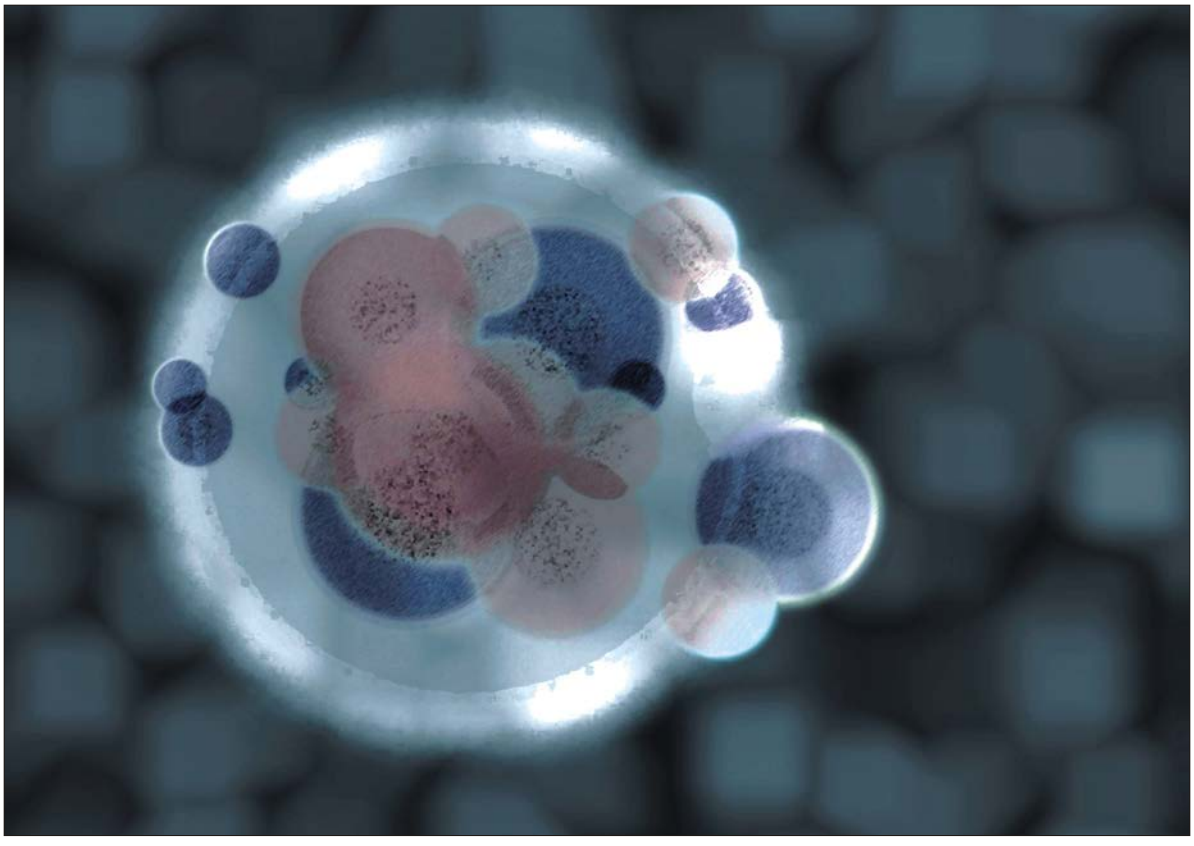

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The Protective Effects of Ethanolic Extract of Garlic and Ascorbic Acid on Cadmium-Induced Oxidative Stress

[1]I.V. Ogungbe and [2]A.O. Lawal
[1]Department of Biochemistry, Delta State University, Abraka, Delta State, Nigeria
[2]Strathclyde Institute of Pharmacy and Biomedical Research, University of Strathclyde, Glasgow, UK

Abstract: The protective effects of ethanolic extract of garlic (*Allium sativum*) and ascorbic acid on cadmium-induced oxidative stress were investigated in the liver and kidney of male rats by monitoring the lipid peroxides profiles, enzymatic and non-enzymatic antioxidants activities. The rats were pretreated with ethanolic extract of garlic (100 mg kg^{-1} body weight) or ascorbic acid (100 mg kg^{-1} body weight) orally for 4 weeks. These rats were also given cadmium (4 g kg^{-1} body weight) intraperitoneally for 3 days at the last week of treatment. Another group of rats were either given extract (100 mg kg^{-1} body weight daily, orally for 4 weeks) or ascorbic acid (100 mg kg^{-1} body weight daily, orally for 4 weeks) or cadmium (4 g kg^{-1} body weight for 3 days) or distilled water. The results showed a significant increase in the activities of catalase and Super Oxide Dismutase (SOD) in the liver and kidney of ethanolic extract of garlic and ascorbic acid pretreated rats compared to control ($p<0.05$). Lipid peroxides levels were significantly reduced in the liver and kidney of pretreated rats compared to control ($p<0.05$). Reduced glutathione (GSH) levels were significantly increased in both organs of ascorbic acid and ethanolic extract of garlic pretreated rats when compared to control ($p<0.05$). The study revealed the potential of ethanolic extract of garlic to prevent oxidative damage induced by acute dose of cadmium.

Key words: Lipid peroxides, cadmium, garlic, glutathione, antioxidant enzymes

INTRODUCTION

Oxidative stress originating from outside the body is a feature of life in the modern world. Tens of thousands of confirmed toxic substances in our external environment are invariably sources of free radical or related oxidants (Kidd, 1993; Kidd, 1996). Sustained oxidative stress from a heavy cumulative burden of oxidants may deplete the body's antioxidant reserves to a point beyond which the antioxidant defenses are overwhelmed (Kidd, 1991). Cadmium is a naturally occurring element; it is present everywhere in the environment. Cadmium is virtually absent at birth in mammals but accumulates with time especially in the liver and kidney, such that up to 75% of the total body burden is found in these organs (Friberg *et al*., 1985; Bellinger *et al*., 2004). Exposure to cadmium can occur from food, water and smoking or at work (Degraeve, 1981). The diet is the major source of human exposure of cadmium (Bellinger *et al*., 2004).

The cadmium concentrations in food samples vary widely but the highest average concentrations are detected in mollusks, kidney, liver, cereals, cocoa and leafy vegetables. The estimates of cadmium dietary intake derived from the world health Organization regional diets based on food balance sheets, ranged from 0.35 to 9.63 μg kg^{-1} bodyweight per day (Bellinger *et al*., 2004). Cadmium exerts its toxic effect via oxidation damage to cellular organelles by inducing the generation of Reactive Oxygen Species (ROS) (Stohls *et al*., 2000; Bagchi *et al*., 1996), which consist mainly of $O_2^{\bullet}$, H_2O_2 and $OH^{\bullet}$. The mechanisms through which this happens are not well understood but reports have indicated that cadmium does this via an indirect phenomenon (Watkin *et al*., 2003). Reactions of these ROS with cellular biomolecules have been shown to lead to lipid peroxidaton, membrane protein and DNA damage (Dally and Hartwig 1997; Nartey *et al*., 1987). This possibly leads to the depletion of the body's endogenous antioxidants which serve as premier source of protection against free radical and other oxidative stressors to which it invariably becomes exposed (Cross *et al*., 1987).

Garlic (*Allium sativum*), a member of the lily family, is a perennial plant that is cultivated worldwide. The protein content of garlic is about 16.8% (dry weight basis), other constituents of garlic include sulfur-containing compounds: allicin, diallyldisulfide, diallyltrisulfide, allin,

Corresponding Author: I.V. Ogungbe, Department of Biochemistry, Faculty of Science, Delta State University, P.M.B. 01, Abraka, Delta State, Nigeria

s-methyl-L-cysteine sulfoxide, vitamins, glucosinates, enzymes like allinase and peroxidase and high concentrations of trace minerals (especially selenium), generally considered to be responsible for most of the pharmacological properties of garlic (Leung, 1980). The numerous health benefits of garlic have been ascribed to its potent antioxidant action (Wei and Lau, 1998), its ability to stimulate immunological responsiveness (Reeve *et al*., 1993) and modulation of prostanoid synthesis (Dimitrov and Bennink, 1997). The strong odor of fresh garlic and its ability to generate unpleasant gastric side effects (Heber, 1997; Moriguchi *et al*., 1997) have caused many to favor dietary garlic supplements. Among the many supplements, Aged Garlic Extract (AGE), obtained by prolonged extraction of fresh garlic at room temperature has a reproducible array of components including S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) which have been analyzed and studied extensively for their high antioxidant content and health-protective potential (Amagase, 1997).

Therefore, the objective of this study was to evaluate the protective effect of ethanolic extract of garlic on cadmium-induced oxidative damage in liver and kidney of male albino rats.

MATERIALS AND METHODS

Plant materials: Garlic (*Allium sativum*) was obtained from a local grocery in Oba's market in Akure, Ondo state of Nigeria. The extraction was carried out as described by kasuga *et al*. (2001). The garlic cloves were peeled, sliced and soaked in a water/ethanol (95%) mixture (3:2) and left to extract for 10 weeks at room temperature. The extract was then obtained by filtering the suspension through Mira cloth into storage tubes and preserved until use at -40°C. The extract was then evaporated to dryness at 25°C and reconstituted in distilled water during usage.

Animals: Laboratory bred adult male albino rats of wistar strain weighing 180-220 g were obtained from Small Animal Breeding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were maintained under standard laboratory conditions to acclimatize for 6 weeks. Commercial pellet diet and water were provided *ad libitum*.

Reagents and chemicals: All chemicals were of analytical grade and chemicals required for sensitive biochemical assays were obtained from Sigma Chemical Co. USA. Double distilled water was used in all biochemical assays.

Dosage and treatment: Ascorbic acid and ethanolic extract of garlic were given to the rats at an oral dose of 100 mg kg^{-1} body weight each per day for 4 weeks. An acute dose of 4 g kg^{-1} body weight of cadmium salt was administered intraperitoneally for 3 days at the last week of treatment. The rats were sacrificed 24 h after the last dose of cadmium salt had been administered.

Experimental procedure: The rats were divided into six groups of 4 animals each. Treatments were done accordingly.

Group 1: Normal control
Group 2: Animals received ethanolic extract of garlic 100 mg kg^{-1} body weight orally.
Group 3: Animals received ascorbic acid 100 mg kg^{-1} body weight orally.
Group 4: Animals received cadmium salt 4 g kg^{-1} body weight intraperitoneally
Group 5: Animals received cadmium salt 4 g kg^{-1} body weight intraperitoneally+Pretreatment with ethanolic extract of garlic 100 mg kg^{-1} body weight orally.
Group 6: Animals received cadmium salt 4 g kg^{-1} body weight intraperitoneally+Pretreatment with ascorbic acid 100 mg kg^{-1} body weight orally.

Tissue decapsulation: The tissues were decapsulated, washed free of blood and adhering tissues and immersed in ice-cold normal saline. They were homogenized immediately and stored in the freezer until required up to a maximum period of 4 days.

Biochemical analysis: Lipid peroxides levels were estimated by assaying malondialdehyde (MDA) formation using the method of Varshey and Kale (1990). Reduced Glutathione (GSH) levels were determined according to the method of Jollow *et al*. (1974). Catalase activity was measured following the decomposition of H_2O_2 as described by Sinha (1972). SuperOxide Dismutase (SOD) activity was determined by following the autooxidation of epinephrine according to the method of Misra and Fridovich (1972). Protein content was estimated by the Biuret method as described by Gornall *et al*. (1949) with some modifications.

Statistical analysis: Data are expressed as mean±SEM. Statistical analysis were performed using student t-test at $p<0.05$ significant levels.

RESULTS

The results revealed a significant increase in lipid peroxidation and decrease in antioxidant enzymes activities and in reduced glutathione level in cadmium treated rats (Group 4) compared to control ($p<0.05$). A significant increase in antioxidant enzymes levels and

Table 1: Effect of ethanolic extract of garlic (*Allium sativum*), ascorbic acid and cadmium on lipid peroxidation, antioxidant enzymes, reduced glutathione and protein levels in the liver of rats

	Groups					
Parameters	1	2	3	4	5	6
Protein (mg mL^{-1} homogenate)	27.25±4.34	28.58±2.09	27.38±3.38	24.20±2.34	27.81±1.26^{b}	23.13±2.37
Lipid peroxides (mmole of MDA/mg protein)	5.33±0.51^{b}	1.19±0.42^{ab}	3.50±0.32^{b}	7.56±0.69^{a}	1.30±0.23^{ab}	4.85±0.72^{b}
Superoxide dismutase (Units/mg protein)	0.94±0.10^{b}	0.68±0.05^{ab}	3.29±0.62^{ab}	0.53±0.03^{a}	1.54±0.06^{ab}	2.24±0.47^{ab}
Catalase (μmole of H_2O_2 decompose/Min/mg protein)	0.56±0.12^{b}	0.64±0.03	0.94±0.09^{ab}	0.41±0.07^{a}	0.67±0.02^{ab}	0.76±0.05^{ab}
GSH (μg mL^{-1} homogenate)	1.05±0.07^{b}	3.11±0.19^{ab}	2.27±0.21^{ab}	0.33±0.02^{a}	2.14±0.19^{ab}	2.20±0.16^{ab}

Values are Mean±SEM of 4 animals in each group, ^{a}Significant difference compare to control ($p<0.05$), ^{b}Sgnificant difference compare to cadmium ($p<0.05$)

Table 2: Effect of ethanolic extract of garlic (*Allium sativum*), ascorbic acid and cadmium on lipid peroxidation, antioxidant enzymes, reduced glutathione and protein levels in the kidney of rats

	Groups					
Parameters	1	2	3	4	5	6
Protein (mg mL^{-1} homogenate)	24.38±3.81	26.33±2.05	22.17±4.97	20.63±5.6	32.38±6.35^{b}	21.88±1.10
Lipid peroxides (mmole of MDA/mg protein)	7.38±0.51^{b}	0.73±0.33^{ab}	2.95±0.18^{ab}	11.4±1.50^{a}	1.42±0.32^{ab}	6.88±0.79^{b}
Superoxide dismutase (Units/mg protein)	1.03±0.10^{b}	0.56±0.16^{ab}	1.93±0.11^{ab}	0.35±0.01^{a}	1.47±0.16^{ab}	1.96±0.53^{ab}
Catalase (μmole of H_2O_2 decompose/Min/mg protein)	0.94±0.01^{b}	1.08±0.02^{b}	2.10±0.23^{ab}	0.43±0.15^{a}	1.01±0.05^{ab}	2.05±0.24^{ab}
GSH (μg mL^{-1} homogenate)	1.08±0.04	2.47±0.13^{ab}	1.71±0.21^{ab}	0.21±0.05^{a}	1.56±0.17^{ab}	1.69±0.19^{ab}

Values are Mean±SEM of 4 animals in each group, ^{a}Significant difference compare to control ($p<0.05$), ^{b}Significant difference compare to cadmium ($p<0.05$)

reduced glutathione level (GSH) were obtained in extract and ascorbic acid pretreated rats when compared to control. Lipid peroxides levels were also significantly decreased in extract and ascorbic acid pretreated rats when compared to control ($p<0.05$) (Table 1).

Superoxide Dismutatse (SOD), catalase and GSH levels were significantly increased in extract and ascorbic acid pretreated rats when compared to control ($p<0.05$). The decrease in lipid peroxides levels in extract and ascorbic acid pretreated rats were also significant when compared to control (Table 2).

DISCUSSION

This study further demonstrated the effect of cadmium as an inducer of oxidative stress by notably increasing lipid peroxidation and reducing the activities of antioxidant enzymes and GSH level in both the liver and kidney of experimental rats exposed to acute dose of cadmium.

Lipid peroxidation has been observed in cadmium toxicity. MDA is a well-known lipid peroxidation indicator and has been found to increase in the liver and kidney after exposure (Shaikh *et al*., 1999). This agrees with our present study which revealed a considerable increase in MDA concentrations in both the liver and kidney of the rats, which is clearly indicative of the severe lipid peroxidation caused by cadmium-induced oxidative stress. Pretreatment with extract and ascorbic acid however significantly decreased lipid peroxidation in both the liver and kidney of rats exposed to cadmium. This agrees with earlier reports that Aged garlic extract inhibits lipid peroxide formation, indicating a potent ROS scavenging effect (Wei and Lau, 1998) and the ability of ascorbic acid to provide antioxidant protection in animals exposed to oxidative stressors (Borek, 1993).

GSH levels were significantly decreased in both the liver and kidney of rats exposed to cadmium in this study. It has been earlier reported that the expression of γ-glutamylcysteine synthase, an enzyme that catalyzes the rate-limiting step in the biosynthesis of GSH can be inhibited by cadmium (Casalino *et al*., 2002). Our present finding is consistent with previous report by Ikediobi *et al*. (2004) that glutathione level decreased in liver cells exposed to cadmium and that of Kidd (1997) that ROS, which can be induced by exposing tissues to cadmium (Berlett and Stadtman, 1997), depletes cellular GSH in liver, kidney, heart and other tissues thus compromising the ability of the tissues to prevent oxidative damage.

Aged Garlic Extract (AGE) have been reported to increase cellular glutathione levels in variety of cells (Liu *et al*., 1992) and ascorbate have been reported to considerably conserve GSH levels *in vivo* (Winkler *et al*., 1994). This agrees with our findings that glutathoine levels were considerably increased in the liver and kidney of ethanolic extract of garlic and ascorbic acid pretreated rats. The ability of the extract to increase GSH levels in these organs could be attributed to the possible presence of organosulfur compounds like s-allylcysteine (SAC) and s-allylmercaptocysteine (SAMC), which could serve as precursors for cysteine synthesis, cysteine being vital in GSH system. SAC and SAMC in AGE have been previous reported to have potent antioxidant activities (Imai *et al*., 1994).

It has been reported that SOD evoked varied responses to cadmium that are concentration-, cell type- and frequency of exposure-dependent (Stohls *et al.*, 2000), however Ikediobi *et al.* (2004) reported that an increase in SOD activity during cadmium exposure is a response to ROS accumulation. Previous studies have also shown that antioxidants SOD and catalase work synergistically in oxygen handling cells for the complete detoxification of superoxide and hydrogen peroxide (Macmillan-Crow and Thompson, 1998), thus an increase in the activity of SOD could activate an increase in catalase activity. Present study agrees with this earlier reports in that notable increase were observed in the activities of SOD and catalase in the liver and kidney of extract and ascorbic acid pretreated rats. This is consistent with earlier works of Borek (1997) and Amagase *et al.* (1996) that antioxidant phytochemicals in garlic may act in single or combined fashion to protect against disease-causing oxidative damage by enhancing the activities of endogenous cellular antioxidant defenses.

In conclusion, r present study revealed that ethanolic extract of garlic like Aged Garlic Extract (AGE) can provide antioxidant protection against oxidative stress induced by cadmium, this is possibly due to the presence of essential antioxidant phytochemicals like those is AGE that have been reported to protect against toxic free radicals thus further underscoring the increased interest in the use of phytochemical-based drugs/formulation from medicinal plants like garlic for the protection against and treatment of complex diseases like arteriosclerosis, hypertension and neurodegerative disorders.

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