



## Effects of garlic extract on cadmium-induced toxicity in wistar albino rat

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### Abstract

The effect of garlic extract on cadmium-induced toxicity in wistar albino rats was examined. The administration of cadmium reduced the total body weight of rats by 3.16% after one-week treatment with 15mg/kg bwt/day. Co-treatment for another 2 weeks with 15mg/kg bwt/day + 0.5ml/100g bwt/day (CdGa) and 15mg/kg bwt/day + 1ml/100g bwt/day (CdGaGa) considerably increased the total body weight of the rats. Cadmium administration increases the malondialdehyde (MDA) levels; reduced reduced-glutathione and catalase activities in kidneys of rats considerably compared the control. Alkaline phosphatase (ALP) activity and bone calcium levels were significantly ( $p \leq 0.05$ ) decreased by the administration of cadmium. The toxic effect caused by the administration of cadmium was shown to be ameliorated by garlic resulting to the reduction of the physiological effects mentioned above. Therefore, the result showed that adequate doses of garlic extract will alleviate the biochemical alterations in kidneys and bones.

**Keywords :** Garlic, Cadmium, Alkaline phosphatase and Malondialdehyde

### Introduction

Cadmium (Cd) is extremely hazardous to life and has been involved in historic poisoning cases of human and animal population (Abdulaziz *et al.*, 2001; Satarug & Moore, 2004), thus becoming a serious threat to living organisms (Chalkley *et al.*, 1998; Satarug & Moore, 2004). Human activities such as mining, production and consumption of cadmium and non-ferrous metals as well as smoking, have accelerated the rate of mobilization and distribution of cadmium far in excess of natural abiotic cycling processes. The recent growth of the study of cadmium toxicity is due to the occurrence of "Itai-Itai" disease in Japan (Jarup, 2002). Dose-response analysis and risk estimates shows that the liver, kidney and bone are mainly affected in chronic or long-term exposure to cadmium (Satarug & Moore, 2004; Akesson *et al.*, 2005; Brzoska & Moniuszko-Jakoniuk, 2005). The kidney carries about 50% of cadmium body

load and the rest is distributed to the liver, bone and other tissues (Jarup, 2002; Akesson *et al.*, 2005). The half-life of cadmium is considerably long (~30years), therefore, its accumulation and distribution in organs renders the kidney, liver, and bone as the primary organs of critical effect (WHO, 1992; Jarup, 2002; Satarug & Moore, 2004). Oxidative damage to lipids, proteins and bones demineralization have been proposed to be the possible cause of cadmium-induced toxicity (Alfen *et al.*, 2000; Brzoska and Moniuszko-Jakoniuk, 2005).

Nutritional deficiencies are believed to have aggravated the concurrent liver, kidney and bone disorders seen in "Itai-Itai" disease patients (Ishihara *et al.*, 2001). Recent studies showed a correlation between cadmium-related kidney and bone diseases, non-occupationally exposed populations to nutritional deficiencies (such as proteins, trace

elements and antioxidants) (Alfen *et al.*, 2000; Asagba *et al.*, 2004). This suggests that nutritional status may greatly influence the metabolic fate and toxicity of cadmium (Asagba *et al.*, 2004). Further cadmium-related bone and kidney toxicities have been observed in people whose dietary cadmium intake were well within the provisional tolerable weekly intake (PTWI) set by the Joint Food and Agriculture Organization/ World Health Organization Expert Committee on Food Additives of 1µg/kg body weight /day (Satarug & Moore, 2004). For centuries, consumption of fruits and vegetables has been attributed to beneficial health effects. Fruits and vegetables generally contain both nutrients and non-nutrients that control and modulate various functions in the body to contribute to the maintenance of a steady state of health and reduce the risk of diseases (Diplock *et al.*, 1998; Prior, 2003). Fruits and vegetables are endowed with phytochemical (biologically active chemicals and other nutrients), that can help in prevention of diseases and have become a major interest to both the scientific community and the public health (Lampe, 2000; Prior, 2003). Outstanding among these health-promoting vegetables is *Allium Sativum* (garlic). Garlic is versatile and widely accepted by almost all cultures. Garlic is rich in organosulphur compounds (Lachiman & Pronek, (2003). The organosulphur compounds are known to exhibit antioxidant and metal-chelating properties as well as modulating inflammatory and detoxification systems (Ferrari *et al.*, 2000; Rice-Evans, 2001; Morales *et al.*, 2006). Apart from garlic's functional effects, its components have been extensively reported to have an array of health benefits translating from their functional roles. These include: Anticarcinogenic effect (Hsing *et al.*, 2002, Donaldson, 2004), anti-diabetic effect (Kumari & Augusti, 2002; Campos *et al.*, 2003; El-Demerdash *et al.*, 2005), anti-asthmatic effect (Augusti & Sheela, 1996; Dorcsch & Wagner, 1991), anti-osteoporotic effect (Susan *et al.*, 2000; Wetti *et al.*, 2005), anti-cataractogenic effect (Spector, 1995),

hypcholesterolaemic effect ( Ali *et al.*, 2000; Matthew *et al.*, 2001).

### Materials and methods

Fresh bulbs of garlic (*Allium sativum* Linn) were obtained from Swali market, Yenagoa, Nigeria. Department of Agricultural studies, Niger Delta University identified, authenticated and confirmed them. The bulbs were carefully dressed and frozen between 0<sup>o</sup> and 4<sup>o</sup>C. About 100mls of cool water (4<sup>o</sup>C) per 100g of garlic were added and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen until used.

Thirty (30) adult male Wistar Albino rats weighing between 200 – 220g were purchased from the College of Health Sciences, Niger Delta University, Bayelsa State. The animals were kept in well-ventilated cages under standard conditions of temperature and humidity and were maintained on normal laboratory chow and water *ad libitum*.

### Experimental design

The animals were divided into five groups with 6 rats in each group. The cadmium solution was prepared by dissolving 2.5g of cadmium sulphate (3cd S04.8H2O) salt in 500ml of distilled water. The extract treated groups were pre-treated for one week with the extract solution and continued for additional 3 weeks during which Cd, CdGa, CdGaGa groups were treated with cadmium solution. The routes of administration were oral.

The kidney homogenized with ice-cold Tri buffer (0.1M, pH 7.4) using Qlink homogenizer. It was done using 3ml buffer to 1g of kidney tissue. The homogenates were centrifuged at 500rpm for 10 minutes at 4<sup>o</sup> C using mistral 3000i centrifuge and the resultant supernatants we stored at – 20<sup>o</sup>c until used for different biochemical assays. The tibio-fibula bones were wet digested with trace pure 69% nitric acid (1g tissue/2ml nitric acid), dilute up to 100ml with double – distilled water and the centrifuged at 3000rpm. The supernatant obtained was used for calcium assay

*Assessment of Lipid Peroxidation in Kidney*

Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535nm. (Buege & Aust, 1978). One volume of the kidney sample and two volumes of stock reagent was mixed in a cocked test tube and heated for 15mins on a boiling water bath. After cooling at room temperature the precipitate was removed by centrifugation at 1000xg for 10minutes and the absorbance of the supernatant was measured at 535nm against blank containing all the reagents except kidney sample.

#### *Assay of reduced glutathione (GSH) in the kidney*

The level of reduced glutathiones in kidney homogenates was determined by the method of Jellow et al (1974).

#### *Assay of catalase activity in kidney.*

Catalase activity was determined according to the method Sinha (1977).

#### *Assay of alkaline phosphatase (ALP) activity*

The assessment of alkaline phosphatase (ALP) was based on the method of Englehardt et al. (1970). ALP activity was measured by monitoring the concentration of p-nitrophenol formed when ALP hydrolyses with p-nitrophenyl phosphate.

#### *Assay of bone calcium content*

Calcium concentrations of the bones were determined using the colorimetric method described by Barnett et al, (1973).

### **Statistical analysis**

Values are means  $\pm$  standard deviation (SD) of 6 rats. Data were analyzed by Independent-sample t test using Microsoft Excel version 2007 for Total weight gain determination.

\*=  $p < 0.05$ , # =  $p < 0.01$  and ¥ =  $p < 0.001$

### **Results and discussion**

Each figure represents the means  $\pm$  SD of 6 rats. Data were analyzed by one -way analysis of variance (ANOVA) plus Fisher least significance difference (LSD) post hoc test to examine difference between groups using Microsoft Excel version 2007

\*=  $p < 0.05$ , # =  $p < 0.01$  and ¥ =  $p < 0.001$  ; a = control versus other groups.

Control = No treatment

Cd = treated with 15mg Cd<sup>2+</sup>/kg bwt/day

Ga = treated with 0.5mlgarlic/100g bwt/day

CdGa = co-treated with 15mg Cd<sup>2+</sup>/kg bwt/day + 0.5mlgarlic/100g bwt/day

CdGaGa = co-treated with 15mg Cd<sup>2+</sup>/kg bwt/day + 1ml garlic/100g bwt/day.

Table.1represents the totalprotein contein of kindeys and bones indifferences in weight of rats and Table.2 shows the enzyme levels and activity in rats treated with cadmium and garlic. Results obtained were discussed as follows:

Chronic cadmium exposure has been reported to have a serious effect on the kidney (kidney injury) and disturbances in calcium metabolism in bone progressing to skeletal damages (WHO, 1992; Jarup, 2002; Ohta & Yamauchi, 2000; Aoshima & Fan, 2003; Akesson *et al.*, 2005; Broska & Moriuszk-Jakoniuk, 2005a). The effect led to several alterations in the kidney such as proximal tubular dysfunction, enzymuria, proteinuria, glucosuria bringing about an end-time renal failure have been noted in animals and humans exposed to cadmium (Akesson *et al.*, 2005). In bone calcium disturbance leading to impartment in calcium metabolism, bone disorders such as osteopenia, osteoporosis, osteomalacia arising from cadmium exposure have been reported (Alfven *et al.*, 2002; Aoshima *et al.*, 2005).

The present study showed that oral administration of varied doses of aqueous extracts of garlic for 3 weeks produced significant chemo-modulatory effects on cadmium induced nephrotoxicity and bone disorders brought about by oral administration of 15mg/kg bwt/day of cadmium for 3 weeks.

Cadmium induced nephropathy has been extensively reported (Asagba *et al.*, 2005), to be associated with systematic and intra-renal oxidative stress and the down regulation of antioxidants evident by the elevated level of reduced glutathione (GSH) as well antioxidants enzymes (Gill, 1990; Bagchi *et al.*, 1996; Sumathi *et al.*, 1996; Shaikh & Tang, 1996; Filho *et al.*, 2000).

Table 1. Total Protein content in Kidneys and Bones the difference in weight of rats

| Experimental groups | Kidney Protein (mg/ml)  | Bone Protein (mg/ml) | Initial weight (g) | Final weight (g)          | Difference in body weight (%) |
|---------------------|-------------------------|----------------------|--------------------|---------------------------|-------------------------------|
| Control             | 26.14±2.76              | 7.28±1.04            | 210.00±10.95       | 246.67±16.33 <sup>#</sup> | 17.45                         |
| Cd                  | 29.14±1.65              | 8.54±1.53            | 210.00±10.95       | 203.33±8.16*              | -3.18                         |
| Ga                  | 35.65±6.86*             | 7.20±2.10            | 210.00±10.95       | 263.33±15.05              | 25.40                         |
| CdGa                | 33.18±7.95 <sup>#</sup> | 8.38±4.07            | 210.00±10.95       | 223.33±26.85              | 8.06                          |
| CdGaGa              | 42.44±12.34             | 7.39±1.80            | 210.00±10.95       | 220.00±33.46              | 8.20                          |

Table 2. Enzyme levels and activity in rats treated with cadmium and garlic

| Experimental groups | Renal MDA levels (µg/g tissue) | Renal GSH levels (µg/g tissue) | Renal catalase activity (kat f) | Bone ALP Activity (U/L)      | Calcium levels (mmol/g tissue) |
|---------------------|--------------------------------|--------------------------------|---------------------------------|------------------------------|--------------------------------|
| Control             | 31.091±8.04                    | 9.62±0.55                      | 34.81±1.62                      | 1701.81±95.65                | 1256.92±35.05                  |
| Cd                  | 74.291±10.12                   | 3.67±0.44 <sup>‡</sup>         | 23.31±1.30 <sup>‡a</sup>        | 1247.40±49.46 <sup>‡</sup>   | 807.16±356.04 <sup>‡</sup>     |
| Ga                  | 21.763±4.83*                   | 11.701±0.70 <sup>‡</sup>       | 35.48±1.54                      | 1869.36±83.80 <sup>*a</sup>  | 1277.10±8.82                   |
| CdGa                | 44.141±7.49 <sup>#</sup>       | 7.48±0.55 <sup>‡a</sup>        | 28.22±1.38 <sup>‡a</sup>        | 1533.92±58.01 <sup>#</sup>   | 1159.02±32.59 <sup>‡</sup>     |
| CdGaGa              | 51.111±5.46 <sup>‡</sup>       | 5.98±0.74 <sup>‡a</sup>        | 27.77±1.48 <sup>‡a</sup>        | 1443.94±104.21 <sup>‡a</sup> | 1184.06±40.85 <sup>‡</sup>     |

In the study, intra-renal malondialdehyde (MDA) level an empirical index of lipid peroxidative injury was measured. Cadmium administration caused marked increase in the intra-renal level of lipid peroxidation by about 74.3% relative to the control. Similar observation are also been reported by (Matties & Freedman, 2001; Asagba *et al.*, 2004). Cadmium treatment severely depleted ( $P<0.001$ ) the renal concentration of reduced glutathione (GSH) by 60.62% and almost the same results have been reported in other studies (Wong and Klaassen, 1981; Singhal *et al.*, 1987; Shaikh & Tang, 1999).

Catalase (CAT) is an important enzyme of cellular anti-oxidant defence system (Aruoma, 1998) and it plays an essential role in reducing oxidative stress. In the study, there was a significant decrease ( $P<0.001$ ) in the renal activity of total catalase in the cadmium alone treated group by 35.68%. A similar observation was also reported (Gill, 1990; Filtho *et al.*, 2000), but Zikic *et al.* (1997) had a contrary report. The discrepancy may be because at the initial stage of oxidative stress, the activity of catalase increases conferring a kind of protection (Augusti & Sheela, 1996). It may be possible because the report of Zikic *et*

*al.* (1997) cadmium exposure lasted for only 24 hours.

Disorders in bone metabolism due to cadmium as reflected in decreased mineralization of bone or change in the rate of bone turn-over and alkaline phosphatase, have been reported by several authors (Ohta & Yamauchi, 2000; Swammathan, 2001; Choi & Rhee, 2003; Brzoska & Moniuszko-Jakoniuk, 2005). Cadmium interferes with bone formation and also collagen synthesis in the bone by inhibiting the activity of mature Osteoblasts from differentiation and thus, reduces the alkaline phosphatase content (Broska & Moniuszko-Jakoniuk, 2005).

Alkaline Phosphatase (ALP) activity, an index of bone formation has been reported to be inhibited in the trabecular bones of cadmium-exposed rats (Swammathan, 2001). Thereafter, Obi and Bode (2002) reported increased ALP activity when rats were exposed to cadmium. The variation of this study and theirs may be due to low dose of cadmium treatment and the post-weaning rats used.

Skeletal injuries due to cadmium exposure at all levels that do not impair kidney function have been demonstrated (Wong & Bhattacharyya, 1993), other studies have demonstrated that cadmium-induced renal



tubular damage progress to a more generalized renal impairment (Alfven *et al.*, 2005a).

The study evident that groups co-treated with cadmium and garlic-extract had improved ALP and the calcium bone content by 9.56% (P<0.01) and 3.34% (P<0.05) respectively compared to the control. Almost the same result was reported (Comparative effects of garlic and onion on cadmium-induced nephrotoxicity and bone disorders in rats). Thus, the results of this study suggest that moderate garlic intake may be useful dietary/nutritional approach in ameliorating cadmium-induced bone dysfunction and its attendant osteomalacia and osteoporosis (Alfven *et al.*, 2000; Broska & Moniuszko-Jakoniuk, 2005).

### Conclusion

In conclusion, it is difficult to escape the convincing fact that garlic may protect against cadmium induced biochemical alterations in the kidney and bone. Since the primary underlying mechanism by which cadmium exerts its toxicity is through the induction of oxidative stress, therefore, the little protection is due to the presence of antioxidant, photochemical and nutrients in the garlic extract. The results of this study allowed a comfortable conclusion that garlic extracts co-treatment with cadmium did ameliorate cadmium nephrotoxicity and bone disorders. It seems likely that consumption of garlic by cadmium exposed humans, the biochemical alterations in the kidney and bone would be circumvented and possibly normalized. Thus, it may be recommended for the reduction of cadmium induced nephropathy and bone disorders by increasing considerably the intake of garlic.

### Acknowledgement

The authors give thanks to God Almighty who is responsible for their existence, giving them wisdom, strength and understanding to carry out this work.

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