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Protective effects of *Allium sativum* on carbon tetrachloride-induced hepatotoxicity in rats

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Abstract

The antihepatoxic effect of *Allium sativum* L. (garlic) and its possible mechanism of action were investigated in rats treated with carbon tetrachloride (CCl_4). The activities of hepatic enzymes; alkaline phosphatase (ALP), alanine aminotransferase (ALT), asparate aminotransferase (AST), and levels of total protein and albumin were determined in serum, whereas the levels of thiobarbituric acid reactive-substances (TBARS) and glutathione (GSH) were assayed in liver homogenate. CCl_4 (0.5ml/kg; i.p.) administered consecutively for 7 day resulted in significant increases in activities of ALP, ALT and AST, and the level of TBARS in the liver. Conversely, CCl_4 administration led to significant decreases in serum concentrations of total protein and albumin, and GSH content in the liver. Concomitant treatment of A. *sativum* (800 mg/kg; orally) or vitamin C (400 mg/kg; orally) with CCl_4 for 7 consecutively days, significantly ($p<0.05$) prevented the CCl_4-induced alterations in the activities of liver marker enzymes as well as serum levels of total protein, albumin, and TBARS and GSH content of liver. These results suggest that A. *sativum* exerts protective action against CCl_4 –induced hepatotoxicity, possibly due to its antioxidant property.

Keywords: *Allium sativum* L. (Liliaceae), Carbon tetrachloride, Garlic extract, Hepatotoxicity, Lipid peroxidation, Vitamin C, Glutathione

1.Introduction

Allium sativum L. Liliaceae (garlic) has been employed as a formidable prophylactic and therapeutic herbal medicinal agent in the folklore of many cultures over the centuries (1). Many health-promoting effects attributed to A. *sativum* include reduction of risk factors for cardiovascular diseases and cancer, antibacterial and anti-parasitic activities, enhanced foreign compound detoxification, resistance to various stresses and anti-aging effect (2, 3). Recent experimental studies have demonstrated that A. *salivum* exhibits several interesting pharmacological properties, including antioxidant free-radical-scavenging activity and prevention of lipid peroxidation (4, 5).

The liver is prone to environmental chemical-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation, its physiological and anatomical structure. Most drugs and environmental chemicals are not intrinsically toxic to the liver but cause injury secondary to the formation of a hepatotoxic chemical metabolite through a process termed bioactivation (6). It has been reported that cytochrome P-450 enzyme mediates reduction of halogenated hydrocarbons such as carbon tetrachloride (CCl_4), which can generate free radical intermediates that can directly cause liver oxidative damage (7, 8). Glutathione (GSH), an important non-vitamin antioxidant, plays a ubiquitous role in the cellular antioxidant defence in all mammalian cells and with a high synthesis rate and concentration in the liver (9). Hence, the antioxidant activity could be an important mechanism in the protection against CCl_4-induced hepatotoxicity (10, 11). Thus, search for medicinal plant with antioxidant property has become a significant focus of study of hepatoprotection. The aim of the present study was to investigate whether administration of aqueous extract of A. *sativum* would prevent CCl_4-induced hepatotoxicity and to assess potential mechanisms for the effect of A. s*ativum* on CCl_4-induced hepatotoxicity.

2. Materials and methods

2.1 Animals

Twenty-four male Sprague-Dawley rats (150-180g) were obtained from the Animal Breeding Centre of the College of Medicine, University of Ilorin, Nigeria. Animals were housed 6 per cage in a standard environmental condition and were allowed free access to commercial pelleted rat chow (Ladokun Feeds Ltd, Ibadan, Nigeria).

2.2 Assay Kits and Chemicals

CCl_4 was obtained from S. D. Fine-Chem. Ltd., (Boistar, India). Vitamin C was obtained from Chemo-Pharma Laboratories Ltd (Lagos, Nigeria), Thiobarbituric acid, 5-5'- Dithro-bis-Z-Nitrobenzoic acid (DTNB) and glutathione (GSH) were supplied by Sigma Chemical Company (St. Louis MO. USA). The kits for alkaline phosphatase, alanine aminotransferase and asparate aminotransferase were purchased from Quinica Clinica

Aplicada, (S. A. Spain). Total protein and albumin kits were supplied by Randox Laboratory Ltd. (Co. Antrim, U.K.). All other chemicals were of analytical grade and were prepared in all glass-distilled water.

2.3 *Allium sativum* Extract

Bulbs of A. *sativum* were collected from Sokoto town of Sokoto State, Nigeria. The materials were botanically authenticated by Prof. F. A. Oladele of the department of Botany, University of Ilorin, Ilorin, Nigeria. The collected materials were thoroughly washed in distilled water. A. *sativum* cloves were peeled, chopped, air dried and pulverized into a fine powder. One hundred grams of powdered material was percolated in 1000ml of distilled water (1:10; w/v) and kept for 12 hours. The percolated mixture was filtered and evaporated at room temperature to dryness. The resultant powder of the extract was stored in refrigerator for further use.

2.4 Administration

The animals were randomly divided into 4 groups of 6 rats each. Group I received appropriate volume of distilled water orally and intraperitoneally (i.p.) consecutively for 7 days. Group II received distilled water (orally) and CCl_4 (0.5 ml/kg; i.p.), simultaneously for 7 days (12). Group III received A. *sativum* extract (800 mg/kg, orally) and CCl_4 (i.p.), simultaneously for 7 days. Group IV received vitamin C (400 mg/kg; orally) and CCl_4 (i.p.) simultaneously for 7 days.

2.5 Tissue preparation

Following 24-hour overnight fast, the rats were sacrificed by cervical dislocation on the 8th day of the experiment. Blood sample was collected from the jugular vein. Serum was obtained by centrifugation at 3000 rpm for 10 min (13). The livers were excised immediately, blotted off blood, rinsed in ice-cold saline and 50% homogenate was prepared in 0.05 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 1000 rpm for 10 minutes at 4^0C. The supernatant was immediately used for the determination of malondialdehyde (MDA) and glutathione (GSH).

2.6 Biochemical measurements

The activities of marker enzymes; alkaline phosphatase (ALP), alanine aminotransferase (ALT) and asparate aminotransferase (AST) were determined in serum using standard assay kits. The levels of total protein and albumin were determined in serum by biuret method and bromcresol green method, respectively (14) using standard assay kits. Liver MDA content was estimated by reactive substances (TBARS) as an index of lipid peroxidation (15).GSH content of the liver was determined using 5-5'-Dithro-bis-z-Nitro-benzoic acid (DTNB) as described by Buetler *et al* (16).

2.7 Statistical analysis

The results are expressed as means ± SEM. The results were analyzed by a One-way Analysis of Variance (ANOVA) using SPSS (SPSS Inc., Chicago, USA). The differences between the means were determined using Duncan's Multiple-range test. Statistical significance was considered at $p<0.05$

3. Results

3.1 Activities of marker enzymes

Administration of CCl_4 for 7 days led to significant ($p<0.05$) increases in the serum alkaline phosphatase, alanine aminotransferase and asparate aminotransferase, which were significantly ($p<0.05$) abrogated by concomitant treatment with A. *sativum* or vitamin C. The effects of A. *sativum* or vitamin C was not significantly different (Table I).

3.2 Serum total proteins and albumin

Administration of CCl_4 produced significant ($p<0.05$) decreases in the serum levels of total proteins and albumin. The changes induced in serum total proteins and albumin were significantly ($p<0.05$) abolished by treatment with A. *sativum* or vitamin C. The effect of A. *sativum* and vitamin C on serum levels of total proteins and albumin were not significantly ($p>0.05$) different (Table 2)

3.3 Liver malondiadehyde (MDA) and glutathione (GSH) levels

The levels of lipid peroxidation index, MDA in the livers of CCl_4-treated rats were significantly ($p<0.05$) higher when compared with control rats. However, compared with control rats, GSH levels were significantly ($p<0.05$) lower in the livers of CCl_4-treated (Table 3). Concomitant treatment with A. *sativum* or vitamin C led to significant ($p<0.05$) abrogation of the alterations in liver content of MDA and GSH. The effect of A. *sativum* and vitamin C were not significantly ($p>0.05$) different (Table 3).

Discussion

Carbon tetrachloride (CCl_4) is an environmental contaminant that has been detected in ambient air sea water, and snow. It is one of the most commonly used environmental chemical in the experimental study of liver injury (8). It has been documented that CCl_4-induced hepatotoxicity is attributed to accumulation of CCl_4 in hepatic parenchymal cells that are activated by cytochrome P-450 dependent monoxygenase trichloromethyl radical ($CCl_3^{\cdot}$) (7, 8). CCl_4 alkylates cellular macromolecules with a simultaneous oxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to generation of lipid peroxides, which in turn causes products like MDA that cause injury to the membrane (7). Hepatotoxicity of CCl_4 is evidenced in these animals by increases in activities of serum alkaline phosphatase, alanine aminotransferase and asparate aminotransferase. It has been documented that increased levels of serum activities of alkaline phosphatase, alanine aminotransferase and asparate aminotransferase are biochemical markers of hepatic injury (17). The observation that concomitant administration A. s*ativum* extract completely abolished the alterations induced by CCl_4 in activities of serum

Table 1: Effect of Allium sativum (AS) and vitamin C (Vit C) on serum activities of liver marker enzymes in CCl_4-induced hepatotoxicity

Parameters	I (Control)	II (CCl_4)	III (CCl_4+AS)	IV (CCl_4+Vit C)
ALP (IU/L)	209.1±18.2[a]	482.9±21.2[b]	245.2±22.3[a]	221.1±16.9[a]
ALT (IU/L)	45.5±7.7[a]	159.9±13.3[b]	62.8±9.3[a]	51.5±6.8[a]
AST (IU/L)	78.1±8.2[a]	281.7±12.8[b]	72.3±10.1[a]	69.9±9.2[a]

Values are presented as means ± SEM for 6 rats per group. The means in the row not sharing a common superscript letter are significantly different ($p<0.05$). ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, asparate aminotransferase

Table 2: Effect of Allium sativum (AS) and vitamin C (Vit C) on serum albumin (Alb) and total protein (TP) in CCl_4-induced hepatotoxicity

Parameters	I (Control)	II (CCl_4)	III (CCl_4+AS)	IV (CCl_4+Vit C)
Alb (mmol/L)	29.7±1.5[a]	13.6±1.8[b]	25.8±2.1[a]	26.3±2.3[a]
TP (mmol/L)	42.2±1.8[a]	30.1±2.5[b]	38.1±3.1[a]	40.9±2.5[a]

Values are presented as means ± SEM for 6 rats per group. The means in the row not sharing a common superscript letter are significantly different ($p<0.05$).

Table 3: Effect of Allium salivum (AS) and vitamin C (Vit C) on malondialdehyde (MDA; µmol/mg protein) and glutathione (GSH: µmol/100g of wet tissue) levels in CCl_4-induced hepatotoxicity

Parameters	I (Control)	II (CCl_4)	III (CCl_4+AS)	IV (CCl_4+Vit C)
MDA	0.91±0.04[a]	1.10±0.05[b]	0.88±0.05[a]	0.86±0.06[a]
GSH	325.5.2±15.2[a]	228.3±13.5[b]	288.2±10.1[a]	311.7±11.3[a]

Values are presented as means±SEM for 6 rats per group. The means in the row not sharing a common superscript letter are significantly different ($p<0.05$).

alkaline phosphatase, alanine aminotransferase and asparate aminotransferase (Table 1), suggests that aqueous extract of A. s*ativum* possesses hepatoprotective action.

Serum albumin is the main plasma protein produced in the liver, a clinically useful indicator of hepatic synthetic function (17). Liver injury is associated with a decline in the concentration of serum albumin, and hypoalbuminemia has been considered to be consequence of a decreased rate of hepatic synthesis of the essential protein in both humans and animals (18). Accordingly, the serum albumin and total protein levels were determined in the present study. The CCl_4-treated rats exhibited significantly lower serum total protein and albumin levels compared with the control rats. However, concomitant treatment with A. s*ativum* increased these serum levels to a control range. Thus, this suggests that A. *sativum* possesses ability to promote protein synthesis that plays a significant contributory role in hepatoprotective activity, which in turn has been shown to enhance proliferative process and the production of hepatocytes (19).

Hepatotoxicity induced by CCl_4 has been attributed to lipid peroxidative degradation of biomembranes (20). MDA is known to be a reliable marker of lipoperoxidation and oxidative stress (15). In this study, elevated content of MDA in liver of CCl_4-treated rats implies excessive generation of reactive oxygen species and activation of lipoperoxidative hepatic damages and/or failure of antioxidant defense mechanisms to prevent generation of excessive free radicals. Treatment with A. s*ativum* significantly prevented increase in MDA induced by CCl_4. Hence, the hepatoprotective activity of A. s*ativum* may be due to its antioxidant activity.

GSH is a principal non-protein thio in living organisms which plays a significant role in coordinating the endogenous antioxidant defence processes in all mammalian cells, with a high synthesis rate and concentration in the liver where it is released into the blood to supply other tissues (9). Studies have suggested that disruption of the structural integrity of cell membranes and subcellular structures induced by free oxygen radicals is associated with altered GSH level (9, 21). Reduction in GSH level in the liver of CCl_4-treated rats and the ability of extract of A. *sativum* to prevent the alteration strongly suggests that lipid peroxidation and oxidative stress-induced by CCl_4 intoxication have been abrogated due to the antioxidant property of A. s*ativum*.

All the effects of A.*sativum* on hepatotoxicity induced by CCl_4 were comparable with those of vitamin C, a well known water-soluble synthetic antioxidant. These observation implies that A. *sativum* ability to protect liver damage induced by CCl_4 seems to be as effective as the water-soluble synthetic antioxidant as previously observed in other related studies (22, 23). A. *sativum* with a natural antioxidant and a synthetic water-soluble antioxidant, (vitamin C) seem to exert hepatoprotective action by enhancing activity of non-vitamin antioxidant defence mechanism in liver.

The component in A. *sativum* that negates CCl_4-induced hepatotoxicity was not identified in this study. Much attention has been given to the numerous sulfur-containing compounds that seem to be important for a number of the medicinal properties of A. *sativum* (1, 2, 3). Allicin, the predominant thiosulfinate in A. *sativum*, has been reported to be responsible for antioxidant and free-radical scavenging activity of A. *sativum* extract (4, 5). In conclusion, these findings suggest that A. *sativum* extract exhibits protective effect against liver injury, possibly through its antioxidant property.

References

1. L. Harris. Book of Garlic. (Reading, MA, Addison – Wesley, 1995).
2. E. Ernst. Pharmatherapeutical **5(2)**, 83-89 (1987).
3. H. Amagase, B.L. Petesch, H. Matsuura, S. Kasuga and Y. Itakura J Nutr B1, 9555-9625, (2001).
4. K. Prasad, V.A. Laxdal, M.Yu and B.L. Raney, Mol Cell Biochem **148**, 183-189 (1995).
5. A. Rabinkov, T. Miron, L. Konstantinovski, M. Wilchek, D. Mirelman and L. Weiner Biochem Biophysics Acta. **1379**, 233-244, (1998).
6. D.A. Vessey, In: Hepatology: a textbook of liver disease, D.B. Zakin and T.D. Goyer (eds). (WB Saunders, Philadelphia, 875-890, (1996).
7. R.O. Recknagel, Life Sci. **33**, 401-408, (1983).
8. D.E. Johnson and C. Kroening. Pharmacol Toxicol **83**, 231-239, (1998).
9. H. Sies Free Radic Biol Med **27**, 916-921, (1999).
10. W. Zhu and P.C. Fung, Free Radicals Biol Med **29(9)**, 870-880 (2000).
11. J. I. Sotelo-Felix, D. Martinez-Fong and M. De la Torre., Euro J Gastroenterol Hepatol **14(9)**, 1001-1006 (2002).
12. P.G.M. Rao and S.G. Rao, V. Kumar. Fitoterapia. **LXIV**, 108-13, (1993).
13. L.A. Olatunji, J.O. Adebayo, O.B. Oguntoye, N.O. Olatunde, V.A. Olatunji and A.O. Soladoye, Pharmaceut Biol, in press, (2005).
14. Gowenlock, A.H., McMurray, J.R., McLauchlan, D.M. In: Varley's Practical Clinical Biochemistry, 6th Ed., CBS publishers, New Delhi, 401-435, (1988).
15. D. D. Kitts, Y.V. Yuan and Godin, D.V. Can J Pharmacol. **76**, 202-209, (1998).
16. E. Buetler, O. Duron and B.M. Kelly, J Lab Clin Med **61**:882, (1963).
17. M.G. Sturgill and G.H. Lambert, Clin Chem **43:8(B)**, 1512-1526, (1997).
18. B. Ruot, F. Bechereau, G. Bayle, D. Breuille and C. Obled Clin Sci. **102**, 107-114, (2002).
19. D. Awang Can Pharmacol J **23**, 749-754, (1993).
20. W. Kaplowitz, T.Y. Aw, F.R. Simon, A. Stolz. Intern Med. **104**, 826-839, (1986).
21. C.L. Bandyopadhyary, D. Das and R. Banerjee. Current Sciences. **77(5)**, 658 – 665 (1999).
22. S.P. IP and K.M. Ko. Biochem Pharmacol **52(11)**, 1687 – 1693 (1996).
23. A. Valenzuelaz, C. Lagos, K. Schmidt, K. Videla, Biochem Pharmacol, **3**, 2209-2212, (1985).