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THE PROTECTIVE EFFECT OF GREEN TEA EXTRACT ON LEAD INDUCED OXIDATIVE AND DAMAGE ON RAT KIDNEY

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ABSTRACT

The role of green tea in protection against nephrotoxicity induced by lead acetate was investigated in rats. Five equal groups, each of ten rats were used. The first group was served as control, the second and third groups were given lead acetate, lead acetate and green tea, respectively, for one month. Pb was given orally at a dose of .4 % w/v, while green tea was given in drinking water at a concentration of (6.6% w/v). Pb administration induced loss of body t and elevation in kidney weight. Significant perturbations of renal function as evidenced via increase in biochemical parameters were observed in treated rats. Renal oxidative damage was observed in Pb-treated rats via augmentation in kidney lipid peroxidation as well as depletion in kidney antioxidant enzymes; catalase, superoxide dismutase and glutathione peroxidase and pathological changes. Co-administration of green tea with lead acetate significantly alleviated these adverse effects. Thus, this study suggests the potent role of green tea in management of injury-induced by lead exposure.

KEYWORDS: **Green tea, Lead, Histopathology, Nephrotoxicity, Oxidative stress.**

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INTRODUCTION

In recent years, the level of heavy metals, particularly lead has increased in air, water and soil in both urban and periurban areas[1]. It is well known that heavy metals induce toxic effects on different systems and apparatuses. Furthermore, because of their long half-life, heavy metals also induce accumulation phenomena, which in turn produce an experimental increase of their concentration in blood and tissues. Among heavy metals, lead represents the main environmental poison. Lead is a non-essential toxic heavy metal widely distributed in the environment and chronic exposure to low levels of lead has been a matter of public health concern in many countries[2]. Lead may be absorbed through the skin, gastrointestinal tract or lungs and distributed to three major compartments-blood, soft tissue and bone. Blood lead is in equilibrium with lead in soft tissue. The soft tissues that take up lead are liver, kidneys, brain and muscle. Lead is not metabolized in the body, but it may be conjugated with glutathione and excreted primarily in the urine. Lead is a multi-targeted toxicant affecting gastro intestinal tract, hematopoietic system, cardiovascular system, central and peripheral nervous system, kidneys, immune system and reproductive system[3]. Lead can damage all tissues, particularly the kidneys and the immune system. Recent evidence suggested that the kidney might also be one of the major organs for chronic lead toxicity. Lead may exert toxic effects on several organ systems, but those in the kidney are the most insidious. Nephrotoxicity results because kidney is the main route of elimination of lead[4]. Effect of lead on renal system is characterized by dysfunction of proximal renal tubules manifested by glycosuria, generalized amino aciduria, hyperphosphaturia, hyperphosphataemia and rickets are noted in acute lead poisoning. Long-term exposure to lead is known to cause irreversible functional and morphological changes, which include interstitial, tubular atrophy, and ultra-structural changes in renal tubule mitochondria[5]. Chronic lead toxicity is caused by the change of renal function parameters. Lead induced oxidative stress contributes to the pathogenesis of lead toxicity for disturbing the delicate prooxidant/antioxidant balance that exists within mammalian cells. Lead exposure cause generation of ROS (Reactive Oxygen Species) and alteration of antioxidant defense in animals and occupationally exposed workers[6]. Nephroprotective agents are the substances which possess protective activity against Nephrotoxicity. Medicinal plants have curative properties due to the presence of various complex chemical substances. Early literatures have prescribed various herbs for the cure of renal disorders[7]. Co-administration of various medicinal plants possessing nephroprotective activity along with different nephrotoxic agents which may attenuate its toxicity. The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood[8]. In addition to this, there is a failure of regulation of fluid and electrolyte balance along with endocrine dysfunction. The renal failure is fundamentally categorized into acute and chronic renal failure[9]. Tea in the form of green tea (GT) or black tea is one of the most widely consumed beverages in the world today second only to water[10]. Since ancient times GT consumption has been known to maintain and improve health. Polyphenols are plant metabolites occurring widely in plant food and exhibit outstanding antioxidant and free radical scavenging properties[11]. GT is an excellent source of polyphenols such as catechins[12], orgallotannins, flavonols, flavandiols, and phenolic acids[13]. In particular GT catechins and their derivatives are known to contribute beneficial health effects ascribed to tea by their antioxidant[8], antimutagenic, and anticarcinogenic properties[14]. GT consumption has been linked to lowering of various forms of cancers[15]. GT constituents also have been shown to have cardioprotective, neuroprotective, antidiabetic, and antimicrobial properties [16]. In addition, GT has been found to be useful in the treatment of arthritis, high cholesterol levels, infection, and impaired immune function[17]. GT consumption also has resulted in improved kidney functions in animal models of renal failure [18]. Hence, the goal of the present study has been to investigate the efficacy of green tea, as a source of water soluble antioxidants on renal function abnormalities of mature rats exposed to an oxidative stress induced by lead.

MATERIALS AND METHODS

ANIMAL TREATMENT

40 male wistar rats (age: 14-16 weeks and about 170-200g body weight) were purchased from Animal House, department of Biology, Algeria University. All animals were conditioned at room temperature (22-25°C) at a natural photoperiod for one week before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 4 weeks. All the procedure performed on animals were approved and conducted in accordance with the National Institute of health Guide (Reg. No. 488/160/1999/CPCSEA). They were randomly divided into 4 groups (10 rats each) as the following: Group I (Control group) receives distilled water as sole drinking source. Group II (GTE group) received green tea extract (6.6% w/v) of beginning of experiment. Group III (Pb group) received lead acetate at dose of 0.4 % w/v in distilled water. Group IV (Pb + GTE group) received mixture of lead acetate and GTE as sole drinking source.The GTE was made by soaking 30 g of instant green tea powder in 500 ml of boiling distilled water for 15 minutes. The solution was filtered to make 6.6% GTE. This solution was provided to rats as their sole source of drinking water. During the experimental duration, body weights were recorded. After 4 weeks, the animals of different groups were sacrificed under light anesthesia (Chloral 10% at 3ml/kg) 1 day after the end of the treatment. Both kidneys were removed, cleaned and weighed for histological and biochemical evaluation.

MEASUREMENT OF BIOCHEMICAL PARAMETERS

The serum creatinine, urea, and uric acid concentrations were measured spectrophotometrically and expressed as mg/dl by using commercial kits (Spinreact, Spain) according to the instructions of the manufacturers.

OXIDATIVE STRESS EVALUATION
RENAL LIPID PEROXIDATION

Malondialdehyde (MDA) occurs in lipid peroxidation and was measured in kidney tissues after incubation at 95 °C with thiobarbituric acid in aerobic conditions (pH 3.4). The pink colour produced by these reactions was measured spectrophotometrically at 532 nm to measure MDA levels[19].

RENAL ANTIOXIDANT ENZYMES
ASSAY OF CATALASE (CAT)

Catalase (CAT) activity was measured using the method of Aeibi[20]. Twenty µl of the supernatant were added to a cuvette containing 780 µl of 50 mM potassium phosphate buffer (pH 7.4) and then the reaction at 25 °C was initiated by adding 200 µl of 500 mM H_2O_2 to make a final volume of 1 ml. The decomposition rate of H_2O_2 was measured at 240 nm for 1 min on a spectrophotometer. A molar extinction coefficient of 0.0041 mM–1 was used to determine the CAT activity. The activity was defined as an nmoles H_2O_2 decrease/min/ mg protein.

ASSAYS OF SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich[21]. The reaction mixture contained 50 mM of kidney homogenates in 0.1 M of potassium phosphate buffer (pH7.4), 0.1 mM EDTA, 13 mM L-methionine, 2 µM riboflavin and 75 µM Nitro Blue Tetrazolium (NBT). The developed blue color in the reaction was measured at 560 nm. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50% and the activity was expressed as units per mg of protein.

ASSAYS OF GLUTATHIONE PEROXIDASE (GPx)

Glutathione peroxidase (GPx) activity was measured according to Flohe and Gunzler[22]. GPx catalyzes the oxidation of reduced glutathione by cumene hydroperoxide. In the presence of reduced glutathione reductase and nicotinamide adenine dinucleotide phosphate reduced form (NADPH), the oxidized reduced 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. The enzyme activity was expressed as nmol of GSH oxidized/min/ mg protein. Protein concentration was determined according the

method described by Lowry et al., [23], using bovine serum albumin (BSA) as a standard.

HISTOLOGICAL AND HISTOCHEMICAL

At the end of the experiment, kidney from each sacrificed rat was dissected out; and trimmed of excess fat. Then, it was fixed in 10% buffered formalin and was processed for paraffin sectioning by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin blocks. Sections of about 5 µm thickness were stained with Harris haematoxylin and eosin (H&E) for histological study[24], and Feulgen's reaction to demonstrate DNA[25].

Statistical analysis

Results were analyzed using a one-way analysis of variance (ANOVA). Comparison within groups was considered statistically significant at $p<0.05$. All the data were expressed as mean ±SD of number of experiments (n=10).

RESULTS

BODY AND ORGAN WEIGHTS

At the end of the experimental course, there was no significant difference in body and relative kidney weights between GTE and untreated rats. However a significant loss of weekly body weight gain accompanied by a significant increase in the relative kidney weights were recorded in rats treated with lead acetate compared to the control. The administration of GTE to Pb treated groups has an ameliorated effect either in the loss of body weight or in the increase of relative Kidney weights (Table1).

RENAL LIPID PEROXIDATION (LPO)

Administration of Pb led to a significant increase ($p < 0.05$) in lipid peroxidation as evidenced by the increase in kidney tissue MDA levels by 44%, when compared to the control group. However, co-administration of GTE to treated rats reduced the augmentation in MDA levels to 19% for Pb-treated rats (Table 2). Results in Table 2 show the influence of lead acetate on the activities of CAT, SOD and GPx. Subacute levels of the tested metal resulted in a state of kidney injury and extensive oxidative damage in rats as manifested by the significant alteration in these enzymes. In fact, in treated rats, a significant depletion was noted in the activities of CAT, SOD and GPx. However, the co-administration of GT mitigated the change in the activities of SOD, GPx and CAT (Table 2).

MARKERS OF RENAL FUNCTION

Serum creatinine, uric acid and urea level, of Pb -treated rats were higher (59%, 56%, 53%), respectively) with control group. However, oral administration of GTE to normal rats produced no changes in thecited above biochemical parameters when compared to the untreated control. Co-administration of GTE to tested groups induced a significant decrease in renal markers (Table3).

HISTOPATHOLOGICAL EXAMINATION
H & E STAIN

The representative pictures of histopathological examination in the kidney tissue are shown in Figure 1 (A-C). Kidney sections from the control group rats and green tea-treated rats showed intact histological structure of glomeruli and renal tubules. However, abnormalities in kidney of treated rats were detected in glomeruli and in convoluted tubules (Figure 1-B) compared to those of controls (Fig. 1A). The main characteristic findings were the appearance of vacuolization and swelling in the endothelium of glomerular tuft**,** swelling in the lining epithelium of tubules and inflammatory cells infiltration in between the degenerated tubules with fibrosis and hyalinosis between the tubules in focal manner. However, the co-administration of the GTE with Pb (Fig. 1C) showed marked improvement in their histological structure in comparison to the treated groups (Pb) alone.

FEULGEN'S REACTION

For demonstrating DNA content, sections were stained with Feulgen's reaction to demonstrate DNA as a magenta colour. Sections of kidney of the control and GTE group showed normal DNA contents (Fig. 2A). The DNA content was reduced as explained by a moderate magenta colour in the cells of renal tissue of rats treated with Pb (Fig. 2B). Rats group treated with (Pb+GTE) showed restoration of DNA content in tissues of kidney to the control group (Fig. 2C).

Table1
Effect of green tea consumption on body weight (g), kidney weight (g), and relative kidney weight (%) of rats treated with lead acetate.

Groups	Body weight (g)	Absolute kidney weight (g)	Relative kidney weight (g/100g body weight
Control group	218.9±3.75	1.27±0.05	0.58±0.012
GTE group	221.9±4.19[b]	1.26±0.0[b]	0.57±0.089[b]
Pb group	173.9±6.44[a]	1.22±0.02[a]	0.71±0.016[a]
Pb+GTE group	192.18±7.40[ab]	1.21±0.04[ab]	0.63±0.011[ab]

Values are means ± S.E.; N (number of animals) = 10; LSD (least significant difference) at the 5% level = 0.01650. [a] Superscript in the same row differ significantly at P < 0.05 with ,control (C). [b] Superscript in the same row differ significantly at P < 0.05 with lead group (Pb).

Table 2
Effect of lead acetate on renal lipid peroxidation and antioxidant enzymes Of male ratsand the ameliorative role of green tea.

Treatmens	LPO (nmoles/mg protein)	CAT (nmol/min/mg protein)	SOD (units/mg protein)	GPx (nmoles of GSH oxidized/min/mg protein)
	3.28±0.19[a]	312.2±14.3ab	18.8±0.80[a]	4.26±0.25[a]
GTE group	3.22±0.18[a]	324.4±9.4[a]	324.4±9.4[a]	4.19±0.24[a]
Pb group	5.87±0.21[d]	198.4±11.8[f]	10.3±0.84[d]	2.58±.09[b]
Pb+GTE group	4.05±0.14[b]	271.4±11.7[cd]	13.5±0.70[bc]	3.78±0.12[a]

Each value is a mean of 10 rats ± S.E.M; [a, b, c, d] values are not sharing superscripts letters (a, b, c, d) differ significantly at p < 0.05.

Table 3
Change in the level renal parameters (mg/dl)

Treatments	Uric acid	Urea	Creatinine
Control group	2.5±0.36[a]	16.37±0.94[a]	0.62±0.12[a]
GTE group	2.4±0.36[a]	17.6±2.91[d]	0.63±0.06[a]
Pb group	3.8±0.16[d]	29.75±4.12[ab]	0.9±0.14[a]
Pb+GTE group	2.99±0.25[c]	19.6±2.5[d]	0.73±0.09[a]

Each value is a mean of 10 rats ± S.E.M; a, b, c, d values are not sharing superscripts letters (a, b, c, d) differ significantly at p < 0.05.

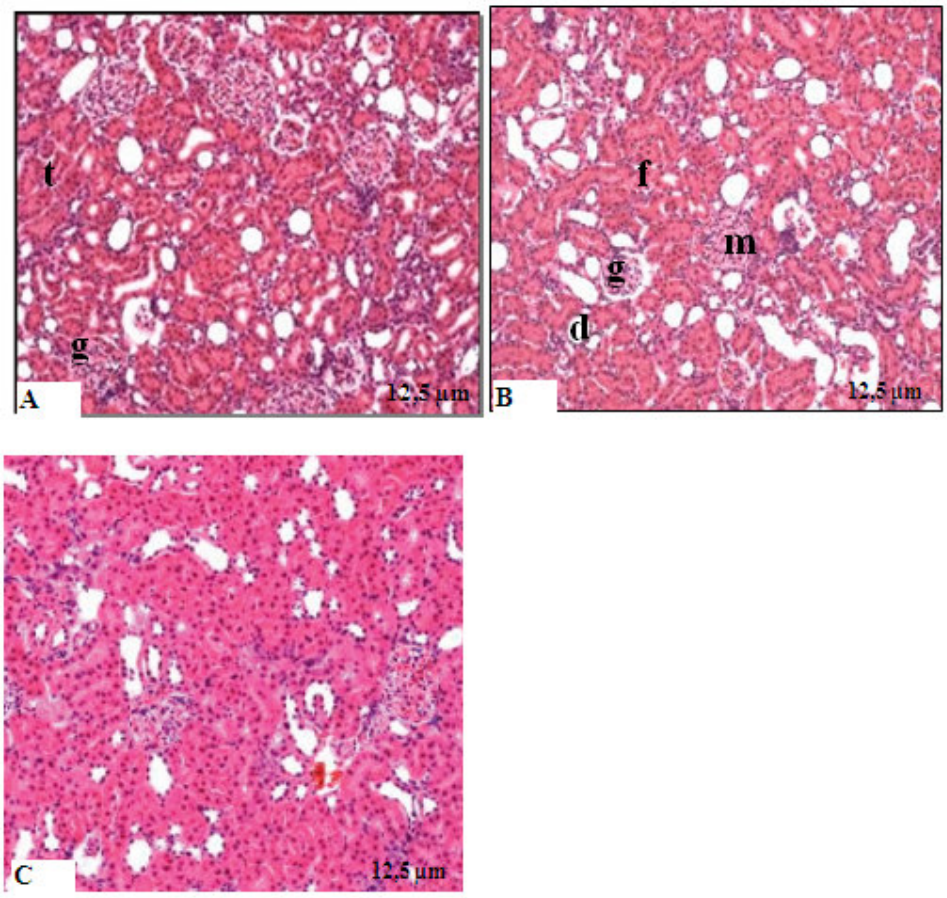

Figure1

Kidney paraffin sections stained by haematoxylin and eosin (H&E) for histopathological changes. Control and GTE group (A) showing intact histological structure of glomeruli (g) and renal tubules (t) (x 200), Pb-treated group (B) showing swelling and vacuolization in the endothelial cells lining the tuft of the glomeruli (g) with fibrosis (f) and hyalinosis (h) between the tubules in focal manner and inflammatory cells infiltration (m) and few fibroblastic cells proliferation (arrow) in between the degenerated tubules (d) in focal manner at the corticomedullary portion (x 200).

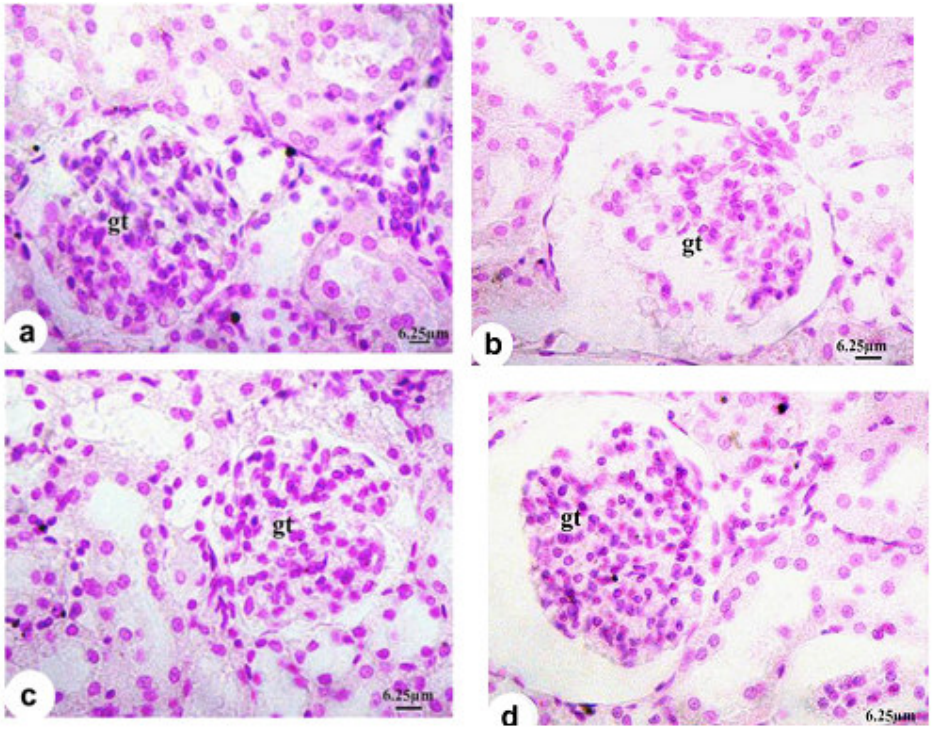

Figure 2

Sections in the kidney stained with Feulgen' reaction. (a): A control rat showing the normal content of DNA. (b): Rats treated with Pb showing a reduction of DNA content. (c): Sections in rats groups treated with (Pb + GTE) and (GTE) showing the restoration of DNA content.

DISCUSSION

Lead is a ubiquitously found environmental and industrial pollutant that has been detected in nearly all phases of environment and biological system. Its persistence in human and animal tissues has quite often been associated with considerable health risks[26]. Several strategies, mechanisms and agents were utilized to prevent Pb nephropathy in animal model[27, 28]. Tea, most widely consumed beverage worldwide since ancient times, is known for its beneficial health effects. In particular, green tea polyphenols, chiefly catechins and their derivatives have been shown to retard various forms of cancers due to its antimutagenic, anticarcinogenic and antioxidant properties[29- 30]. It was also found to be cardioprotective, neuroprotective, antidiabetic and antibacterial besides other health benefits[27; 16]. In toxicological studies, body, organ and relative organ weights are important criteria for evaluation of organ toxicity[31]. In the present study, oral administration of Pb resulted in a significant reduction in the body weight gain, and an increase in the relative kidney weight. The reduction in body weight gains may be due to the combined action of cholinergic and oxidative stress[30] and/or due to the increased degradation of lipids and proteins as direct effects of organophosphate compound exposure[30]. Moreover, the increase in kidney weight could be attributed to the relationship between kidney weight increase and various toxicological effects or to the reduction in body weight gain of experimental animals[31; 32].These results are consistent with many previous investigators with Pb and other metals[31] Co-administration of GTE improved body and kidney weights of intoxicated rats. In the present study, the oral administration of Pb to adult male rats provoked an increase in serum creatinine urea, and uric acid levels of rats. It is well known that the kidney is the main site of elimination of xenobiotics[33].These findings reflect the diagnosis of renal failure[34].The potentially reactive derivatives of oxygen, ascribed as ROS such as O_2, H_2O_2 and OH are continuously generated inside the

human body as a consequences of exposure to a lot of exogenous chemicals in our ambient environment and/or a number of endogenous metabolic processes involving redox enzymes[35]. Under normal circumstances, the ROS generated are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present[36]. Harmful effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species. However, owing to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is hampered favoring the ROS upsurge that culminates in oxidative stress [36]. The ROS readily attack and induce oxidative damage to various biomolecules including proteins, lipids, mitochondria, lipoproteins and DNA[37]. Oxidative stress affects many cellular functions by various mechanisms such as alteration in gene expression through activation of transcription factor NF-kB or induction of permeability transition in mitochondria with lethal consequences[37]. Our results revealed that the effect of exposure to Pb for 4 weeks, in rats induced nephrotoxicity evidenced by histopathological observation and biochemical parameters perturbations in kidney of rats. This treatment has a negative effect on renal lipid peroxidation as well as the renal antioxidant defense system.A significant increase in the LPO level following administration of the Pb observed in the present study. Since ROS are highly reactive and can oxidize cellular macromolecules (e.g. lipids, DNA, nucleic acid and proteins) which may lead to genetic alterations. Lipid peroxidation is linked with excessive generation of ROS, which may be contributed by exogenous or endogenous sources and is the most destructive process in the living cells has been implicated in causing a wide range of biological effects such as increase membrane rigidity, osmotic fragility, decreased cellular deformation, reduced erythrocyte survival, and membrane fluidity[38- 39]. Lipid peroxidation products, such as malondialdehyde and 4-hydroxy-2-nonenal (the most cytotoxic) cross link the membrane, damage the DNA and are mutagenic leading to functional changes[40]. Therefore, we used lipid peroxidation as a marker of oxidative stress and studied the effect of Pb administration on renal lipid peroxidation. Treatments of animals with Pb lead to the induction of lipid peroxidation, as monitored by measuring the rate of production of Thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde equivalents, reflecting the formation of activated species in rat kidney. Accumulation of lipid peroxide is believed to be a major contributor to the loss of cell function under oxidative stress conditions[40]. This further indicate that renal injury induced by Pb in present study is the result of oxidative stress that arise as a result of excessive generation of ROS, which have been reported to attack various biological molecules including lipids and causing lipid peroxidation.Results of the current study revealed that green tea extract (GT) reversed the elevation of lipid peroxidation. Hence, it is possible that the mechanism of green tea extract may be attributed to epicatechins (antioxidant present in green tea) that scavenge a wide range of free radicals including the most active hydroxyl radical, which may initiate lipid peroxidation. Therefore, it may decrease the concentration of lipid free radicals[41]. Moreover, it was reported previously that it chelates metal ions, especially iron and copper, which, in turn inhibit generation of hydroxyl radicals and degradation of lipid hydroperoxides[42].Nephrotoxicity could also be explained by the impaired antioxidant enzyme activities in the kidney of the rats. Indeed, the antioxidant enzymes SOD, GPx and CAT limit the effects of oxidant molecules in tissues and act in the defense against oxidative cell injury by means of their being free radical scavengers[43]. These enzymes work together to eliminate active oxygen species. In this respect, SOD accelerates the dismutation of H_2O_2, also termed as a primary defense, as it prevents further generation of free radicals whereas, CAT helps in the removal of H_2O_2 formed during the reaction catalyzed by SOD[43]. In the current study, our results indicated that Pb exposure inhibited SOD, CAT and GPx activities in kidney of rat. This depletion may be due to the decreased synthesis of enzymes or oxidative inactivation of enzyme protein. Our histopathological data substantiate kidney dysfunction. Indeed, the renal histoarchitecture of the Pb treated rats showed swelling in the endothelium of

glomerular tuft, swelling in the lining epithelium of tubules and inflammatory cells infiltration in between the degenerated tubules. Most of the biochemical alterations accompanied by histopathological changes were alleviated following GTE administration. This could be attributed to the antioxidant capacity of GTE that reduce the lipid peroxidation which in turn restore the integrity of the cell membrane and improve the disturbance in permeability. Since the oxidative damage as the central mechanism of metals toxicity occurs primarily through production of reactive oxygen species (ROS), including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules, eventually damaging membranes and other tissues[44; 45]. The use of antioxidants to counteract the formed ROS is the corner stone in alleviation of such hazards. So, the major nutraceutical compounds in green teas are tea catechins that have the most effective antioxidant activity. Tea catechins are an efficient free radical scavenger due to their one electron reduction potential[46-47]. In addition, tea contains minerals that function as co-factors in antioxidant enzymes: zinc, selenium and manganese. Polyphenols have additional mechanisms in which they reduce oxidation level besides direct role as antioxidants: (1) Binding of metal ions such as iron and copper and preventing their participation in oxidation reactions (leading to the formation of hydroxyl radical). (2) Prevention of redox sensitive transcription factors activation that amongst others things serve as mediators of inflammatory reactions. (3) Suppression of oxidation stimulants such as induced nitric oxide synthase (iNOS), cyclooxygenase 2 (COX- 2), lipoxygenase 2 (LOX-2) and xanthine oxidase. (4) Induction of antioxidant enzymes such as glutathione S - transferase and super oxide dismutase[35].

CONCLUSION

The results of present study show that lead acetate treatment caused oxidative damage, biochemical and histopathological alterations in the kidney of male rats. In contrast GT reduces oxidative damage by virtue of its antioxidant properties thus improving the structural integrity of cell membrane and eventually alleviates the histopathological changes as well as the biochemical perturbations. Based on our present observations, we propose that GT may provide a cushion for prolonged therapeutic option against toxins-induced nephrotoxicity without harmful side effects.

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