SCIENCE SET
OPEN ACCESS PUBLISHERS

Review Article

Journal of Clinical and Biomedical Advances

The Effects of Aqueous Extract of Garlic (Allium sativum) on the Histomorphology of the Coronary Artery of Adult Female Swiss Albino Rats

Ojo Gideon B[1, 3*], Adefusi Temiloluwa O[1], Balogun A Olubunmi[1], Ojo Femi T[2] and Dawodu Simon E[3]

[1]*Anatomy Program, College of Health Sciences, Bowen University, Iwo, Nigeria*
[2]*Department of Biology, School of Science, Federal College of Education Special, Oyo, Nigeria*
[3]*Department of Medical Biology and Genetics, Near East University, Lefkosa, Cyprus*

[*]Corresponding author: Ojo Gideon B, Anatomy Program, College of Health Sciences, Bowen University, Iwo, Nigeria.

Submitted: 11 October 2023 **Accepted:** 18 October 2023 **Published:** 24 October 2023

Citation: *Ojo Gideon B, Adefusi Temiloluwa O, Balogun A Olubunmi, Ojo Femi T and Dawodu Simon E (2023) The Effects of Aqueous Extract of Garlic (Allium sativum) on the Histomorphology of the Coronary Artery of Adult Female Swiss Albino Rats. J Clin Bio Med Adv 2(4), 01-09.*

Abstract

Garlic has been widely utilized as a curative material for treating many ailments and infections; however, its usefulness has been overused and its adverse effects have been disregarded. The purpose of this study is to determine the negative effects of too much garlic on the coronary arteries which transport blood to the cardiac muscles, allowing all parts of the heart to operate properly.

Group A received normal saline, Groups B, C, and D divided into subgroups 1 and 2 received different dosages of raw aqueous garlic extract below the (LD50) of garlic. Groups B, C, and D rats received 10 - 15 mg/100g, 20 - 25mg/100g, and 28 - 30mg/100g body weight of extracts respectively. Before administration, each extract was dissolved in 1ml of oral saline. Subgroups 1 and 2 received treatment for 7 and 10 days, respectively. The animals were weighed, sacrificed, the hearts were taken, and histological sections of coronary arteries produced for Hematoxylin and Eosin stain and Verhoeff Van Gieson stain.

They were examined under a microscope and photomicrographs were taken. Statistical analyses were carried out using one-way analysis of variance (ANOVA). At the P 0.05 level, values were considered substantially different.

Results showed the severity of these effects were observed with increased doses of excess garlic extract, validating the possibility of serious risks with excessive garlic use. These effects included the accumulation of collagen fibers on the adventitia of the coronary arteries, the buildup of plaque in the arteries' lumen, and anteromedial degeneration.

Keywords: Garlic, Coronary Artery, Extract, Collagen Fibers.

Introduction and Background

Allium sativum L. commonly known as garlic is a species in the onion family Alliaceae. It has a characteristic pungent spicy flavor that mellows and sweetens considerably with cooking. It could either be eaten raw or cooked, or has been used throughout recorded history because of its potential medical properties [1, 2]. Garlic is rich in antioxidants, which help destroy free radicals – particles that can damage cell membranes, interact with genetic material, and possibly contribute to the aging process as well as the development of a number of diseased conditions, including cardiovascular diseases (which would be extensively considered here) and cancer [1, 2].

The leaves of garlic plants are neither inflated like onion leaves nor tubular like those of bunching onions. Instead, they are flat, with a crease down the middle and are held erect in two opposite ranks. Most varieties stand about 1 – 2 ft (0.3 – 0.6 m) tall at maturity. Garlic plants produce an underground bulb that usually is divisible into 4 – 20 segments, called cloves [3].

Allicin is the main constituents of Allium sativum; it is very reactive in lowering serum cholesterol level. The transformation of allicin into the biological active allicin molecule upon crushing of a garlic clove is extremely rapid [4, 5]. Diallyl disulfide (DADS), an active constituent of garlic is known for its anti-hyperlipidemic properties. However, a study reported that garlic powder preparation did not significantly affect plasma lipid levels [6].

Protection from vampires is just one of the many uses of garlic. Until quite recently, most civilizations used it medicinally and only their poor ate it, while the priests and upper-class citizens scorned its strong odor [3].

The coronary arteries, the first branches of the aorta, supply the myocardium and epicardium. The right and left coronary arteries arise from the corresponding aortic sinuses at the proximal part of the ascending aorta, just superior to the aortic valve, and pass around opposite sides of the pulmonary trunk. The coronary arteries supply both the atria and the ventricles; however, the atrial branches are usually small and not readily apparent in the cadaveric heart. The ventricular distribution of each coronary artery is not sharply demarcated [7]. Blood flowing in the coronary arteries supplies the cardiac muscles and prevents necrosis in the cardiac tissues.

Aims and Objectives

1. To successfully prepare aqueous garlic extract.
2. To examine the effects of varying doses of garlic on the microanatomy of the coronary artery.
3. To determine the changes in the components of the coronary vessels.

Justification

Allium sativum (Garlic) is used to treat various disease cases especially cardiovascular diseases which has led to its widespread prescription and use. Often times, garlic has been used indiscriminately and without caution which may cause series of adverse effects on the carefree users. This research work seeks to reveal the dangerous effects of high dosage of garlic on the coronary vessels surrounding the heart.

Literature Review

Garlic (Allium Sativum)

Allium sativum, also known as garlic, is a plant in the onion genus that can be classified as a food or a medicinal herb. It is related to the onion, shallot, leek, chive, and rakkyo [8, 9]. It is a common plant product grown all over the world [8]. Garlic is native to Central Asia, and has long been a staple in the Mediterranean region, as well as a common condiment in Asia, Africa, and Europe [10]. It was utilized for food flavoring and traditional medicine by the ancient Egyptians [11]. Ayu is a Yoruba word, Tafarnuwa is a Hausa word, Da Suan is a Chinese word, and Skorda is a Greek word.

Uses of Garlic

Throughout history, garlic has played vital dietary and therapeutic roles. The Avesta, a compilation of Zoroastrian Holy Scriptures that was most likely composed in the sixth century BC contains some of the first references to this medicinal herb [12]. Garlic was also used as a medication by the Sumerians and the ancient Egyptians. There is some evidence that garlic was offered to athletes during the first Olympics in Greece to increase stamina [13]. Garlic was advised by ancient Chinese and Indian medicine to improve breathing and digestion, as well as to treat leprosy and parasitic infection [14]. Garlic was also used in the treatment of several ailments during the medieval times. It was suggested that garlic be used to cure arthritis, toothache, persistent cough, constipation, parasitic infestation, snake and insect bites, gynecologic illnesses, and infectious ailments (as an antibiotic). With the advent of the Renaissance, Europe paid special attention to the health advantages of garlic. Garlic has piqued the interest of modern medicine due to popular belief in its health-promoting properties. Garlic preparations are sold alongside prescription medications in various Western nations [15]. Garlic has significant epidemiologic evidence supporting its therapeutic and preventative functions. Several experimental and clinical studies demonstrate that garlic and its derivatives have numerous beneficial effects. These effects have generally been related to the reduction of risk factors for cardiovascular disease, the decrease of cancer risk, the antioxidant effect, the antimicrobial action, the promotion of detoxification of foreign chemicals, and hepatoprotection [16].

Biological Activities of Garlic

Allium sativum is an important plant which has been found to have the following effects;

Effects on Cardiovascular Diseases

Garlic consumption has been shown to reduce blood pressure, prevent atherosclerosis, cut serum cholesterol and triglycerides, inhibit platelet aggregation, and increase fibrinolytic activity [17].

Blood Pressure Lowering Effect: Several clinical trials have shown that garlic reduces blood pressure in more than 80% of patients with high blood pressure [18]. Garlic is thought to reduce blood pressure because the nitric oxide (NO) system is more active than the Renin-Angiotensin-Aldosterone System. Both NO and H2S were evaluated in diabetic rats and it was demonstrated that prolonged administration of garlic normalized both gaseous molecules and lowered BP [19].

It was discovered that both aged and raw garlic produced the same quantity of NO, showing that nitric oxide synthase (NOS) is the key to decreasing blood pressure. The activation of NOS was increased by both types of garlic. An increase in NO causes the blood arteries to relax, resulting in a reduction in blood pressure. However, studies have indicated that both AGE and RG require at least 3 - 4 weeks of daily administration before their activity is shown, implying that there may be a separate mechanism for Blood Pressure lowering [20].

Lipid Lowering Effect: Low Density Lipoprotein (LDL) oxidation was reduced and High-Density Lipoproteins (HDL) were elevated, which could be one of the protective mechanisms of garlic's favorable effects on cardiovascular health [21]. A meta-analysis of 39 primary studies on the effect of 2 months of garlic preparation administration on total cholesterol and triglycerides was done [22]. The findings reveal that garlic can reduce total serum cholesterol by 176 mg/dL and low-density lipoprotein cholesterol by 96 mg/dL in persons with high total cholesterol levels (>200 mg/dL). At the age of 50, an 8% reduction in total blood cholesterol is related with a 38% reduction in the incidence of coronary events. High - density lipoprotein cholesterol levels improved only marginally, and triglyceride levels were unaffected [22].

Anti-Atherosclerotic Effect: Garlic's ability to lower lipid content in artery membranes has been linked to its ability to prevent atherosclerosis. Allicin, S - allyl cysteine, found in aged garlic extract, and diallyl disulfide, found in garlic oil, are the primary chemicals responsible for the anti-atherosclerotic activity [23, 24].

Anti-Cancer Effect: Garlic-derived diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) have been found to have anticancer properties [25]. In U937 leukemia cells,

DATS cytotoxicity is mediated by the production of ROS (reactive oxygen species) and subsequent activation of the ROS-dependent caspase pathway [26]. DATS has been proven in vitro to inhibit apoptosis in many human cancer cell lines and to provide considerable protection against cancer in animal tumor models, including colorectal cancer [27]. Another study found that DADS therapy reduced tumor cell motility and invasion, acting as a dietary source to lower the risk of cancer spreading [28]. Allicin (diallyl thiosulfate), the most well-known physiologically active component of freshly crushed garlic extract, has been shown to inhibit colon cancer cell proliferation [29].

Anti-Inflammatory Effect: Garlic treatment effectively reduced liver inflammation and damage caused by Eimeria papillata infections in one trial [30]. Garlic oil's anti-inflammatory effect is primarily achieved through blocking the cytoskeleton's construction and disassembly processes [31]. According to one study, thiochromone, a Sulphur molecule derived from garlic, decreases neuroinflammation and amyloid genesis by inhibiting NF - B activity and hence could be used to treat inflammation-related neurodegenerative illnesses such as Alzheimer's [32].

Anti-Bacterial Effect: In vitro research has revealed that varying doses of garlic extract have antibacterial effects on human dental plaque microbiota [33]. Ciproflaxin and garlic extract synergize, but not ampicillin and garlic extracts [34]. Garlic cloves and ginger rhizomes extracted with 95% ethanol have been shown to have anti-bacterial action against multi-drug clinical pathogens and can be utilized to avoid drug resistant microbial illnesses.

Anti-Fungal Effect: Allicin (diallyl - dithiosulfinate), which is generated by the garlic enzyme alliinase from alliin, has been demonstrated to have broad antifungal specificity. One study found that allicin from garlic has antifungal action, specifically against Candida albicans [35]. Ajoene, another component of garlic, is responsible for many of the plant's pharmacological effects, particularly its antifungal impact [36]. In the treatment of mice intratracheally infected with Paracoccidioides brasiliensis, this chemical is more effective when combined with antifungal medications (sulfametoxazol/trimethoprim) [37].

Anti-Viral Effect: Human Cytomegalovirus (HCMV), Influenza B virus, Herpes simplex virus type 1, Herpes simplex virus type 2, Para influenza virus type 3, vaccinia virus, vesicular stomatitis virus, and human Rhinovirus type 2 are among the viruses that are sensitive to garlic extracts [38]. One study found that Allicin-containing supplements can help reduce common cold virus assaults [39]. In the case of HIV, ajoene is hypothesized to work by blocking integrin-dependent mechanisms [40].

Immunomodulatory Effect: Garlic extract inhibits the proliferation of interleukin (IL) - 2 and interferon (INF) - gene expression in activated cells in a dose - dependent manner [41]. Garlic extracts inhibited macrophage infection in vitro by inducing nitric oxide (NO) production [42]. Allicin can reduce immune-mediated liver damage in mice, likely due to its immunomodulatory effects on T cells and adhesion molecules, as well as its inhibition of NF - B activation [43].

Effect on Reproductive System: In a study conducted by Qian and colleagues, garlic delivery to rats resulted in a decrease in sperm quality and functionality. The amount and preparation of garlic will result in a decrease in specific seminal parameters. Garlic crude extract increases the percentage of empty seminiferous tubules. It has been found to cause membrane breakdown and irreversible immobilization of sperm while decreasing sperm viability [44]. Testicular morphological changes were observed in another investigation after male adult rats were administered 50mg kg - 1 garlic powder for 45 - 75 days [45]. In contrast to prior studies that indicated garlic had a deleterious impact on male reproductive function, Hammami and El May (2012) discovered that garlic relieved male sexual dysfunction. The key difference, and possibly the explanation for the disparities in outcomes, could be the absence of standardization between research models and the varying doses of garlic administered to test individuals [46].

Adverse and Toxic Effects of Garlic

Animal Studies: Higher concentrations of garlic extract have been shown to be clastogenic in mice, which was appreciably reduced at lower concentrations. Prolong feeding of high levels of raw garlic in rats has resulted in anemia, weight loss and failure to grow due to lyses of red blood cells. Raw garlic juice at high dose has resulted in death of rats due to stomach injury. Surviving rats exhibited swelling of the liver, hypertrophy of the spleen and adrenal glands, and the decrease of erythrocyte count with various morphological changes after 3 and 8 days. Aqueous garlic extract (200gm/drinking water) for 10 days exhibited significantly higher levels of aspartate aminotransferase (AST) due to liver injury [47].

The liver's histopathology revealed localized nonspecific damage with inflammatory cell infiltration in hepatocytes. Chen et al. (1999) found that giving rats fresh garlic homogenate for 7 days resulted in a significant decrease in liver catalase activity at doses of 2 and 4 gm/kg. A laboratory ultrastructural research demonstrated a severe loss of normal cellular architecture in the heart, liver, and kidneys following 30 days of feeding raw garlic homogenate at a level of 1000mg/kg/day [47].

Allicin (100mg/kg/day) fed to rats for 15 days increased the activity of liver lipase and alpha glucal phosphorylase while decreasing glucose-6-phophatase activity. The precise mechanism of garlic-induced changes in cell structure and function is unknown. Garlic powder has also been linked to some cases of poisoning. Chronic treatment of garlic powder (50 mg/day) inhibited spermatogenesis in rats. Garlic's anti-androgenic action is reflected in lower levels of sialic acid in the testes, epididymis, and seminal vesicles, as well as decreased leydig cell function [47]. Higher concentrations of garlic powder (200 mg/ml) or allicin isolated from garlic caused significant cell damage in the porta hepatis zone of isolated perfused rat liver, which were not detected at lower concentrations. Another in vitro investigation found that diallyl sulfide (an oxidized product of allicin) at 5mM dramatically reduced cell viability in the liver. Garlic oil at a level of 100mg/kg following a 24-hour fast has also been proved to be fatal. The cause of death appears to be acute pulmonary edema with significant congestion. Garlic oil and Diallyl sulfide (200mg/kg b.w.) greatly inhibited rat body weight gain [47].

Ajoene, a natural chemical generated from garlic and found in different types of garlic oil, is an inhibitor as well as a substrate

of human glutathione reductase and is believed to enhance oxidative stress in the cell. All of the toxicity reports mentioned above cannot be fully explained, but the sulphoxides present in garlic extract can spontaneously exchange with tri - table SH - groups of enzymes and other proteins in the body at physiological pH and temperature, inhibiting their activity. Garlic has been shown to inhibit alkaline phosphatase, papain, and alcohol dehydrogenase enzymes. Toxicity may be caused by enzyme interactions with garlic components [47].

Human Studies: Clinical investigations involving garlic and its preparations revealed relatively minimal negative effects. The majority of the reported side effects were generic. The most common complaint was gastrointestinal discomfort and nausea. The majority of these instances had allergic contact dermatitis, which has been described in people who work with garlic. There have also been isolated cases of allergic conjunctivitis, rhinitis, and bronchospasms caused by garlic inhalation or consumption [47].

Bloating, headache, dizziness, and intense perspiration were also noted as side effects. Ingestion of fresh garlic and garlic powder may interact with anticoagulants or platelet aggregation inhibitors, resulting in a potentially fatal bleeding in one case [47].

Research Methodology

Extract Preparation: Fresh garlic bulbs (A. sativum L.) were purchased from Bodija market in Ibadan, Oyo state, Nigeria. If was taken to the department of plant biology, Bowen University, Iwo, Osun state, Nigeria where it was identified and a sample placed at their herbarium. The garlic bulbs were peeled and were dried and washed thoroughly, the total weight of peeled garlic was about 2500g. The peeled garlic bulbs were blended using a home blender (electronic model blender) to obtain an aqueous extract from the garlic bulbs. The total weight of garlic (2500g) was blended to obtain aqueous extract. The weight of the blended garlic was about 2842g.

Sieving was done to extract the pure aqueous garlic extract and the residue was mashed to extract any other liquid left in the blended garlic. Using a filter paper, funnel and graduated container. The clear aqueous extract gotten was about a litre. The aqueous extract was kept in the graduated container and put in the refrigerator while the residue was disposed after the filtration.

Apparatus: Knife, weighing balance, glass beaker, sensitive weighing balance, home blender, refrigerator, plastic cages, cannula, 5ml syringe, slide, microscope, paraffin wax, sterile lancet, specimen bottle.

Animal Care and Management

40 Swiss albino rats with weight ranging between 100 – 150g were used for the experiment. The animals were purchased from the department of Animal science, Faculty of Agriculture, Bowen University, Iwo and kept in cages in the animal house located at the College of health sciences, Bowen University, Iwo, Osun state and acclimatized for one week and four days. The animals were fed with standard pellet feed and given water liberally, the rats were kept in a well-ventilated room at room temperature.

Grouping, Administration of Extract and Animal Sacrifice

The animals were divided into 4 groups A, B, C and D with 10 rats in each group. Group A was the control group, groups B, C and D were the experimental groups. Groups B, C and D were divided into subgroups B1, C1 and D1 and subgroups B2, C2 and D2.

Group A rats were given a single dose of normal saline orally (1ml per animal).

Group B, C and D rats were administered orally with garlic extract that revolved around the lethal dose (LD50) of garlic in rats which is between 20mg– 30mg/ml/100g body weight.

Group B rats were given garlic extract that was below the lethal dose (LD50) with subgroup B1 given for 7days and subgroup B2 given for 10days. The garlic administered was measured and given according to relative body weight causing the garlic dosage to range between 10mg-15mg/100g body weight of garlic extract in 1ml of normal saline. Each of them was given 1ml of the raw aqueous garlic extract in normal saline using an oral cannula.

Group C rats were given garlic extract that was about the lethal dose (LD50) with subgroup C1 given for 7days and subgroup C2 given for 10days. Garlic administered was measured and given according to relative body weight causing the garlic dosage to range between 20mg-25mg/100g body weight of garlic extract in 1ml of normal saline. Each of the rats was given 1ml of the raw aqueous garlic extract in normal saline using an oral cannula.

Group D rats were given garlic extract that was a little above the lethal dose (LD50) of garlic with subgroup D1 given for 7days and subgroup D2 given for 10days. Garlic administered was also measured and given according to relative body weight to range between 28mg-30mg/100g body weight of garlic extract in 1ml of normal saline. Each of the rats was given 1ml of the raw aqueous garlic extract in normal saline using an oral cannula.
After seven days, five animals from the control group along with all the animals in subgroups B1, C1 and D1 were sacrificed, after anaesthetizing with chloroform/diethyl ether in an air tight jar. The heart of each animal was harvested and weighed using a sensitive digital weighing balance. The harvested hearts were then placed in a specimen bottle containing neutral buffered formalin.

How Relative Weight of Organ was calculated:

$$\text{Relative Weight of Heart} = \frac{\text{Weight of Heart}}{\text{Weight of Animal}} \times 100$$

Histological Procedure

Histological study was carried out following fixation in 10% neutral buffered formalin solution (NBF Solution), dehydration, infiltration, embedding, sectioning and staining using the routine Hematoxylin and Eosin to reveal the myocardium and blood vessels in the heart and Verhoeff Van Gieson stain to reveal the connective tissues of the heart such as the intercalated disks and gap junctions. Permanent photomicrographs were taken using a binocular microscope with the third eye piece for digital camera attachment. The camera attached to the microscope was connected to a personal computer for color adjustment, also brightness and contrast. Images viewed through the eyepiece were adjusted to the best resolution.

Statistical Analysis was done and values were expressed in form of their means and standard error of the mean (SEM). One-way analysis of variance (ANOVA, GraphPad Prism 7.00) was used to determine differences in means between groups. Values were considered significantly different at the level of $P < 0.05$.

Results

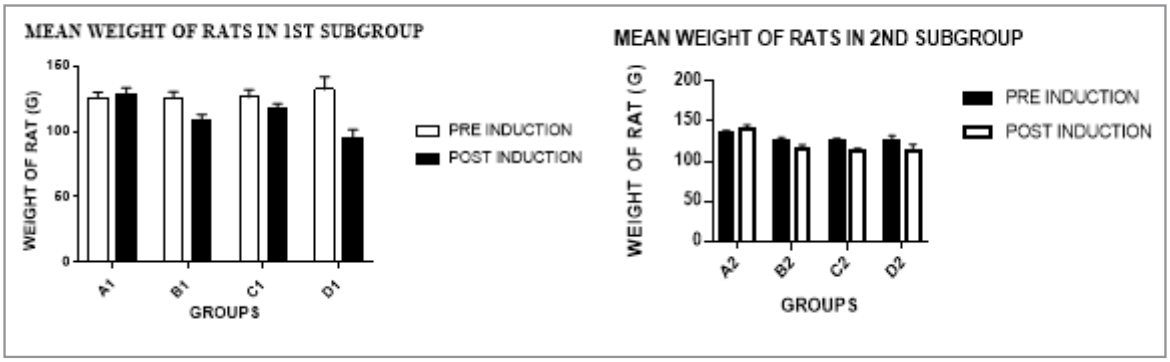

CHART 1: The mean body weight of rats before and after induction with excess amount of garlic revolving around the lethal dose. Group A1 – Control group, Group B1 – Below lethal dose, Group C1 – Lethal dose, Group D1 – Slightly above lethal dose. The rats in Groups B1, C1 and D1 reduced in weight after induction with their various doses of garlic while that of Control group A1 increased because it was not induced with garlic and the mean body weight of rats before and after induction with excess amount of garlic revolving around the lethal dose. Group A2 – Control group, Group B2 – Below lethal dose, Group C2 – Lethal dose, Group D2 – Slightly above lethal dose. Rats in Groups B2, C¬2 and D2 decreased in weight after induction with their different doses of garlic while the rats in Control group A1 increased in weight because they were not induced.

CHART 1: Histological Analysis

Table 1: MEAN VALUE OF BODY WEIGHT IN RATS FOR SUBGROUP 1 AND 2

Group	Body Weight Before Induction	Body Weight After Induction	Group	Body Weight Before In-duction	Body Weight After Induction
A_1	125.6 ± 4.214	128.4 ± 4.946	A_2	134.0 ± 4.370	139.6 ± 5.776
B_1	125.2 ± 5.152	109.0 ± 3.975*	B_2	125.2 ± 4.352	115.8 ± 4.521*
C_1	126.0 ± 5.891	118.2 ± 3.169	C_2	124.2 ± 4.329	112.0 ± 4.012*
D_1	132.0 ± 9.566	95.0 ± 6.588*	D_2	125.6 ± 6.313	112.0 ± 9.268*

Data are presented as mean ± standard error of mean. Asterisk denotes significant p values. *Significant $p < 0.05$ from control group. The initial mean body weight of rats in groups B1, C1 and D1 do not differ significantly from the initial weight of rats in the control group A1 by $p < 0.05$ but the mean body weight of rats in groups B1 and D1 after induction differ significantly from the control group A1 by $p < 0.05$ while that of group C1 ¬after treatment does not differ significantly from the control group A1 by $p < 0.05$.

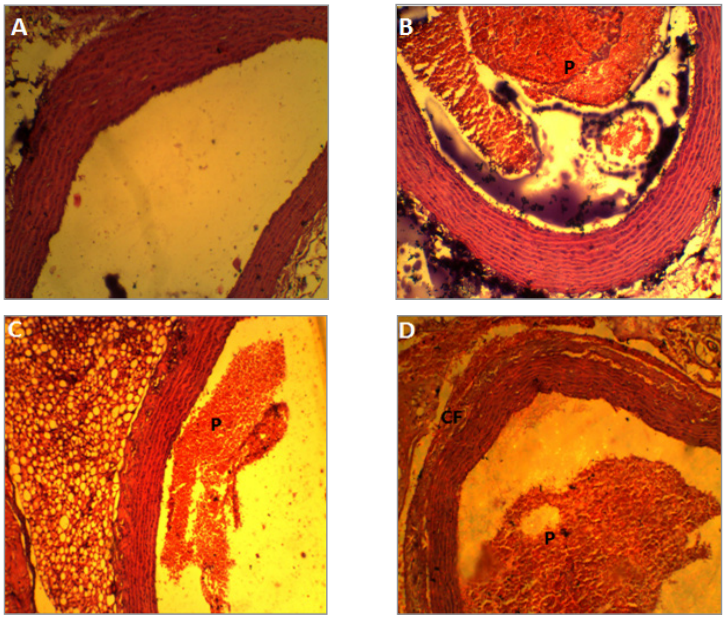

PLATE 1: Photomicrograph of coronary artery after inducing with excess garlic for 7 days. H & E. MagX100.

a) Coronary artery from Control rat shows no abnormal histomorphology.
b) Coronary artery from rat given below lethal dose of garlic for 7 days shows formation of acid stained plaque (P) inside the lumen of the artery
c) Coronary artery from rat given lethal dose of garlic for 7 days also shows formation of acid stained plaque (P) in the lumen of the artery.
d) Coronary artery from rat given above lethal dose of garlic for 7 days shows increased collagen fibers (CF) on the outer layer of the artery and acid stained plaque (P) in the lumen of the artery.

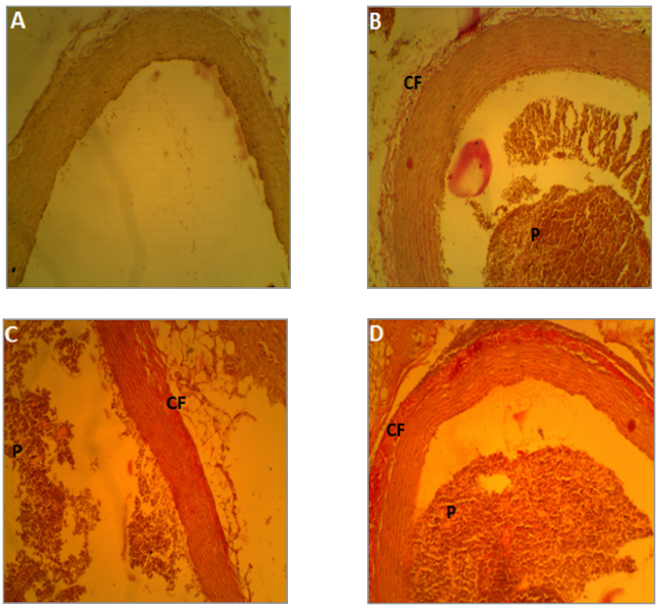

PLATE 2: Photomicrograph of coronary artery after inducing with excess garlic for 7 days. VVG (Special Stain). MagX100.
a) Coronary artery from Control rat shows no abnormal histomorphology.
b) Coronary artery from rat given below lethal dose of garlic for 7 days shows little increase in adventitial collagen fibers (CF) and plaque (P) in the lumen of the artery.
c) Coronary artery from rat given lethal dose of garlic for 7 days shows buildup of plaque (P) in the lumen and little amount of collagen fibers (CF) on the adventitia.
d) Coronary artery from rat given above lethal dose of garlic for 7 days shows intima degeneration, increased amount of adventitial collagen fibers (CF) and formation of plaque (P) in the lumen of the artery.

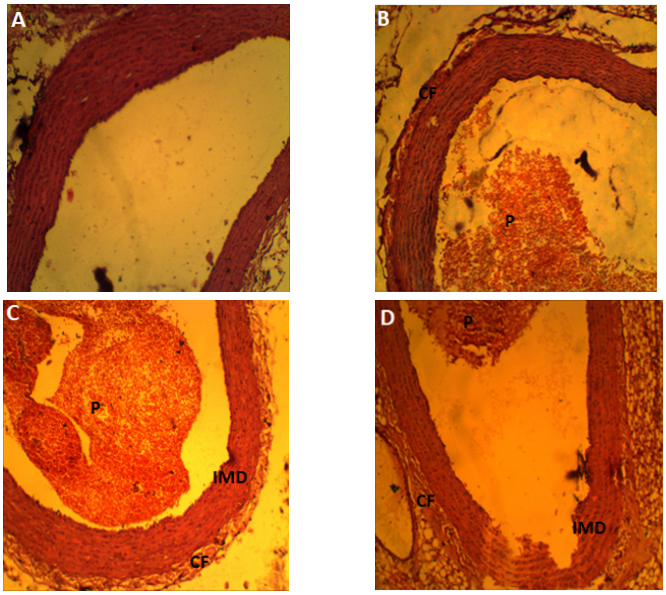

PLATE 3: Photomicrograph of a coronary artery after inducing with excess garlic for 10 days. H & E. MagX100.
a) Coronary artery from Control rat shows no sign of abnormal histology
b) Coronary artery from rat given little below lethal dose of garlic for 10 days shows little increase in the amount of collagen fibers on the adventitial layer of the artery and plaque buildup in the lumen of the artery.

c) Coronary artery from rat given lethal dose of garlic for 10 days shows thicker layer of collagen fibers on the adventitia, mild intimomedial degeneration (IMD) and plaque in the lumen of the artery
d) Coronary artery from rat given above lethal dose of garlic for 10 days shows severe intimomedial degeneration and collagen accumulation on the adventitia with plaque in the lumen of the artery.

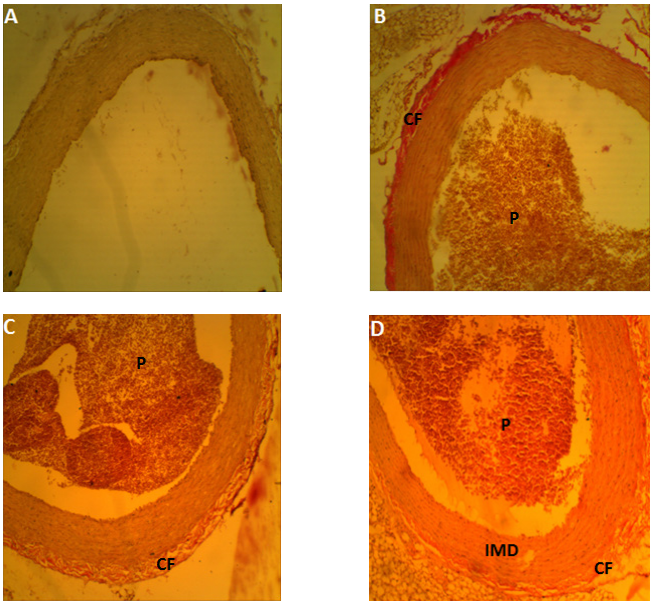

PLATE 4: Photomicrograph of coronary artery after inducing with excess garlic for 10 days. VVG (Special Stain). MagX100.
a) Coronary artery from Control rat shows no abnormal histomorphology.
b) Coronary artery from rat given little below lethal dose of garlic for 10 days shows collagen fibers (CF) accumulation on the adventitial layer and plaque (P) in the lumen of the artery
c) Coronary artery from rat given lethal dose of garlic for 10 days shows increased collagen fibers (CF) on the adventitia and plaque in the lumen
d) Coronary artery from rat given above lethal dose of garlic for 10 days shows excess collagen fibers (CF) on the adventitial layer, plaque (P) in the lumen and intimomedial degeneration (IMD).

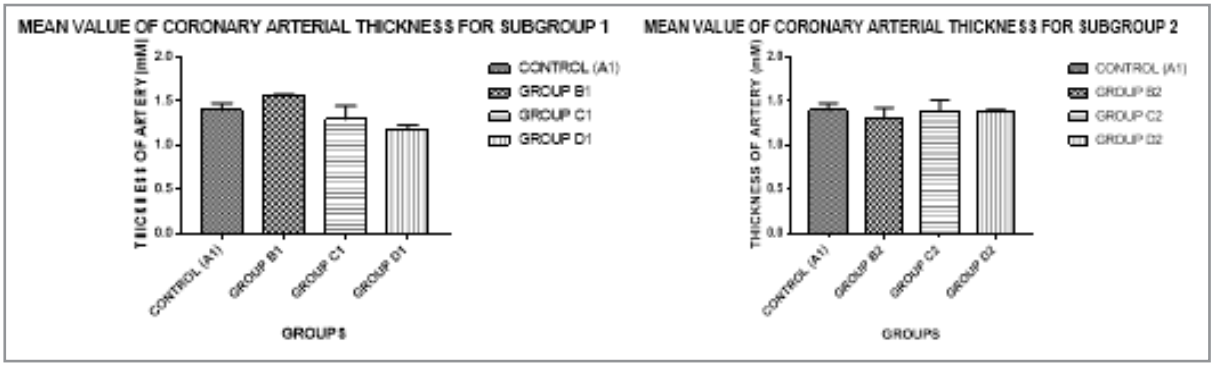

Chart 2: This graph shows the variation in mean thickness of the arterial walls of the rats in different groups in subgroup 1& 2

Table 2: THICKNESS OF CORONARY ARTERIES MEAN VALUE OF CORONARY ARTERIAL THICKNESS FOR RATS IN SUBGROUP 1 AND 2.

Group	Mean Thickness (mM)	Group	Mean Thickness (mM)
A_1 (Control)	1.400 ± 0.046	A_2	1.400 ± 0.046
B_1	1.561 ±0.010	B_2	1.316 ± 0.065
C_1	1.296 ± 0.089	C_2	1.388 ± 0.075
D_1	1.179 ± 0.029*	D_2	1.386 ± 0.011

Data are presented as mean ± standard error of mean. Asterisk denotes significant p values.
* Significant $p < 0.05$ from control group. Only arterial walls from group D1 rats show a significant change (reduction) in the thickness of the arteries in comparison to the control group.

Discussion, Conclusion and Recommendations

In this study, the administration of various doses of excess aqueous garlic extract was found to result in weight loss of the experimental animal which is similar to the research work done in an article published by [47]. In the coronary arteries, it resulted in the increase in collagen fiber layers on the tunica adventitia which could have resulted in arterial stiffness; a situation where several degenerative changes in the arteries contribute to increased stiffening over time, including mechanical fraying of lamellar elastin structures within the wall of the arteries, increase in the content of arterial collagen proteins, partially as a compensatory mechanism against the loss of arterial elastin and partially due to fibrosis [48]. It also caused a decrease in the diameter of the lumen of the arteries, intimal degeneration; characterized by aneurysm formation following mucin deposition in the intima and media with elastic tissue degeneration and accumulation of dark staining masses in the lumen of the arteries such as is present when an animal has dyslipidemia which is a major marker for atherosclerosis [49]. The thickness of the arteries was affected and would have been a great study point but due to the fact that sectioned slides were not sectioned in the same orientation and could not provide the same artery for viewing on all sectioned slides, the thicknesses could not be accurately compared. All of the histomorphological changes that occurred in the coronary arteries increased in severity as the dosage got more concentrated which implies that coronary arteries from rats in groups B showed milder disruptions while those in groups D showed the most severe disruptions in histomorphological architecture.

During the course of the research work, it was noticed that the experimental rats seemed to begin to adapt to the aqueous garlic extract after the first 7 days of administration because there was a little increase in weight in rats sacrificed after 10 days than in rats sacrificed after 7 days, this could be due to the degeneration of Allicin because the choky smell in the garlic extract reduced even though it was still somewhat present. All the noticeable changes in histomorphology of the coronary arteries help point out that despite all the health benefits garlic or garlic supplements could provide, excess garlic could be very dangerous to the vascular system besides other adverse effects that occur in the body such as the swelling of the liver, hypertrophy of the spleen and adrenal glands and decrease in erythrocyte count with various morphological changes after 3 to 8 days [47].

It was noted during the course of the research work that no research has been done in this area regarding the adverse effects of beneficial plant extracts and its effect on the arterial system before so the results are prone to further evaluation and correction.

This study has shown that despite the numerous benefits of garlic, there are a lot of hidden dangers attached to the excessive use of garlic and garlic supplements and this study revealed findings about the adverse effects that various excess doses of aqueous garlic extract could cause in the blood vessels using the coronary arteries as a case study. These adverse effects could lead to tissue necrosis, degeneration of cells and dysfunction or death of the organ supplied by the blood vessel and death of the organism altogether. So, avoid excess garlic and stay healthy!

This study revealed findings about the adverse effects that various excess doses of aqueous garlic extract could cause in the blood vessels using the coronary arteries, Further studies should be carried out on the side effects of garlic extracts such as garlic oil, aqueous garlic extract, garlic powder or methanolic extract on the blood vessels and for longer periods of time as the research work was carried out for a maximum of ten days. I'll also recommend that the experiment should be carried out again so as to validate my results and provide more sources for reference in case of further research work in this area.

References

1. Katzer G (2005) Spice Pages. Garlic (Allium sativum).
2. Tattelman E (2005) Health effects of Garlic. AmFam Physician 72: 103-106.
3. Metwally EI, El-Denary ME, Omar AMK, Naidoo Y, Dewir YH (2012) Bulb and vegetative characteristics of garlic (Allium sativum L.) from in vitro culture through acclimatization and field production African. Journal of Agricultural Research 7: 5792-5795.
4. Kerst I, Keusgen M (1999) Stabilization and pharmaceutical use of allinase. Pharmizie 54: 289-293.
5. Oyebadejo, Samson, Eno-obong Bassey, Ajayi Oyewunmi, Victor Archibong and Ekaete Usoro (2014) Histopathological study of the liver of alloxan induced diabetic rats and macerated Allium sativum (garlic) Ameliorative effect. Asian Journal of Biomedical and Pharmaceutical Sciences 4:72-77.
6. Christopher DG, Chattergee LM, Carlson JJ (2001) Effect of garlic preparation on plasma lipid levels in moderator hypercholesteromia adults. Atherosclerosis 154: 213-220.
7. Moore Keith L, Dalley Arthur F, Agur Anne MR (2014) Clinical Oriented Anatomy. 7th ed. Philadelphia, Pa Lippincott Williams and Wilkins 145-148.
8. Eric Block (2010) Garlic and other Alliums. The lore and the Science. Royal Society of Chemistry. 190-197.
9. Beaumont PDA, Moneret-Vautrin C, Dzviga JL (2013) Major Food Anaphylaxis with Onion and Garlic: Insufficiency of prick tests and allergen-specific ages. About five cases / Food Anaphylaxis onion and garlic. Lack of Skin Prick tests and specific ige to allergenic source. 5 boxes Rev Fr Allergol 53:446-449
10. Marion Eugene Ensminger, Audrey H Ensminger (1994) Foods & Nutrition Encyclopedia. CRC Press ISBN 0-8493-8980-1: 750.
11. Gualtiero Simonetti (1991) SIMON & SCHUSTER'S GUIDE TO HERBS AND SPICES (Nature Guide Series) ISBN 0-671-73489-X.
12. Dannesteter J (2003) AVESTA: AVESTA: VENDIDAD (English): Fargard 20. American Edition, New York, The Christian Literature Company.
13. Lawson LD, Bauer R (1998) Garlic A Review of its medicinal effects and indicated active compounds. In: Phytomedicines of Europe. Chemistry and Biological activity Washington DC. American Chemical Society 177-209.
14. Rivlin RS (1998) Patient with hyperlipidemia who received garlic supplements. Lipid management. Report from the Lipid Education Council 3: 6-7.
15. Bayan L, Koulivand P H and Gorji A (2014) Garlic a review of potential therapeutic effects. Avicenna Journal of Phytomedicine 4: 1-14.
16. Aviello G, Abenavoli L, Borelli F, Capasso R, Izzo AA, et al. (2009) Garlic empiricism or science? Natural Product Community 4: 1785-1796.
17. Chan JY, Yuen AC, Chan RY, Chan SW (2013) A Review of the cardiovascular benefits and antioxidant properties of allicin. Phytotherapy Research 27: 637- 646.
18. Stabler SN, Tejani AM, Huynh F and Fowkes C (2012) Gar-

lic for the prevention of cardiovascular morbidity and mortality in hypertensive patients. Cochrane database systemic review 8.
19. Elkayam A, Mirelman D, Peleg E, Wilchek M, Miron T, et al. (2003) The effects of allicin on weight in fructose induced hyperinsulinemic, hyperlipidemic, hypertensive rats. American Journal of Hypertension 16: 1053-1056.
20. Carlo Valente, Guillaume A, Stefan S Du Plessis (2014) Garlic and its Effects on Health with Special Reference to the Reproductive system.
21. Rahman K, Lowe GM (2006) Significance of Garlic and its constituents in cancer and cardiovascular disease. Journal of Nutrition 136: 736-740.
22. Reid K, Frank OR and stocks NP (2013) Aged garlic extract reduces garlic supplements. Lipid management. Report from the Lipid Education Council 3: 6 -7.
23. Gebhardt R, Beck H (1996) Differential inhibitory effects of garlic – derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte culhue. Lipids 31: 1269-1276.
24. Yu -Yan Y, Liu L (2001) Cholesterol lowering effect on garlic extracts and organosulfur compounds. Human and Animal studies. Journal of Nutrition 131: 989 – 993.
25. Lai KC, Kuo CL, Ho HC, Yang JS, MaC. Y,Lu H. F (2012) Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells in vitro and in vivo. Phytomedicine 19: 625-630.
26. Choi YH, Park HS (2012) Apoptosis induction of U937 human leukemia cells by diallyl trisulfide induces through generation of reactive oxygen species. Journal of Biomedical Science 19: 50.
27. Yu CS, Huang AC, Lai KC, Huang YP, Lin MW et al (2012) Diallyl trisulfide induces apoptosis in human primary colorectal cancer cells. Oncol Rep 28: 949- 954.
28. Park HS, Kim GY, Choi IW, Kim ND, Hwang HJ, Choi YW (2011) Inhibition of matrix metalloproteinase activities and tightening of tight junctions by diallyl disulfide in AGS human gastric carcinoma cells. Journal of Food Science 76: 105-111.
29. Bar-Chen W, Golan T, Peri I, Ludmer Z, Schwartz B (2010) Allicin purified from fresh garlic cloves induces apoptosis in colon cancer cells via Nrf2. Nutrition Cancer 62: 947-957.
30. Ban JO, Lee, DH, Kim MS, Cho et al (2012) Antiobesity effects of a sulphur compound thiacremonone mediated via down-regulation of serum triglyceride and glucose levels and lipid accumulation in the liver of db/db mice. Phytotherapy Research 10: 3729.
31. Dhkil MA, Abdel-Baki AS, Wunderlich F, Sies H, Al-Quraishy S (2011) Anticoccidial and anti – inflammatory activity of garlic in murine Eimeria papillata infections. Veterinary Parasitology 175: 66-72.
32. Lin GH, Lee YJ, Choi DY, Han SB, Jung JK (2012) Anti – amyloidogenic effect of thiacremonone through anti – inflammation in vitro and in vivo models. Journal of Alzheimer's disease 29: 659-676.
33. Houshmand B, Mahjour F and Dianat O (2013) Antibacterial effect of different concentrations of garlic (Allium sativum) extract on dental plaque bacteria. Indian Journal of Dental Research 24: 71.
34. Zain al-abdeen SS, Abdullah IT, Al-Salihi SS (2013) The synergism effect of aqueous garlic extract and ciproflaxin against some multi-resistant bacteria. Journal of Microbiology Biotechnology Research 3: 136- 142.
35. Appel E, Vallon – Eberhard A, Rabinkov A, Brenner O, Shin I et al (2010) Therapy of murine pulmonary aspergillosis with antibody – alliinase conjugates and alliin. Antimicrobial agents Chemotherapy 54: 898-906.
36. Ledezma E and Apitz-Castro R (2006) Ajoene, the main active compound of garlic (Allium sativum): a new antifungal agent. Rev Iberoam Micol 23: 75-80.
37. Thomaz L Apitz-Castro R, Marques, A. F, Travassos, L. R and Taborda C. P (2008) Experimental paracoccidioidomycosis: alternative therapy with ajoene, compound from Allium sativum, associated with sulfamethoxazole/trimethoprim. Med Mycol 46: 113-118.
38. Ankri S and Mirelman, D (1999) Antimicrobial properties of allicin from garlic. Microbial infections 2: 125-129.
39. Josling P (2001) Preventing the common cold with a garlic supplement: a double – blind placebo – controlled survey. Adv Ther 18: 189-193.
40. Tatarintsev A. V, Vrzhets P. V, Ershov D. E, Shchegolev A. A, Turgiev A. Set al (1992) The ajoene blockage of integrin – dependent processes in an HIV infected cell system. Vestn Ross Akad Med Nauk 6-10.
41. Hanieh H, Narabara K, Tanaka Y, Gu Z, Abe A et al (1992) Immunomodulatory effects of Alliums and Ipomoea batata extracts on lymphocytes and macrophages functions in white leghorn chickens. in vitro study. Animal Science Journal 83: 68 – 76.
42. Gamboa-Leon MR, Aranda-Gonzalez I, Mut-Martin Garcia-Miss MR and Dumonteil E (2007) In vivo and In vitro control of Leishmania Mexicana due to garlic-induced NO production. Scand Journal of Immunology 66: 508-514.
43. Bruck R, Aeed H, Brazovsky E, Noor T and Hershkoviz R (2005) Allicin, the active component of garlic, prevents immune – mediated, concanavalin A – induced hepatic injury in mice. Liver International 25: 613 - 621.
44. Ogbuewu IP, Unamba-Oparah IC, Odoemenam VU, Etuk IF and Okoli IC (2011) The otentiality of medicinal plants as the source of new contraceptive principles in males. New American Journal of Medical Sciences; 3: 255 -263.
45. Dixit VP and Joshi S (1982) Effects of chronic administration of garlic (Allium sativum Linn) on testicular function. Indian Journal Experimental Biology 20: 534 -536.
46. Hammami B, Mahjour F and Dianat O (2013) Antibacterial effect of different concentrations of garlic (Allium sativum) extract on dental plaque bacteria. Indian Journal of Dental Research 24: 71.
47. Shah, Aparna. (2016). Adverse and Toxic effects of garlic, biology discussion. http://www.biologydiscussion.com/human-body/adverse-and-toxic-effects-of-garlic/25184 Date retrieved: 19th June 2021
48. Dietz J (2007) "Arterial stiffness and extracellular matrix". Adv Cardiol. 44: 76-95.
49. Viljoen Charle, Szymanski Patryk, Osman Naeem, Henning Kristin Lorenc, ScholtZ Paul, (2016) Intimomedial mucoid degeneration causing aortic and renal artery aneurysms in a young adult. Cardiovascular Journal of Africa 27(1): 49-52.