The Effects of Green Tea Extract on Liver and Kidney Functions in The Sprague–Dawley Male Rats Treated with Lead Nitrate

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Abstract

Green tea has become a subject of interest because of its beneficial effects on human health, health hazards from increased Pb exposure as a result of industrial and environmental pollution are recognized. In this study, the effect of green tea extract (GTE) on lead nitrate induced toxicity was conducted to investigate the biochemical and histopathological effects on liver and kidney in Sprague–Dawley rats. Four groups of rats were used in this study, 6 animals in each group. First group was given distilled water, second group was given green tea, third group was given lead and forth group was given Lead and GTE orally to the rats with drinking water for 8 weeks, blood sample were collected and then animals sacrificed for histological test. Liver enzymes ALT, AST and ALP, and creatinin , urea for the kidney in the serum, determinations indicated the protective effects of green tea extract. Histopathological studies of liver and kidney revealed that supplementation of green tea extract resulted in mild tissue damage, decrease the inflammation, an enhanced Immunological reaction and regenerative capacity of the cells.

Key words: Green tea extract, lead nitrate, liver, kidney, rats, histopathological changes.

الخلاصة

الشاي الأخضر مثير للاهتمام بسبب تأثيراته المفيدة في صحة الإنسان والرصاص الناتج من زيادة التلوث البيئي والصناعي بات خطرا على الصحة العامة، لذا في هذه الدراسة تم دراسة تأثير الشاي الأخضر في سمية نترات الرصاص لمعرفة التأثيرات البايوكيميائية والتأثيرات النسجية المرضية في الكبد والكلية في ذكور الجرذان البيض نوع Sprague–Dawley ،قسمت الحيوانات عشوائيا إلى أربع مجاميع في كل مجموعة ٦ حيوانات ، المجموعة الاولى سيطرة جرعت بالماء المقطر ،المجموعة الثانية جرعت بالشاي الأخضر المجموعة الثالثة جرعت بالرصاص،المجموعة الرابعة جرعت بالشاي الاخضر والرصاص مع ماء الشرب لمدة 8 اسابيع، ثم جمعت عينات الدم وقتلت الحيوانات لغرض الفحص النسيجي ، بينت دراسة إنزيمات الكبد ALT,AST,ALP واليوريا والكرياتين للكلية في مصل الدم فعالية مستخلص الشاي الاخضر في حماية وظائف الكبد والكلية من أضرار الرصاص.اما الدراسة النسجية المرضية للكبد والكلية أثبتت أن إعطاء الشاي الأخضر ساهمت في تقليل الأضرار النسيجية والالتهاب وتحفيز التفاعل المناعي وتحسين قابلية الخلايا على إعادة التركيب النسجي الطبيعي .

Introduction

Humans are exposed to lead: through deteriorating paint, household dust, bare soil, air, drinking water, food, ceramics, home remedies, hair dyes and other cosmetics. Lead exposure as a result of industrial and environmental pollution are recognized. It has been found to produce wide range of biochemical and physiological dysfunctions [1]. Lead has been associated with various forms of cancer, nephrotoxicity, central nervous system effects and cardiovascular diseases in humans [11]. Immediately after absorption, lead enters into the blood, which is then distributed particularly to the liver and kidneys, and is then stored in the bones as well as cause damage of liver and kidneys [2]. Liver, responsible for maintaining the body's metabolic homeostasis has been considered as the target organ for the toxic effects of Lead [3], followed by kidney [4].

Pathogenesis of lead toxicity is multifactorial, as lead causes oxidative stress by inducing the generation of reactive oxygen species (ROS) [6]. Herbal medicines derived from plant extracts are being increasingly utilized to treat wide variety of clinical diseases. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities [9]. The increasing interest in the health properties of tea extract and its main catechin polyphenols have led to a significant rise in scientific investigation for prevention and therapeutics in several diseases [19]. Green tea extract (GTE) treatment was able to reverse the impairment in liver and kidney functions and architecture and reduced the negative impact on serum lipid profile [20]. Green tea contain a number of components including catechins, saponins and flavonoids, and it has been demonstrated that catechins can reduce cellular oxidation [15]. Flavonoids are known to be effective in removing individual oxygen and free radicals from the lipids peroxidation step [16]. Nephrotoxicity results because kidney is the main route of elimination of lead [21]. The present study was conducted to investigate the biochemical and histopathological effects of Lead toxicity on liver, kidney of rats. Moreover, the chelating property of GTE to reduce Lead burden in rat tissues was detected.

Materials and Methods

Sixteen healthy male Sprague–Dawley rats (about 170–200 gram (g) body weight) were purchased from Animal House, College of medicine, Babylon University. All animals were conditioned at room temperature at a natural photoperiod for 2 weeks before experiment execution. A commercial balanced diet and tap water were provided. The duration of experiment was 4 weeks. The rats were randomly divided into 4 groups (4 rats each) as the following: (Control groups) one received distilled water as sole drinking source (Group I). Other one received Green tea (GTE group) (Group II). Group III (Lead-group) received 0.5% lead nitrate in distilled water (7). Group IV (GTE +Lead group) received mixture of lead nitrate and Green tea, as sole drinking source. The Green tea made, by soaking 15 gram of instant green tea powder in 1 L of boiling distilled water for 5min (28). The solution was filtered to make (1.5% GTE, W/V). This solution was provided to rats as their sole source of drinking water. The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment. The blood sample from each rat was collected from the heart in heparinized tubes. They were centrifuged at 5000 round per mint (rpm) for 10 mint (min) to obtain plasma samples for biochemical analyses [5].

Histopathological Examination

Specimens from liver and kidney tissues were taken from all rat groups after scarification. Specimens were collected and fixed in 10% neutral buffered formalin, dehydrated in ascending grades of ethanol alcohols, cleared in xylol, casting, blocking, cutting at 2–5-Mμ (micrometer) thickness and stained using the routine pathological technique [22].

Statistical Analysis

The results were expressed as mean ± standard error (SE).

Differences between groups were assessed by one-way analysis of variance (Bonferroni test) using the Prism version5 software package for Windows. The level of significance was accepted with P +0.05 [5].

Results

Liver Function Evaluation:-

The plasma levels of bioindices of liver functions in differentially treated groups are given. The liver enzymes, aspartate transaminase (AST), alanine transaminase (ALT), and alanine transaminase (ALP) I/U/L(international unit per liter) activities were significantly elevated in Lead-treated rats in comparison with controls. These enzymes were significantly reduced in Lead + GTE-treated rats comparing with Lead-treated rats, as in Figures (1), (2), and (3).

Kidney Function Evaluation

The serum analyzed concentration of creatinine and urea milligram per 100 ml (mg/100 ml)in the treated groups are shown in figures 4 and 5 ,significant elevated were observed for creatinine and urea concentration in the groups which treated with lead compared with control groups while the level of creatinine and urea are significantly lower in the groups which treated with green tea and lead compare with control groups.

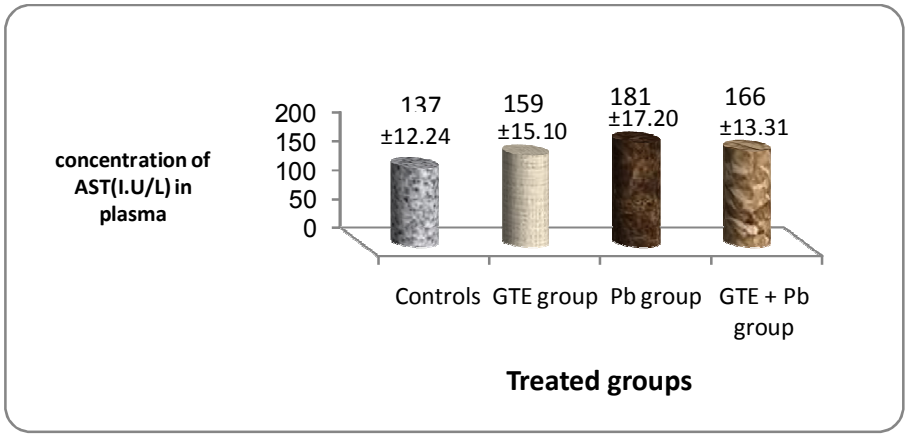

Figure 1: Concentration of (AST) aspartate transaminase liver enzyme indifferent treated group s:controls groups; controls &GTE (green tea) group; Lead(lead nitrate) group, GTE +Lead(green tea +lead nitrate) group.

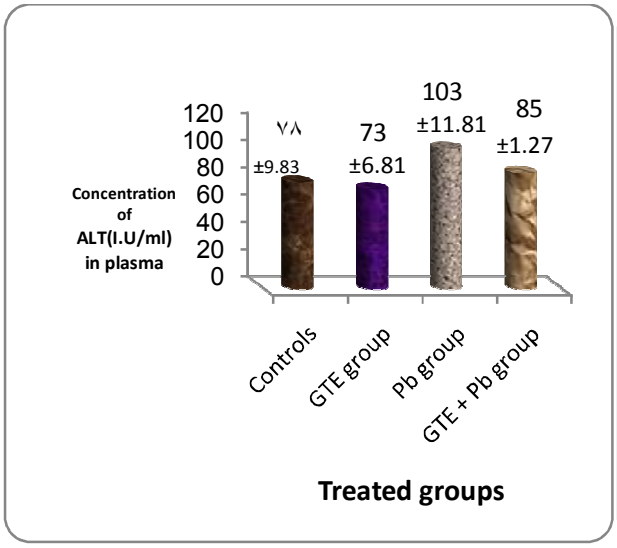

Figure 2: Concentration of (ALT) alanine transaminase liver enzyme in different treated groups :control groups ; GTE (green tea) group; Lead (lead acetate) group, GTE +Lead (green tea + lead nitrate) group.

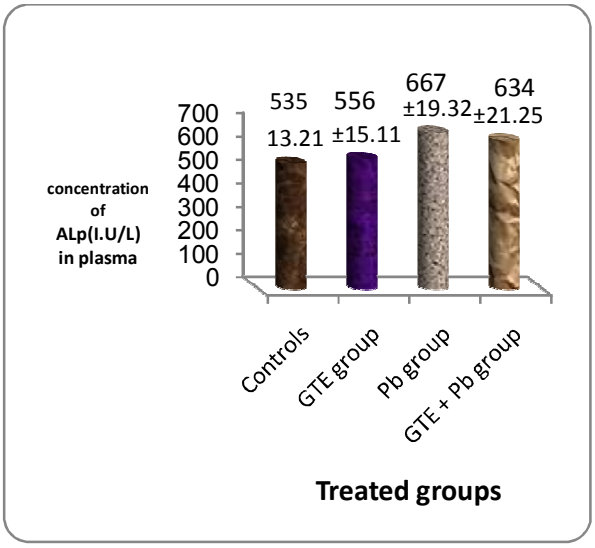

Figure 3: Concentration of (ALp) alanine transaminase liver enzyme in different treated groups :controls groups; controls and GTE (green tea) group; Lead (lead acetate) group, GTE + Lead (green tea +lead nitrate) group.

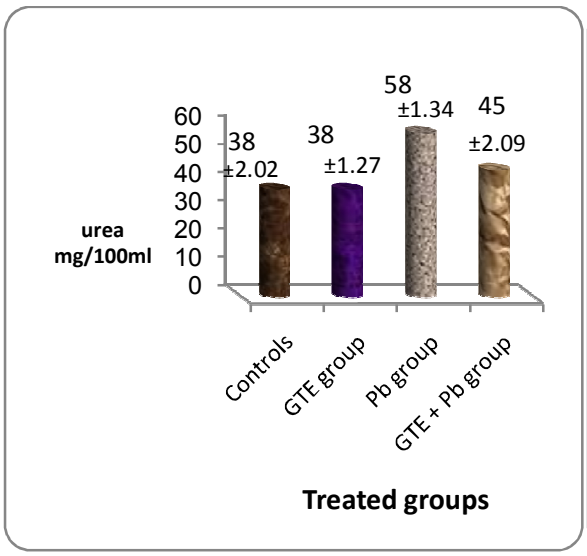

Figure 4: Concentration of urea in blood plasma in different treated groups: controls groups ; controls and GTE (green tea) group; Lead (lead acetate) group, GTE + Lead(green tea + lead nitrate) group.

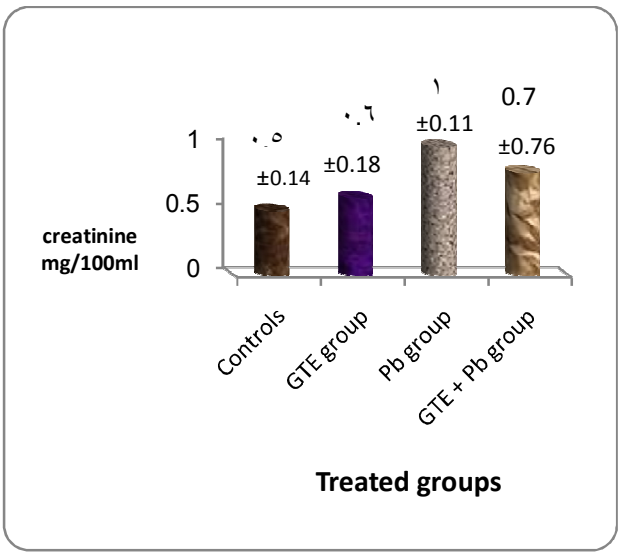

Figure 5: Concentration of creatinine in blood plasma in different treated groups: controls groups; controls and GTE (green tea) group; Lead (lead acetate) group, GTE + Lead (green tea + lead nitrate) group.

Histological examination

Macroscopic Changes in Liver:

The liver of the male albino rats, intoxicated with lead nitrate alone, showed congestion of central vein with distraction endothelial cells, diffuse necrosis, the necrotic cells replaced by mononuclear leucocytes, fatty change and hydropic degeneration of hepatocytes, hypertrophy of kupffer cells (Figures 2 and 3).

On the other hand, rats treated with Lead + GTE showed mild congestion of central vein and few distraction of endothelial cells, hydropic degeneration, pale and vacuolated nuclei (no necrosis and cells infiltration) binucleated hepatocytes (indication of proliferation), (Figure 4 and 5). No detectable macroscopic changes showed in control groups (Figure 1).

Macroscopic Changes in Kidney:

Kidney of male rat intoxicated with lead nitrate alone showed; accumulation of inflammatory cells as lymphatic nodules, hemocidrine precipitate, early renal tubules dilation, multinucleated gaint cell, hypertrophy of blood vessels, and hydropic necrosis diffuse type of coagulative necrosis,atrophy of glomeruli ,cast and cystic dilated of renal tubules ,diffuse hemosidrine (Figures 7 and 8).

On the other hand, rats treated with Lead + GTE showed; mild inflammatory cells, normal renal tubules and glomeruli with mild degree of necrosis, nearly normal kidney just hydropic and fatty change besides apoptotic necrotic cells (Figure 9). No detectable macroscopic changes showed in control groups (Figure 6).

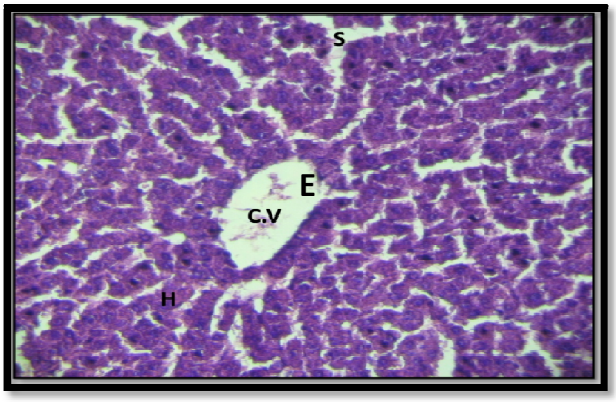

Figure 1: Normal Liver of male rat showing: liver lobule forms radiated ,a polygonal mass of liver plates that anastomose freely (H), limiting the space occupied by the sinusoids (S), (centrolobular) vein (C.V) endothelial cells (E) (H and E, X400).

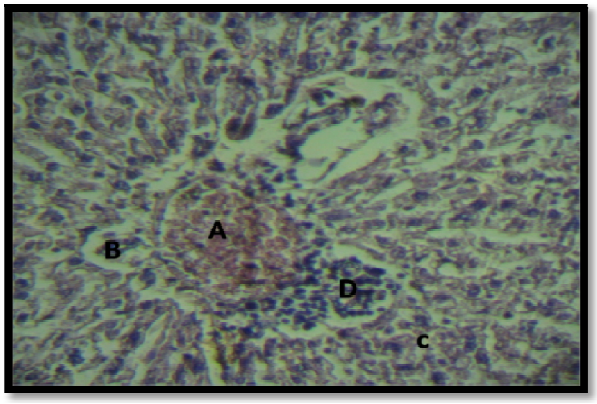

Figure 2: Liver of male rat intoxicated with lead acetate alone showing: congestion of central vein(A), distraction of endothelial cells (B), diffuse necrosis (C), the necrotic cells replaced by mononuclear leucocytes (D) (H and E,X200).

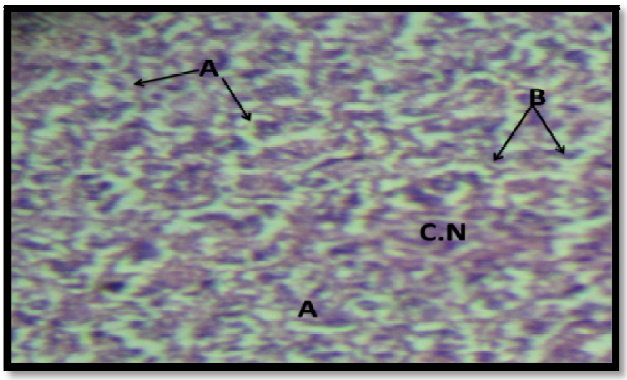

Figure 3: Liver of male rat intoxicated with lead acetate alone showing: fatty change and hydropic degeneration of hepatocytes (A), hypertrophy of kupffer cells (B), and coagulative necrosis (C.N) (H & E, X400).

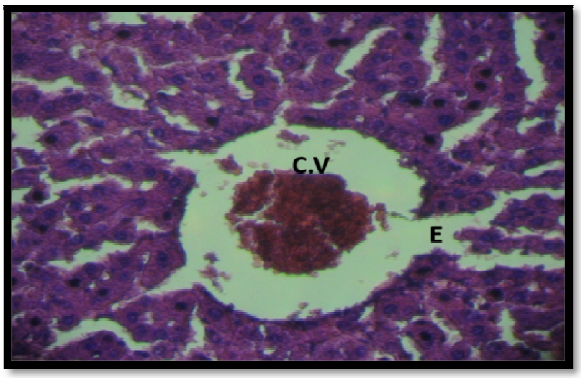

Figure 4: Liver of male rat treated with GTE + Lead showing: mild congestion of central vein (C.V) and little distraction of endothelial cells (E). (H and E, X400).

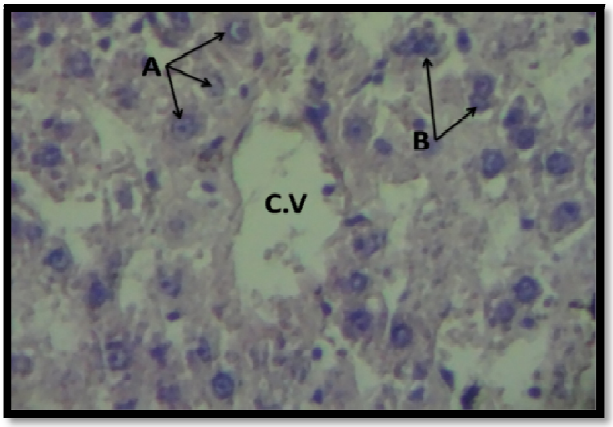

Figure 5: Liver of male rat treated with GTE + Lead showing: (A) hydropic degeneration, pale and vacuolated nuclei (no necrosis and cells infiltration), (B) binucleated hepatocytes (indication of proliferation) with no congestion of central vein(C.V) H & E, X400.

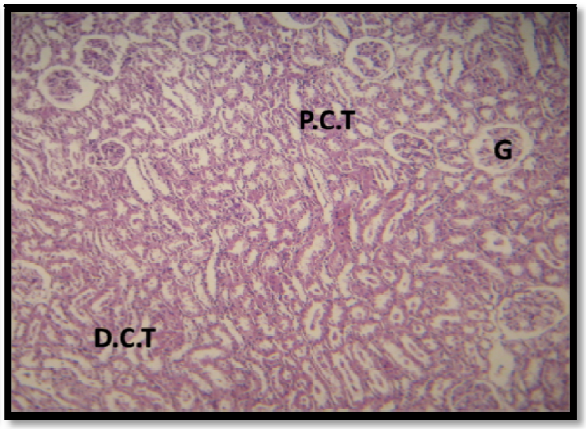

Figure 6: Tissue section of control kidney of male rat (cortex region) contain glomeruli (G) with proximal convoluted tubule (P.C.T) and distal convoluted tubules (D.C.T) (H & E, X100).

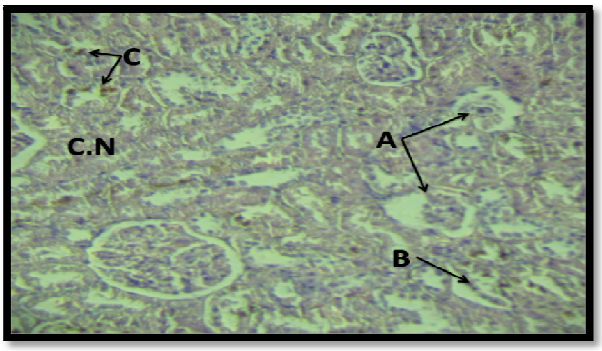

Figure 7: Kidney of male rat intoxicated with lead acetate alone showing; accumulation of inflammatory cells as lymphatic nodules (A), hemocidrine precipitate(B), early renal tubules dilation (C) multinucleated giant cell(D) ,hypertrophy of blood vessels (H.B) and hydropic necrosis (H.N) (H & E, X200).

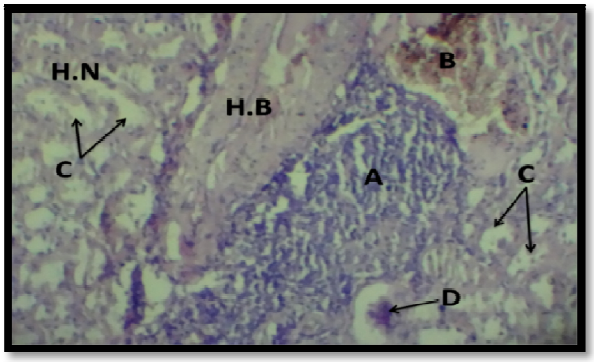

Figure 8: Kidney of male rat treated with lead showing: diffuse type of coagulative necrosis (C.N), atrophy of glomeruli (A), cast and cystic dilated of renal tubules (B), diffuse hemosidrine (C) (H & E, X200)

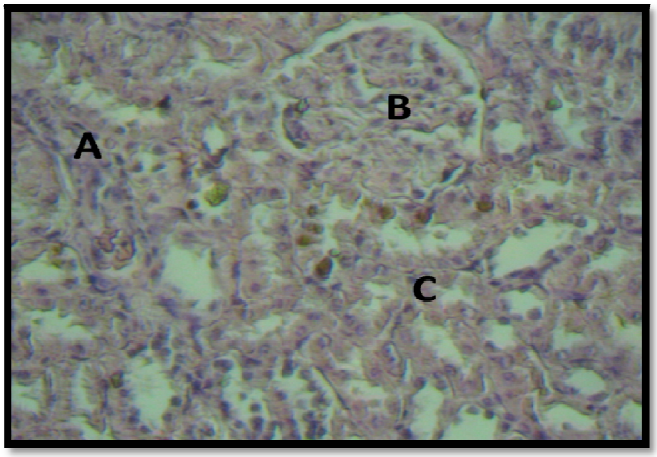

Figure 9: Kidney of male rat treated with GTE+ Lead showing: mild inflammatory cells (A), normal renal tubules and glomeruli with mild degree of necrosis (B), nearly normal kidney just hydropic and fatty change besides apoptotic necrotic cells (C) (H & E, 400).

Discussion

Green tea extract have led to a significant rise in scientific investigation for prevention and therapeutics in several diseases [10]. Lead is a ubiquitously found environmental and industrial pollutant that has been detected in nearly all phases of environment and biological system [5].

In this study the effects of Pb burden may be from reactive oxygen species (ROS), Lead binds to form a stable complex with mitochondria in the liver and kidneys, mitochondria source and target of (ROS) [25]. Chronic ingestion of lead leads to a significant decrease in liver glutathione syntheses (GSH) levels [26]. Other studies have indicated that an increase in reactive oxygen species (ROS) in the liver ,which play an important role in inducing apoptosis under physiological and pathological conditions and leads to a significant increase in DNA damage and apoptosis [25]. Lead exposure was found to attenuate antioxidant potential of liver, which was however augmented when supplemented with green tea extract [5]. The liver's supply of antioxidants, resulting in liver injury, rats were chronically intoxicated with alcohol for 4 weeks, green tea prevented damage to their livers, free radical production and liver injury was so greatly reduced [13].

Exposure to lead alone reduced the antioxidant capacity of the liver, this was reversed to some degree by ingestion of green tea extract, the levels of liver enzymes ALT, AST and ALP and serum protein determinations showed that green tea has a protective effect on liver function in the face of lead [14].

The liver enzyme assays indicated that Lead nitrate ingestion induced a significant elevation of plasma ALT, AST and ALP levels at 4 weeks of Lead treatment. These enzymes are regarded as markers of liver injury. In addition, ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. Moreover, elevated ALP activity, which was used as a marker of liver adaptation to damaging factors, has been reported frequently in Lead-exposed animals [27].

Plasma Creatinine and blood urea nitrogen levels were found to be reduced by the administration of green tea polyphenols in alloxan-induced diabetic rats [17]. Diabetic

rats treated with green tea showed a significant reduction in creatinine level, but blood urea statistically did not change (18). Reducing the antioxidant defense system of cells interfering with some essential metals [12]. Green tea extract (GTE) due to its content of catechins reveals strong antioxidative activity, which is manifested by its ability to inhibit free radical generation, scavenge free radicals and chelate transition metal ions that catalyze free radical reactions [19].

Kidneys are particularly susceptible to the effect of toxic agents like lead that can cause renal damage and even renal failure Its persistence in human and animal tissues has quite often been associated with considerable health risks [23]. The findings of the present study might suggest that lead inclusion bodies may represent accumulation of both extra cellular and intracellular materials as a result of cellular metabolic disturbance and alterations in nuclear membrane permeability [24]. Histopathological studies of liver revealed that supplementation of green tea extract resulted in mild degeneration and congestion of the blood vessels and an enhanced regenerative capacity.

In addition congestion, mononuclear inflammatory cells infiltration and apoptotic necrotic cells and sever inflammation (nephritis) has been noticed in some other cases. Ingestion of Lead is one of the primary causes of its hepatotoxic effects [8]. The molecular understanding of Lead effects on hepatic drug metabolizing enzymes, cholesterol metabolism, oxidative stress, and hepatic hyperplasia suggest a potential role for Lead in damaging extrahepatic systems, including the cardiovascular system. While, group treated with lead nitrate and green tea extract showed mild degeneration of the hepatocytes without necrosis and binucleated cells that represent good sign of regeneration.

The reduction of Lead concentrations in the studied tissues of the rats treated with GTE combined with Lead may be due to its chelating property. We suggest one possibility that GTE complexes with Lead ion that decreases its lipophilicity, and thus its gastrointestinal absorption. The chelating agents form an insoluble complex with Lead to remove it from Lead-burdened tissues [4]. If GTE were able to chelate toxic lead ions in the body and allow them to be flushed out by the kidneys then that health effect could be demonstrated in a relatively straightforward way [7].

Conclusion

The beneficial effect of GTE in the improving and reduction of Pb burden in the tissue organs(the sever inflammation become mild inflammation), thus potentially reducing Pb toxicity and tissue damage this fact we reach as that pb +GTE cause Immunological reaction. This study provides Pb. GTE reduced tissue Pb burden, the oral supplementation of GTE to Pb-intoxicated rats augmented the antioxidant potential by affecting the antioxidant enzyme activities beside reducing the tissue injury of liver and kidney cells.

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