity. Therefore Alternate hypothesis is accepted.

RECOMMENDATION

This work will serve as a foundation for further studies in the following areas to unravel some observations pointed out in this work. The use of aggregometer to measure the adhesive aggregation of platelets and to study the platelets. The mechanism through which *Allium cepa* (White onion bulb) brings about long claws and undesirable circular motion. The phytochemical analysis of *Allium cepa* (White onion bulb) and their effect on hemopoietic activities.

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