

HISTOMORPHOLOGICAL AND THROMBOGENIC STATUS OF WISTAR RATS FED WITH *ALLIUM SATIVUM* (GARLIC) IN OXIDATIVE STRESS INDUCED WITH CABON 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 (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 CCl_4 . G5 received 150mg/kg of Vitamin E twice daily for 2weeks and then treated with a single dose of 1ml/ kg of 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 histomorphological 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.

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

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 stroke¹. 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 vivo¹

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 Europe²⁻⁴.

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 II⁴. 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 Compounds⁵. These “magic” drugs are well known for their: fibrinolytic effects⁶, hemodynamic and haemostatic effects^{6&7}, platelet effects⁸, immunologic effects⁸, lipid-lowering effects⁹, anti-atherosclerotic effects¹⁰, anti-oxidative effects¹¹, anti-cancer effects¹², vascular effects⁷, anti-microbial effects¹³ and hepatoprotective effects⁹ among other health benefits.

Aim of Study

This study aimed at ascertaining the Status of Carbon Tetrachloride (CCl₄) 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 protective effect of *Allium sativum* on platelet count of 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 CCl₄ (Ritesh *et al.*, 2015) 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 CCl₄ 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 CCl₄ to induce oxidative stress

Materials and sources

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 4⁰c.

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 (ILAR, 1996).

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 (CCl₄)

Carbon tetrachloride (CCl₄) in its concentrated form was obtained from a local laboratory in Lagos State, Nigeria. CCl₄ was diluted in groundnut oil prior to administration in order to neutralize its hepatotoxicity level. CCl₄ was dissolved in groundnut oil in the ratio 1:1 v/v. Oxidative damage was induced in rats at a dose of 1mL/kg CCl₄¹⁵.

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 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

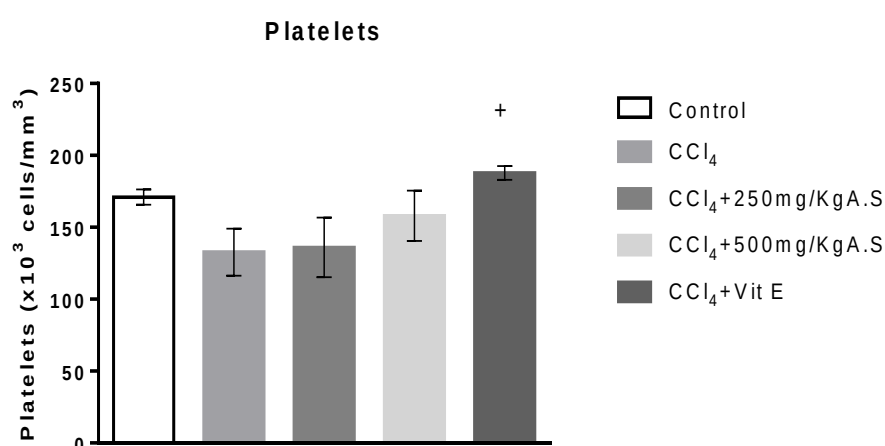
Results of the study were presented as mean $\pm$ Standard error of mean (SEM) of sample size. Mean values among and between groups were compared statistically by one way analysis of variance (ANOVA), followed by post hoc Turkey's test for multiple comparison using Statistical package for social sciences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

156 statistically significant. Data was further subjected to LSD post hoc test and differences
157 between means accepted significant at $p < .05$

158 **Results**

159 This study showed the effect of aqueous extract of *Allium sativum* on some biomarkers of
160 oxidative stress, following administration of Carbon Tetrachloride (CCl_4)

161 **CHAT 1:** Status of Platelet count (PC) in rats treated with Carbon Tetrachloride (CCl_4)
162 induced oxidative stress after administration of *Allium sativum*

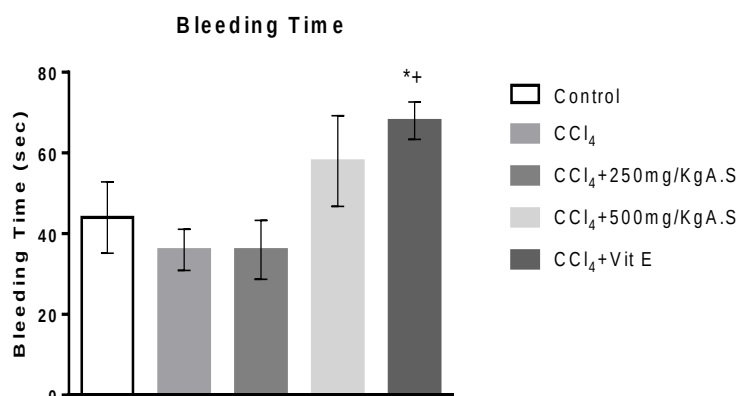


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164 Insignificant increase in PC ($p < .05$) as compared to rats treated with CCl_4 . A.S = *Allium*
165 *sativum*, CCl_4 = Carbon Tetrachloride

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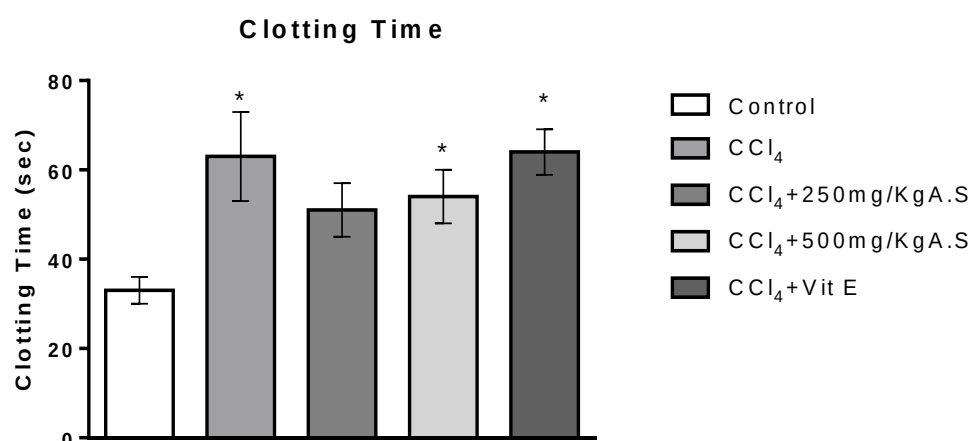
167 **Chat 2** Effect of *Allium sativum* on Bleeding Time of rats treated with Carbon
168 Tetrachloride (CCl_4)



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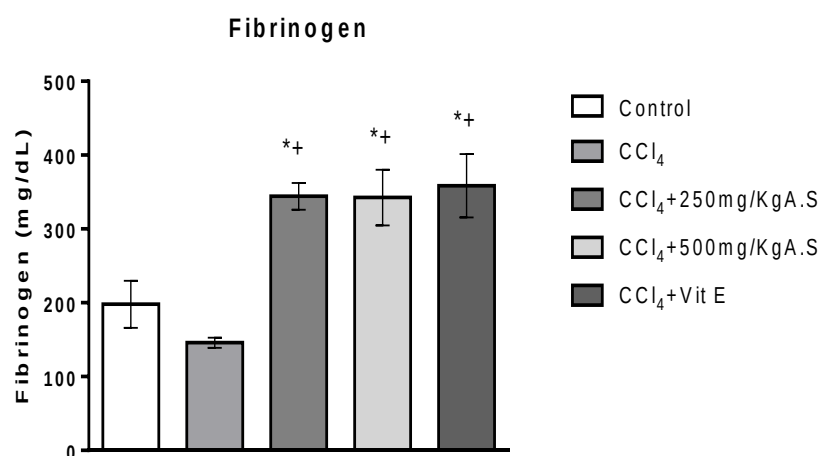
CCl₄ decreased the bleeding time, while aqueous administration of Allium sativum caused a dose dependent increase in bleeding time of the rats. A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

CHAT 3: Effect of Allium sativum on Clotting Time of rats treated with Carbon Tetrachloride (CCl₄)



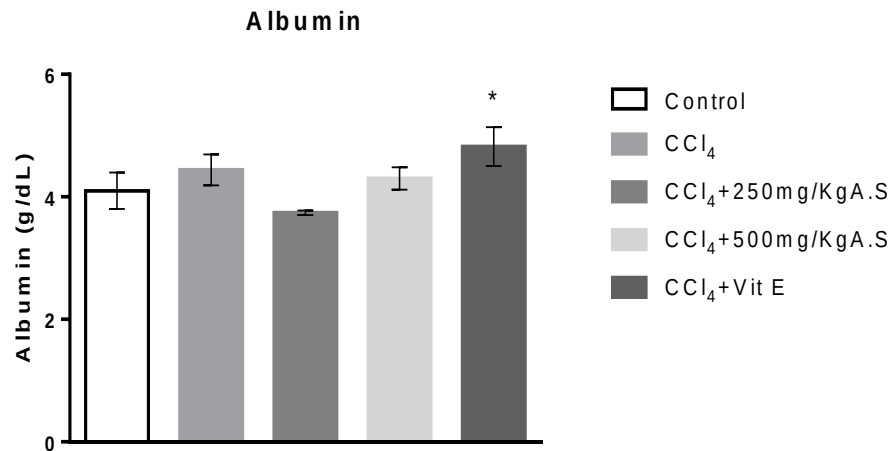
CCl₄ significantly ($p < .05$) increased clotting time. In contrast, Allium sativum decreased the clotting time in a dose dependent manner with statistical significance Vitamin E significantly ($p < .05$) increased the clotting time. A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

CHAT 4: Effect of Allium sativum on Fibrinogen of rats treated with Carbon Tetrachloride (CCl₄)



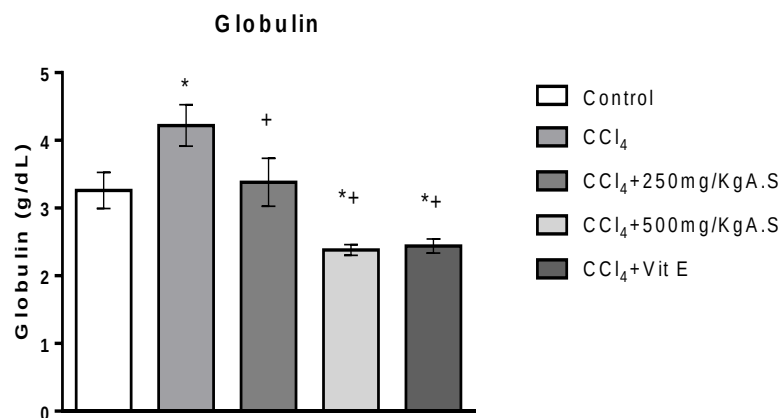
Compared with control, CCl₄ decreased the fibrinogen concentration with no significance. Allium sativum and Vitamin E administration increased the fibrinogen level with statistical significance ($p < 0.05$). A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

CHAT 5: Effect of *Allium sativum* on Albumin Level of rats treated with Carbon Tetrachloride (CCl₄)



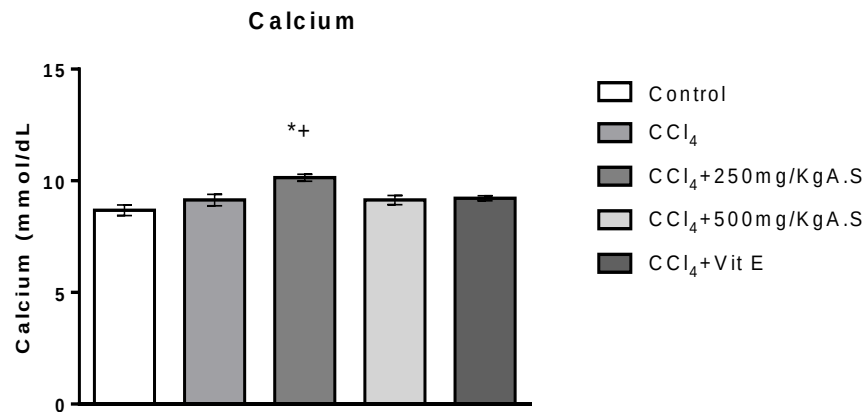
*CCl₄ caused an insignificant increase in the albumin level of rats, while *Allium sativum* significantly decreased albumin level ($p < 0.05$); though not in a dose dependent manner. A.S = *Allium sativum*, CCl₄ = Carbon Tetrachloride*

CHAT 6: Effect of *Allium sativum* on Globulin level of rats treated with Carbon Tetrachloride (CCl₄)



**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 CCl₄ treated rats. A.S = *Allium sativum*, CCl₄ = Carbon Tetrachloride*

CHAT 7: Effect of *Allium sativum* on Calcium level of rats treated with Carbon Tetrachloride (CCl₄)



Compared to control, CCl₄ 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*, CCl₄ = Carbon Tetrachloride.

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

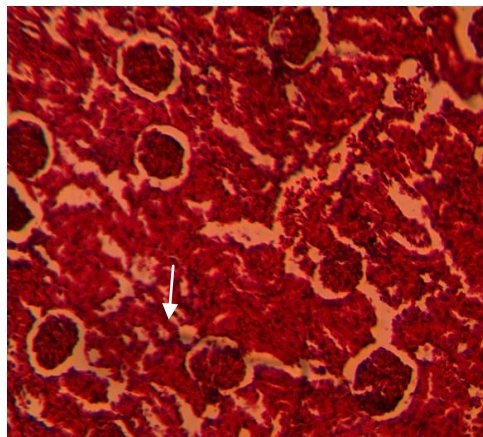


PLATE I

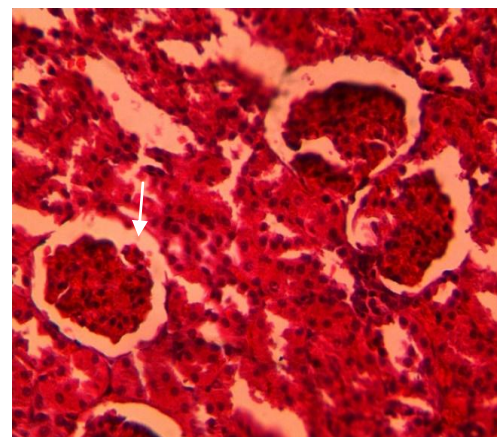
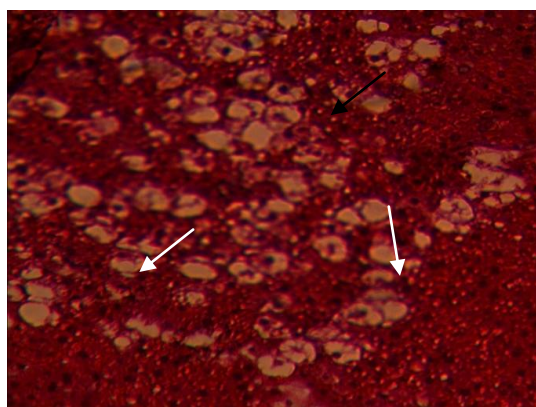


PLATE II

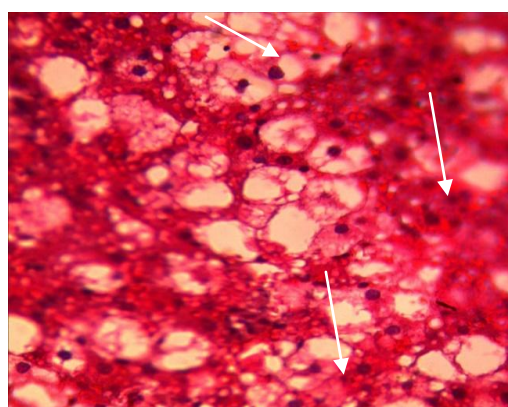
PLATE I: Normal control rat kidney composed of Glomeruli (Arrow head) with H & E staining x 200

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 CCl₄ (G₂) after 2weeks.



(a)



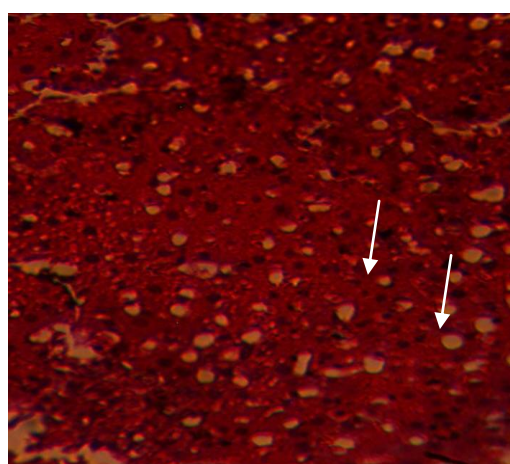
(b)

PLATE III

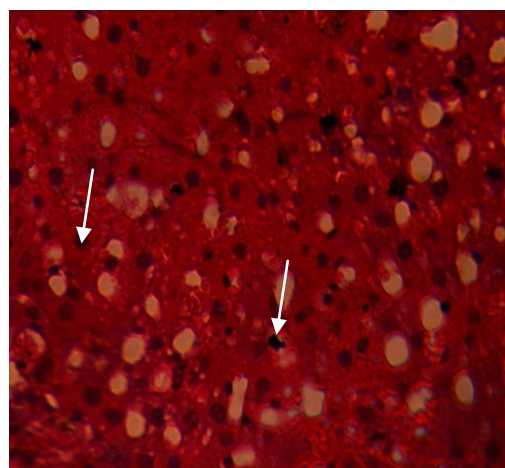
PLATE IV

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_3) of *Allium sativum* extract daily for 2weeks.



(a)



(b)

PLATE V

PLATE VI

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_4 after 2weeks.

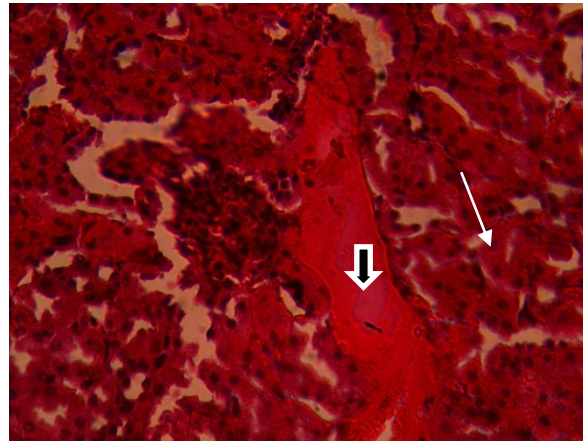


PLATE VII

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).

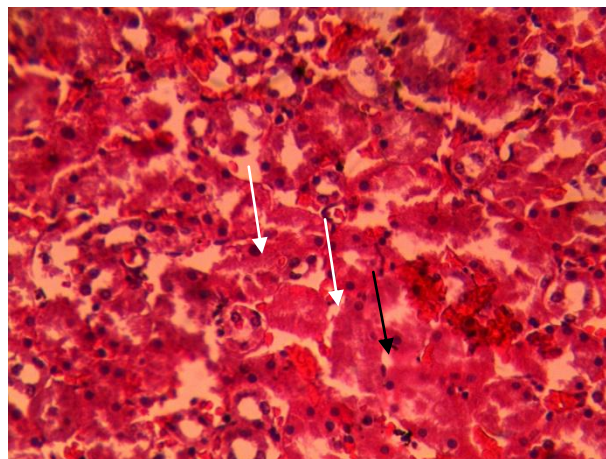


PLATE VIII

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. Bordia and associates have shown that garlic has a protective action against decrease in fibrinolytic activity and increase

in clotting time in patients with myocardial infarction and coronary artery disease. Normally, fibrinolysis occurring in the body maintains the fluidity of the circulating blood by dissolving and removing deposits of fibrin. Perhaps an important function of fibrinolysis is to remove very minute clots from tiny peripheral vessels that eventually would become occluded were there no way to cleanse them. Defects in coagulation and fibrinolytic activity are main factors for the development of thrombus. Garlic, when used continuously produces constant lytic activity, probably due to decrease in cholesterol and an increase in the plasminogen activity (Sainani et al 1979). Fibrinolysis of fibrin, releases fibrin degradation products which have anticoagulant activity. Increased plasmin may lower factor V, VIII, IX and can cause increase in clotting time (Kwann 1972).

The commonly used thrombolytic agents like streptokinase and urokinase are expensive, antigenic, and effective only on parenteral use and cannot be used for a long time. These drawbacks are absent with garlic therapy. In the present study, we found that ingestion of raw garlic for two months had brought about increase in fibrinolytic activity and increase in clotting time in normal individuals. No side effect was noted with garlic. In addition, it is cheap and can be taken orally. So, daily use of simple dietary substance like garlic may be useful for prevention of thromboembolic phenomenon.

Although variable data were obtained in this study, these results taken together imply that AGE probably exerts its inhibitory effect on platelet aggregation either by suppressing the influx of calcium ions by chelating calcium within platelet cytosol or by altering other intracellular second messengers within the platelets (Allison et al., 2006).

These results suggested that the anticoagulant action of DAT-rich garlic oil was due to inhibition and/or inactivation of thrombin. In addition, DAT-rich garlic oil benefits blood anticoagulation factors, which might further prevent the development of thrombus formation (Kung-chi 2007).

More so Garlic caused increase in fibrinogen level. This is consistent with Kung-chi 2007. This researchers earlier found out that the intake of garlic oil at high dose significantly **increases plasma fibrinogen concentration** and affected the levels of several hematological parameters such as erythrocyte count, hemoglobin and platelets

Thus, AGE exerts selective inhibition on platelet aggregation and adhesion, platelet functions that may be important for the development of cardiovascular events such as myocardial infarction and ischemic stroke (Steiner and Li, 2001).

Increased platelet aggregation plays a pivotal role in the etiology of cardiovascular disease. Upon platelet aggregation, an increase in free cytoplasmic Ca^{2+} results in the inhibition of soluble guanylyl cyclase (sGC) and adenylyl cyclase (AC), leading to a decrease in cyclic guanosine-5'-monophosphate (cGMP) and cAMP, respectively. This leads to the activation of the glycoprotein IIb/IIIa (GPIIb/IIIa) fibrinogen receptor, resulting in platelet shape change. Aged garlic extract (AGE) decreases platelet aggregation; however, the mechanisms involved are not clearly defined (Khalid et al., 2014). These results indicate that AGE inhibits platelet aggregation by increasing cyclic nucleotides and inhibiting fibrinogen binding and platelet shape change (Khalid et al., 2014).

Societal 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

In this study Garlic increased antioxidant enzymes SOD, GPx, CATALASE but significantly reduced MDA and TBARs in CCL4 induced oxidative stress. Though it did not alter calcium concentration, it caused a decrease in Globulin but increased platelets, fibrinogen, albumin levels, clotting and bleeding time in rats induced with oxidative stress. Garlic in this study has therefore been seen to be associated with a favorable dose dependent improvement in oxidative biomarkers and maintenance of thrombogenic indices. It is therefore observed that Garlic significantly improves thrombogenic indices in CCL4 - induced oxidative stress

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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