PROTECTIVE ROLE OF DIALLYLTETRASULFIDEAN ACTIVE NUTRACEUTICAL FROM GARLIC ON CADMIUM-INDUCED RENAL TOXICITY IN RATS

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Abstract: Generation of free radical with subsequent oxidative stress has been involved in biochemical and molecular mechanisms of Cadmium (Cd) toxicity. The aim of this study was to investigate the nutraceutical effects of diallyltetrasulfide (DTS), (*an organosulfer compound derived from garlic*) on Cd induced renal toxicity in rats. Renal toxicity was induced by subcutaneous injection of Cd chloride (3mg/kg/bw/day). Forty Albino rats were used in this study; the first group was injected subcutaneously with isotonic saline and received intragastric corn oil. The second group received intragastric DTS 40 mg/Kg/bw/day. The third group was injected with the same dose of Cd chloride. The forth group was injected with Cd chloride followed by intragastric DTS. At the end of the experiment (30 days) urine samples were collected, rats were sacrificed and blood samples (4ml) were collected for biochemical analysis. Significant increased levels of urea, creatinine, advanced oxidative protein products and 8-hydroxy-2'-deoxyguanosine ($p < 0.001$) as well as significant decreased levels of serum total protein and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) were observed in the third group (Cd intoxicated rats) ($p < 0.001$). Significant decreased ($p< 0.01$) levels of urea, creatinine , advanced oxidative protein products and 8 hydroxyguanosine as well as significant increased levels ($p< 0.01$) of antioxidant enzymes in the forth group after intragastric administration of DTS . The data confirmed the nutraceutical property of 40 mg/Kg/bw/day DTS as an antioxidant that ameliorates oxidative stress and revealed that it is efficiently protects kidneys from Cd-induced oxidative damage.

Keywords: *Cadmium; Diallyltetrasulfide; Antioxidant enzymes; Nutraceuticals; Renal toxicity; Oxidative stress*

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INTRODUCTION

Cadmium (Cd) is an environmental and industrial pollutant that poses increasing risks to populations in many parts of the world (Diawara et al., 2006; Cackovic et al., 2009). With chronic exposure, Cd accumulates in the epithelial cells of the proximal tubule of the kidney and glomerulus which is believed to be irreversible at advanced stages (Ahn et al., 1999). As a result of the extensive use of Cd in industry and its extensive dissemination in the environment, numerous studies have focused on the identification of the early stages of Cd-induced kidney injury in exposed human populations (Prozialeck et al., 2007; Edwards and Prozialeck 2009). It has been demonstrated that Cd stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals (Waisberg et al. 2003). Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis (Casalino et al. 2002, Waisberg et al. 2003).

Recent trends in controlling and treating diseases tend to favor natural antioxidant compounds rather than synthetic ones (Craig and Beck 1999).

Garlic (*Alium sativum*) is a bulbous plant, whose bulb has a strong taste and characteristic odor, arising from allicin and other oil-soluble sulfur components.

Typical volatiles in crushed garlic and garlic essential oil include Diallyltetrasulfide (DTS) diallyltrisulfide, 3-vinyl-1,2-dithiin (Fenwick and Hanley, 1985) and E, 2-ajoene (Block et al.,1984). Garlic, is a commonly worldwide used food, and its nutraceutical properties have been documented, it is known for its anti-carcinogenic (Hussain et al.,1990), hypolipidemic (Bordia et al., 1975), anti-atherosclerotic (Bordia et al., 1980) and an antioxidant against free radicals (Banerjee et al 2003, Morihara et al.,2006). Therefore, the aim of this research was to investigate the nutraceutical role of DTS in preventing -CdCl2-induced abnormalities in experimental animals. The changes in the biochemical parameters associated with the oxidative stress in male albino rats will be

also assessed in order to ascertain the possible use of "garlic" as protective herbal medication in kidney disease.

MATERIALS AND METHODS

Chemicals

Diallyltetrasulfide and Cadmium chloride ($CdCl_2$) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA).

Experimental Animals

Forty male Albino rats (weighing about 170-200 g) were used in this study. The animals were kept under good ventilation and daily received a standard diet (Rogers et al. 1979) and water ad libitum throughout the experimental period (30 days). The experiment was carried-out in accordance with the national regulations of animal welfare and Institutional Animal Ethical Committee (IAEC), National Research Center.

Experimental Design

Rats were divided into four groups of 10 each.

Group 1: 'Control' group received standard diet, intragastrica corn oil and subcutaneously injected with isotonic saline solution.

Group 2: received standard balanced diet and intragastrica DTS 40 mg/Kg/bw/day

Group 3: received standard diet and subcutaneously injected with $CdCl_2$ (3mg/kg/bw/day)

Group 4: received standard diet, subcutaneously injected with $CdCl_2$ and intragastrically DTS 40 mg/Kg/bw/day.

Rats were placed in metabolic cages, after 30-day period urine samples were collected to measure Isoprostane $F_2$and 8-OHdG,blood samples were obtained from the retro-orbital plexus of rats after being lightly anesthetized with ether. Blood sample from each rat was divided into two sub-samples; first sub-sample underwent centrifugation for 15 min at 2500 rpm. and the separated serum was used to assess urea, creatinine, total protein and advanced oxidative protein products, the other sub-sample was collected in heparinized tubes to measure the levels of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase).

ANALYSIS OF THE BIOCHEMICAL INDICES

Assessment of kidney function

Urea and creatinine were determined according to analysis protocols set by Palton & Crouch (1977) and Heinegard & Tiderstrom (1973) respectively.

Measurement the activities of antioxidant enzymes

Glutathione peroxidase (GPx) activity was performed according to the method of Kraus and Ganthen (1980). Glutathione peroxidase catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$ the decrease in absorbance at 340 nm was measured Werns and Lucchesi, 1990 . Superoxide dismutase (SOD) was measured at 560 nm as the rate of suppression of reduction of nitrotetrazolium blue and for 1 unit of activity, the amount of protein was taken which provided 50% inhibition of nitrotetrazolium blue reduction under standard conditions (Fridovich,1974). Catalase was measured at 510 nm as interacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxides remaining H_2O_2 reacts with 3, 5-Dichloro -2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample (Aebi,1984)

Evaluation of urinary 8-OHdG

Protocol for urinary 8-OHdG analysis was modified from the method described by Kim et al., (2001). Briefly; 8-OHDG was extracted from 1 ml urine. The eluents were dried under ultra-pure N_2 stream and reconstituted in 5 ml deionized water for injection in HPLC.

HPLC condition: HPLC column for 8-OHDG was C18 (250 ×4.6 mm, particle size 5µm). The mobile phase consists of acetonitrile / methanol/ phosphate buffer (25/10/965). Phosphate buffer was prepared by dissolving 8.8 of potassium dihydrogen phosphate in 1000 ml deionized water and PH was adjusted at 3.5, the buffer then filtered 2 times before using.

Flourate 1ml/min and using electrochemical detector with cell potential 600 mv.

Measurement of Urinary F2- isoprostane

Urinary F2- isoprostane concentration was determined by enzyme immunoassay by a kit derived from Cayman, Ann Arbor, USA (Montuschi et al., 2004)

Quantitative determination of advanced oxidation protein products (AOPP)

This is performed according to the method of Witko-Sarsat et al., 1996. The kit was supplied from immunodiagnostic AG, Germany. The assay is based on the spectroscopic analysis of modified proteins at 340nm. Samples assayed for AOPP were placed in each well of a 96- well microtiter plate. The chloramines-T (CT) absorbance at 340 nm being linear within the range of 0-100 µmol/L. AOPP concentrations were expressed as CT equivalents.

Statistical Analysis

Statistical analysis of the results was carried out using the statistical package for social sciences- SPSS (V.9.04, Echo Soft Corporation, USA, 1998). The data were analyzed by means of one-way analysis of variance (ANOVA) and were expressed as the mean ± S.D, *p*-values less than 0.05 were considered statistically significant.

RESULTS

Rats fed on standard diet supplemented with DTS did not show any significant changes in the majority of the parameters examined.

There is a significant increase in serum levels of urea and creatinine and decrease in serum levels of total protein (p value< 0.001.) in rats which were subcutaneously injected with $CdCl_2$, administration of DTS to the Cd Cl_2-intoxicated rats significantly restored these parameters (Table 1).

As regard the antioxidant enzymes there are significant decreased in their activities after subcutaneous injection with CdCl2. However, supplementation with DTS ameliorated CdCl2 adverse effects as evidenced by a significant increase in their concentrations (Table 2).

It is shown in (Table 3), that there is a significant increase of serum levels of advanced oxidative

protein products (AOPP) as well as urinary 8-hydroxy-2'-deoxyguanosine (8-OHydG) and Isoprostane F_2 in rats which were subcutaneously injected with $CdCl_2$, administration of DTS significant decrease their levels.

Table 1. Effect of diallyltetrasulfide (DTS) and cadmium chloride ($CdCl_2$) on kidney function tests in the studied groups

Parameter	Control	DTS	$CdCl_2$	$CdCl_2$+ DTS
Urea (mg/dl)	52.31 ± 3.15	51.29 ± 3.18	86.38 ± 5.21[a]	65.33 ± 3.52[b]
Creatinine (mg/dl)	2.16 ± 0.15	2.05 ± 0.13	3.6 ± 0.18[a]	2.78 ± 0.28[b]
T. Protein(g/dl)	9.83 ± 0.85	9.41 ± 0.77	5.01 ± 0.57[a]	7.3 ± 0.41[b]

n = 10 for each groups.

Data are expressed as mean ±SD. $p< 0.05$

a=Significantly different from controls; DTS; CdCl2+ DTS

b=Significantly different from controls; DTS; CdCl2

Table 2. Effect of diallyltetrasulfide (DTS) and cadmium chloride ($CdCl_2$) on the activities of antioxidant enzymes in the studied groups

Parameter	Control	DTS	$CdCl_2$	$CdCl_2$+ DTS
SOD(U/ml)	51.61 ± 6.78	57.05 ± 6.92	19.41 ± 4.84[a]	38.75 ± 2.94[b]
CAT(U/L)	347.88 ± 45.78	386.98 ± 41.7	130.35 ± 10.52[a]	273.63 ± 26.32[b]
GPx(mU/ml)	121.29 ± 4.75	121.7 ± 5.19	80.22 ± 4.88[a]	100.24 ± 4.77[b]

n = 10 for each groups.

Data are expressed as mean ±SD. $p < 0.05$

a= Significantly different from controls; DTS; CdCl2+ DTS

b= Significantly different from controls; DTS; CdCl2

SOD : Superoxide Dismutase

CAT: Catalase

GPx: Glutathione Peroxidase

Table 3. Effect of diallyltetrasulfide (DTS) and cadmium chloride ($CdCl_2$) on levels of AOPP, 8-OHydG and Isoprostane F_2 in the studied groups

Parameter	Control	DTS	$CdCl_2$	CdCl2+ DTS
AOPP (nmol/l)	69.83 ± 9.64	65.42 ± 9.28	151.1 ± 9.85[a]	94.41 ± 7.75[b]
8-OHydG (nmol/ml)	0.53 ± 0.13	0.55 ± 0.12	1.30 ± 0.21[a]	0.91 ± 0.12[b]
Isoprostane F_2(ng/ml)	0.59 ± 0.12	0.63 ± 0.13	1.48 ± 0.14[a]	0.93 ± 0.12[b]

n = 10 for each group.

Data are expressed as mean ±SD. $p< 0.05$

a=Significantly different from controls; DTS; CdCl2+ DTS

b= Significantly different from controls; DTS; CdCl2

AOPP: Advanced Protein End Product

8-OHydG: 8-hydroxy-2'-deoxyguanosine

DISCUSSION AND CONCLUSION

Cd has been recognized as one of the most toxic environmental and industrial pollutants (Diawara et al., 2006). Chronic Cd exposure is associated with a number of adverse health effects which are attributable to distinct pathological changes in variety of tissues and organs, reflecting multiplicity of Cd targets and toxicities (Satarug and Moore,2004). Cd may induce oxidative damage by disturbing the prooxidant-antioxidant balance in the tissues together with the production of ROS, which may act as a signaling molecule in the induction of cell death (Waisberg et al., 2003). With chronic exposure, Cd accumulates in the epithelial cells of the proximal tubule of the kidney and causes a generalized dysfunction of the proximal tubule (Satarug et al.,2006). As a result of the extensive use of Cd in industry and its extensive dissemination in the environment, numerous studies have focused on the identification of the early stages of Cd-induced kidney injury (Prozialeck et al., 2007; Edwards and Prozialeck 2009).

Garlic has been used as a folk remedy for a variety of ailments since ancient times. In the past few years, it has been found in certain models that garlic preparations including aged garlic extract and garlic oil, prevented cardiovascular diseases (Kleijnenet al., 1989), liver damage (Nakagawa et al., 1989), aging (Moriguchi et al., 1994) and renal damage (Wongmekiat and Thamprasert, 2005;Kabasakal et al., 2005) which are considered to be associated with oxygen radical and lipid peroxidation. The diallylsulfer compounds in garlic have also been reported for its capacity to decrease oxidative stress and to preserve the renal tissue from nephrotoxicants (Pdraza-Chaverri et al., 2003)

The present study was undertaken to assess the possible nutraceutical protective role of DTS against the toxic effect Cd on kidney by examining different biochemical parameters of oxidative damage in the serum of male rats.

Results revealed that administration of Cd subcutaneously 3mg/kg/bw/day caused significant elevated serum levels of urea and creatinine that is an indicator of impairment kidney function($p < 0.01$).

Elevated urea usually indicates

glomerular damage, while creatinine is a metabolite of creatine and is excreted completely in the urine viaglomerular filtration, an elevation of its level in the blood is, thus, an indication of impaired kidney function, similar results were previously shown by Bulan et al.,2008. Administration of DTS of intragastric 40 mg/Kg/bw/day induced a clear improvement in kidney functions, perhaps due to the antioxidant properties of garlic in scavenging free radicals leading to reduced lipid peroxidation and modulating the oxidative stress and detoxifying enzyme system (Saravanan and Prakash, 2004; Popova and Popove, 2005)

There were significant changes in the activity of AOS enzymes during the treatment of rats with Cd. SOD , GSH-Px and CAT activities were significantly decreased, this is probably a consequence of the intracellular accumulation of ROS with subsequent development of kidney injury, similar results were observed by Renugadevi and Prabu (2008) who reported decreased (was this decrease significant or not, if yes what was the p-value, please) in non-enzymatic as well as enzymatic antioxidants in cadmium treated rats .This suggests a role of free radicals in causing cellular damage during exposure to Cd. The decreased activity of GSH-Px can be explained by competition of Cd-metallothioneins and GSH-Px for sulfur-containing amino acids (Waisberg*etal.* 2003). Studies of other authors have shown that Cd inhibits the activity of majority of enzymes involved in AOS (Casalino et al. 2002) inducing an increased production of free radicals, lipid peroxidation, and destruction of cell membranes (Ognjanović *et al.* 2003). Cd also inhibits the activities of many enzymes by binding to their sulfhydryl groups or by inhibiting the protein synthesis (Waisberg et al. 2003).

Treatment with DTS significantly increased ($p< 0.01$) serum levels of SOD and GSH-Px activities, as well as CAT. By concomitant exposure of rats both to Cd and DTS, the activities of SOD, CAT and GSH-Px remain at the level of the control values, indicating that DTS eliminates the toxic effects of Cd on the activity of these enzymes.

8- Hydroxy-2 –deoxyguanosine (8-OHdG), a hydroxylated-product at the C-8 position of 2 –deoxyguanosineproduced by reaction with ascorbic acid in the presence of oxygen was first

reported by Kasai and Nishimura (1984). It has been established that the hydroxyl radical, singlet oxygen, or peroxynitrite is responsible for the formation of 8-OHdG (Halliwell and Aruoma 1993). At present, 8-OHdG is one of the most commonly used markers for evaluation of cellular oxidative stress. The results of the present study revealed increased excretion of urinary 8-OHdG after Cd administration ($P<0.01$), this may be due to the generation of ROS by Cd exposure which may lead to direct or indirect oxidative DNA damage (Sidorczuk et al., 2009).

It can be concluded from the present study that Cd exposure causes nephrotoxicity associated with marked alterations of enzymatic antioxidants as well as markers of oxidative DNA damage. The results showed usefulness of DTS, as potent nutraceutical compound derived from garlic which act as an antioxidant that ameliorates oxidative stress and loss of cellular antioxidants. Furthermore, it is suggested that DTS 40 mg/Kg/bw/day efficiently protects kidneys from Cd-induced oxidative damage. Nevertheless, more studies are needed in order to explore the exact cellular mechanisms underlying the protective effects of DTS.

BIOGRAPHY

Professor Raafat Awadallah is a professor of medical biochemistry, his researches has included work on the protective effect of natural plants sources and drugs on experimental animal models subjected to induced diseases.

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