

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

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

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

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. 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 Europe¹¹⁻¹³.

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 II¹⁴. 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 Compounds¹⁵. These “magic” drugs are well known for their: fibrinolytic effects¹⁶, hemodynamic and hemostatic effects¹⁷, platelet effects¹⁸, immunologic effects¹⁸, lipid-lowering effects¹⁹, anti-atherosclerotic effects¹³, anti-oxidative effects²¹, anti-cancer effects (Milner, 2001), vascular effects¹⁷, anti-microbial effects²², haematological effects¹⁶ and hepatoprotective effects¹⁹ among other health benefits.

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

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. Access 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 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 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 Misra and Fridovich (1972); Aline *et al.* (2013), 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 adrenochrome

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 Okhawa *et al.* (1979); Aline *et al.* (2013). 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

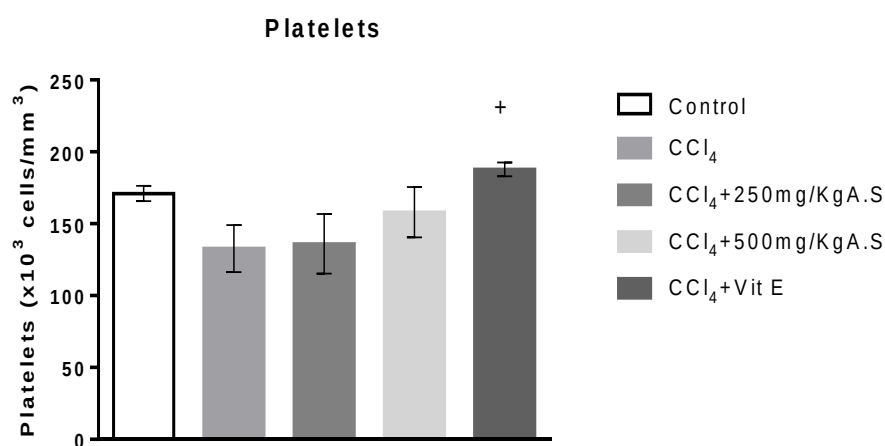
Results of the study were presented as mean $\pm$ Standard error of mean (SEM) of sample size. Mean values among and between groups were compared statistically by one way analysis of variance (ANOVA), followed by post hoc Turkey's test for multiple comparison using

185 Statistical package for social sciences (SPSS version 20). $P < .05$ was considered to be
 186 statistically significant. Data was further subjected to LSD post hoc test and differences
 187 between means accepted significant at $p < .05$.

188 **Results**

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

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

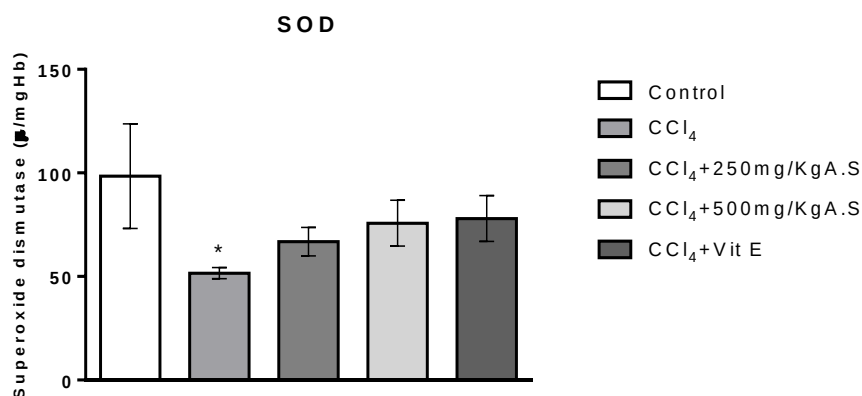


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194 Insignificant increase in PC ($p < .05$) as compared to rats treated with CCl_4 . A.S = *Allium*
 195 *sativum*, CCl_4 = Carbon Tetrachloride

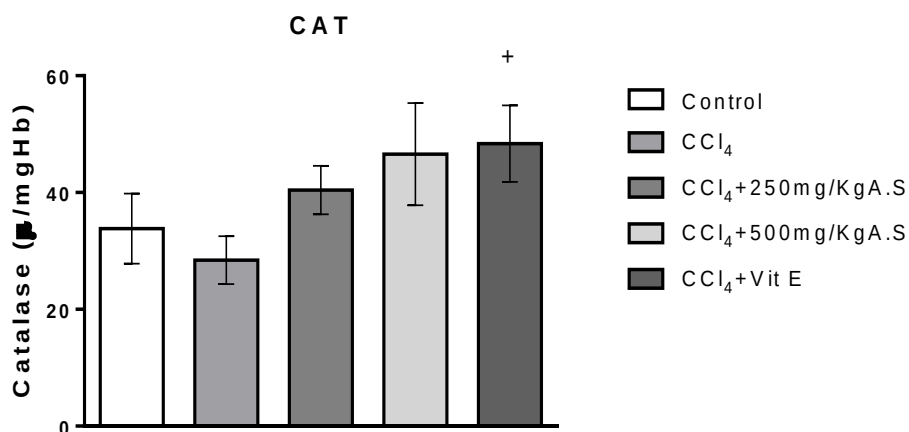
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197 **Chat 2** Status of SOD levels of rats treated with Carbon Tetrachloride (CCl_4) induced
 198 oxidative stress after administration of *Allium sativum*



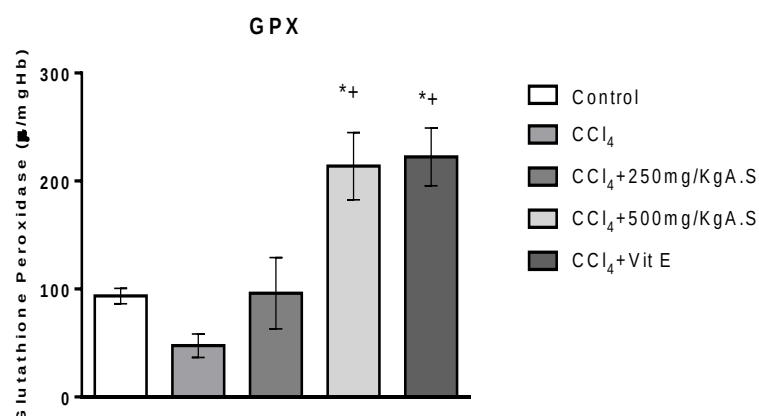
*CCl₄ significantly ($p < .05$) decreased SOD activities. This was reversed in a dose dependent manner, following administration of graded doses of *Allium sativum*. Vitamin E also attenuated the effect of *CCl₄* with increasing changes in the SOD level. A.S = *Allium sativum*, *CCl₄* = Carbon Tetrachloride*

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



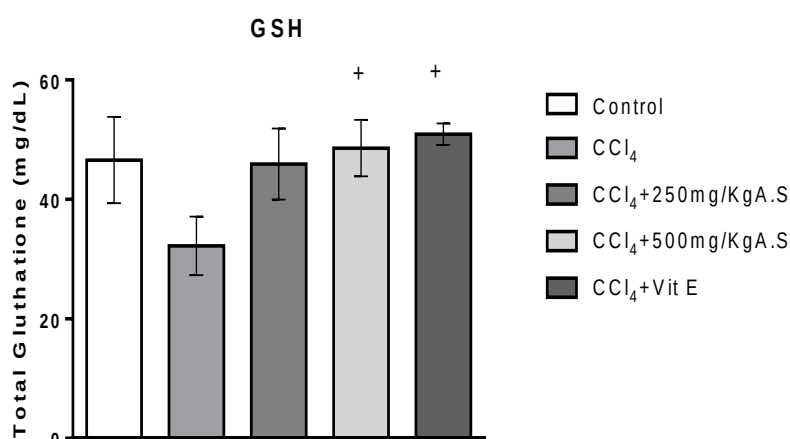
*CCl₄ caused an insignificant decrease in the Catalase levels. This was reversed by a dose dependent increase in administration of graded doses of *Allium sativum* on *CCl₄* treated rats. Vitamin E caused a significant ($p < .05$) increase in Catalase activities. A.S = *Allium sativum*, *CCl₄* = Carbon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

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



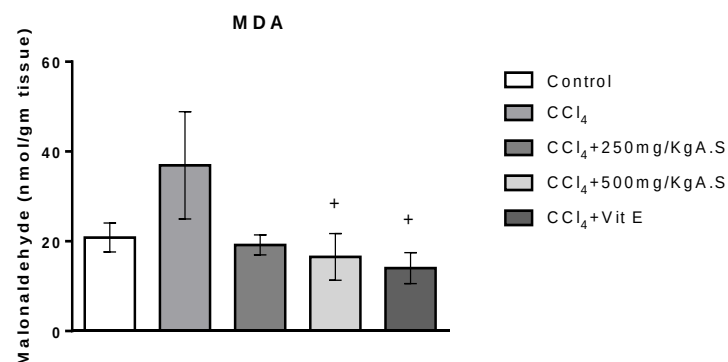
CCl₄ caused an insignificant decrease in GPx activities of blood, with dose dependent reversal observed in CCl₄ treated rats administered with graded doses of Allium sativum. Vitamin E and 500mg/Kg Allium sativum caused significant ($p < .05$) increase the GPx level. A.S = Allium sativum, CCl₄ = Carbon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

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



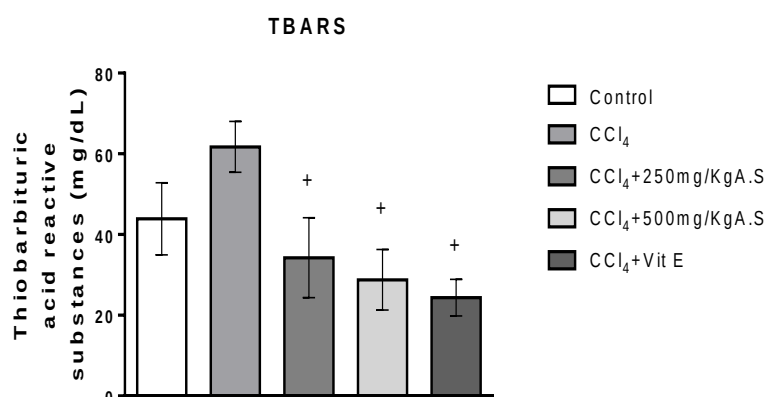
CCl₄ insignificantly decreased the Total GSH level. Allium sativum and Vitamin E significantly attenuated the effect ($p < .05$). A.S = Allium sativum, CCl₄ = Carbon Tetrachloride

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 decreased MDA and TBARS. It is therefore seen to be associated with a favourable improvement in oxidative biomarkers. Augusti & Sheela also proposed that antioxidant effect of S-allyl cysteine sulfoxide (isolated product from garlic) may have contributed to its beneficial effect as anti-diabetes (Augusti and Sheela 1996). 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 insulin (Mathew and Augusti, 2003). This finding is consistent with works carried out by Abdultawab and Ayuob in 2013, who concluded that Garlic normalises oxidative stress in alloxan-induced diabetic rats biochemically.

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 aggregation (Allison *et al.*, 2006).

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. Result from this study indicate that garlic decreases platelet aggregation; however, the mechanisms involved are not clearly defined (Khalid et al., 2014). However, it is likely that it (garlic) achieves this by increasing cyclic nucleotides and inhibiting fibrinogen binding and platelet shape change (Khalid et al., 2014). This finding is consistent with Kung-chi's 2007 report 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.

282 Societal benefit of Study

283 From this study, the following can be deduced:

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

290

291 Conclusion

292 Within the ambient of vulnerability to possible human, statistical and/or logical errors,
 293 this study has establish the series of alterations that oxidative stress markers may undergo,
 294 following treatment with garlic extract in carbon-tetrachloride administered wistar rats. Study
 295 ascertained that Garlic increased antioxidant enzymes SOD, GPx, CATALASE but
 296 significantly reduced MDA and TBARs in CCL4 induced oxidative stress.

297

298 Recommendations

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

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

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304 References

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- 306 1. Osman, H.G., Gabr, O.M., Lotfy, S. and Gabr, S. (2007) Serum levels of bcl-2 and
- 307 cellular oxidative stress in patients with viral hepatitis. Indian Journal of Medical
- 308 Microbiology, 25, 323-329.

2. Niki, E. (2009) Lipid peroxidation: Physiological levels and dual biological effects. *Experimental and Molecular Pathology*, 47, 469-484.
3. Turgay, M., Durak, I., Erten, S., Ertugrul, E., Devrim, E. and Avci, A. (2007) Oxidative stress and antioxidant parameters in a Turkish group of patients with active and inactive systemic lupus erythematosus. *APLAR Journal of Rheumatology*, 10,101-106.
4. Schaffer, F. O and Buettner, G. R. (2001). Redox environment of the cell as viewed through the redox state of the glutathione disulphide/glutathione couple. *Free Radical in Biology and Medicine*. **30** (11): 1191 – 1212.
5. Schneider, C. (2005): Chemistry and Biology of Vitamin E. *Molecular and Nutritional Food Research*. 49 (1): 7 – 30.
6. Sosa Torres ME, Saucedo-Vazquez JP, Kroneck P.M (2015). “Chapter 1, Section 3 The dark side of dioxygen” In: Kroneck PM, Torres ME. Sustaining Life Planet Earth: Metalloenzymes Mastering Dioxygen and Other Chewy Gases. Metal Ions in Life Sciences. 15 pp. 1-12.
7. Valko, M. Morris, H. and Cronin, M. T. (2005). “Metals, toxicity and oxidative stress.” *Current Medical Chemistry*. **12** (10): 1161 – 1208.
8. Rojas P., Serrano-García N., Medina-Campos O. N., Pedraza-Chaverri J., Maldonado P. D., Ruiz-Sánchez E. (2011); S-Allylcysteine, a garlic compound, protects against oxidative stress in 1-methyl-4phenylpyridinium- induced parkinsonism in mice. *The Journal of Nutritional Biochemistry*. 22(10):937–944.
9. Garrido, N., Meseguer, M., Simon, C., Pellicer, A. and Remohi, J. (2004) Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male fertility. *Asian Journal of Andrology*, 6, 59-65.
10. Anu Rahal, Amit Kumar , Vivek Singh , Brijesh Yadav , Ruchi Tiwari, Sandip Chakraborty, and Kuldeep Dhama (2014). Oxidative Stress, Prooxidants, and Antioxidants: The Interplay, *BioMed Research International* Volume 2014, Article ID 761264, pp 1-19.
11. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.T.; Mazur, M.; Telser, J (2007) values of garlic: A review *International Journal of Medicine and Medical*
12. Vecchia C (2006). Onion and garlic use and human cancer. *Am. J. Clin.* vol. 39, no. 7, pp. 841–852.
13. Ray B., Chauhan N. B., Lahiri D. K. (2011), The ‘aged garlic extract’ (AGE) and one of its active ingredients S-allyl-L-cysteine (SAC) as potential preventive and

therapeutic agents for Alzheimer's disease (AD) Current Medicinal Chemistry.18(22):3306–3313.

14. Rahman K (2007). "Effects of garlic on platelet biochemistry and physiology". Mol Nutr Food Res. **51** (11): 1335–44.

15. Banerjee S. K., Maulik S. K. (2002), Effect of garlic on cardiovascular disorders: Nutrition Journal.; 1(4) 1475-2891.

16. Christen, Y. (2000). Oxidative stress and Alzheimer disease. *American Journal of Clinical Nutrition*. **71** (2): 621 -629.

17. Block, E. (2010). Garlic and Other Alliums: The Lore and the Science. Royal Society of Chemistry J. Nutrition Science. 11 (5): 82–99.

18. Ashraf R. Khan R.A. Ashraf I, Qureshi A.A. (2013); Effects of Allium sativum (garlic) on systolic and diastolic blood pressure in patients with essential hypertension. J Pharm Sci 26(5) pp.859-863.

19. Ide N. And Lau B, (1999), Aged Garlic extract attenuates intracellular oxidative stress . Phytomedicine, Vol 6. Pp. 125-131.

20. Bohnen NI, Kaufer DI, Hendrickson R, Ivanco LS, Lopresti BJ, Koeppe RA, Meltzer CC, Constantine G, Davis JG, Mathis CA, Dekosky ST, Moore RY (2005). Degree of inhibition of cortical acetylcholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease. J Neurol Neurosurg Psychiatry.76:315-319.

21. Halliwell, Barry (2007). "Oxidative stress and cancer: have we moved forward?" (PDF). Biochem. J . 401 (1): 1–11

22. Jung F, Jung EM, Pundir G and Kieseewetter H (1990). Effect of different garlic preparations on the fluidity of blood, fibrinolytic activity and peripheral microcirculation in comparison with placebo. *Planta Med*, 56, 668.

23. Ritesh K.R. Suganyaa A., Dileepkumarb H.V., Rajashekara Y., T.Shivanandappab, toxicology report. (2015). A single acute hepatotoxic dose of CCl4 causes oxidative stress in the rat brain. Toxicology Reports 2 891–913.