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The Protective Effect of Green Tea Extract against Lead Toxicity in Rats Kidneys

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Abstract

Background: Previous reports claimed that lead enhances oxidative stress and induces renal dysfunction. Antioxidant and free radicals scavenger properties of green tea extract (GTE) can play a protective role against harmful effects of lead on the kidneys.

Objectives: (1) to assess the effects of lead on renal function and oxidative stress, (2) to investigate the effects of GTE on oxidative stress and lead-induced renal dysfunction.

Methods: 60 male Sprague-Dawley rats were divided into four equal groups (N= 15 rat for each); control group (received distilled water), GTE–group (received GTE as 1.5% w/v), Pb–group (received 0.4% lead acetate in distilled water), Pb + GTE-group (received both Pb and GTE). Lead concentration in kidney tissue was measured by atomic absorption spectrometer. Serum levels of total proteins, lipid peroxides (LPO), glutathione (GSH), glutathione S-transferase (GST), were measured using colorimetric methods and compared between different study groups.

Results: Sprague-Dawley rats treated with lead and GTE (GTE + Pb group) showed significantly increased total protein (6.9 ± 0.4 vs 4.5 ± 0.3 g/dL, $P < 0.01$) and improved renal functions as indicated by lower levels of urea (35.9 ± 3.0 mg/dL) and creatinine (0.4 ± 0.03 mg/dL) compared to the Pb-control group (48.6 ± 2.6 mg/dL and 0.7 ± 0.05 mg/dL respectively, $P < 0.01$). GTE + Pb group also showed decreased levels of lead (1.9 ± 0.1 ppm) and LPO (1.3 ± 0.1 nmol/mg protein) compared to the Pb- group (2.5 ± 0.1 ppm, $P < 0.001$ and 1.7 ± 0.2 nmol/mg protein, $P < 0.05$ respectively). Alternatively, GSH was significantly increased in GTE + Pb group (13.4 ± 0.8 nmol/mg protein) compared to the Pb-group (8.3 ± 0.7 nmol/mg protein, $P < 0.001$).

Conclusion: Lead can compromise renal function by interfering with redox state in the renal tissues. The treatment with GTE can attenuate oxidative stress and lead burden on renal function.

Keywords: Green Tea Extract, Kidney, Lead, oxidative stress.

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INTRODUCTION

Lead is a common environmental pollutant especially in industrial countries [1]. Abnormally high level of lead in human body fluids can result in detrimental effects on the renal, nervous, gastrointestinal and reproductive systems [2]. Lead toxicity was proved to enhance oxidative stress [3, 4] probably by increasing reactive oxygen species (ROS) production [5, 6], altering antioxidant enzyme levels and activities [4, 7].

Implications of the role of the oxidative stress in pathogeneses of lead toxicity are further supported by recent researches which prove the ability of antioxidants to ameliorate lead poisoning [8]. Herbs rich in natural antioxidants like Green tea (GT) (*Camellia sinensis*) are supposed to attenuate harmful effects of lead toxicity [9,10]. Polyphenolic compounds like Catechins are abundant in GT and act as a potent antioxidant especially in prevention of lipid hydroperoxidation and metal-catalyzed free-radicals formation [11, 12]**.** Current researches uncover several useful consequences of antioxidant properties of GT like cardioprotective, hepatoprotective, neuroprotective, anticarcinogenic and antimicrobial effects [13, 14].

The present study aimed to investigate the biochemical effects of lead on the renal cells of adult male Sprague-Dawley rats and the possible effects of GTE on oxidative stress and lead-induced renal dysfunction.

MATERIAL AND METHODS:

Chemicals:

Thiobarbituric acid, butylated hydroxytoluene, reduced glutathione, sodium sulphate, sodium nitrite, epinephrine, lead acetate, naphthylethylenediamine dihydrochloride, sulphanilamide, 5',5'-dithiobis-2-nitro-benzoic acid and 1 chloro-2,4 dinitrobenzene were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Animals and Experiment:

Sixty healthy male Sprague-Dawley rats (170-200 gram) were purchased from Animal House, Faculty of Pharmacy, King Saud University, KSA. All animals were conditioned at room temperature (25-27 °C) at a natural photoperiod for one week before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The animals were treated according to the principles outlined in the NIH Guide for the care and use of laboratory animals. The duration of experiment was 6 weeks. The animals were randomly divided into 4 groups (15 rats per each) as the following; Group I (*Control* group) received distilled water as sole drinking source. Group II (*GTE - group*) received green tea (GTE) (1.5% w/v). Group III *(Pb - group)* received 0.4% lead acetate in distilled water [15], Group IV (*Pb + GTE group)* received mixture of lead acetate and GTE. The solutions used in groups II, III and IV from were the only source of drinking water for the rats all through the experiment. GTE was made according to [16], by soaking 15 g of instant green tea powder in one liter of boiling distilled water for 5 minutes. The solution was filtered to make 1.5% GTE.

Biochemical Analysis:

The animals of different groups were anesthetized using diethyl ether and sacrificed one day after the end of treatment. The kidney was excised immediately for biochemical evaluation. Kidney was divided into two parts: one gram of kidney tissue digested and used for assessment of lead. The second part was homogenized in ice-cold 100mM phosphate buffer (pH 7.4) using Potter-Elvehjem homogenizer fitted with a Taflon Plunger. Homogenates were centrifuged and the resulting supernatants were divided into aliquots and stored at – 80 ^{o}C. The blood sample from each rat was collected from orbital vein in two heparinized tubes and centrifuged. Separated plasma divided into aliquots and kept at -20^{o}C until biochemical analyses.

Kidney tissue samples were carefully weighed, placed in polypropylene tubes, and digested in 1ml of concentrated HNO_3 in a shaking water bath at 60°C for 30min. This treatment ensures complete destruction of organic matter [17]**.** After digestion, 100ul aliquot was taken from clear solution and diluted (1:5 v/v) with deionized water. Calibration curves were constructed by adding known amounts of lead standard (E. Merck). Analysis of diluted samples of blood and digested tissue were injected into atomic absorption spectrophotometer (Perkin-Elmer Model 400, Shelton, CT, USA) according to Villeda-Hernandez *et al* [18]. Hollow cathode lamps of lead were used at wavelength of 283.3nm. The protein concentration in tissue lysates was measured by colorimetric method of Bradford (1976)[19]. The levels of lipid peroxidase were measured using thiobarbituric acid reactivity (TBARS). The product of the reaction between malondialdehyde (MDA) and thiobarbituric acid was measured as described by Thayer[20]. Glutathione (GSH) concentrations were determined chemically as described by Dutta et al [21]**.** The plasma level of total antioxidant capacity (TAC) was measured by specific ELISA assay kits (Biodiagnostic, Giza, Egypt) according to manufacturer protocol. Superoxide dismutase (SOD) activity was determined according to its ability to inhibit auto-oxidation of epinephrine at alkaline medium [22]**.** Glutathione S-transferase (GST) activity was chemically determined using 1-chloro-2,4-dinitrobenzene substrate [23]**.**

RESULTS

Biochemical Changes:

The control and GTE groups shows comparable (P > 0.05) concentrations of lead in the kidneys (0.9 ± 0.05

vs 0.98 ± 0.05 ppm), total protein (7.6 ± 0.5 vs 7.9 ± 0.5 g/dL), urea (30.8 ± 1.0 vs 30.9 ± 1.2 mg/dL) and creatinine (0.5 ± 0.04 vs 0.4 ± 0.02 mg/dL) (table – 1). Levels of GSH (15.2 ± 0.6 nmol/mg protein) and GST (80.6 ± 1.4 mM/min/ g protein) were significantly increased in GTE group compared to the controls (13.1 ± 0.9 nmol/mg protein and 72.8 ± 2.5 mM/min/ g protein, $P < 0.05$). In contrast, LPO level (0.7 ± 0.03 nmol/mg protein) was significantly less in the GTE group compared to control group (0.8 ± 0.03 nmol/mg protein, $P < 0.05$) (table – 2).

Sprague-Dawley rats treated with 0.4% lead acetate showed significant reduction in total protein (4.5 ± 0.3 vs 7.9 ± 0.5 g/dL, $P < 0.001$) and deterioration in renal functions as indicated by higher levels of urea (48.6 ± 2.6 mg/dL) and creatinine (0.7 ± 0.05 mg/dL) in Pb – group compared to the control group (30.9 ± 1.2 mg/dL, $P < 0.001$ and 0.4 ± 0.02 mg/dL, $P < 0.05$ respectively) (table – 1). Pb–group showed increased levels of lead (2.5 ± 0.1 ppm) and LPO (1.7 ± 0.2 nmol/mg protein) compared to the control group (0.9 ± 0.05 ppm and 0.8 ± 0.04 nmol/mg protein respectively, $P < 0.001$). Alternatively, GSH, GST and SOD were significantly decreased in Pb–Group (8.3 ± 0.7 nmol/mg protein, 64.3 ± 2.6 mM/min/g protein and 1.4 ± 0.2 mU/mg protein) compared to the control group (13.1 ± 0.9 nmol/mg protein, 72.8 ± 2.5 mM/min/g protein and 2.6 ± 0.2 mU/mg protein respectively, $P < 0.05$) (table – 2).

Sprague-Dawley rats treated with lead and green tea (GTE + Pb group) showed significantly increased total protein (6.9 ± 0.4 vs 4.5 ± 0.3 g/dL, $P < 0.01$) and improved renal functions as indicated by lower levels of urea (35.9 ± 3.0 mg/dL) and creatinine (0.4 ± 0.03 mg/dL) in GTE + Pb group compared to the Pb group (48.6 ± 2.6 mg/dL and 0.7 ± 0.05 mg/dL respectively, $P < 0.01$) (table – 1). GTE + Pb group showed decreased levels of lead (1.9 ± 0.1 ppm) and LPO (1.3 ± 0.1 nmol/mg protein) compared to the Pb- group (2.5 ± 0.1 ppm, $P < 0.001$ and 1.7 ± 0.2 nmol/mg protein, $P < 0.05$ respectively). Alternatively, GSH was significantly increased in GTE + Pb Group (13.4 ± 0.8nmol/mg protein) compared to the Pb-group (8.3 ± 0.7 nmol/mg protein, $P < 0.001$) (table – 2).

Pb correlate positively with lipid peroxidase (LPO) ($r = + 0.58$, $P < 0.05$) and negatively with glutathione (GSH) ($r = - 0.59$, $P < 0.05$)

Variables	**Controls** (N = 15) M±SE	**GTE- Group** (N = 15) M±SE	**Pb – Group** (N = 15) M±SE	**GTE+Pb Group** (N = 15) M±SE
Total protein (g/dL)	7.6 ± 0.5	7.9 ± 0.5	4.5 ± 0.3***	6.9 ± 0.4 ¥¥
Urea (mg/dL)	30.8 ± 1.0	30.9 ± 1.2	48.6 ± 2.6 ***	35.9 ± 3.0 ¥¥
Creatinine (mg/dL)	0.5 ± 0.04	0.4 ± 0.02	0.7 ± 0.05 *	0.4 ± 0.03 ¥¥

Table (1) Plasma levels kidney function tests and total proteins in different studied groups

*$P < 0.05$; ***$P < 0.001$ for comparison between either Pb-treated or GTE-treated groups with the control group using Student's T-test.

¥¥ $P < 0.01$; for comparison between Pb+ GTE group with Pb-treated group.

	Controls (N = 15) M±SE	**GTE-Group** (N = 15) M±SE	**Pb –Group** (N = 15) M±SE	**GTE+ Pb Group** (N = 15) M±SE
Pb levels (ppm) in the kidney	0.9 ± 0.05	0.98 ± 0.05	2.5 ± 0.1***	1.9 ± 0.1¥¥¥
LPO (nmol / mg protein)	0.8 ± 0.04	0.7 ± 0.03*	1.7 ± 0.2***	1.3 ± 0.1¥
GSH (nmol/mg protein)	13.1 ± 0.9	15.2 ± 0.6*	8.3 ± 0.7***	13.4 ± 0.8¥¥¥
GST (mM/min/ g protein)	72.8 ± 2.5	80.6 ± 1.4*	64.3 ± 2.6*	67.5 ± 4.0
SOD (mU/mg protein)	2.6 ± 0.2	2.1 ± 0.2	1.4 ± 0.2***	1.6 ± 0.2

Table (2) Lead concentrations (ppm) in kidney, tissue levels of lipid peroxides, glutathione and enzymes activities of glutathione S-transferase and superoxide dismutase in different treated groups of male rats.

*$P < 0.05$; ***$P < 0.001$ for comparison between either Pb-treated or GTE-treated groups with the control group using Student's T-test.

¥ $P < 0.05$; ¥¥ $P < 0.01$; ¥¥¥ $P < 0.001$ for comparison between Pb+GTE group and Pb-treated group.

DISCUSSION

The results of the current study revealed three main findings: firstly, Sprague-Dawley rats treated with lead showed significant reduction in total protein, deterioration in renal function and enhanced oxidative stress. Secondly, administration of green tea to rats attenuates oxidative stress in both healthy and lead-treated rats. Thirdly, treatment of Sprague-Dawley rats with green tea does not affect total protein level and renal function of healthy rats; yet, it minimizes harmful effects of lead on renal function and total protein levels. By the end of the last century, an exposure-response relation between lead and renal dysfunction was demonstrated by Ehrlich *et al* in South African battery factory workers [24]. However, adverse renal changes

lead workers seem to be dose dependent [25]. Lead-associated tissues injury in vital organs mostly results from the effects of lead on membranes, DNA, and antioxidant defense systems of cells [26]. Mohammad *et al* assessed antioxidant status in lead-exposed residential and commercial painters of Lucknow city in Uttar Pradesh, India [27]. Results showed that painters had a significant decrease antioxidant enzymes compared to controls. It is worth mentioning that same to the current study, Mohammad *et al* monitored lipid peroxidation by measuring TBARS and demonstrate higher plasma concentration of MDA in painters than in controls. Another similarity between the findings of Mohammad *et al* and the present study is the significant changes observed in reduced and oxidised glutathione levels [27]. The finding of the current study is further supported by the results Patil and his group who assessed the effect of lead exposure on the activity of superoxide dismutase in battery manufacturing workers who were occupationally exposed to lead over a long period of time [28]. The study obviously showed disparity between pro-oxidants and antioxidants in lead exposed battery manufacturing workers resulting in enhanced lipid peroxidation and decreases erythrocyte SOD.

The current study gives an evidence for potential therapeutic effect on the harmful consequences of lead toxicity on the kidneys. Similar protective effects of green tea on organs at risk of lead toxicity were demonstrated over the last few years. Administration of green tea significantly alleviated lead-induced depressed concentration of reduced glutathione and SOD activity in rats' brain tissues [29]. Supplementation of green tea extract was also proved to augment antioxidant potential of liver after being attenuated by lead exposure [30]. The protective effect of green tea is probably attributed to presence of catechins, which binds with metal ions to form insoluble complex ionic salt used to remove the lead metal. Catechins also inhibit the arachidonic acid cascade and normalize bone metabolic disorders in lead poisoned rats [12,8]. Decrease of lead concentrations in tissues of the rats treated with GTE combined with lead may be due to its chelating property. Alternatively, GTE may combine with lead ions forming complexes with decreased lipophilicity, and thus diminished gastrointestinal absorption.

In conclusion, the present study adds further evidence for the detrimental effect of lead on the kidneys induced by augmented oxidative stress. In addition, the study proposed a protective role of green tea against lead-induced renal dysfunction by readjusting antioxidant/oxidant balance.

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