

STATUS OF CARBON TETRACHLORIDE INDUCED OXIDATIVE STRESS MARKERS IN WISTAR RATS FED WITH *ALLIUM SATIVUM* (GARLIC)

Abstract:

Concerns over the safety of medicinal drugs are rife, following consumption of some Herbal preparations with their underlying pathogenesis remaining cryptic. The awareness of the far reaching effects of herbal preparations is germane to continued use of plants. For instance, free radicals from day to day release of metabolites can lead to numerous damages to cardiovascular and other body systems. The situation can be minimized through dietary inclusion of food nutrients with anti-oxidant properties. In this study, the status of oxidative stress markers 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. G1 (normal control) - received 1ml/kg of groundnut oil G2 (negative control) - received single dose of 1ml/kg Carbon tetrachloride (CCl_4) after 2weeks. G3 and G4 - got 250 and 500mg/kg *Allium sativum* extract twice daily for 2weeks respectively and then treated with a single dose of 1ml/kg CCl_4 . G5 got 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. Following the period of administration, the rats were euthanized via cervical dislocation. Blood samples were collected directly from the heart (by cardiac puncture) for laboratory analysis. With results expressed as mean $\pm$ Standard deviation, evaluation of data for significance was done, using one way analysis of variance (ANOVA). From the results, Garlic increased antioxidant enzymes SOD, GPx, CATALASE but significantly reduced MDA and TBARs. Though garlic administration did not alter calcium concentration, it however caused a decreased globulin but increased platelets. Garlic in this study has therefore been seen to be associated with a favourable dose dependence improvement in oxidative stress markers.

Keywords: Oxidative Stress, *Allium Sativum*, anti-oxidant enzymes, Carbon Tetrachloride

INTRODUCTION

Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies. Though defined as harmful due to free radicals' attack of biological molecules like lipids, proteins, and DNA, Oxidative stress also has a useful role in physiologic adaptation and in the regulation of intracellular signal transduction^{1&2}. Therefore, a more

40 useful definition of oxidative stress may be “a state where oxidative forces exceed the
41 antioxidant systems due to loss of the balance between them.” In recent times, the biomarkers
42 that can be used to assess oxidative stress in vivo have been of attracting interest to
43 researchers³.

44 Oxidative stress has been implicated in the progression of major health problems by its
45 inactivation of metabolic enzymes, damaging important cellular components, and oxidizing
46 of nucleic acids, leading to cardiovascular diseases, eye disorders, joint disorders,
47 neurological diseases (Alzheimer’s disease, Parkinson’s disease and amyotrophic lateral
48 sclerosis), atherosclerosis, lung and kidney disorders, liver and pancreatic diseases, cancer,
49 ageing, disease of the reproductive system including male and female infertility, etc⁴⁻⁷.

50 In humans, the toxic effect of reactive oxygen and nitrogen species is balanced by the
51 antioxidant action of non-enzymatic antioxidants, as well as by antioxidant enzymes. Such
52 antioxidant defenses are extremely important as they represent the direct removal of free
53 radicals (pro-oxidants)⁸⁻¹⁰, thus providing maximal protection for biological sites. These
54 systems not only assert with the problem of oxidative damage, but also play a crucial role in
55 wellness, health maintenance, and prevention of chronic and degenerative diseases.

56 Apart from having a history of human consumption and use for over 7,000 years, Garlic
57 has been known to show some anti-oxidant activity. This food spice is native to central
58 Asia, and has long been a staple in the Mediterranean region, as well as a frequent seasoning
59 in Asia, Africa, and Europe¹¹⁻¹³.

60 Overtime, Garlic has been used for medicinal purposes. Louis Pasteur for instance, noted
61 garlic’s antibacterial activity in 1858. Subsequently, garlic was used as an antiseptic to
62 prevent gangrene during World War I and World War II¹⁴. The potency of onion (*Allium*
63 *cepa*) and garlic (*Allium sativum*) as medicinal plants is due to their high content of vitamins,
64 trace elements, amino acids and several organo-sulphur Compounds¹⁵. These “magic” drugs
65 are well known for their: fibrinolytic effects¹⁶, hemodynamic and haemostatic effects¹⁷,
66 platelet effects¹⁸, immunologic effects¹⁸, lipid-lowering effects¹⁹, anti-atherosclerotic
67 effects¹³, anti-oxidative effects²¹, anti-cancer effects (Milner, 2001), vascular effects¹⁷, anti-
68 microbial effects²², haematological effects¹⁶ and hepato-protective effects¹⁹ among other
69 health benefits.

70 From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were
71 available, such markers would be useful for physicians to gain an insight into the pathological
72 features of various diseases and assess the efficacy of drugs.

Aim of Study

This study aimed at ascertaining the status of Carbon Tetrachloride induced oxidative stress markers in wistar rats fed with *allium sativum* (garlic). Specifically, Study attempted to:

- i. Assess the effect(s) of dose-dependent administration of *Allium Sativum* on biomarkers of oxidative stress
- ii. 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 each (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 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 Methods

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 Garlic 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 (Sumiyoshi *et al.*, 1984). 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 CCl₄²³.

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.

Biochemical Analysis

Different biochemical assays; Superoxide Dismutase (SOD), Reduced glutathione (GSH), Lipid peroxidation (Malondialdehyde, MDA), and protein concentration were conducted according to various principles, and methods described by Misra and Fridovich.

Determination of superoxide dismutase activity

Assay principle: According to Aline *et al.* (2013)²⁷, this method was based on the ability of superoxide dismutase to inhibit the autoxidation of adrenaline (epinephrine) at pH 10.2 because, superoxide (O_2^-) radical generated by the xanthine oxidase reaction causes the oxidation of epinephrine to adrenochrome²⁷

Determination of reduced glutathione activity

This method was based upon the development of a relatively stable (yellow) colour when 5', 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. The chromophoric product resulting from the reaction of DTNB with the reduced glutathione, 2-nitro-5-thiobenzoic acid possesses a characteristic absorbance at 412nm and the amount of reduced glutathione in the sample is proportional to the absorbance at this wavelength.

Determination of lipid peroxidation

The malondialdehyde (MDA) content of the lipid peroxidation was measured according to the method described by Anu *et al.* (2014). This assay principle is based on the fact that lipid peroxidation generates unstable lipid peroxides, which decompose to form a complex series of compounds including reactive carbonyl compounds. The polyunsaturated fatty acid peroxides produced, generate malondialdehyde (MDA) upon decomposition. MDA form a 1:2 adduct with thiobarbituric acid (TBA) that that give rise to a pink colour product when heated in acidic pH, with a maximum absorbance of 532nm.

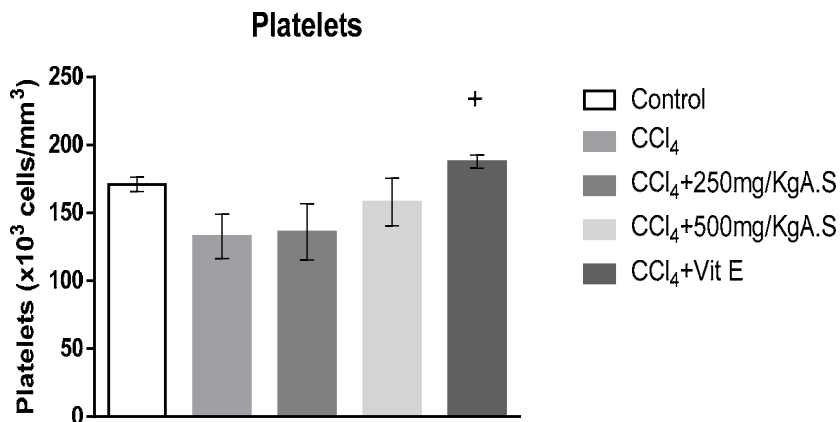
Analytical Approach

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 LSD post hoc test and differences between means accepted significant at $p < .05$.

Results

187 This study showed the effect of aqueous extract of *Allium sativum* on some biomarkers of
188 oxidative stress, following administration of Carbon Tetrachloride (CCl₄)

189 **CHAT 1:** Status of Platelet count (PC) in rats treated with Carbon Tetrachloride (CCl₄)
190 induced oxidative stress after administration of *Allium sativum*

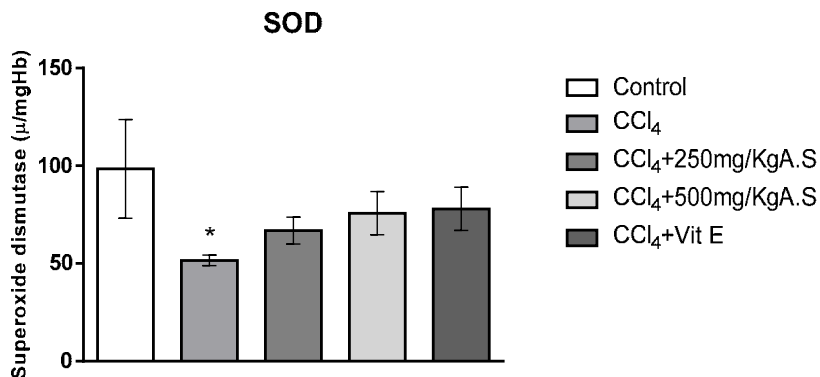


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192 Insignificant increase in PC ($p < .05$) as compared to rats treated with CCl₄. A.S = *Allium*
193 *sativum*, CCl₄ = Carbon Tetrachloride

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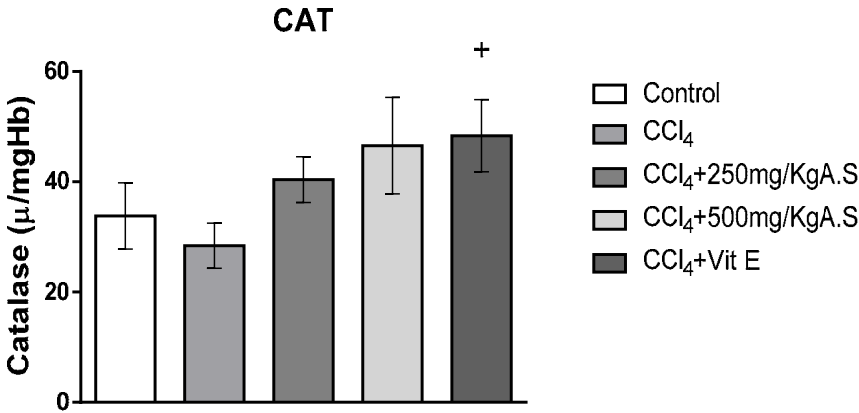
195 **Chat 2** Status of SOD levels of rats treated with Carbon Tetrachloride (CCl₄) induced
196 oxidative stress after administration of *Allium sativum*



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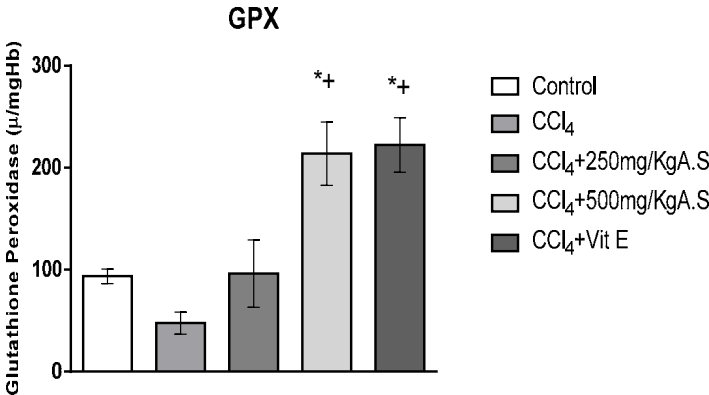
198 CCl₄ significantly ($p < .05$) decreased SOD activities. This was reversed in a dose dependent manner,
199 following administration of graded doses of *Allium sativum*. Vitamin E also attenuated the effect of
200 CCl₄ with increasing changes in the SOD level. A.S = *Allium sativum*, CCl₄ = Carbon
201 Tetrachloride

CHAT 3: Status of Catalase level in rats treated with Carbon Tetrachloride (CCl₄) induced oxidative stress after administration of *Allium sativum*



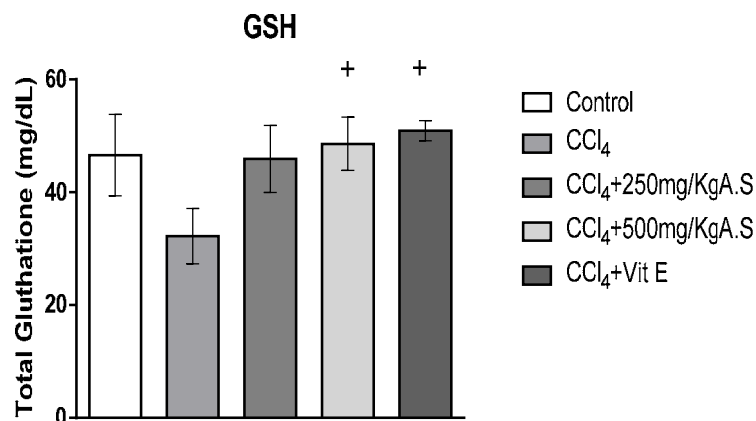
CCl₄ caused an insignificant decrease in the Catalase levels. This was reversed by a dose dependent increase in administration of graded doses of Allium sativum on CCl₄ treated rats. Vitamin E caused a significant ($p < .05$) increase in Catalase activities. A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

CHAT 4: Status of Glutathione Peroxidase (GPx) level in rats treated with Carbon Tetrachloride (CCl₄) induced oxidative stress after administration of *Allium sativum*



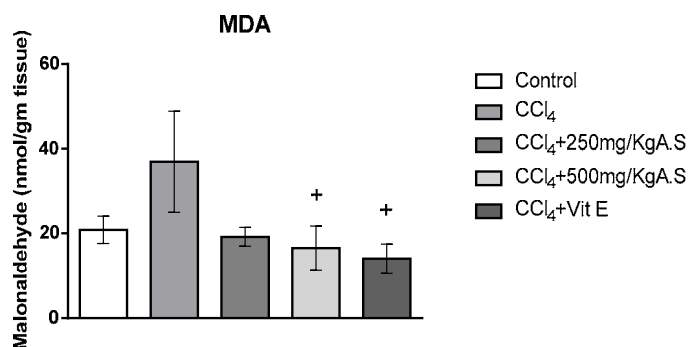
CCl₄ caused an insignificant decrease in GPx activities of blood, with dose dependent reversal observed in CCl₄ treated rats administered with graded doses of Allium sativum. Vitamin E and 500mg/Kg Allium sativum caused significant ($p < .05$) increase the GPx level. A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

CHAT 5: Status of Glutathione (GSH) activity in rats treated with Carbon Tetrachloride (CCl₄) induced oxidative stress after administration of *Allium sativum*



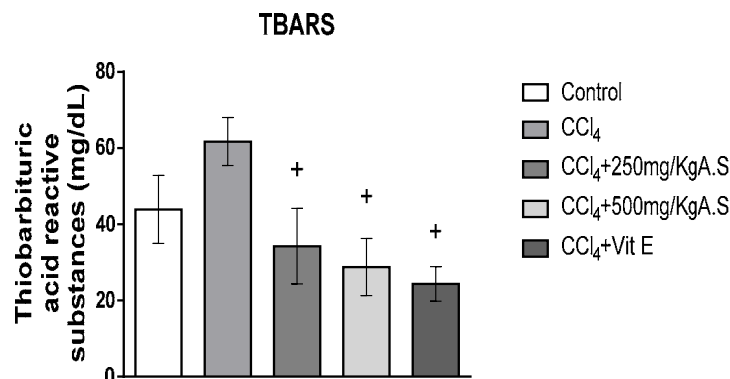
CCl₄ insignificantly decreased the Total GSH level. Allium sativum and Vitamin E significantly attenuated the effect ($p < .05$). A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

CHAT 6: Status of Malonaldehyde (MDA) activity in rats treated with Carbon Tetrachloride (CCl₄) induced oxidative stress after administration of *Allium sativum*



CCl₄ increased MDA activities in the rats. The increased MDA level was reversed by decreasing the MDA level of CCl₄ treated rats with increased dose of Allium sativum. Vitamin E caused a significant ($p < .05$) decrease in MDA level of rats treated with CCl₄. A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

CHAT 7: Status of Thiobarbituric Acid (TBARS) activity in rats treated with Carbon Tetrachloride (CCl₄) induced oxidative stress after administration of *Allium sativum*



CCl₄ increased the TBARS level of the rats. The CCl₄ induced TBARS increase was decreased in a dose dependent manner after administration of graded doses of Allium sativum. Vitamin E also decreased TBAR activities significantly ($p < .05$). A.S = Allium sativum, CCl₄ = Carbon Tetrachloride.

Discussion

Garlic in this study increased antioxidant enzymes SOD, GPx and Catalase, but caused a decrease in MDA and TBARS levels. It was therefore seen to be associated with a favourable improvement in oxidative biomarkers. Augusti & Sheela had proposed that antioxidant effect of S-allyl cysteine sulfoxide (isolated product from garlic) may have contributed to its beneficial effect as anti-diabetes²⁴. Another proposed mechanism is due to spare insulin from sulphydryl group. Inactivation of insulin by sulphydryl group is a common phenomenon. Garlic (allicin) can effectively combine with compounds like cysteine and enhance serum insulin²⁵. This finding is consistent with the works carried out by Anu Rahal *et al.*, in 2013, who concluded that Garlic normalises oxidative stress in alloxan-induced diabetic rats biochemically¹⁰.

Though Garlic did not alter blood calcium concentration, it caused a decrease in Globulin while increasing total platelets in rats induced with oxidative stress. The unaltered calcium level could be due to protective effect of garlic as anti-oxidative stress, or a possible increase in Calcium abolished by the presence of Aged garlic extract (AGE). It is likely that AGE

works in a synergistic manner and exerts multiple effects on the biochemical pathways involved in platelet aggregation¹⁸.

Increased platelet aggregation plays a pivotal role in the aetiology 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. Results from this study indicate that garlic decreases platelet aggregation; however, the mechanisms involved are not clearly defined^{12 & 13}. However, it is likely that it (garlic) achieves this by increasing cyclic nucleotides and inhibiting fibrinogen binding and platelet shape change¹². This finding is consistent with Lau, 2006 report²⁶ who found that intake of garlic oil at high dose significantly increases plasma fibrinogen concentration and affected the levels of several haematological parameters such as erythrocyte count, haemoglobin and platelets.

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

Within the ambient of vulnerability to possible human, statistical and/or logical errors, this study has establish the series of alterations that oxidative stress markers may undergo, following treatment with garlic extract in carbon-tetrachloride administered wistar rats. Study ascertained that Garlic increased antioxidant enzymes SOD, GPx, CATALASE but significantly reduced MDA and TBARs 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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