

# HISTOMORPHOLOGICAL AND THROMBOGENIC STATUS OF WISTAR RATS FED WITH *ALLIUM SATIVUM* (GARLIC) IN OXIDATIVE STRESS INDUCED WITH CARBON TETRACHLORIDE

## Abstract:

Though concerns over the safety of medicinal drugs are rife following consumption of some herbal preparations, the underlying pathogenesis remains however cryptic. Awareness of the far reaching effects of herbal preparations is germane to their continued use in traditional medicine. Blood vessel occlusion for example can lead to numerous damaging cardiovascular complications. This situation can be avoided through dietary inclusion of herbs with pre-requisite thrombogenic properties. Garlic is one of the few herbs with the potency of keeping the blood in a fluid state. In this study, the status of thrombogenic indices due to oxidative stress induced with carbon tetra-chloride in wistar rats fed with *Allium sativum* (garlic) was investigated. 30 Wistar rats were randomly assigned into five groups of six animals each ( $n = 6$ ): G1, G2, G3, G4 and G5. While G1 (normal control) received 1ml/kg of groundnut oil, G2 (negative control) received single dose of 1ml/kg of Carbon tetrachloride ( $\text{CCl}_4$ ) after 2weeks. G3 and G4 respectively got 250 and 500mg/kg of *Allium sativum* extract twice daily for 2weeks and then treated with a single dose of 1ml/kg  $\text{CCl}_4$ . G5 received 150mg/kg of Vitamin E twice daily for 2weeks and then treated with a single dose of 1ml/kg of  $\text{CCl}_4$  to induce oxidative stress. The rats were then euthanized with Blood samples collected for haematological analysis. Selected organs were also harvested and observed for histomorphologic changes. Evaluation of data for statistical significance was done, using one way analysis of variance (ANOVA). While kidney had some loss of nuclei, increased cytoplasmic eosinophilia, and congested blood vessel, hepatocytes showed some round to oval nuclei interspersed by sinusoids. Also, though garlic administration did not alter blood calcium concentration, it caused a decreased globulin but increased platelets, fibrinogen, bleeding and clotting time. Garlic in this study therefore was seen to be associated with a favourable dose dependence improvement in oxidative biomarkers and maintenance of thrombogenic indices.

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**Keywords:** Biomarkers, Oxidative stress, Anti-oxidant, *Allium Sativum*, Carbon Tetrachloride

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## INTRODUCTION

Thrombus formation is a complex process involving both cellular and molecular (protein) components. Platelets are responsible for maintaining haemostasis and for preventing excessive bleeding. These cells aggregate along with other plasma components and blood cells to form blood clots. Undesirable platelet aggregation may lead to life-threatening conditions such as stroke<sup>1</sup>. Thrombogenicity is the property of a material to induce the

formation of a thrombus, which results in partial or complete occlusion of a blood vessel. This tendency to cause platelet aggregation and perturb plasma coagulation can serve as an in vitro measure of a material's likelihood to be thrombogenic in vivo<sup>1</sup>

Apart from having a history of human consumption and use for over 7,000 years, Garlic has been known to show some anti-oxidant activity, plus prevent haemorrhage. This food spice is native to central Asia, and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe<sup>2-4</sup>.

Overtime, Garlic has been used for medicinal purposes. Louis Pasteur for instance, noted garlic's antibacterial activity in 1858. Subsequently, garlic was used as an antiseptic to prevent gangrene during World War I and World War II<sup>4</sup>. The potency of onion (*Allium cepa*) and garlic (*Allium sativum*) as medicinal plants is due to their high content of vitamins, trace elements, amino acids and several organo-sulphur Compounds<sup>5</sup>. These "magic" drugs are well known for their: fibrinolytic effects<sup>6</sup>, hemodynamic and haemostatic effects<sup>6&7</sup>, platelet effects<sup>8</sup>, immunologic effects<sup>8</sup>, lipid-lowering effects<sup>9</sup>, anti-atherosclerotic effects<sup>10</sup>, anti-oxidative effects<sup>11</sup>, anti-cancer effects<sup>12</sup>, vascular effects<sup>7</sup>, anti-microbial effects<sup>13</sup> and hepatoprotective effects<sup>9</sup> among other health benefits.

## **Aim of Study**

This study aimed at ascertaining the Status of Carbon Tetrachloride (CCl<sub>4</sub>) Induced Oxidative Stress in Wistar rats fed with *Allium sativum*. Specifically, study attempted to:

- i. Investigate the protective effect of *Allium sativum* on serum Calcium level of wistar rats
- ii. Ascertain the effect of *Allium sativum* on the thrombogenic properties of blood of wistar rats
- iii. Examine the effect of *Allium sativum* on histology of selected visceral in wistar rats

## **Methodology**

### **Scope of Study**

Study was conducted in the Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria. Due to the sensitive and invasive nature of the study, Wistar rats were used as choice of experimental model.

### **Study Design**

The experimental rats were randomly divided into five groups of six rats (G1, G2, G3, G4 and G5). Each group of rats were separately housed in standard cages. The first group, G1 (normal control) was given 1ml/kg of groundnut oil. While G2 (negative control) was treated with a single dose of 1ml/kg CCl<sub>4</sub><sup>15</sup> once after 2weeks, G3 and G4 were respectively administered 250 and 500mg/kg of *Allium sativum* extract orally twice daily (morning and evening) for 2weeks and then treated with a single dose of 1ml/kg CCl<sub>4</sub> once after 2weeks garlic administration. G5 got 150mg/kg of Vitamin E twice daily for 2weeks and then treated with a single dose of 1ml/kg of CCl<sub>4</sub> to induce oxidative stress

### **Materials and Method**

#### **Procurement and Preparation of Animals**

30 adult female wistar rats of approximately the same age and a body weight of 100–250g were obtained and kept under a 12:12hr light-dark cycle at room temperature in the animal house of Delta State University, Abraka, Delta State, Nigeria. All animals were allowed to adapt to the environment for two weeks after their arrival before the experiment started. All animals were housed in standard cages in a clean and neat surrounding with *ad libitum* access to water and standard rat diet. Animal handling was performed with regard to CPCSEA guidelines, and Delta State University, Abraka, Nigeria rules.

#### **Preparation of Plant's extract**

Fresh garlic extract was prepared on a daily basis prior to administration. The extract was made by crushing garlic, following 1g to 10ml of water for the low dose and 2g to 10ml of water for the high dose. An aqueous extract was prepared by homogenizing the bulbs in pestle and mortar using distilled water. It was then filtered with the help of muslin cloth and Wattmann filter paper. The extract was administered immediately after preparation as it is known that aqueous garlic lose some of its active components if left for a long time without refrigerating at 4<sup>0</sup>c.

### **Ethical Clearance**

All experimental procedures were performed in strict accordance with the recommendations and Guide for the Care and Use of Laboratory Animals of the Delta State University (Delsu). Ethical clearance was sought and approved by the Research and Ethics committee of Delsu. Study also adhered to the code of conduct stipulated by the Institute for Laboratory Animal Research.

### **Acute Toxicity Study**

The dose selection of *Allium Sativum* was based on acute toxicity studies, carried out according to OPPTS (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure. The influence of garlic extract on the chronic toxicity test was examined orally in Wistar rats for 6 months. There were no toxic symptoms due to garlic extract even at dose level of 2000 mg/kg for 5 times a week. The animals were fasted overnight prior to the studies. Mice were divided into two groups of three each. Test dose of 2 g/kg body weight and 5 g/kg body weight were given orally to either group of mice. Mice were observed for 72 h for mortality.

### **Induction of Oxidative Damage with Carbon tetrachloride (CCl<sub>4</sub>)**

Carbon tetrachloride (CCl<sub>4</sub>) in its concentrated form was obtained from a local laboratory in Lagos State, Nigeria. CCl<sub>4</sub> was diluted in groundnut oil prior to administration in order to neutralize its hepatotoxicity level. CCl<sub>4</sub> was dissolved in groundnut oil in the ratio 1:1 v/v. Oxidative damage was induced in rats at a dose of 1mL/kg CCl<sub>4</sub><sup>15</sup>.

### **Procedure**

#### **Preparation of Stock Solutions of Garlic Extract**

##### **Low dose (250mg/kg)**

1g of garlic was weighed with electronic weighing balance, homogenized in pestle and mortar using 10ml of distilled water and filtered with Wattmann filter paper. This gave stock solutions of 100mg/ml.

### **High dose (500mg/kg)**

2g of garlic was weighed with electronic weighing balance, homogenized in pestle and mortar using 10ml of distilled water and filtered with Wattmann filter paper. This gave stock solutions of 200mg/ml.

### **Blood Sample and Organ Collection**

At the end of the 14 days, the animals were euthanized by cervical decapitation and blood was collected from the superior vena cava using a 5ml syringe. The sample was centrifuged at 3000rpm for 15minutes and the sera collected were stored frozen (at -20°C) and later used for haematological analysis. Worth mentioning is that for each group of rats, before euthanizing, a lancet was used to inflict minimal injury, and the bleeding and clotting time were obtained with the aid of a stop watch.

### **Organ Collection**

The animals were dissected and the testes, kidneys, as well as and liver were removed, cleared of adherent tissues and weighed immediately using an electronic weighing balance. Of each animal euthanized, one organ each was used for histological studies.

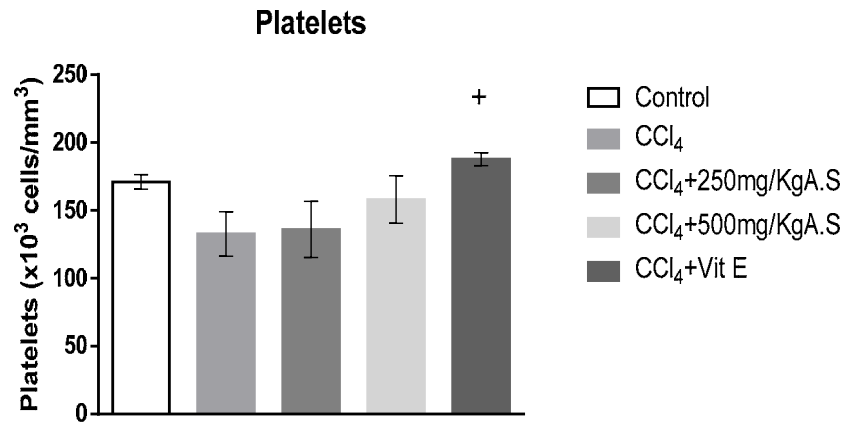
### **Analytical Approach**

Results of the study were presented as mean  $\pm$  Standard error of mean (SEM) of sample size. Mean values among and between groups were compared statistically by one way analysis of variance (ANOVA), followed by post hoc Turkey's test for multiple comparison using Statistical package for social sciences (SPSS version 20).  $p < .05$  was considered to be statistically significant. Data was further subjected to **least significant difference** (LSD) post hoc test and differences between means accepted significant at  $p < .05$

### **Results**

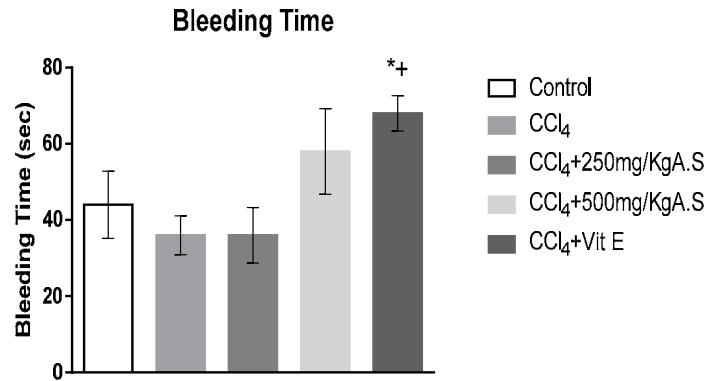
This study showed the effect of aqueous extract of *Allium sativum* on some biomarkers of oxidative stress, following administration of Carbon Tetrachloride (CCl<sub>4</sub>)

**CHART 1:** Status of Platelet count (PC) in rats treated with Carbon Tetrachloride (CCl<sub>4</sub>) induced oxidative stress after administration of *Allium sativum*



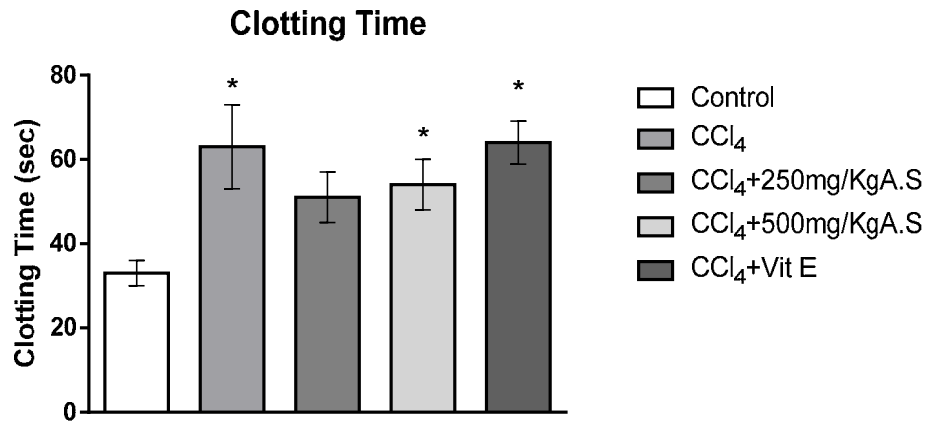
Insignificant increase in PC ( $p < .05$ ) as compared to rats treated with CCl<sub>4</sub>. A.S = *Allium sativum*, CCl<sub>4</sub> = Carbon Tetrachloride

**CHART 2** Effect of *Allium sativum* on Bleeding Time of rats treated with Carbon Tetrachloride (CCl<sub>4</sub>)



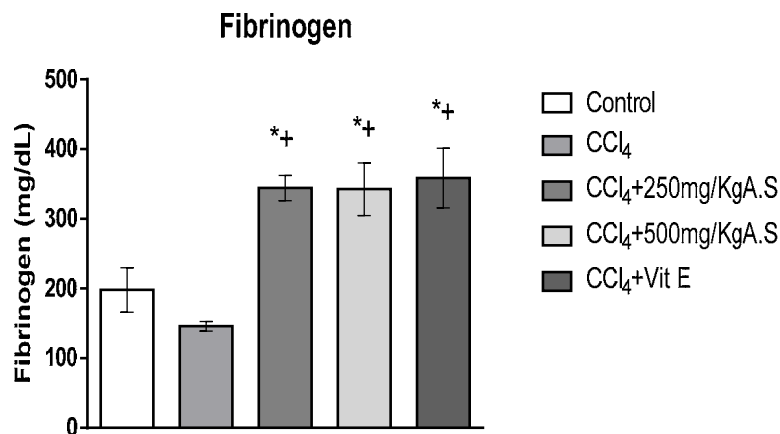
CCl<sub>4</sub> decreased the bleeding time, while aqueous administration of *Allium sativum* caused a dose dependent increase in bleeding time of the rats. A.S = *Allium sativum*, CCl<sub>4</sub> = Carbon Tetrachloride

**CHART 3:** Effect of *Allium sativum* on Clotting Time of rats treated with Carbon Tetrachloride (CCl<sub>4</sub>)



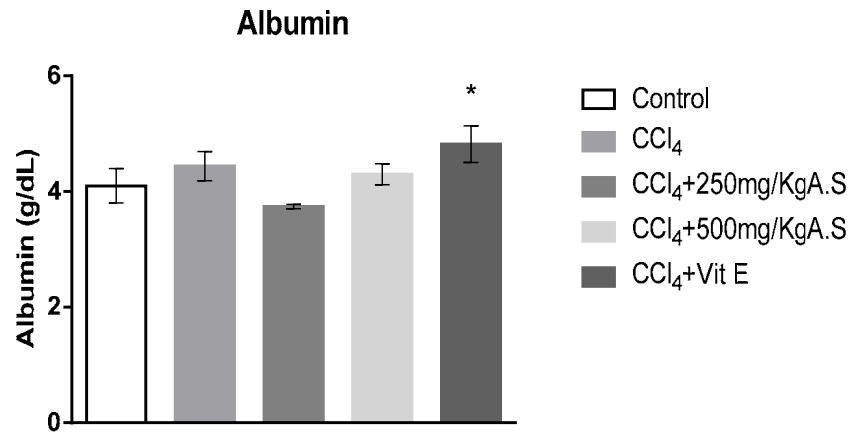
CCl<sub>4</sub> significantly ( $p < .05$ ) increased clotting time. In contrast, *Allium sativum* decreased the clotting time in a dose dependent manner with statistical significance. Vitamin E significantly ( $p < .05$ ) increased the clotting time. A.S = *Allium sativum*, CCl<sub>4</sub> = Carbon Tetrachloride

**CHART 4:** Effect of *Allium sativum* on Fibrinogen of rats treated with Carbon Tetrachloride (CCl<sub>4</sub>)



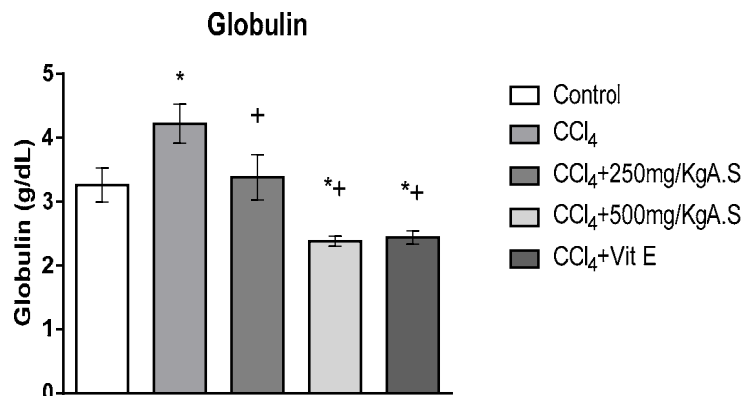
Compared with control, CCl<sub>4</sub> decreased the fibrinogen concentration with no significance. *Allium sativum* and Vitamin E administration increased the fibrinogen level with statistical significance ( $p < 0.05$ ). A.S = *Allium sativum*, CCl<sub>4</sub> = Carbon Tetrachloride

**CHART 5:** Effect of *Allium sativum* on Albumin Level of rats treated with Carbon Tetrachloride (CCl<sub>4</sub>)



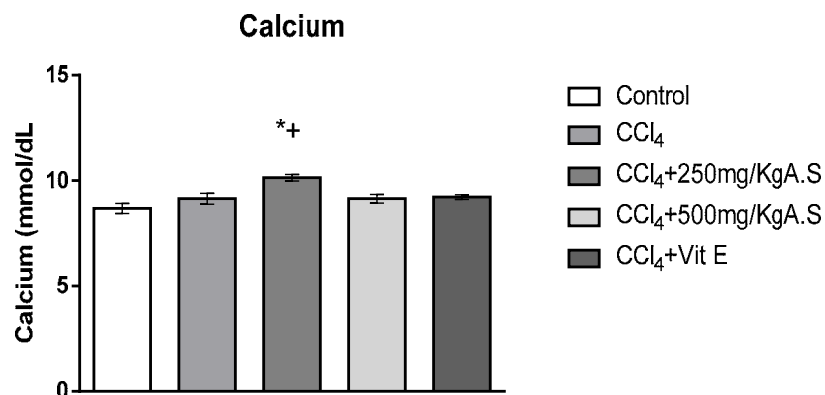
CCl<sub>4</sub> caused an insignificant increase in the albumin level of rats, while *Allium sativum* significantly decreased albumin level ( $p < 0.05$ ); though not in a dose dependent manner. A.S = *Allium sativum*, CCl<sub>4</sub> = Carbon Tetrachloride

**CHART 6:** Effect of *Allium sativum* on Globulin level of rats treated with Carbon Tetrachloride (CCl<sub>4</sub>)



*Allium sativum* significantly ( $p < .05$ ) increased globulin level in a dose dependent manner. Vitamin E also caused a significant decrease in globulin level of the CCl<sub>4</sub> treated rats. A.S = *Allium sativum*, CCl<sub>4</sub> = Carbon Tetrachloride

**CHART 7:** Effect of *Allium sativum* on Calcium level of rats treated with Carbon Tetrachloride (CCl<sub>4</sub>)



Compared to control, CCl<sub>4</sub> caused an insignificant increase in calcium level of rats. 250mg/Kg of *Allium sativum* caused a significant ( $p < 0.05$ ) increase in Calcium level, which was attenuated in higher dose (500mg/Kg). A.S = *Allium sativum*, CCl<sub>4</sub> = Carbon Tetrachloride.

**FIGURE 1:** Comparing photomicrograph of kidney histology for Normal control (Group 1) rats.

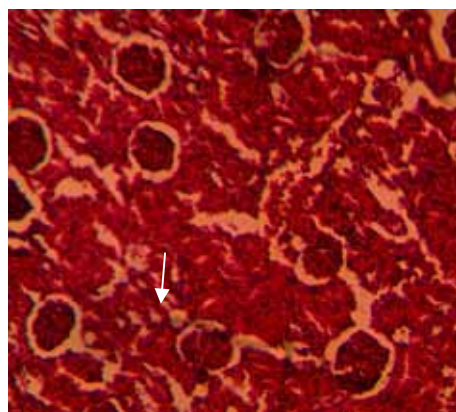


**PLATE I**

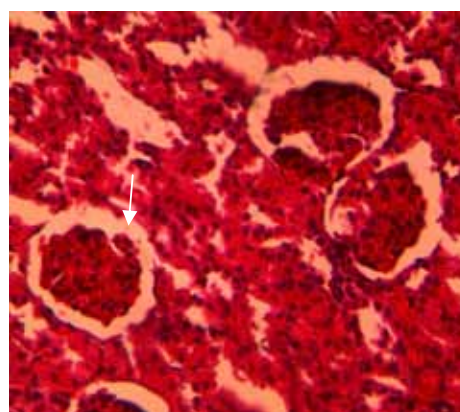
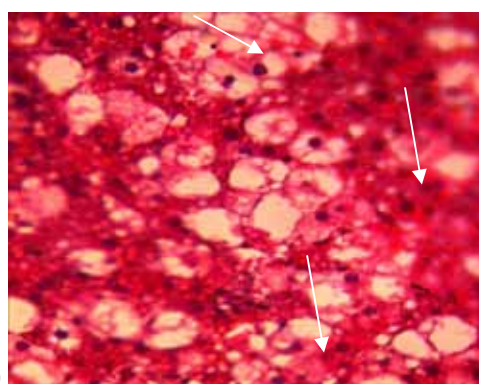
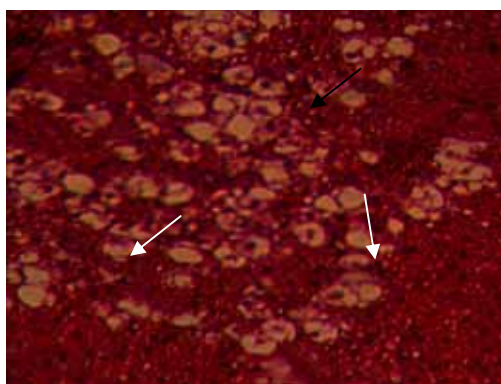


**PLATE II**

**PLATE I:** Normal control rat kidney composed of Glomeruli (Arrow head) with H & E staining x 200

**PLATE II:** Normal control rat kidney composed of Renal Tubules (Thin Arrow) with H & E staining x 400.

**FIGURE 2:** Showing photomicrograph of hepatocytes histology for rats treated with 1ml/kg CCl<sub>4</sub> (G<sub>2</sub>) after 2weeks.



(a)

(b)

**PLATE III**

**PLATE IV**

Above figure shows hepatocytes containing fat vacuoles with clear intracytoplasmic spaces (thin arrow) H & E staining (a) x 200 and (b) x400 magnifications

**FIGURE 3:** Showing photomicrograph of hepatocytes histology for rats treated with 250mg/kg (G<sub>3</sub>) of *Allium sativum* extract daily for 2weeks.

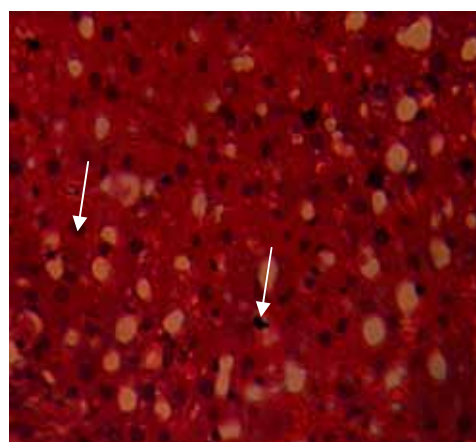
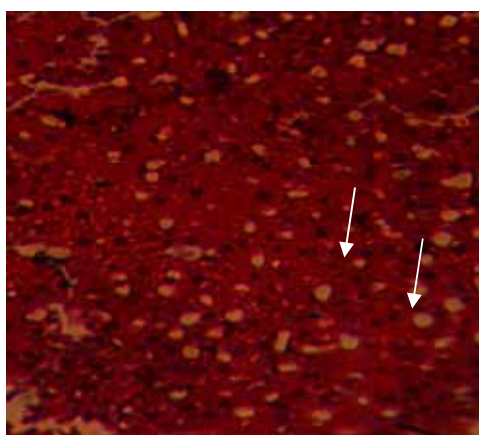


**PLATE V**

**PLATE VI**

(a)

(b)

Above figure shows hepatocytes containing fat vacuoles with clear intracytoplasmic spaces (thin arrow). Haematoxylin and eosin. (a) x 200 and (b) x400 magnification.

**FIGURE 4:** Showing photomicrograph of kidney histology for group rats treated with single dose of 1ml/kg of CCl<sub>4</sub> after 2weeks.

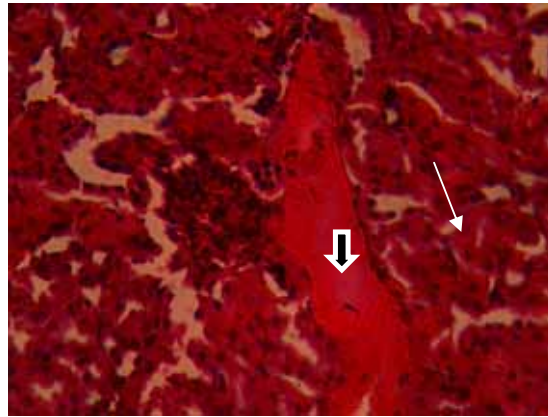


**PLATE VII**

**PLATE VII:** Group II (Negative control) rats kidney composed of glomeruli (thin arrow), tubules (thick arrow) and congested blood vessel with H & E staining x 400

**FIGURE 5:** Showing photomicrograph of kidney histology for group 3 treated rats (250mg/kg *Allium sativum* extract daily for 2weeks).

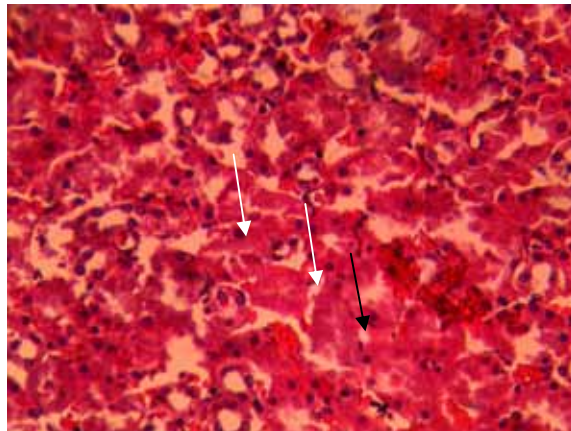


**PLATE VIII**

**PLATE IV:** kidney of Group III rats composed of tubules with loss of nuclei and increased cytoplasmic eosinophilia (thin arrow) and congested blood vessel. H & E staining x 400.

## Discussion

Garlic in this study caused a decrease in Globulin but increased platelets, fibrinogen, bleeding and clotting time in rats induced with oxidative stress. **Studies** have shown that garlic has a protective action against decrease in fibrinolytic activity and increase in clotting

265 time in patients with myocardial infarction and coronary artery disease<sup>16</sup>. Normally,  
266 fibrinolysis occurring in the body maintains the fluidity of the circulating blood by dissolving  
267 and removing deposits of fibrin. Perhaps an important function of fibrinolysis is to remove  
268 very minute clots from tiny peripheral vessels that eventually would become occluded were  
269 there no way to cleanse them. Defects in coagulation and fibrinolytic activity are main factors  
270 for the development of thrombus. Garlic, when used continuously produces constant lytic  
271 activity, probably due to decrease in cholesterol and an increase in the plasminogen activity<sup>16</sup>.  
272 Fibrinolysis releases fibrin degradation products which have anticoagulant activity. Increased  
273 plasmin may lower factor V, VIII, IX and can cause increase in clotting time<sup>17</sup>. The  
274 commonly used thrombolytic agents like streptokinase and urokinase are expensive,  
275 antigenic, and effective only on parenteral use and cannot be used for a long time. These  
276 drawbacks are absent with garlic therapy<sup>17</sup>.  
277 In the present study, we found that ingestion of raw garlic for two months had brought about  
278 increase in fibrinolytic activity and increase in clotting time in normal individuals. No side  
279 effect was noted with garlic. In addition, it is cheap and can be taken orally. So, daily use of  
280 simple dietary substance like garlic may be useful for prevention of thromboembolic  
281 phenomenon.

282 Although variable data were obtained in the study, results taken together imply that aged  
283 garlic extract (AGE) probably exerts its inhibitory effect on platelet aggregation, either by  
284 suppressing the influx of calcium ions by chelating calcium within platelet cytosol or by  
285 altering other intracellular second messengers within the platelets<sup>2</sup>. Some preparations appear  
286 to be anti-oxidative, whereas others may stimulate oxidation. These additional biological  
287 effects attributed to AGE may be due to compounds, such as S-allylcysteine, S-  
288 allylmercaptocysteine, N<sup>α</sup>-fructosyl arginine and others, formed during the extraction process.

289 Although not all of the active ingredients are known, ample research suggests that several  
290 bioavailable components likely contribute to the observed beneficial effects of garlic<sup>2&3</sup>.

291 These results suggested that the anticoagulant action of rich garlic oil was due to inhibition  
292 and/or inactivation of thrombin. In addition, rich garlic oil benefits blood anticoagulation  
293 factors, which might further prevent the development of thrombus formation<sup>17</sup>.

294 More so Garlic caused increase in fibrinogen level. This is consistent with findings of Kung-  
295 chi in 2007<sup>18</sup>. This researchers earlier found out that the intake of garlic oil at high dose  
296 significantly increases plasma fibrinogen concentration and affected the levels of several  
297 haematological parameters such as erythrocyte count, haemoglobin and platelets

298 Thus, AGE exerts selective inhibition on platelet aggregation and adhesion; platelet functions  
299 that may be important for the development of cardiovascular events such as myocardial  
300 infarction and ischemic stroke.

301 Increased platelet aggregation plays a pivotal role in the etiology of cardiovascular disease.  
302 Upon platelet aggregation, an increase in free cytoplasmic  $\text{Ca}^{2+}$  results in the inhibition of  
303 soluble guanylyl cyclase (sGC) and adenylyl cyclase (AC), leading to a decrease in cyclic  
304 guanosine-5'-monophosphate (cGMP) and cAMP, respectively. This leads to the activation  
305 of the glycoprotein IIb/IIIa (GPIIb/IIIa) fibrinogen receptor, resulting in platelet shape  
306 change. Aged garlic extract (AGE) decreases platelet aggregation; however, the mechanisms  
307 involved are not clearly defined<sup>17&18</sup>. These results indicate that AGE inhibits platelet  
308 aggregation by increasing cyclic nucleotides and inhibiting fibrinogen binding and platelet  
309 shape change<sup>18</sup>.

310

311 **Benefit of Study**

From this study, the following can be deduced:

- i. Garlic has high fibrinolytic activity and can maintain blood in fluid state, and therefore useful as nutritional supplement for patients suffering from Diabetes and or prone to cardiovascular disorders
- ii. Garlic has both prophylactic and protective effects on haemostasis
- iii. Garlic has a protective action against alteration in thrombogenic parameters in oxidative stress.

## Conclusion

This study has shown, that compared to control, Carbon Tetrachloride (CCl<sub>4</sub>) caused an insignificant increase in calcium level of rats, while *Allium Sativum* significantly increased it. This was however attenuated at higher dose. More so, a significant and an insignificant increase were respectively seen in the plasma globulin and albumin levels following administration of CCl<sub>4</sub>. However, fibrinogen levels and bleeding time had a significant decrease, with platelet counts and clotting times increase following administration of *Allium Sativum*.

## Recommendations

From this study, the following recommendation is advice for the furtherance and improvement of this work

- i. Effects of the constituents of Garlic on thrombogenic indices
- ii. Effect of *Garlic* on thrombogenic indices in diseases that induce oxidative stress

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