

Reversal of Cadmium Induced Oxidative Stress and its Bio-Accumulation by Culinary Herbs *Murraya koenigii* and *Allium sativum*

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Abstract: In the present study, the effect of culinary herbs *Murraya koenigii* and *Allium sativum* was studied on cadmium induced oxidative stress and its accumulation. Cadmium produces peroxidative damage to tissues predominantly by binding to the sulfhydryl groups of tissues and accumulates in the tissues and generates free radicals, depletes reduced glutathione (GSH) levels and alters calcium homeostasis. Cadmium administration resulted in very large quantity accumulation predominantly in kidney followed by liver and least in breast muscle and produced peroxidative damage as indicated by increase in TBARS, reduction in GSH concentrations in liver and kidney and increase in catalase and Superoxide Dismutase (SOD) of erythrocytes. *Murraya koenigii* leaves and *Allium sativum* cloves powder administration through feed reduced the cadmium load completely from the breast muscle, 80% from liver and 45% from kidney and reversed the antioxidant enzymes of RBC, i.e., catalase and SOD, non-enzymatic antioxidants GSH and lipid peroxidation marker TBARS of liver and kidney. In conclusion, oral administration of *Murraya koenigii* leaves and *Allium sativum* cloves powders prevented cadmium induced peroxidation of tissues but also reduced tissue cadmium concentrations.

Key words: *Murraya koenigii*, *Allium sativum*, cadmium accumulation, tissue peroxidation, stress, India

INTRODUCTION

Cadmium is toxic heavy metal and very large amount cadmium is being released into the environment by anthropogenic activity. It is reported that increased concentrations of cadmium in agricultural soils are known to come from application of phosphate fertilizers, sewage sludge, waste water and pesticides (Limei *et al.*, 2008). Further, it is also established that mining activities at mines, smelting of metalliferous ores with high cadmium content and industrial application of cadmium in pigments, plastic stabilizers, nickel cadmium batteries resulted in wide spread agricultural soil pollution (Liao *et al.*, 2005).

The dispersed cadmium in the soil can persist for decades and can be taken up by plants from soil since, this metal is water soluble and easily and efficiently transfers from soil to plants. This may affect the target species if there is intake of feed ingredients from cadmium contaminated plant sources (Satarug *et al.*, 2003). Cadmium accumulates in biological system because of its

long biological half life (10-30 years) (Jarup, 2002). Chronic exposure to low doses of cadmium results in accumulation in kidney and liver, hence liver and kidney are the primarily target organs of cadmium toxicity. It was reported that oxidative stress has been proposed mechanism for cadmium toxicity because there was decline of natural antioxidant potential in kidney and liver (Bagchi *et al.*, 1997).

Further, it causes depletion of physiological antioxidants like reduced glutathione (GSH) levels (Quig, 1998). Increased production of ROS and depletion of antioxidant status *in vivo* resulted in the development of oxidative stress that signifies with lipid peroxidation, DNA damage, depletion of sulfhydryls and altered calcium homeostasis (Stosh and Bagchi, 1995). It has also been documented that cadmium treatment increased the hepatic levels of thiobarbituric acid reacting substances which were the indicative of oxidative stress and depletion of glutathione (GSH), natural antioxidant levels (Newairy *et al.*, 2007). The people of Indian subcontinent use the *Murraya koenigii* leaves and *Allium sativum*

cloves in the preparation of various food items for their characteristic flavour and taste. The antioxidant substances like dimeric carbazone alkaloids (Tachibana *et al.*, 2003) and antioxidant protein from curry leaves (apc) (Ningappa and Srinivas, 2008) are responsible for the radical scavenging ability and inhibition of lipoxygenase activity.

Feeding of garlic powder as feed additive to broiler chicks (Tollba and Hassan, 2003) has showed significant increase in live body weight and garlic juice (El-Demerdash *et al.*, 2005) decrease in concentration of TBARS in various organs and also decrease in ALT, creatinine and urea in plasma. In the present study an attempt has been made to find out whether culinary antioxidants *Murraya koenigii* and *Allium sativum* reduces the peroxidation only by scavenging the free radicals or by hastening the elimination of cadmium from the body.

MATERIALS AND METHODS

Chicken: A total of 60 male broiler chicks (Cobb strains) of a day old age were procured from the Venkateswara Hatcheries, Hyderabad, AP, India and reared in a battery brooder. These chicks were randomly divided into 4 groups consisting of 15 in each group. All the birds were provided with respective feed and water *ad libitum* throughout the experiment.

Birds of all groups were vaccinated with new castle disease (ND) vaccine on 7th and 28th day and Infectious Bursal Disease (IBD) vaccine on 10th day. Weekly body weights of all birds were recorded from the day of hatch till the completion of experiment. Before commencing the study, permission form institutional animal ethics committee was obtained.

Experimental design: The treatment schedule of various groups of birds follows as:

Group I: Birds were fed with basal diet throughout the experiment (1-42 days).

Group II: Birds were fed with basal diet mixed with 100 ppm cadmium as cadmium chloride up to 28 days (4 weeks) from 29th day onwards only basal diet was fed.

Group III: Upto 28th day basal diet mixed with 100 ppm cadmium from 29th day onwards fed with basal diet mixed with 0.1% powder of *Murraya koenigii* leaves.

Group IV: Upto 28th day basal diet mixed with 100 ppm cadmium from 29th day onwards fed with basal diet mixed with 0.1% powder of *Allium sativum* cloves.

Cadmium toxicity: Cadmium administration to induce oxidative toxicity at the rate of 100 ppm cadmium as cadmium chloride. Cadmium dose selected as per the Uyanik *et al.* (2001) who reported that at 100 mg kg⁻¹ of cadmium significantly altered the performance, biochemical parameters and antioxidant parameters.

Blood and tissue collection form birds: Blood samples were collected from wing veins on 28th and 42nd day from all the birds with anticoagulant (Alsever's solution) in each group for assay of superoxide dismutase and catalase of erythrocytes and without anticoagulant to separate serum immediately and aliquoted for assays. Birds were sacrificed by cervical dislocation at the end of 6th week that is on the same day of blood collection, liver and kidney were collected. Liver after excised out, thoroughly washed in ice cold saline (0.9%) and perfused with ice cold saline via portal vein.

Liver and kidney tissue samples homogenate (10%) was made in ice cold 0.2 M Tris Hcl buffer (pH 7.2). Kidney after excised out washed thoroughly with ice cold saline (0.9%) and prepared in to 10% homogenate with 0.2 M Tris Hcl buffer (pH 7.2) cytosolic sample of liver and kidney homogenate was obtained by centrifuging at 10,000 g for 30 min at 4°C. In addition, to liver and kidney breast muscle also collected for the estimation of cadmium levels.

Biochemical analysis: Catalase (CAT) activity in fresh blood (erythrocytes) was estimated by decomposition of H₂O₂ by Calliborne (1985) method and expressed as μM of H₂O₂ decomposed/min. Super Oxide Dismutase (SOD) activity was determined by inhibition of auto oxidation of pyrogallol at pH 8.2 of Marklund and Marklund (1984)'s method. The enzyme activity was expressed in terms of units g⁻¹ protein.

One unit of the enzyme corresponds to the amount of enzyme that inhibits the pyrogallol auto oxidation reaction by 50%. Liver and kidney reduced Glutathione (GSH) content were determined by reaction of reduced Glutathione (GSH) with 5-5 Dithiobis-2-nitrobenzoic acid (DTNB) by Moron *et al.* (1979)'s method and activity expressed in mg of GSH g⁻¹ protein.

Liver and kidney Lipid Peroxidation (LPO) levels were assessed by determining the amount of TBARS, i.e., Malandialdehyde (MDA) produced during the lipid peroxidation following Subramanian *et al.* (1988)'s method and the concentration is expressed in nanomoles of MDA g⁻¹ protein. Liver function marker like ALT was estimated by method prescribed by Bergmeyer *et al.*

(1986) and kidney function markers creatinine and BUN were estimated by methods of Apple *et al.* (1986) and Wybenga *et al.* (1971), respectively. Total protein in liver and kidney homogenate was quantified by the procedure of (Lowry *et al.*, 1951) using bovine serum albumin as the standard.

Cadmium estimation in tissues: Cadmium concentration of various samples of liver, kidney and breast muscle were determined with atomic absorption spectrophotometer (NOVAA 300). Dry ashing procedure was used for the mineral analysis in organs.

About 5 g of wet tissue was dried at 100°C for 2 h. Dried samples were transferred to a cool muffle furnace and the temperature was slowly raised to 450°C and ashed overnight, then dissolved in 2 mL HNO₃ plus double distilled water and concentration of cadmium was determined (Uyanik *et al.*, 2001).

Statistical analysis: The data values were expressed as mean±SE and were subjected to statistical analysis by applying one way ANOVA using Statistical Package for Social Sciences (SPSS) 10th version. Differences between means tested using Duncan's multiple comparison test and significance was set at p<0.05.

RESULTS

Body weight gain: Groups (II-IV) treated with cadmium up to 28 days showed significant reduction in body weights and feed consumption, following treatment with culinary herbs *Murraya koenigii* and *Allium sativum* from 4-6th week in groups III and IV, the body weights and feed consumption were increased compared to cadmium control group II (Table 1).

Blood antioxidant enzymes: Cadmium treated groups showed significant higher (p<0.05) values of erythrocyte super oxide dismutase and catalase compare to the control group I at the end of 4th week. Where as in group III and IV treated with culinary herbs *Murraya koenigii* and *Allium sativum* from 4-6th week the values were significantly reduced in compared to the cadmium control group (Table 2).

Non-enzymatic antioxidants and lipid peroxidation of liver and kidney: In cadmium control toxic group II exhibited significant decreases in GSH and increase in TBARS concentration of liver and kidney tissues when compared with basal diet control group. Whereas, culinary herbs *Murraya koenigii* and *Allium sativum* treated groups (III and IV) showed significant increase in the liver and kidney tissue GSH concentration and decrease in the TBARS concentration (Table 3).

Liver and kidney toxicity markers: At the end of 4th week serum levels of Alanine Transaminase (ALT), Blood Urea Nitrogen (BUN) and creatinine concentration was significantly increased in cadmium treated groups II and IV, following treatment with culinary herbs *Murraya koenigii* and *Allium sativum* in groups III and IV from 4-6th week there was significant decrease in ALT, BUN and creatinine activity compare to cadmium control group II at the end of 6th week (Table 4).

Cadmium concentration in various tissues: The cadmium concentration (µg/g wet tissue) in kidney is very high, followed by liver and less concentration in breast muscle tissues of cadmium control group compared to basal diet control group. Following treatment with culinary herbs *Murraya koenigii* and *Allium sativum* the concentration

Table 1: Effect of culinary herbs *Murraya koenigii* and *Allium sativum* on average body weights of various groups of chicken

Groups	Body weights (g)		Feed consumption (g)	
	4th week	6th week	4th week	6th week
I	949.7±13.9 ^a	1551.9±20.02 ^{ab}	803.3	710.1
II	592.8±14.08 ^{ba}	824.41±10.76 ^{bb}	430.8	433.5
III	515.9±9.41 ^a	1176±20.65 ^{bb}	355.7	932.6
IV	570.4±28.28 ^{ba}	1037.7±33.95 ^{bb}	476.2	628.0

Values are Mean±SEM (n = 15) One way ANOVA; Means with different alphabets as superscripts differ significantly (p<0.05); Capital alphabets (Horizontal comparison) Small alphabets (Vertical comparison)

Table 2: Effect of culinary herbs *Murraya koenigii* and *Allium sativum* on cadmium induced altered erythrocyte antioxidant enzymes

Groups	SOD (U g ⁻¹ protein)		CAT (µM of H ₂ O ₂ decomposed/min/mg of protein)	
	4th week	6th week	4th week	6th week
I	42.74±0.42 ^a	45.96±0.73 ^{ab}	2.55±0.02 ^a	3.17±0.01 ^{ab}
II	82.68±0.58 ^{ba}	91.43±0.65 ^{bb}	5.89±0.01 ^{ba}	6.63±0.16 ^{bb}
III	82.71±0.62 ^{ba}	61.44±0.55 ^{bb}	5.92±0.02 ^{ba}	4.30±0.003 ^{bb}
IV	82.72±0.54 ^{ba}	60.21±0.48 ^{bb}	5.92±0.02 ^{ba}	4.2±0.04 ^{bb}

Values are Mean±SEM (n = 15) One way ANOVA; Means with different alphabets as superscripts differ significantly (p<0.05); Capital alphabets (Horizontal comparison) Small alphabets (Vertical comparison)

Table 3: Effect of culinary herbs *Murraya koenigii* and *Allium sativum* on cadmium induced tissue lipid peroxidation and GSH levels

Groups	TBARS (nmoles of MDA/g of protein)		GSH (mg of GSH g ⁻¹ protein)	
	Liver	Kidney	Liver	Kidney
I	141.93±3.48 ^a	150.92±7.85 ^a	72.41±2.83 ^a	75.58±1.45 ^a
II	180.99±4.17 ^b	236.10±18.64 ^c	31.96±2.09 ^b	37.65±0.71 ^b
III	153.84±9.35 ^a	172.02±5.58 ^{ab}	53.72±2.91 ^{ab}	59.74±3.96 ^d
IV	151.43±5.26 ^a	171.72±5.12 ^{ab}	60.70±1.77 ^{ab}	54.68±5.77 ^{de}

Values are Mean±SEM (n = 15) One way ANOVA; Means with different alphabets as superscripts differ significantly (p<0.05); Small alphabets (Vertical comparison)

Table 4: Effect of culinary herbs *Murraya koenigii* and *Allium sativum* on cadmium induced bnormal liver and kidney function biomarkers

Groups	ALT (IU L ⁻¹)		BUN (mg dL ⁻¹)		Creatinine (mg dL ⁻¹)	
	4th week	6th week	4th week	6th week	4th week	6th week
I	16.75±0.83 ^{AB}	16.44±0.69 ^{AB}	4.00±0.03 ^{AB}	6.58±0.84 ^{AB}	0.50±0.02 ^{AB}	0.60±0.01 ^{AB}
II	56.42±3.41 ^{ba}	58.76±1.88 ^{ba}	7.46±0.18 ^{ba}	9.55±0.17 ^{ba}	0.69±0.01 ^{ba}	1.28±0.01 ^{ba}
III	58.73±2.96 ^{ba}	29.73±1.65 ^{bb}	7.47±0.16 ^{ba}	8.50±0.4 ^{bb}	0.69±0.02 ^{ba}	0.95±0.01 ^{bb}
IV	58.13±2.00 ^{ba}	37.07±2.67 ^{bb}	7.42±0.14 ^{ba}	8.15±0.38 ^{bb}	0.69±0.01 ^{ba}	0.89±0.01 ^{bb}

Values are Mean±SEM (n = 15) One way ANOVA; Means with different alphabets as superscripts differ significantly (p<0.05); Capital alphabets (Horizontal comparison) Small alphabets (Vertical comparison)

Table 5: Effect of culinary herbs *Murraya koenigii* and *Allium sativum* on tissue cadmium levels (µg g⁻¹ wet tissue) in different groups of broiler chicken

Groups	Cadmium concentration (ppm)		
	Liver	Kidney	Breast muscle
I	0.415±0.02	0.385±0.01	0.54±0.01
II	67.28±1.87	75.10±2.89	8.11±0.75
III	14.25±0.92	41.01±2.45	0.33±0.02
IV	13.11±0.79	41.81±3.42	0.422±0.02

Values are Mean±SEM (n = 15) One way ANOVA; Means with different alphabets as superscripts differ significantly (p<0.05); Small alphabets (Vertical comparison)

reduced significantly (p<0.05) to approximately 20, 55 and 4.6%, respectively of their cadmium treated group (Table 5).

DISCUSSION

Cadmium accumulates in tissues predominantly by binding to sulfhydryl groups of proteins (Stosh and Bagchi, 1995) and produces toxicity by inducing Reactive Oxygen Species (ROS) production through fenton reaction (Liochev, 1999), elevating cytoplasmic Ca²⁺ levels by inhibiting Ca²⁺ export from cytoplasm (Goyer and Clarkson, 2001) and reducing the antioxidant defense systems (Wim and Detmar, 2004), there by produces oxidative stress.

Mammalian cells possess elaborate defense mechanism for free radical detoxification. Non-enzyme molecules including thiols and disulfide bonding (balance between GSH-reduced glutathione and GSH-oxidized glutathione) play important roles among all antioxidant defense systems. The GSH donates electrons to the superoxide anion and hydroxyl radical and hence prevents lipid peroxidation, DNA strand breakage and oxidation of any organic molecules.

Birds treated with cadmium (Group II-IV) showed significant higher values of erythrocyte SOD and catalase compared to the control group I at the end of 4th week which indicates increased production of ROS by cadmium ions. After treatment with culinary herbs *Murraya koenigii* and *Allium sativum* (Groups III and IV) from 4-6th week the erythrocyte SOD and CAT values were reduced significantly compared to the cadmium control group II.

In liver and kidney tissues, the concentration of GSH was reduced in cadmium control group II compared to basal diet control group I which indicates the attachment of cadmium to SH groups of GSH and other proteins. Alteration of erythrocyte SOD and CAT and liver and kidney tissue GSH indicates disturbance of cellular antioxidants and increasing the production of oxygen free radicals which in turn causes lipid peroxidation that is indicated by increase in concentration of TBARS in liver and kidney tissues of cadmium control (Group II).

Increase in lipid peroxidation and other effects caused by oxygen free radicals produces damage to liver and kidney tissues which is revealed by increase in serum ALT enzyme, BUN and creatinine concentration.

Culinary herbs *Murraya koenigii* and *Allium sativum* are known for their antioxidant properties. Results of present study revealed that these antioxidant culinary herbs reduced the erythrocyte SOD and CAT activity and increase in GSH and decrease in TBARS of liver and kidney tissue which reveals the sparing of tissue GSH that binds to cadmium ions and scavenging the ROS there by sparing the SOD and CAT. Once, the lipid peroxidation reduced TABARS concentration was reduced and liver and kidney tissues were regenerated as indicated by reversion of ALT for liver and creatinine and BUN for kidney.

Cadmium accumulation in various organs viz. liver, kidney and breast muscle was analyzed. Cadmium accumulated in very large quantity in kidney followed by liver and less but significant higher quantity in breast muscle of cadmium control group II compared to basal diet control group I. Culinary herbs *Murraya koenigii* and *Allium sativum* supplementation from 4th onwards, the cadmium elimination from the body was increased and cadmium concentration was reduced to 20% for liver, 55% for kidney and 4.6% for breast muscle in compared to cadmium control group II. The reduction in cadmium level is almost complete in breast muscle about 80% in liver and 45% in kidney.

CONCLUSION

In this study, the culinary herbs *Murraya koenigii* and *Allium sativum* administration at the rate of 0.1% in feed significantly reversed the cadmium induced oxidative damage and level of cadmium in the body of chickens and restored the liver and kidney functions and body weight and feed consumption.

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REFERENCES

- Apple, F., C. Bandit, A. Prosch, G. Erlandson, V. Holmstrom, J. Scholen and M.K. Googins, 1986. Creatinine clearance: Enzymatic vs Jaffe determinations of creatinine in plasma and urine. *Clin. Chem.*, 32: 388-390.
- Bagchi, D., P.J. Vuchetich, M. Bagchi, E.A. Hassoun, M.X. Tran, L. Tang and S.J. Stohs, 1997. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. *Free Radic. Biol. Med.*, 22: 471-478.
- Bergmeyer, H.U., M. Horder and R. Rej, 1986. International Federation of Clinical Chemistry (IFCC) scientific committee, analytical section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzyme, part 3. IFCC for alanine aminotransferase. *J. Clin. Chem. Clin. Biochem.*, 24: 481-495.
- Calliborne, A.L., 1985. Assay of Catalase and Peroxidase. In: *Handbook of Oxygen Radical Research*, Greenwald, R.A. (Ed.). CRC Press, Boca-Raton.
- El-Demerdash, F.M., M.I. Yousef and N.I. El-Naga, 2005. Biochemical study on the hypoglycemic effects of onion and garlic in alloxan induced diabetic rats. *Food Chem. Toxicol.*, 43: 57-63.
- Goyer, R.A. and T.W. Clarkson, 2001. *Casarett and Doulls Toxicology*. McGraw-Hill, New York.
- Jarup, L., 2002. Cadmium overload and toxicity. *Nephrol. Dial. Trans.*, 17: 35-39.
- Liao, X.Y., T.B. Chen, H. Xie and Y.R. Liv, 2005. Soil as contamination and its risk assessment in areas near the industrial districts of chenzhou city, Southern China. *Environ. Int.*, 31: 791-798.
- Limei, Z., L. Xiaoyong, C. Tongbin, Y. Xiulan, X. Hua, W. Bin and W. Lixia, 2008. Regional assessment of cadmium pollution in agricultural lands and the potential health risk related to intensive mining activities. *J. Environ. Sci.*, 20: 696-703.
- Liochev, S.I., 1999. The Mechanism of Fenton-like Reactions and their Importance for Biological Systems. A Biologists View. In: *Metal Ions in Biological Systems*, Sigel, A. and H. Sigel (Eds.). Marcel Dekker Inc., USA., pp: 1-39.
- Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Marklund, S. and C. Marklund, 1984. Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.*, 7: 469-474.
- Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione S transferase in rat lung and liver. *Biochem. Biophys. Acta*, 582: 67-68.
- Newairy, A.A., A.S. El-sharaky, M.M. Badreldeen, S.M. Eweda and S.A. Sheweita, 2007. The hepato protective effects of selenium against cadmium toxicity in rats. *Toxicology*, 242: 23-30.
- Ningappa, M.B. and L. Srinivas, 2008. Purification and characterization of approximately 35 kDa antioxidant protein from curry leaves (*Murraya koenigii* L.). *Toxicol In vitro*, 22: 699-709.
- Quig, D., 1998. Cysteine metabolism and metal toxicity. *Alternative Med. Rev.*, 3: 262-270.
- Satarug, S., J.R. Baker, S. Urbenjapol, M. Haswell-Elkins, P.E.B. Reilly, D.J. Willians and M.R. Moore, 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. *Toxicol. Lett.*, 137: 65-83.
- Stosh, S.J. and D. Bagchi, 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biol. Med.*, 18: 321-336.

- Subramanian, K.A., M. Manohar and V.I. Mathan, 1988. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. *Biochim. Biophys. Acta*, 962: 51-58.
- Tachibana, Y., H. Kikuzaki, N.H. Lajis and N. Nakatani, 2003. Comparison of antioxidative properties of carbazole alkaloids from *Murraya koenigi* leaves. *J. Agric. Food Chem.*, 51: 6461-6467.
- Tollba, A.A.H. and M.S.H. Hassan, 2003. Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions. Black cummin (*Nigella sativa*) or garlic (*Allium sativum*). *Poult. Sci.*, 23: 327-340.
- Uyanik, F., M. Eren, A. Atasever, G. Tuncoku and A.H. Kolsuz, 2001. Changes in some biochemical parameters and organs of broilers exposed to cadmium and effect of Zinc on cadmium induced alteration. *Israel J. Vet. Med.*, 56: 128-134.
- Wim, W. and B. Detmar, 2004. Cadmium induced apoptosis in C6 glioma cells: Influence of oxidative stress. *Biometals*, 17: 65-78.
- Wybenga, D.R., J. Di Giorgio and V.J. Pileggi, 1971. Manual and automated methods for urea nitrogen measurement in whole serum. *Clin. Chem.*, 17: 891-895.