Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Research Article

HEPATOPROTECTIVE ACTIVITIES OF *ALLIUM CEPA* IN CADMIUM-TREATED RATS

IGE S.F[1], AKHIGBE R.E[1], EDEOGHO O[1], AJAO F.O[1], OWOLABI O.Q[2], OYEKUNLE O.S[1], AJAYI A.F[1]

[1]Department of Physiology, [2]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. Email: funkeige2006@yahoo.com, akhigbemcroy@yahoo.com

Received: 29 June 2010, Revised and Accepted: 15 May 2011

ABSTRACT

Cadmium (Cd) has been associated with organ toxicity. This study aimed at investigating the mechanism of Cd-induced hepatotoxicity and the role of *Allium cepa* extract (AcE) in preventing Cd-induced hepatic damage. Group 1 (control) received only distilled water. Group 2 (Cd-treated) received cadmium sulphate (1.5mg/100kgBW/day) orally for 4 weeks. Group 3 (AcE- pre-treated) received AcE (1.0mml/100kgBW/day) orally for 8 weeks, followed by a 4 week course of cadmium sulphate (1.5mg/100kgBW/day) orally. Group 4 (AcE- co-treated) received cadmium sulphate (1.5mg/100kgBW/day) orally for 4 weeks and AcE (1.0mml/100kgBW/day) orally for 8 weeks simultaneously. Group 5 (AcE- post-treated) received cadmium sulphate (1.5mg/100kgBW/day) orally for 4 weeks, followed by AcE (1.0mml/100kgBW/day) orally for 8 weeks. Administration of Cd led to significant decrease in body weight gain, hepatic SOD activity, total serum protein and significant increases in hepatic malondialdehyde (MDA) level, and serum alkaline aminotransferase (ALT), and alkaline aspartate aminotransferase (AST) activities. Cd administration was also associated with disruption of the normal hepatic architecture. These alterations were ameliorated by AcE administration. The results of the present study demonstrate that oxidative stress is a mechanism by which Cd induces hepatic damage. The elevated plasma activities of ALT and AST are associated with disruption of the normal liver architecture and consequent leakage of cytoplasmic enzymes into the circulation. AcE ameliorated Cd-induced hepatic damage by maintaining the integrity of the hepatocytes and scavenging reactive oxygen species (ROS), thus restoring hepatic lipid peroxidation (LPO) status.

Keywords: Cadmium, *Allium cepa,* Liver, Oxidative stress, Serum liver enzymes, Weight

INTRODUCTION

Several studies have demonstrated the effect of cadmium (Cd) on various organ-systems in the body. It has been reported that Cd induces nephrotoxicity, testicular damage, lung damage, osteomalacia, lungs and prostate carcinoma, hepatotoxicity, and body weight loss [1,2,3,4,5].

It has also been documented that Cd could modify the activities of numerous enzymes in mammalian cells [6, 7]. Among the enzymes are serum alkaline aminotransferase, ALT, aspartate aminotransferase, AST, and alkaline phosphatase, ALP [8,9].

Cd-related alteration in an enzyme activity might be due to the metal interference with the enzyme essential thiols, enzyme-substrate complex formation, or mechanisms regulating the transcription of the relevant gene, such as those coding for metallothionein, heme oxygenase, several heat shock proteins, and other proteins[10].

Allium cepa, a medicinal plant, has been reported to be antihelmintic, antiparasitic, diuretic, and antioxidant [1,11,12,13].

The present study was aimed at assessing the mechanism of Cd-induced hepatotoxicity and the hepatoprotective potential of *Allium cepa* extract, AcE, in rats.

MATERIALS AND METHODS

Experimental animals

The study was carried out in the Animal House of the Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. Thirty male Wistar rats weighing between 130 and 160g were used. They were divided into 5 groups (groups 1, 2, 3, 4, and 5), each group consisting of six rats. They were housed in standard rat cages under laboratory conditions with 12:12h light/dark cycle at 25°C ± 2. Rats were fed ad lib'itum.

Treatments

After two weeks of acclimatization, rats were treated. Group 1 (control) received only distilled water. Group 2 (Cd-treated) received cadmium sulphate (1.5mg/100kgBW/day) orally for 4 weeks. Group 3 (AcE- pre-treated) received AcE (1.0ml/100kgBW/day) orally for 8 weeks, followed by a 4 week course of cadmium sulphate (1.5mg/100kgBW/day) orally. Group 4 (AcE- co-treated) received cadmium sulphate (1.5mg/100kgBW/day) orally for 4 weeks and AcE (1.0ml/100kgBW/day) orally for 8 weeks simultaneously. Group 5 (AcE- post-treated) received cadmium sulphate (1.5mg/100kgBW/day) orally for 4 weeks, followed by AcE (1.0ml/100kgBW/day) orally for 8 weeks. Treatments were discontinued 24 hours prior to the end of the experiment to rule out the effect of acute treatment. Rats were weighed throughout the course of experiment. The study was conducted in accordance with the American Physiological Society's "Guiding Principles for Research Involving Animals".

Preparation of AcE

AcE was prepared as reported by Nelson *et al* [14]and Ige *et al* [1]. Fresh *Allium cepa* bulbs were thoroughly rinsed, dried, and blended into paste. Juice was then filtered and squeezed out of it. The filtrate juice was prepared weekly following the same procedure and stored below 4°C to preserve its potency.

Blood Sample Collection

Blood was collected from 12hour-fasted animals under ether anaesthesia into heparinized specimen bottles via cardiac puncture. Measurements of total serum protein, total serum bilirubin, and serum liver enzymes were done immediately.

Determination of total serum protein, total serum bilirubin, and serum liver enzymes

Total serum protein and bilirubin were assayed using a standard enzymatic calorimetric method with standard laboratory kits (LABKIT, Barcelona-Spain) as reported by Akhigbe *et al* (2008)[15]. Serum liver enzymes (ALT, AST, and ALP) were also determined using standard kit (Biosystems S.A Barcelona-Spain).

Determination of liver superoxide dismutase (SOD) and malondialdehyde (MDA) levels

At the end of the experiment, the livers were excised, cleaned and weighed. A portion of each liver was washed in normal saline (0.9% NaCl), homogenized in phosphate buffer (1g tissue/4ml). The homogenate were then centrifuged and aliquots of the supernatant were obtained for biochemical analysis. Liver SOD and MDA levels were determined as reported by Fridovich [16] and Varshney and Kale [17] respectively.

Histologic analysis

Some portion of each liver was fixed in 10% formaldehyde for 48 hours for histological analysis. The tissues were processed and embedded in paraffin wax. 6µm thick sections were obtained and stained by haematoxylin and eosin (H&E) method and examined under light microscope to determine the morphological changes.

Statistical analysis

Analyses of data were done using the SPSS software (SPSS Inc, Chicago, USA). Differences among groups were determined using analysis of variance (ANOVA), followed by multiple comparison by two-tailed t-test. P< 0.05 was considered statistically significant. Data are expressed as mean ± SEM

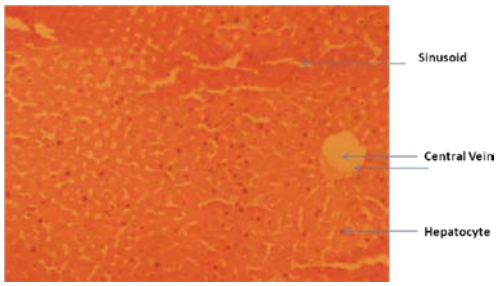
RESULTS

Table 1 shows body weight gain and liver weight in the five groups of rats. Body weight gain was significantly lower in Cd-treated rats compared with all other rats. However, body weight was similar in the control, AcE pre-treated, AcE co-treated, and AcE post-treated rats. There was no significant difference in the liver weight of the five groups.

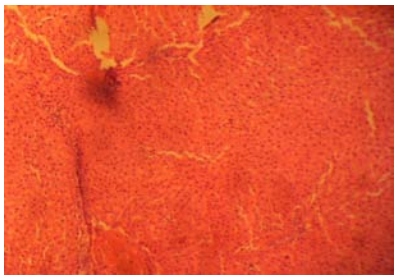
Liver SOD activity and MDA level were not significantly different in the control, AcE pre-treated, AcE co-treated, and AcE post-treated rats. However, Cd-treated rats showed a significantly lower SOD activity and higher MDA level when compared with all other groups (Table 2)

Table 3 shows total serum protein, total bilirubin, and serum liver enzyme activities in the five groups of rats. Cd-treated, and AcE pre-treated rats had significantly lower total serum protein when compared to other groups. Total serum bilirubin and ALP activities were comparable among the five groups. ALT activities were not significantly different in control and AcE pre-treated rats. However, ALT activity was significantly higher in Cd-treated rats when compared to all other groups. AcE co-treated and AcE post-treated rats showed significantly lower activities of ALT. AST activities were significantly higher in Cd-treated and AcE pre-treated rats, but significantly lower in AcE co-treated rats when compared to control and AcE post-treated rats.

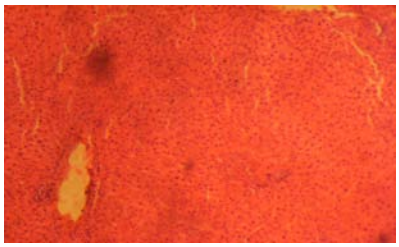
Histologic studies showed that the control rats had normal hepatocytes and central veins. However, Cd-treated had severe cellular degeneration, necrosis, and hepatocytes and localized fatty degenerations. AcE pre-treated rats showed liver with moderate vascular and hepatocytes degeneration. These effects were mild in AcE post-treated rats. AcE co-treated rats showed a normal hepatocytes and central veins.



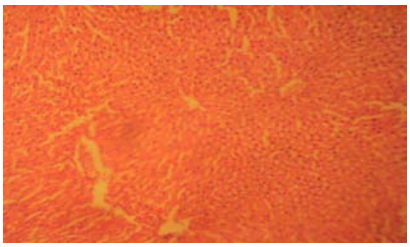
Group 1 (control)



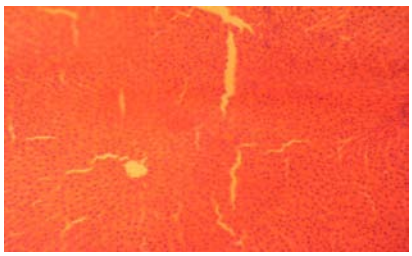
Group 2 (Cd-treated)



Group 3 (AcE pre-treated)



Group 4(AcE co-treated)



Group 5 (AcE post-treated)

Fig. 1: Hepatic changes following administration of cadmium and *Allium cepa* (AcE) extract in rats

Table 1: Effect of AcE on cadmium-induced weight changes

VARIABLES	GROUP1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Body weight Gain (g)	37.17±1.31	6.22±2.66**	32.62±6.35	30.22±3.16	30.27±4.68
Liver weight(g)	6.05±0.42	4.45±2.58	5.13±3.11	7.78±2.35	8.72±3.35

*p<0.05 versus control, **p<0.05 versus other groups

Table 2: Effect of AcE and cadmium on lipid peroxidation parameters

VARIABLES	GROUP1	GROUP2	GROUP3	GROUP4	GROUP5
Hepatic SOD Activity (*U*)	3.80±0.56	0.48±0.43**	0.83±1.58	4.20±0.61	6.17±1.14
Hepatic MDA Level (u/mg)	0.0028±0.0004	0.0055±0.0005**	0.0024±0.0003	0.0014±0.0007	0.0015±0.00058

*p<0.05 versus control, **p<0.05 versus other groups

Table 3: Effect of AcE and cadmium on some liver function parameters

VARIABLES	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Total serum Protein (g/dl)	5.18±0.31	2.57±0.56**	3.17±0.54**	4.47±0.43	5.92±0.31
Total serum Bilirubin (mg/dl)	0.37±0.02	0.60±0.523	0.63±0.91	0.50±0.53	0.41±0.85
ALT (IU/L)	24.17±2.54	69.33±4.50**	25.50±4.20	18.20±0.20*	15.00±1.14**
AST (IU/L)	31.33±1.17	86.50±12.72**	70.33±17.02*	23.30±0.43**	34.17±1.16
ALP (IU/L)	93.00±13.46	74.34±14.97	61.50±14.96	88.33±11.85	89.17±20.6

*p<0.05 versus control, **p<0.05 versus other groups

DISCUSSION

Cd is a toxic heavy metal widely used in various industries. Cd has been associated with several tissue damage [1,3,4]. However, liver, which is one of the most important organs of the body system, helps to detoxify toxic substances such as Cd by generating detoxifying enzymes [17]. This study thus shows the mechanisms by which liver response to Cd exposure.

Previous studies reported that organ toxicity can be revealed by reduction in the weight of the organ following exposure [5,19]. However, the results from this study showed comparable liver weight among the five groups of rats. Though, there was a slight decrease in the liver weight of Cd-treated rats, this decrease was not statistically significant. This confirms results of the study of Ige *et al* [1]. In agreement with previous studies of Anderson *et al* [19], and Goyer [20], this study revealed a significant decline in body weight gain in Cd-treated rats. The decrease body weight gain observed in Cd-treated rats indicates a toxic effect of Cd on hepatocytes with consequent depression of cellular metabolism and growth [21].

Results from this study showed that Cd significantly reduced hepatic SOD activity and increase hepatic MDA level. This is in agreement with previous studies which reported similar changes in SOD activities and MDA levels in kidney tissues following Cd treatment [1,3,22,23,24,25]. This study seems to be the first to report lipid peroxidation (LPO) status in hepatic tissues. The diminution of SOD activities in Cd-treated rats might be due to their response to reactive oxygen species (ROS) generated under oxidative stress [26]. It might also be a consequence of an irreversible autocatalytic process in which the sustained increase in ROS would finally lead to cellular death [27]. The decrease in SOD activities and generation of ROS are basis for rise in hepatic MDA levels. These alterations were prevented by AcE administration. This confirms the antioxidant potentials of AcE reported by Ige *et al* [1]. AcE with antioxidant potential scavenges products of LPO and restores the antioxidant defense systems of the liver.

Histopathologic studies revealed that alterations occurred in the hepatic architecture of all treated rats except AcE co-treated rats. Cd-treated rats showed severe cellular degeneration, necrosis, and hepatocytes and localized fatty degenerations. AcE pre-treated rats showed a moderate alteration in hepatic architecture, while AcE post-treated rats showed a mild alteration. These morphological hepatic changes may be due to the decline activities of hepatic SOD and an increase hepatic MDA seen in Cd-treated rats. This is in agreement with the study of Lora and Tetsuo [2]. The normal hepatic architecture seen in control and AcE co-treated rats could be associated with the normal LPO status seen in them.

This study revealed that total serum protein is significantly decreased in Cd-treated and AcE pre-treated rats. Similar observation has been reported by Hristev *et al* [9]. It is a known fact that albumin is solely synthesized in the liver and also to some extent, α and β globulins. Fibrinogen, involved in blood coagulation, is also known to be synthesized in the liver [28]. If this is responsible for the reduction in total serum protein seen in Cd-treated and AcE pre-treated rats following cellular degeneration with resultant suppression of hepatic synthetic function, then, there would be an impaired coagulating property of the liver leading to a raise in total serum bilirubin. However, observation from this study showed a comparable total serum bilirubin level in all the five groups of rats. This could suggest that the decrease levels of total serum protein seen might be due to increase catabolism as reflected by reduced body weight gain in Cd-treated rats and not a depressed hepatic protein synthesis or the oxidative stress-induced reduction in total serum protein was not severe enough to cause impairment of the conjugating function of the liver.

The commonest enzymes employed as indicators of hepatocellular damage are ALT, AST, and ALP. Damage to the liver results in increase plasma activities of these enzymes. Increases in these enzymes activities are proportional to the extent of the hepatic damage [28]. Serum activities of ALT and AST were increased significantly following Cd treatment, thus suggesting hepatic tissue damage. This is similar to previous studies [8,9,29]. The increase in these enzymes activities could be associated with oxidative damage of hepatocytes reflected by the histologic study of the liver architecture and a rise in MDA level of the Cd-treated rats. However, AcE ameliorated this effect. This is similar to previous study of Mantawy [30]. Serum ALP activities were comparable in the five groups. This suggests that Cd-induced hepatotoxicity is not associated with serum ALP activity.

CONCLUSION

In conclusion, this study conducted in a male Wistar rat model demonstrated that hepatoprotective effect of Cd is due to cumulative oxidative damage. The observed increase in the activities of ALT and AST is due to leakage of cytoplasmic enzymes into the circulation following disruption of the normal architecture of the liver. The mechanism by which AcE ameliorates Cd-induced hepatotoxicity is its ability to maintain the integrity of the hepatocytes and scavenging ROS.

REFERENCES

1. Ige SE, Salawu EO, Olaleye SB, Adeeyo OA, Badmus J, and Adeleke AA. Onion (*Allium cepa*) extract prevents cadmium-induced renal dysfunction. Indian Journal of Nephrology 2009; 19 (4): 140-144
2. Lora E. Rikans and Tetsuo Yamano. Mechanisms of cadmium-mediated acute hepatotoxicity. J Biochem Toxicol 2000;14:110-117

3. Dino Manca, Anne C Richard, Bertin Trottier and Gaston Chevalier. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of chloride. Toxicology 2002;67 (3) 303-323
4. Jadranka Dundjersrki, Jelena Predic, Aleksandra Cvoro, and Gordona Matic. Rat liver tyrosine aminotransferase activity and induction by dexamethasone upon cadmium intoxication. Arch Biol Sci 2003;55 (1-2):3-7
5. Nadir Rencuzogullan and Suat Erdogan. Oral administration of lycopene reverses cadmium-suppressed body weight loss and lipid peroxidation in rats. Biol Trace Elem Res 2007;118:175-183
6. Zhang GH, Yamaguchi M, Kimura S, Higham S, and Kraus-Friedmann N. Effects of heavy metal on rat liver microsomal Ca^{2+}-ATPase and Ca^{2+} sequestering. J Biol Chem 1990;265:2184-2189
7. Long GJ. Cadmium perturbs calcium homeostasis in rat osteosarcoma (ROS 17/2.8) cells a possible role for protein kinase C. Toxicol Lett 1997;91:91-97
8. Morgan R, Kundomal Y, Hupp E. Serum aspartate aminotransferase levels following cadmium and/or ^{60} Co radiation. Journal of Environmental Science and Health. 1993;Part A 28 (9):2029-2039
9. Hristev Hristo, Penkov Dimo, Hallak Abdulkarim, Kirova Maria, Baykov Bayko, Bliznakov Atanas. Serum protein changes in rabbits after chronic administration of lead and cadmium. Journal of Central European Agriculture 2008;9 (1):157-162
10. Beyersmann D, and Hechtenberg S. Cadmium, gene regulation and cellular signaling in mammalian cells. Toxicol Appl Pharmacol 1997;144:247-261
11. Guarrera PM. Traditional antihelmintic, antiparasitic and repellent uses of plants in central Italy. Journal of ethnopharmacology 1999;68 (1-3,15):183-192
12. Helen A, Krishnakumar K, Vijayammal PL, and Augusti KT. Antioxidant effect of onion oil (*Allium cepa* Linn) on the damages induced by nicotine in rat as compared to alpha-tocopherol. Toxicology 2000;116:61-68
13. Prakash D, Singh BN, and Upadhyay G. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). Food chemistry 2007;102 1389-93
14. Nelson CA, Reginald A O, Okoro N, and Janet K. Antibacterial activity of *Allium cepa* (Onions) and *Zingiber officinale* (Ginger) on *staphylococcus aureus* and *pseudomonas aeruginosa* isolated from High Vaginal Swab. Int J Trop Med 2007;3(2):1-5
15. Akhigbe R.E, Azeez O.M, Ige S.F, Oyeyipo I.P, Ajao F.O, Soladoye A.O. Hemorheological effects of long-term administration of combined oral contraceptive in rats. Int J Pharmacol 2008;4(5):403-406
16. Fridovich I. Superoxide dismutases. Adv Enzymel Relat Area Mol Biol 1986 58:61-97
17. Varshney R, and kale RK. effects of calmodulin antagonists on radiation-induced Lipid peroxidation microsomes.Int J Radiat Biol 1990;58:733-43
18. Ramammourthy Gopal and Rajangam Udayakumar. Enzymatic and non-enzymatic antioxidant activities of Enicostemma littorale in p-DAB induced hepatocarcinoma in rats. Int J Pharmacol 2008;4 (5) 369-375
19. Anderson H, Larsen S, Splid H, and Christenson ND. Multivariate statistical analysis of organ weights in toxicity studies. Toxicology 1999;136:67
20. Goyer RA and Cherian MG. Toxicology of metals Biochemical aspects. In *Handbook of Experimental Pharmacology*. New York Springer-Verlag, 1991;vol. 11:Pp 189–213.
21. Hispard F, Vaufleury A, Martin H, Devaux S, Cosson RP, Scheifler R, Richert L, Berthelot A, and Badot P. Effects of subchronic digestive exposure to organic or inorganic cadmium on biomarkers in rat tissues. Ecotoxicology and Environmental Safety 2007;24:45-50
22. Sarkar S, Yadar P, Trived R, Bansal AK, and Bhatnagar 0. Cadmium-induced lipid peroxidation and status of the antioxidant system in rat tissues. J Trace Elem Med Biol 1995;9 (3) 144-9
23. Bagchi D, Bagchi M, Hassoun E A, and Stohs SJ. Cadmium-induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague-dawley rats. Biological Trace Element Research 1996;52 (2) 143-154
24. Elisabetta Casalino, Cesare Sblano, and Clemente Landriscina. Enzyme Activity alteration by cadmium administration to rats The possibility of Iron involvement in lipid peroxidation. Archives of Biochemistry and Biophysics 1997;346(2) 171-179
25. Shaikh ZA, Vu TT and Zaman K. Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicology and Applied pharmacology 1999;154 (3) 256-263(8)
26. Fabiana C, Ester G, Leda O, Nora F, Alcira B, and Elba V. On the promoting action of tamoxifen in a model of hepatocarcinogenesis induced by p-dimethylaminobenzene in CF1 mice. Int J Biochem Cell Biol 2001;33:681-690
27. Pigeolet E, Corbisier P, Houbion A, Lambert D, Michiels C, Raes M, Zachary MD, and Remacle J. Glutathione peroxidase, superoxidase dismutase and catalase inactivation by peroxides and oxygen derived free radicals. Mech Ageing Dev 1990;51:283-297
28. Chatterjea MN, and Rana Shinde. Liver function tests. In Textbook of Medical Biochemistry. Jaypee Brothers Medical Publishers Ltd, New Delhi(2005)
29. Fatma M El-Demerdash, Mokhtar I Yusuf, Fatma S Kedwany, and Hoda H Baghdadi. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats protective role of vitamin E and β-carotene. Food and Chemical Toxicology 2004;42 (10):1563-1571
30. Mantawy MI. Potential effect of *Allium cepa* and *Allium sativum* on haemolymph of Biomphalaria alexandrina, the intermediate host of Schistosoma mansoni. J Egypt Soc Parasitol 2001;31(3):711-23.