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Onion and garlic extracts lessen cadmium-induced nephrotoxicity in rats

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Abstract Cadmium (Cd) is a well-known nephrotoxicant inducing kidney damage via oxidative stress. Since kidney is the critical target organ of Cd toxicity, this study was designed to evaluate the protective effects of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) aqueous extracts on Cd-induced renal oxidative stress in male Wistar rats. The control group received double distilled water alone and Cd group was challenged with $3CdSO_4 \cdot 8H_2O$ (as Cd) (1.5 mg/100 g bw/day per oral) alone. Extract-treated groups were pre-treated with varied doses (0.5 ml and 1.0 ml/100 g bw/day per oral) of onion and/or garlic extract for 1 week after which they were co-treated with Cd (1.5 mg/100 g bw/day per oral) for 3 weeks. The results showed that the levels of renal lipid peroxidation (LPO) and glutathione-S transferase (GST) were significantly ($P < 0.001$) increased in rats that received Cd alone relative to the control group. More so, the levels of renal glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and Na^+/K^+-ATPase were significantly ($P < 0.001$) decreased in rats that received Cd alone. Treatment of Cd-intoxicated rats with varied doses of onion and/or garlic extract significantly ($P < 0.05$) restored the alterations in these parameters relative to the group that received Cd alone. While treatment with high dose of onion extract exerted a significant dose-dependent restoration of these parameters, treatment with high dose of garlic elicited a pro-oxidant effect, relative to their respective low dose. Our study suggests that onion and garlic extracts may exert their protective effects via reduction in LPO and enhanced antioxidant defense. These extracts may, therefore, be useful nutritional option in alleviating Cd-induced renal damage.

Keywords Onion · Garlic · Cadmium · Kidney · Nephrotoxicity · Protection

Introduction

Cadmium (Cd) is a widespread environmental pollutant remarkably notorious in its ability to cause kidney damage (Jarup et al. 1998). Its toxicity has been widely studied and reported (Satarug and Moore 2004). Anthropogenic activities such as mining, production and consumption of Cd and non-ferrous metals have accelerated the rate of mobilization and distribution of Cd from nonbioavailable geological matrices into biologically accessible situations far in excess of natural abiotic cycling process. Couple with cigarette smoking, these have predisposed animal and human populations to both subtle and direct exposure pathways with an attendant increase in Cd-related pathologies (Satarug and Moore 2004).

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Cd is a cumulative nephrotoxicant whose toxicity may occur even at concentrations lower than the set provisional tolerable weekly intake (PTWI) of 7 μg/kg body weight per week (Satarug and Moore 2004; Akesson et al. 2005). It preferentially accumulates and persists in the kidneys (biological half-life of 10–30 years) due to lack of an active biochemical mechanism for elimination coupled with renal reabsorption (Satarug and Moore 2004). This is so because Cd induces metallothionein (MT) synthesis and readily binds to it to form CdMT complex. Because of its low molecular weight (~7 kDa), CdMT is freely filtered through renal glomerulus into the proximal tubular reabsorption into proximal tubular cells. Upon catabolisation, 'free' Cd is released to react with sensitive sites in the cell (WHO 1992; Nordberg et al. 2007).

The pathobiochemical mechanism of Cd-induced renal damage is mainly via induction of oxidative stress (Manca et al. 1991; Shaikh et al. 1999). Cytosolic Cd indirectly generates reactive oxygen species (ROS) capable of depleting endogenous antioxidant status and inflicting peroxidative damage on biologic membrane lipids and a variety of transport proteins, including Na^+/K^+-ATPase (Stohs and Bagchi 1995; Thevenod and Friedmann 1999). Na^+/K^+-ATPase, the cornerstone of kidney transport machinery, provides the necessary electrochemical gradients of Na^+ and K^+ to maintain body fluid volume and thus plays a crucial role in homeostasis (Ferraille and Doucet 2001). The dysfunction of the enzyme may lead to severe pathological consequences. Cd has been reported to decrease renal Na^+/K^+-ATPase activity suggesting that the enzyme may be involve in the pathogenesis of Cd-induced nephropathy (Thevenod and Friedmann 1999; Asagba et al. 2004).

Attempts have been made to minimize the severity of Cd toxicity via enhanced sequestration (chelation) and elimination using different agents (Nordberg 1984). However, in case of chronic exposure, when Cd is bound to metallothionein (MT), chelation therapy is only weakly effective (Nordberg 1984). Antioxidant therapies have also been reported to exert protective effects on Cd toxicity (Kowalczyk et al. 2003; El-Demerdash et al. 2004; Morales et al. 2006; Asagba et al. 2007; Massadeh et al. 2007). Furthermore, some studies have provided evidence linking the severity of cadmium-related pathologies to nutritional deficiencies (Tewari et al. 1986; Alfven et al. 2000; Asagba et al. 2004). These suggest that dietary factors may play a key role in the severity of Cd toxicity.

In recent times, doubts about the efficacy and safety of chelating agents have prompted the search for alternative, safer and affordable therapy in medicinal plants. This has potentiates the evaluation of functional vegetables as a phytotherapeutic approach. In this regard, attention has been drawn to the functional health effects of *Allium cepa* Linn (onion) and *Allium sativum* Linn (garlic). Garlic and onion are versatile and widely accepted vegetables because of their culinary and medicinal properties. While garlic is primarily endowed with organosulfur compounds, onions, in addition to organosulfur compounds, are rich in flavonoid (anthocyanins and quercetin) (Augusti 1996; Griffiths et al. 2002). Flavonoids and organosulfur compounds are known to exhibit antioxidant and metal-chelating properties as well as modulate lipid and carbohydrate (glucose) metabolisms, inflammatory and detoxification systems (Augusti 1996; Ferrali et al. 1997; Griffiths et al. 2002; El-Demerdash et al. 2005; Morales et al. 2006; Massadeh et al. 2007; Pari et al. 2007).

In view of these considerations, the functional effects of onion and garlic in the areas of antioxidant effects and xenobiotic detoxification afford these vegetables a potential nutritional option in the modulation of Cd toxicity.

Materials and methods

Preparation of extract

Fresh bulbs of onion and garlic were purchased from the local Sasa Market in Ibadan, Nigeria. Among the three local varieties of onion identified at National Institute of Horticultural Research (NIHORT), Ibadan, the Kano Red was preferably selected because of its reported high antioxidant potentials and pungency (Denton and Ojeifo 1990). Only a single variety of garlic was identified. The bulbs were carefully dressed and frozen (0 and 4°C). About 100 ml of chilled, distilled water per 100 g of onion and/or 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.

Animals and treatments

This study used fifty-six healthy adult male Wistar rats weighing 212 ± 5 g. The animals were purchased from the Institute of Medical Research and Training (IMRAT), University College Hospital (UCH), Ibadan. They were housed in well-ventilated plastic cages under standard conditions. Feed (Bendel Feed and Flour Mill, Ltd, Edo State) and water were provided ad libitum. All animal experiments were conducted in accordance with the *Ethical Norms on Animal Care and Use* approved by IMRAT and Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan.

After 2 weeks of acclimatization, animals were divided into nine groups of six rats each and were treated accordingly (Table 1). The extract-treated groups were pre-treated with their respective extracts (0.5 ml and 1.0 ml/100 g bw/day orally) for 1 week and continued for additional 3 weeks during which Cd, CdOn, CdOnOn, CdGa, CdGaGa and CdOnGa groups were treated with $3CdSO_4 \cdot 8H_2O$ (1.5 mg/100 g bw/day). The dose (1 ml/100 g bw) of onion and garlic chosen was based on the result of a pilot study and the study of El-Demerdash et al. (2005). The extracts and Cd solution were administered once daily by gavage and 2 h apart. At the end of the experimental period of 4 weeks, the final body weights of the animals were determined and animals were fasted 12 h prior to when they were sacrificed by cervical dislocation.

Collection of sample

All rats were carefully dissected and the kidneys excised, cleared of adhering connective tissues, weighed and homogenized in ice-cold 1.15% KCl (1 g tissue/3 ml) using a Potter-Elvehjem type homogenizer. The homogenates were then centrifuged at 5,000 rpm for 15 min at 4°C and aliquots of the supernatant were used for biochemical assays.

Biochemical analysis

The total protein content of homogenized kidney was determined by the method of Lowry et al. (1951). Lipid peroxidation, as measured by malondialdehyde (MDA) content, was assayed by the TBA method of Beuge and Aust (1978). Reduced glutathione level was assayed by the method of Jollow et al. (1974) based on the reaction with 5,5'-dinitro bis (2-nitrobenzoic acid) (DTNB). SOD (EC 1.15.1.1) activity was measured by the method of Misra and Fridovich (1972). CAT (EC 1.11.1.6) activity was estimated by the method of Sinha (1971) by measuring the rate of decomposition of hydrogen peroxide (H_2O_2). The method of Habig et al. (1974) was followed to assay the activity of GST (EC 2.5.1.18) based on the rate of increase in the conjugate formation between GSH and 1-chloro-2,4-dinitrobenzene (CDNB). The Na^+/K^+-ATPase activity was determined by the method of Adam-Vizi and Seregi (1982). The inorganic phosphate determination was based on the method developed by Stewart (1974). Three samples of the onion and garlic were analysed for Cd in the fresh forms. Samples were mashed in a porcelain mortar to give a homogenized paste. To 10 g of each sample, 20 ml HNO_3 was added and allowed to stand for 15 min. The mixture was heated until the liquid reduced to 5 ml. After cooling, 20 ml HNO_3, 10 ml H_2SO_4 and 8 ml H_2O_2 were added and the content evaporated to 5 ml and allowed to cool. To eliminate residual acid, 10 ml deionized H_2O was

Table 1 Experimental design

Group	Treatment
Control	Distilled water (0.5 ml/100 g bw/day)
Cd	Cd only (1.5 mg/100 g bw/day)
On	Onion extract (0.5 ml/100 g bw/day)
Ga	Garlic extract (0.5 ml/100 g bw/day)
CdOn	Cd (1.5 mg/100 g bw/day) + Onion extract (0.5 ml/100 g bw/day)
CdOnOn	Cd (1.5 mg/100 g bw/day) + Onion extract (1 ml/100 g bw/day)
CdGa	Cd (1.5 mg/100 g bw/day) + Garlic extract (0.5 ml/100 g bw/day)
CdGaGa	Cd (1.5 mg/100 g/bw/day) + Garlic extract (1 ml/100 g bw/day)
CdOnGa	Cd (1.5 mg/100 g bw/day) + Onion extract (0.5 ml/100 g bw/day) + Garlic extract (0.5 ml/100 g bw/day)

Notes: $n = 6$ for each treatment group; Cd = Cadmium sulphate octahydrate; $3CdSO_4 \cdot 8H_2O$. Agents were administered daily by gavage

added and boiled for 10 min (this was repeated twice). After cooling, the digest was filtered into 25 ml volumetric flask and made up to mark with deionized H_2O. The digestion solution was analysed for Cd by means of a flame atomic absorption spectrophotometer (Unicam 969). Standard Cd solutions of 0.2, 0.4, 0.6, and 0.8 μg/g were made from standard Cd solution (100 ppm). Water was added to 10 ml of standard Cd solution, and the same procedure as for the samples was followed to get a standard volume of Cd concentration by AAS.

Statistical analysis

All results are expressed as mean ± SD. Data were analyzed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test using SPSS 11 software (SPSS Inc, Chicago). Statistical significance was considered at $P < 0.05$.

Results

The effect of onion and garlic extract on body weight gain of Cd-exposed rats is presented in Table 2. During the experiment, Cd group had a slight decrease in the mean body weight gain whereas the remaining groups had increase in their body weight gains, which was significant ($P < 0.01$) in the control animals and those treated with onion or garlic extract alone. There were no clinical signs of morbidity in any of the groups studied except in the group that received Cd plus higher dose of garlic extract (CdGaGa) where two of the rats showed symptomatic diarrhea on the third week of the treatment.

Table 2 Effects of onion and garlic treatments on body weight gain of cadmium-exposed rats

Group	Body weight		
	Initial (g)	Final (g)	Sig.
Control	212.83 ± 8.38	245.83 ± 16.25	#
Cd	210.00 ± 10.95	203.33 ± 8.16	NS
On	211.67 ± 8.16	269.17 ± 21.54	#
Ga	210.00 ± 9.94	266.67 ± 12.11	#
CdOn	212.50 ± 7.58	214.83 ± 5.15	*
CdOnOn	211.67 ± 9.31	222.50 ± 6.89	#
CdGa	213.00 ± 7.74	219.33 ± 8.68	*
CdGaGa	210.83 ± 9.17	209.17 ± 14.97	NS
CdOnGa	212.67 ± 6.12	218.83 ± 7.63	*

Notes: Values are means ± SD. $n = 6$ for each treatment group. Data were analysed by paired samples t test and comparison was done between initial weight and final weight. $^{*}P < 0.05$, $^{\#}P < 0.01$, $^{\dagger}P < 0.001$, NS = not significant

The effect of onion and garlic extract on the weight of kidney of Cd-exposed rats is presented in Fig. 1. Rats treated with Cd alone had a significant ($P < 0.001$) decrease in kidney weight compared to control rats. However, exposure of rats to onion or garlic extract alone had no significant effect on kidney weight. Treatment of Cd-exposed rats with varied doses of onion and/or garlic extract significantly increased kidney weight in the CdOn—($P < 0.05$), CdOnOn—($P < 0.01$), CdGa ($P < 0.05$), CdGaGa—($P < 0.05$), CdOnGa—($P < 0.01$) treated groups relative to group that received Cd alone.

The level of renal MDA is presented in Fig. 2. Renal level of MDA (an empirical index of lipid peroxidation) was markedly ($P < 0.001$) increased in rats challenged with Cd alone relative to the control group. However, renal MDA level was considerably ($P < 0.05$) reduced in rats treated with onion or garlic alone compare to control. Cd-intoxicated rats treated with varied dose of extracts showed a remarkable ($P < 0.001$) decrease in their renal levels of MDA relative to Cd group. High dose of onion extracts exerted a dose-dependent reduction in the renal level of MDA in Cd-challenged rats whereas high dose of garlic extract showed a trend to lower level of renal MDA, though not significant.

The level of renal GSH is presented in Fig. 3. Cadmium administration markedly ($P < 0.001$) depleted renal level of GSH relative to control whereas a marked ($P < 0.001$) increase was observed in rats that received onion or garlic extracts alone. All Cd-challenged rats treated with extracts showed a significant ($P < 0.001$) increase in their renal levels of GSH relative to the rats treated with Cd alone. Treatment of Cd-challenged rats with high dose of onion caused a significant ($P < 0.001$) dose-dependent increase in the renal level of GSH while treatment with high dose of garlic exerted a significant ($P < 0.001$) dose-dependent decrease.

The activities of renal SOD and CAT are presented in Figs. 4 and 5 respectively. The renal activities of SOD and CAT were significantly ($P < 0.001$) reduced in rats that received Cd alone relative to control. Treatment with onion alone significantly

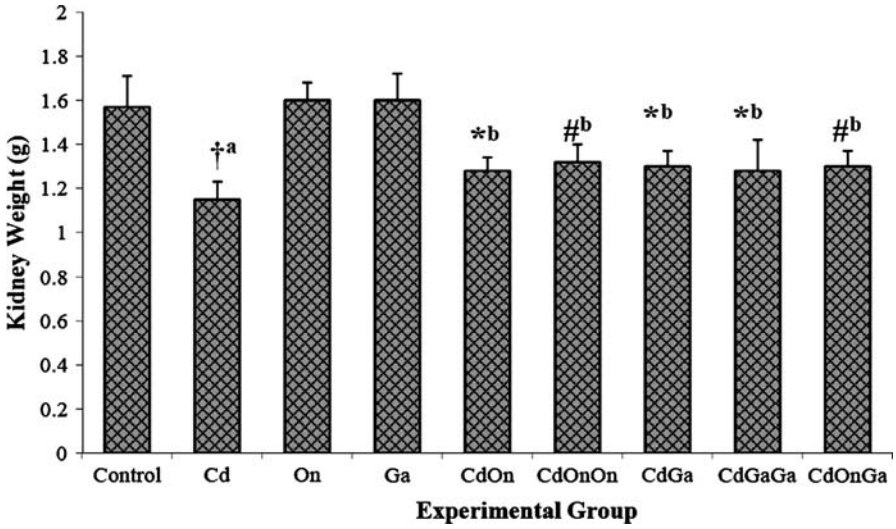

Fig. 1 Effects of onion and garlic treatments on kidney weight of cadmium-exposed rats. *Notes*: Bars are means ± SD. $n = 6$ for each treatment group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test. $^{*}P < 0.05$, $^{\#}P < 0.01$ and $^{\dagger}P < 0.001$; a = control vs. Cd, On, Ga; b = Cd vs. CdOn, CdOnOn, CdGa, CdGaGa, CdOnGa; c = CdOn vs. CdOnOn; d = CdGa vs. CdGaGa

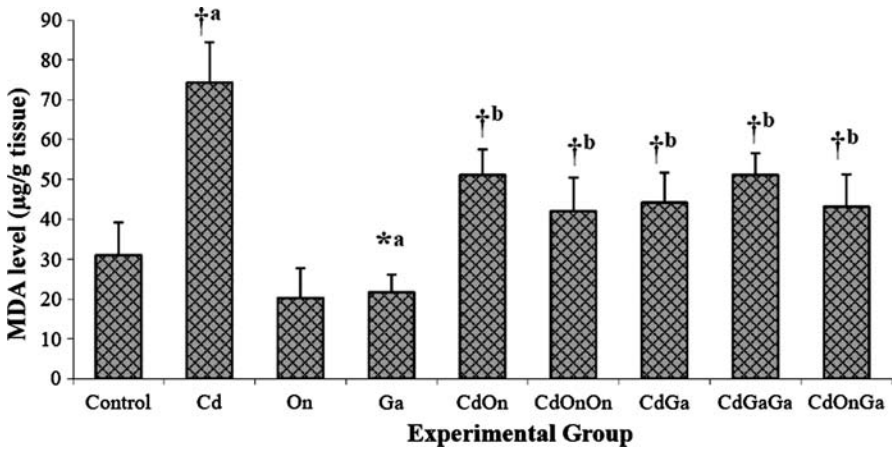

Fig. 2 Effects of onion and garlic treatments on level of renal lipid peroxidation in cadmium-exposed rats. *Notes*: Bars are means ± SD. $n = 6$ for each treatment group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test. $^{*}P < 0.05$, $^{\#}P < 0.01$ and $^{\dagger}P < 0.001$; a = control vs. Cd, On, Ga; b = Cd vs. CdOn, CdOnOn, CdGa, CdGaGa, CdOnGa; c = CdOn vs. CdOnOn; d = CdGa vs. CdGaGa

enhanced SOD ($P < 0.001$) and CAT ($P < 0.05$) activities whereas treatment with garlic alone failed to exert significant effect on the activities of these enzymes. Relative to rats challenged with Cd alone, treatment of Cd-challenged rats with varied dose of both extracts significantly ($P < 0.001$) relieved the inhibitory effect of Cd on renal activities of SOD and CAT. However, CdOn-treated rats had significant ($P < 0.05$) increase in renal CAT activity. Treatment of Cd-challenged rats with high dose of onion extract exerted a significant ($P < 0.001$) dose-dependent increase in the activities of renal SOD and CAT while treatment with high dose of garlic extract exerted a decrease, which was only significant ($P < 0.05$) with respect to renal SOD activity.

The activity of renal GST is presented in Fig. 6. The activity of renal GST was significantly increased in rats that received Cd alone ($P < 0.001$) and garlic alone ($P < 0.01$) while rats treated with onion extract alone had a slight increase relative to control. Treatment of Cd-intoxicated rats with varied dose of both extracts significantly ($P < 0.001$) brought the activity of renal GST close to the control values. Treatment of Cd-challenged rats with high dose of onion extract

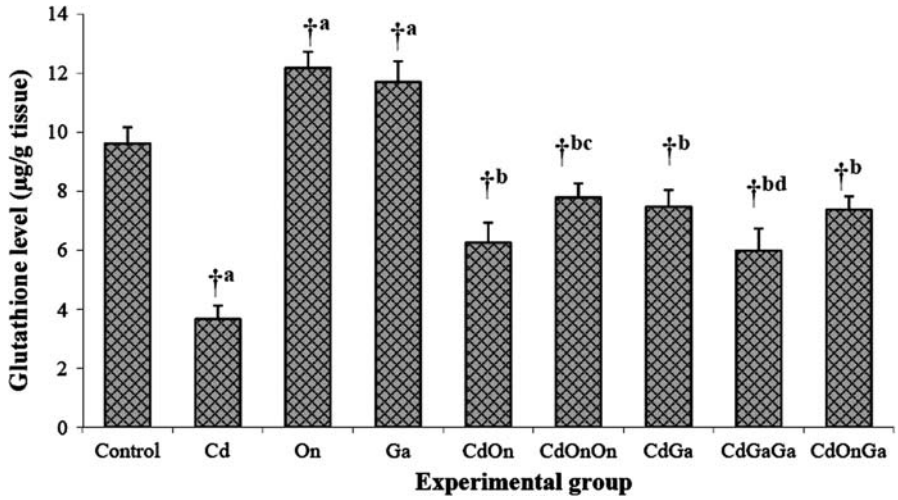

Fig. 3 Effects of onion and garlic treatments on level of renal glutathione in cadmium-exposed rats. *Notes*: Bars are means ± SD. $n = 6$ for each treatment group. Data were analysed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test. $^{*}P < 0.05$, $^{\#}P < 0.01$ and $^{\dagger}P < 0.001$; a = control vs. Cd, On, Ga; b = Cd vs. CdOn, CdOnOn, CdGa, CdGaGa, CdOnGa; c = CdOn vs. CdOnOn; d = CdGa vs. CdGaGa

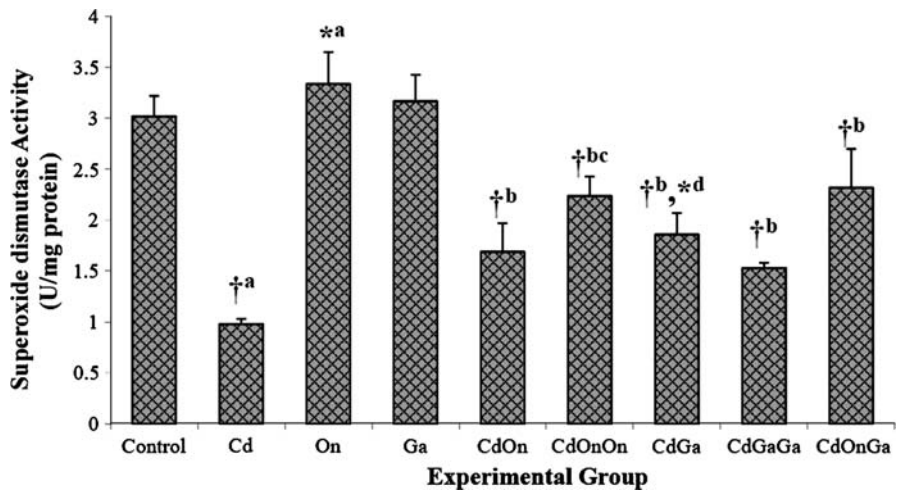

Fig. 4 Effects of onion and garlic treatments on activity of renal superoxide dismutase in cadmium-exposed rats. *Notes*: Bars are means ± SD. $n = 6$ for each treatment group. Data were analysed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test. $^{*}P < 0.05$, $^{\#}P < 0.01$ and $^{\dagger}P < 0.001$; a = control vs. Cd, On, Ga; b = Cd vs. CdOn, CdOnOn, CdGa, CdGaGa, CdOnGa; c = CdOn vs. CdOnOn; d = CdGa vs. CdGaGa

caused a significant ($P < 0.001$) dose-dependent reduction in renal GST activity whereas a treatment with high dose of garlic extract exerted negligible increase.

The activity of renal Na^+/K^+-ATPase is presented in Fig. 7. Rats treated with Cd alone had a significant ($P < 0.001$) reduction in renal Na^+/K^+-ATPase activity relative to the control rats. The activity of renal Na^+/K^+-ATPase was significantly enhanced in rats treated with onion extract alone ($P < 0.01$) but treatment with garlic extract alone exerted negligible increase. Treatment of Cd-challenged rats with varied dose of onion and garlic extracts significantly ($P < 0.001$) enhanced the activity of renal Na^+/K^+-ATPase relative to the rats treated with Cd alone. Also, the activity of renal Na^+/K^+-ATPase followed a significant ($P < 0.001$) dose-dependent increase and decrease for high dose of onion and garlic extracts respectively. The result of the AAS analysis showed that the onion and garlic bulbs used in this study contain 0.017 ± 0.003 µg/g and 0.044 ± 0.004 µg/g respectively.

Discussion

Changes in body and kidney weights are not unconnected with chemical toxicity (Timbrell 1991). The observed changes in these parameters in rats that received Cd alone are indications of Cd toxicity, which

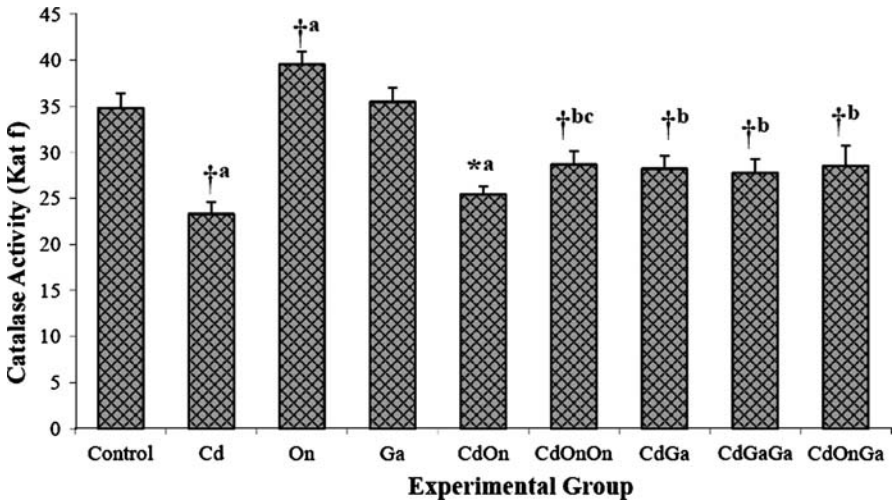

Fig. 5 Effect of onion and garlic treatments on activity of renal catalase in cadmium-exposed rats. *Notes*: Bars are means ± SD. $n = 6$ for each treatment group. Data were analysed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test. $^{*}P < 0.05$, $^{\#}P < 0.01$ and $^{\dagger}P < 0.001$; a = control vs. Cd, On, Ga; b = Cd vs. CdOn, CdOnOn, CdGa, CdGaGa, CdOnGa; c = CdOn vs. CdOnOn; d = CdGa vs. CdGaGa. Catalase feiahigkeit or "Kat f" is equivalent to µmole H_2O_2 consumed/min/mg protein

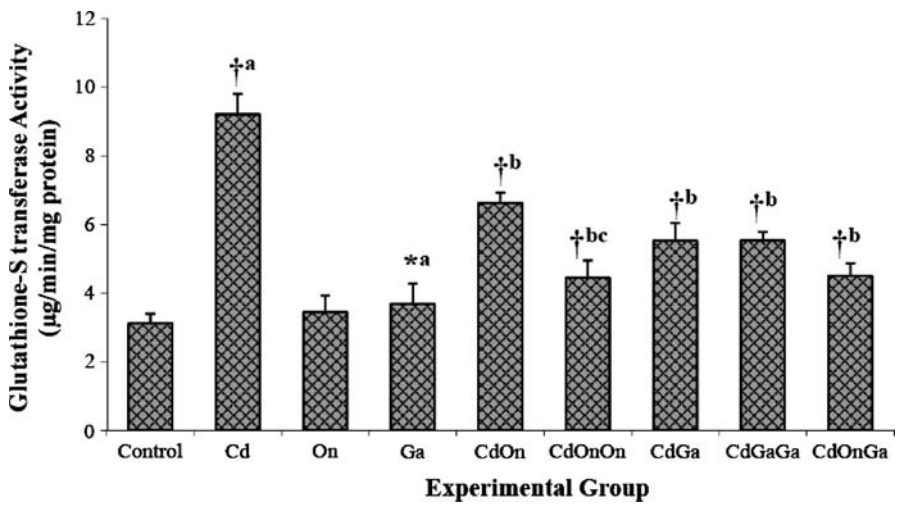

Fig. 6 Effects of onion and garlic treatments on activity of renal glutathione-S transferase in cadmium-exposed rats. *Notes*: Bars are means ± SD. $n = 6$ for each treatment group. Data were analysed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test. $^{*}P < 0.05$, $^{\#}P < 0.01$ and $^{\dagger}P < 0.001$; a = control vs. Cd, On, Ga; b = Cd vs. CdOn, CdOnOn, CdGa, CdGaGa, CdOnGa; c = CdOn vs. CdOnOn; d = CdGa vs. CdGaGa

could possibly have resulted from Cd-associated pathologies in renal tubular epithelium, such as calcuria, magnesuria and proteinuria as well as bone demineralization and anemia (Leffler et al. 1990; Horiguchi et al. 1996; Alfven et al. 2000). The partial restoration of these parameters in Cd-exposed rats treated with extracts of onion and garlic may suggest a protective role for the extracts. The positive influence of extracts on these parameters is also evident in rats treated with onion or garlic extract alone. The impact of onion or garlic on weight gain (economic performance) in animals has been reported (El-Wafa et al. 2002; Kamal and Daoud 2003; El-Demerdash et al. 2005).

Cd is an oxidative stressor known to be associated with systemic and intra-renal oxidative stress (Gill et al. 1990; Manca et al. 1991; Stohs and Bagchi 1995; Shaikh et al. 1999). Cd has been shown to enhance ROS formation, capable of depleting endogenous antioxidant status and inflicting peroxidative damage on the vulnerable membrane lipids of renal cells (Stohs and Bagchi 1995). In this study, Cd

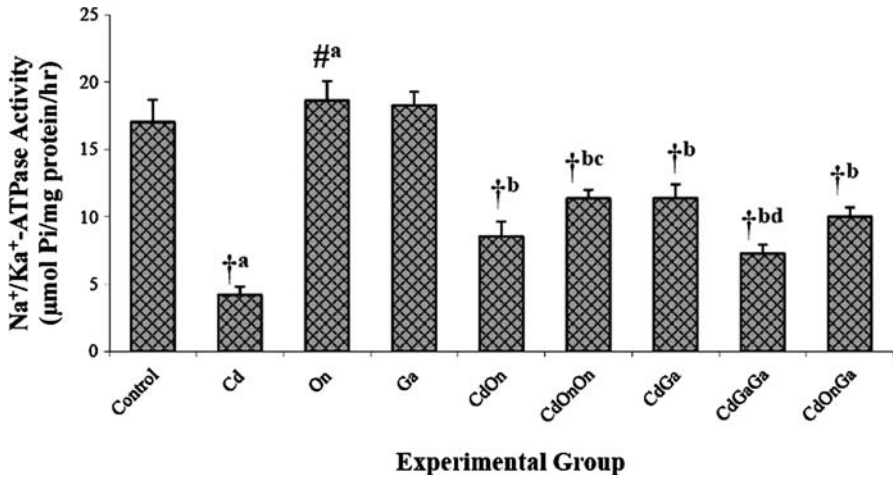

Fig. 7 Effects of onion and garlic treatments on activity of renal Na^+/Ka^+-ATPase in cadmium-exposed rats. *Notes*: Bars are means ± SD. $n = 6$ for each treatment group. Data were analysed by one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test. $^*P < 0.05$, $^{\#}P < 0.01$ and $^{\dagger}P < 0.001$; a = control vs. Cd, On, Ga; b = Cd vs. CdOn, CdOnOn, CdGa, CdGaGa, CdOnGa; c = CdOn vs. CdOnOn; d = CdGa vs. CdGaGa

administration caused a marked increase in the intra-renal levels of LPO and GST and marked decrease in GSH, SOD and CAT. Several authors have reported similar findings (Gill et al. 1990; Ognjanovic et al. 1995; Sarkar et al. 1997; Shaikh et al. 1999; Asagba et al. 2004; Pari et al. 2007). Increased GST activity might be one of the defense mechanisms in these animals to detoxify Cd-generated free radicals. The elevated levels of GST in Cd-intoxicated rats are consistent with their respective levels of LPO, suggesting a state of oxidative stress (Grose et al. 1987; Ognjanovic et al. 1995; Sarkar et al. 1997).

Cellular GSH is highly sensitive to oxidative stress, acting as the first line of defense in Cd toxicity (Singhal et al. 1987). The depletion of renal level of GSH Cd group may be the consequence of enhanced GSH utilization to conjugate Cd, counteract ROS and lipid peroxidative products (Singhal et al. 1987; Sen 1997). More so, Cd has been reported to inhibit a variety of thiol-containing enzymes, which may include γ-glutamyl cysteine synthetase, the rate-limiting enzyme in the biosynthesis of GSH (Singhal et al. 1987; Jinna et al. 1989).

SOD and CAT are integral parts of cellular antioxidant defense system and they play a crucial role in circumventing oxidative stress. The significant decrease in the renal activities of these enzymes in the Cd group may partly be the consequence of an overwhelming oxidative stress evident by a marked increase in the level of LPO. Cytosolic Cd is capable of oxidative modification of the enzymatic proteins via indirect enhancement of ROS formation and direct Cd-enzyme interaction that may perturb enzyme topography critical for catalytic activity (Casalino et al. 2002).

Based on the doses administered in this study, the protective effects of onion and garlic extracts on Cd-induced oxidative stress was evident as marked reduction in the renal level of LPO. This may be associated with their widely reported antioxidant properties (Augusti 1996; Griffiths et al. 2002). Bioactive phytochemicals and nutrients in onion and garlic have been reported to exert antioxidant effects both in-vitro and in-vivo (Augusti 1996; Augusti and Sheela 1996; Ferrali et al. 1997; Griffiths et al. 2002; Kumari and Augusti 2002; Nuutila et al. 2003; Pari et al. 2007). It is possible that the antioxidant components of the extracts might act as sacrificial antioxidants, sparing the depletion of endogenous GSH, SOD and CAT occasioned by Cd-induced oxidative stress. The ability of onion and garlic components to enhance SOD and CAT activities in carbon tetrachloride-induced toxicity has been demonstrated (El-Manakly et al. 1998).

Additionally, onion and garlic are good sources of dietary cysteine, a rate-limiting substrate in the biosynthesis of GSH (Sen 1997). Its bioavailability may augment the biosynthesis of GSH. Furthermore, dietary polyphenols have been reported to up-regulate the expression of γ-glutamyl cysteine synthetase, the

rate-limiting enzyme in GSH biosynthesis (Moskaug et al. 2005). Put together, these might be rational mechanisms by which the extracts augmented the endogenous level of GSH.

Besides having direct chemopreventive roles, onion and garlic may also initiate renal cells to produce their own chemical oxidative defense mechanisms via induction of phase II drug metabolizing enzymes such as GST and p-nitrophenol UGT (Guyonnet et al. 1999; Teyssier et al. 2001). The down-regulation of GST activity in Cd-intoxicated rats treated with extracts may not have resulted from inhibition by the extracts since the enzyme activity tended to be slightly high in rats treated with the extracts alone relative to control rats. Since both extracts caused a marked reduction in LPO level, it is possible that the extracts exerted a reduction in Cd-induced oxidative stress which may thus normalize renal GST activity.

Quercetin (a flavonoid component of onion) has been reported to exert synergic action with Cd in up-regulating the expression of metallothionein (MT), an antioxidant and metal chelating protein (Morales et al. 2006). Its reported over-expression after Cd and quercetin co-administration may add credence to the protective effect of onion extract against Cd-induced oxidative damage on renal tissues observed in this study. More so, Pari et al. (2007) reported that diallyl tetrasulfide, an organosulfur component of garlic, protected the kidney from Cd toxicity via reduction in LPO, enhanced antioxidant status and metal chelation.

In this study, Cd administration severely decreased renal Na^+/K^+-ATPase activity. Similar observation has been reported by other investigators (Jinna et al. 1989; Thevenod and Friedmann 1999; Asagba et al. 2004). Studies have demonstrated that ROS-induced inactivation of renal Na^+/K^+-ATPase is amenable to treatment with antioxidant (Beltowski et al. 2005; Murugavel and Pari 2007). Apparently, our study shows that Cd-induced inhibition of Na^+/K^+-ATPase is amenable to attenuation by both extracts probably via reduction in plasma membrane lipid peroxidative and oxidative damage to Na^+/K^+-ATPase, and enhanced antioxidant defense.

It is pertinent to note that though both extracts elicited a general reduction in LPO and augmentation of GSH, SOD, CAT and Na^+/K^+-ATPase levels in the renal tissues of Cd-challenged rats, onion extracts elicited a remarkable dose-dependent restoration of these parameters while high dose of garlic extract appears to elicit a prooxidant effect (Banerjeea et al. 2001), alongside the pre-existing Cd-induced oxidative stress. It may not be possible at this stage to say with exactitude how much of the protection is due the antioxidant components and how much to nutrients in these extracts. However, if these impressive benefits are imputable to the antioxidants, then the protective substances may primarily be the antioxidant phytochemicals and appreciable amounts of glutathione, vitamin C and E, selenium, zinc, magnesium, manganese and others reported to be present in both onion and garlic (Rodrigues et al. 2003; Fenwick and Hanley 1990). Presumably, there could as well be synergistic interactions.

Whether the findings of this study translate to human health benefits is inconclusive in view of conflicting reports on plasma antioxidant status in human model treated with either onion or garlic extracts alone. O'Reilly et al. (2001) failed to observe any significant change in antioxidant status of human subjects after consumption of onions. On the other hand, Boyle et al. (2000) reported a transient decrease in some biomarkers of oxidative stress following ingestion of onions. Also, Durak et al. (2004) reported that intake of garlic extract of 1 ml/kg bw/day for 6 months lowered plasma erythrocyte MDA levels in atherosclerotic patients even in the absence of changes in antioxidant enzyme activities. These latter observations may suggest in-vivo antioxidant potential for the extracts and dose used in this study in human model. While it may not be assured that the protective effects of both extracts stems from antioxidant actions alone, the findings of this study suggest that high onion and modest garlic intake may offer a measure of protection against Cd-induced oxidative damage in the kidney.

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References

Adam-Vizi V, Seregi A (1982) Receptor independent stimulatory effect of noradrenalin on Na^+/K^+-ATPase in rat brain homogenate, role of lipid peroxidation. Biochem Pharmacol 31:2231–2236. doi:10.1016/0006-2952(82)90106-X

Akesson A, Lundh T, Vahter M, Bjellerup P, Lidfeldt J, Nerbrand C et al (2005) Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. Environ Health Perspect 113:1627–1631

Alfven T, Elinder CG, Carlsson MD, Grubb A, Hellstrom L, Person B et al (2000) Low level cadmium exposure and osteoporosis. J Bone Miner Res 15:1579–1586. doi:10.1359/jbmr.2000.15.8.1579

Asagba SO, Adaikpoh MA, Kadiri H, Obi FO (2007) Influence of aqueous extract of *Hibiscus sabdariffa* L. petal on cadmium toxicity in rats. Biol Trace Elem Res 115:45–57. doi:10.1385/BTER:115:1:47

Asagba SO, Eriyamremu GE, Adaikpoh MA, Ezeoma A (2004) Levels of lipid peroxidation, superoxide dismutase, and Na^+/K^+ ATPase in some tissues of rats exposed to a Nigerian-like diet and cadmium. Biol Trace Elem Res 100:75–86. doi:10.1385/BTER:100:1:075

Augusti KT, Sheela CG (1996) Antiperoxide effect of S-allyl cysteine sulfoxide, insulin-secretagogue, in diabetic rats. Experentia 52:115–120. doi:10.1007/BF01923354

Augusti KT (1996) Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Indian J Exp Biol 34:634–640

Banerjeea SK, Maulika M, Manchandab SC, Dindac AK, Dasd TK, Maulika SK (2001) Garlic-induced alteration in rat liver and kidney morphology and associated changes in endogenous antioxidant status. Food Chem Toxicol 39:793–797. doi:10.1016/S0278-6915(01)00018-7

Beltowski J, Jamroz-Wisniewsska A, Borkowska E, Nazar J, Marciniak A (2005) Antioxidant treatment normalizes renal Na^+/K^+-ATPase activity in leptin-treated rats. Pharmacol Rep 57:219–228

Beuge JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310. doi:10.1016/S0076-6879(78)52032-6

Boyle SP, Dobson VL, Duthie SJ, Kyle JAM, Collins AR (2000) Absorption and DNA protective effects of flavonoid glycosides from an onion meal. Eur J Nutr 39:213–223. doi:10.1007/s003940070014

Casalino E, Calzaretti G, Sblano C, Landriscina C (2002) Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology 179:37–50. doi:10.1016/S0300-483X(02)00245-7

Denton L, Ojeifo IM (1990) Onion production practices and their improvement in Nigeria. Onion Newsl Tropics 2:10–13

Durak I, Aytac B, Atmaca Y, Devrim E, Avei A, Erol C et al (2004) Effect of garlic extract consumption on plasma and erythrocyte antioxidant parameter in atherosclerotic patients. Int J Vitam Nutr Res 74:199–203. doi:10.1024/0300-9831.74.3.199

El-Demerdash FM, Yousef MI, Abou EI-Naga NI (2005) Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 43:57–63. doi:10.1016/j.fct.2004.08.012

El-Demerdash FM, Yousef MI, Kedwany FS, Bahadadi HH (2004) Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats, protective role of vitamin E and beta-carotene. Food Chem Toxicol 42:1563–1571. doi:10.1016/j.fct.2004.05.001

El-Manakhly EM, Youssef SA, Hassieb MM (1998) The protective effect of garlic against carbon tetrachloride induced hepatotoxicosis in male rats. Some histopathologic and histochemical studies. Egypt J Comp Pathol Clin Pathol 11:35–45

El-Wafa SA, Sedki AA, Ismail AM (2002) Response of growing rabbits to diet containing black seed, garlic or onion as natural feed additives. Egypt J Rabbit Sci 12:69–83

Fenwick RG, Hanley AB (1990) Chemical composition. In: Brewster JL, Rabinowitch HD (eds) Onions, allied crops volume III, biochemistry, food science, minor crops. CRC Press Inc, Boca Raton, FL

Ferraille E, Doucet A (2001) Sodium potassium-adenosine triphosphatase-dependent sodium transport in the kidney. Hormonal control. Physiol Rev 81:346–395

Ferrali M, Signorini C, Caciotti B et al (1997) Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity. FEBS Lett 416:123–129. doi:10.1016/S0014-5793(97)01182-4

Gill KD, Sandhir R, Sharm G, Pal R, Nath R (1990) Perturbations in lipid peroxidation and antioxidation enzymes on cadmium exposure to growing rats. J Trace Elem Exp Med 3:79–89

Griffiths G, Trueman L, Crowther T, Thomas B, Smith B (2002) Onions–a global benefit to health. Phytother Res 16:603–615. doi:10.1002/ptr.1222

Grose EC, Richards JH, Jaskot RH, Menache MG, Graham JA, Dauterman WC (1987) Glutathione peroxidase and glutathione transferase activities in rat lung and liver following cadmium. Toxicology 44:171–179. doi:10.1016/0300-483X(87)90147-8

Guyonnet D, Siess MH, Le Bon AM, Suschetet M (1999) Modulation of phase II enzymes by organosulfur compounds from *Allium* vegetables in rat tissues. Toxicol Appl Pharmacol 154:50–58. doi:10.1006/taap.1998.8574

Habig WH, Pabst MJ, Jakoby WB (1974) The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Horiguchi H, Sato M, Konno N, Fukushima M (1996) Long-term cadmium exposure induce anaemia through hypoinduction of erythropoietin in the kidneys. Arch Toxicol 71:11–19. doi:10.1007/s002040050352

Jarup L, Berglund M, Elinder C, Nordberg G, Vahter M (1998) Health effects of cadmium exposure – a review of literature and a risk estimate. Scand J Work Environ Health 24(Suppl. l):1–52

Jinna RR, Ahanandsalim KJ, Desiough D (1989) Protection against cadmium toxicity and enzyme inhibition by dithiothreitol. Cell Biochem Funct 7:110–112

Jollow DJ, Michell JR, Zampaglione N, Gillete J (1974) Bromobenzene-induced liver necrosis, protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11:151–169

Kamal AM, Daoud JR (2003) Effect of onion and/or garlic as feed additives on blood, tissue constituents and growth performance in Muscovy ducks. Vet Med J Giza 51:161–175

Kowalczyk E, Kopff A, Pijalkowski P et al (2003) Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium. Acta Biochem Polon 50:543–548

Kumari K, Augusti KT (2002) Anti-diabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (*Allium cepa* Linn) as compared to standard drugs in alloxan diabetic rats. Indian J Exp Biol 40:1005–1009

Leffler P, Jin TY, Nordberg GF (1990) Cadmium-metallothionein-induced kidney dysfunction increases magnesium excretion in the rat. Toxicol Appl Pharmacol 103:180–184. doi:10.1016/0041-008X(90)90274-X

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Manca D, Richard A, Trottier B, Chevallier G (1991) Studies on lipid peroxidation in rat tissues following administration of cadmium chloride. Toxicology 67:303–323. doi:10.1016/0300-483X(91)90030-5

Massadeh AM, Al-Safi SA, Momani IF, Alomary AA, Jaradat QM, Alkofahi AS (2007) Garlic (*Allium sativum* L.) as a potential antidote for cadmium and lead intoxication: cadmium and lead distribution and analysis in different mice organs. Biol Trace Elem Res 120:227–234. doi:10.1007/s12011-007-8017-3

Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Morales AI, Vicente-Sanchez C, Jerkic M, Santiago JM, Sanchez-Gonzalez PD, Perez-Barriocanal F et al (2006) Effect of quercetin on metallothionein, nitric oxide synthases and cyclooxygenase-2 expression on experimental chronic cadmium nephrotoxicity in rats. Toxicol Appl Pharmacol 210:128–135. doi:10.1016/j.taap. 2005.09.006

Moskaug JO, Carlsen H, Myhrstad MCW, Blomhoff R (2005) Polyphenols and glutathione synthesis regulation. Am J Clin Nutr 81:277S–283S

Murugavel P, Pari L (2007) Diallyl tetrasulfide modulates the cadmium-induced impairments of membrane-bound enzymes in rats. J Basic Clin Physiol Pharmacol 18:37–48

Nordberg GF (1984) Chelating agents and cadmium toxicity: problems and prospects. Environ Health Perspect 54: 213–218. doi:10.2307/3429809

Nordberg GF, Nogawa K, Nordberg M, Friberg L (2007) Cadmium. In: Nordberg GF, Fowler BF, Nordberg M, Friberg L (eds) Handbook on the toxicology of metals. Elsevier, Amsterdam, pp 445–486

Nuutila AM, Puuponen-Pimia R, Aarni M, Oksman-Caldentey K (2003) Comparison of antioxidant activities of onion and garlic and extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chem 81:485–493. doi:10.1016/S0308-8146(02)00476-4

Ognjanovic B, Zikic RV, Stajn A, Saicic ZS, Kostic MM, Petrovic VM (1995) The effects of selenium on the antioxidant defense system in the liver of rats exposed to cadmium. Physiol Res 44:293–300

O'Reilly J, Mallet AI, McAnlis GT et al (2001) Consumption of flavonoids in onions and black tea: lack of effect on F2-isoprostanes and autoantibodies to oxidized LDL in healthy humans. Am J Clin Nutr 73:1040–1044

Pari I, Murugavel P, Sitasawad SL, Kumar KS (2007) Cytoprotective and antioxidant role of diallyl tetrasulfide on cadmium-induced renal injury, an in-vivo and in-vitro study. Life Sci 880:650–658. doi:10.1016/j.lfs.2006.10.013

Rodrigues AS, Fogliano V, Graziani G, Mendes S, Vale AP, Gonçalves C (2003) Nutritional value of onion regional varieties in Northwest Portugal. Electron J Environ Agric Food Chem 2:519–524

Sarkar S, Yadav P, Bhatnagar D (1997) Cadmium-induced lipid peroxidation and the antioxidant system in rats erythrocytes: the roles of antioxidant. J Trace Elem Med Biol 11:8–13

Satarug S, Moore MR (2004) Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environ Health Perspect 112:1099–1103

Sen CK (1997) Nutritional biochemistry of cellular glutathione. Nutr Biochem 8:660–676. doi:10.1016/S0955-2863(97)00113-7

Shaikh ZA, Vu TT, Zaman K (1999) Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol Appl Pharmacol 154:256–263. doi:10.1006/taap.1998.8586

Singhal RK, Anderson ME, Meister A (1987) Glutathione, a first line of defense against cadmium toxicity. FASEB J 1:220–223

Sinha KA (1971) Colorimetric assay of catalase. Anal Biochem 47:389–394. doi:10.1016/0003-2697(72)90132-7

Stewart DJ (1974) Sensitive automated methods for phosphate and (Na^+ plus K^+)-ATPase. Anal Biochem 62:349–364. doi:10.1016/0003-2697(74)90167-5

Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 18:321–336. doi:10.1016/0891-5849(94)00159-H

Tewari PC, Jain VK, Ashquin M, Tandon SK (1986) Influence of protein deficiency on cadmium toxicity in rats. Arch Environ Contam Toxicol 15:409–415. doi:10.1007/BF01066408

Teyssier C, Amiot MJ, Mondy N, Auger J, Kahane R, Siess MH (2001) Effect of onion consumption by rats on hepatic drug metabolizing enzymes. Food Chem Toxicol 39:981–987. doi:10.1016/S0278-6915(01)00056-4

Thevenod F, Friedmann JM (1999) Cadmium-mediated oxidative stress in kidney proximal tubule cells induces degradation of Na^+/K^+-ATPase through proteasomal and endo-/lysosomal proteolytic pathways. FASEB J 13: 1751–1761

Timbrell JA (1991) Principle of biochemical toxicology. London, Taylor and Francis