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Original Article

Effect of Ginkgo Biloba Extract (EGb 761) on Lead-induced Nephrotoxicity in Wistar Albino Rats.

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Abstract

Background: Lead is a well-known omnipresent environmental toxin that is harmful to almost any system of the body. Kidneys being the major site of excretion, are very susceptible to its toxic effects. The damage usually involves the proximal convoluted tubules (PCTs) and glomeruli, mainly because of the oxidative stress induced by lead. Ginkgo biloba extract (EGb 761), being known to have natural anti-oxidative properties, was used in this study to see whether it ameliorates the lead-induced nephrotoxic effects of not.

Methodology: Twenty male Wistar albino rats were divided into four equal groups. Group A was kept as control and given nothing, Group B was given 8 mg/kg body weight lead acetate intraperitoneally, Group C was given EGb 761 100 mg/kg body weight orally and Group D received both lead acetate as well as EGb 761 simultaneously in the above-mentioned doses. The treatment continued daily for 42 days, after which the rats were sacrificed and histomorphological and immunohistochemical parameters were studied from the extracted kidneys.

Results: Lead-induced histomorphological changes, including widened PCTs, contracted glomeruli, decreased and pyknotic nuclei and widened urinary spaces, were all reverted to near control in Group D animals. EGb 761 itself did not produce changes in Group C rats except that expression of laminin was marginally decreased. Overall, the laminin expression was not much affected in any of the groups.

Conclusion: The present study suggests an ameliorating effect of EGb 761 on lead-induced nephrotoxicity in albino rats. At the same time other major paralogues of laminin need to be incorporated into the study to see the specific paralogue which is affected in lead-induced nephrotoxicity.

Keywords

Lead Acetate, Ginkgo Biloba, Nephrotoxicity, Laminin.

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Introduction

Lead is a pervasive toxic metal that is one of the major environmental pollutants. Globally, in the last five millennia, about 300 metric tons of lead has been added to the environment, mostly during the last 500 years[1]. In the U.S domestic market only, about 1.6 metric tons of lead is consumed annually[2]. The lead is mainly incorporated in animals, including humans, through gastrointestinal and pulmonary tracts[3]. As a toxin, it can alter the anatomy and physiology of several systems of the body. Kidneys being the major organ of excretion, are the most frequent target of lead toxicity, and the major changes occur in the PCTs of the kidneys[4]. One of the most important factors in producing lead toxicity is its competitive behavior with calcium, which is an important constituent of a battery of enzymes[5]. lead-induced reduction in alkaline phosphatase, acid phosphatase and different types of ATPases in the PCTs of kidneys results in decreased absorptive and secretory functions of PCTs, adding to the deterioration in the histomorphometric parameters[6].

Oxidative stress is one of the major mechanisms involved in lead toxicity. Accumulation of reactive oxygen species and other free radicals as a result of an imbalance between prooxidants and antioxidants leads to increased lipid peroxidation and structural damage to proteins, carbohydrates and DNA of cells[7-9]. The PCTs of the kidneys are particularly vulnerable to damage by the reactive free radicals liberated as a result of lead-induced toxicity[10].

Antioxidants are stable molecules that can neutralize free radicals by donating electrons[11]. Many of the phytochemicals are rich in antioxidants and hold free radical scavenging properties[12]. Use of antioxidants in conditions where oxidative stress is the main mechanism of tissue injury can be beneficial in reverting or ameliorating the anatomical and physiological disturbances in the target tissues. Lead-induced nephrotoxicity has been previously shown to be ameliorated by berberis vulgaris, garlic extract, black grapes and costus[13-16]. EGb 761 is a standardized extract prepared from the leaves of the only living species in the plant division Ginkgophyta[17]. It contains about 24 % flavones (kaempferol, quercetin and isorhamnetin) and 6 % terpene lactones (ginkgolides and bilobalides), and has been studied extensively for its antioxidant properties[18].

The positive effect of EGb 761 has been shown in cases of dementia/ cognitive impairment, cardiovascular disease, psychiatric disorders, sexual dysfunction, vertigo, tinnitus, vitiligo, macular degeneration, glaucoma and altitude sickness[19]. There have been promising results in the experimental use of EGb 761 in gentamycin-induced nephrotoxicity[20], renal ischemia-reperfusion injury[21] and endotoxin-induced oxidative renal tissue damage[22].

Previously, in an endpoint study, we have shown the ameliorating impact of EGb 761 on lead-induced nephrotoxicity in albino rats in relation to their body weight, absolute and relative kidney weight, number and diameter PCTs, and number and diameter of nuclei in the PCTs[23,24]. The present investigation aims to explore the time course changes in body weight of lead-induced nephrotoxic model of albino rats along with architectural changes in the basement membrane, and amelioration of these changes by EGb 761.

Methodology

Experimental Setup

Twenty male Wistar albino rats weighing 250-300 gm were obtained from the Department of Laboratory Animal Sciences, Dow University of Health Sciences were recruited in the study and divided into four groups, A, B, C and D. The rats were placed under observation for a week for acclimatization. Group A animals were kept as control. Group B animals were given 8 mg/kg body weight lead acetate intraperitoneally on a daily basis for 42 days. Group C and D animals were given EGb 761 (100 mg/kg body weight orally) and EGb 761+ lead acetate (100 mg/kg body weight orally+ 8 mg/kg body weight intraperitoneal) respectively on a daily basis for 42 days.

Histological Investigation

At the end of the experiment, all the rats were euthanized using a carbon dioxide gas chamber. The kidneys were retrieved and fixed in 10 % neutral buffered formalin. The tissues were then processed in the automatic tissue processor (Excelsior AS) overnight. Subsequently, the tissues were embedded in paraffin blocks, and 4 µm sections were taken by the microtome. Finally, the sections were stained with hematoxylin and eosin (H&E), Masson trichrome and periodic acid-Schiff (PAS). The slides were photographed using a digital camera microscope (Optica B3), and computerized morphometry was performed using ImageJ software. The number and diameter of nuclei were observed in H&E stained sections, the diameter of PCTs and urinary space was measured in PAS stained sections, and fibrosis was measured in Masson's trichrome stained sections.

Quantifying laminin expression

To monitor the expression of laminin, sections were deparaffinized and rehydrated, followed by antigen retrieval by microwaving in sodium citrate buffer (pH 6.0). This was followed by treatment with 3 % hydrogen peroxide to inhibit endogenous peroxidase activity and then blocked with ≤ 10 EU/mg animal serum. The samples were then probed with anti-rat primary laminin polyclonal antibody (1:75) and then with goat anti-rabbit IgG secondary antibody for 10 minutes. Finally, the sections were incubated with Diaminobenzidine (DAB), counterstained with H&E and mounted in Dibutylphthalate polystyrene xylene (DPX). Expression of laminin was measured with Immunohistochemistry (IHC) profiler plug-in incorporated in ImageJ software[25].

Statistical Analysis

GraphPad Prism version 5.01 was used for data analysis. Kolmogorov-Smirnov test was used to check the nature of the distribution of data. Comparison between different groups was made using Student's t-test for normally distributed data, whereas the Wilcoxon-Mann-Whitney test was employed for skewed data. Statistical significance was kept at a $p \leq 0.05$.

Results

Time course treatment showed a significant drop in body weight in group B (lead-treated) and group D (lead +EGb 761-treated) rats compared to group A (control) and group C (EGb 761-treated). Moreover, the weight of group A (control) and group C (EGb 761-only treated rats) tend to increase comparably (Figure 1). This shows that there was no apparent toxicity caused by EGb 761, and holistically, EGb 761 did not show any amelioration in relation to the lead-induced decrease in body weight.

Different anatomical parameters were observed by histological examination (Figure 2). Exposure of lead significantly increased the tubular diameter ($p<0.0001$) and urinary space ($p=0.0009$) in rats. Conversely, nuclear count ($p=0.0003$) and diameter ($p=0.0014$) decreased in the lead-exposed animals compared with control rats. A marginal decrease in the tubular diameter ($p=0.0187$) and urinary space ($p=0.0216$) was observed in EGb 761 treated rats compared to the control group. However, no significant difference was found in the nuclear count and nuclear diameter between the two groups. Treatment of EGb 761 along with lead (group D) showed restoration of tubular diameter, urinary space, nuclear count and diameter, as the difference in these attributes were found statistically significant with the lead-treated animals (group B) but insignificant with the control group (group A). Tubular diameter showed a negative ($p=0.0036$) and positive ($p<0.0001$) correlation with the nuclear count and urinary space, respectively. This points out that the trends examined in the study are relatable (Figure 3). Expression of laminin protein as a marker for basement membrane did not reveal any significant difference, suggesting lead may not have disturbed the basement membrane or at least not via its one of the main structural components, laminin protein (Figure 4).

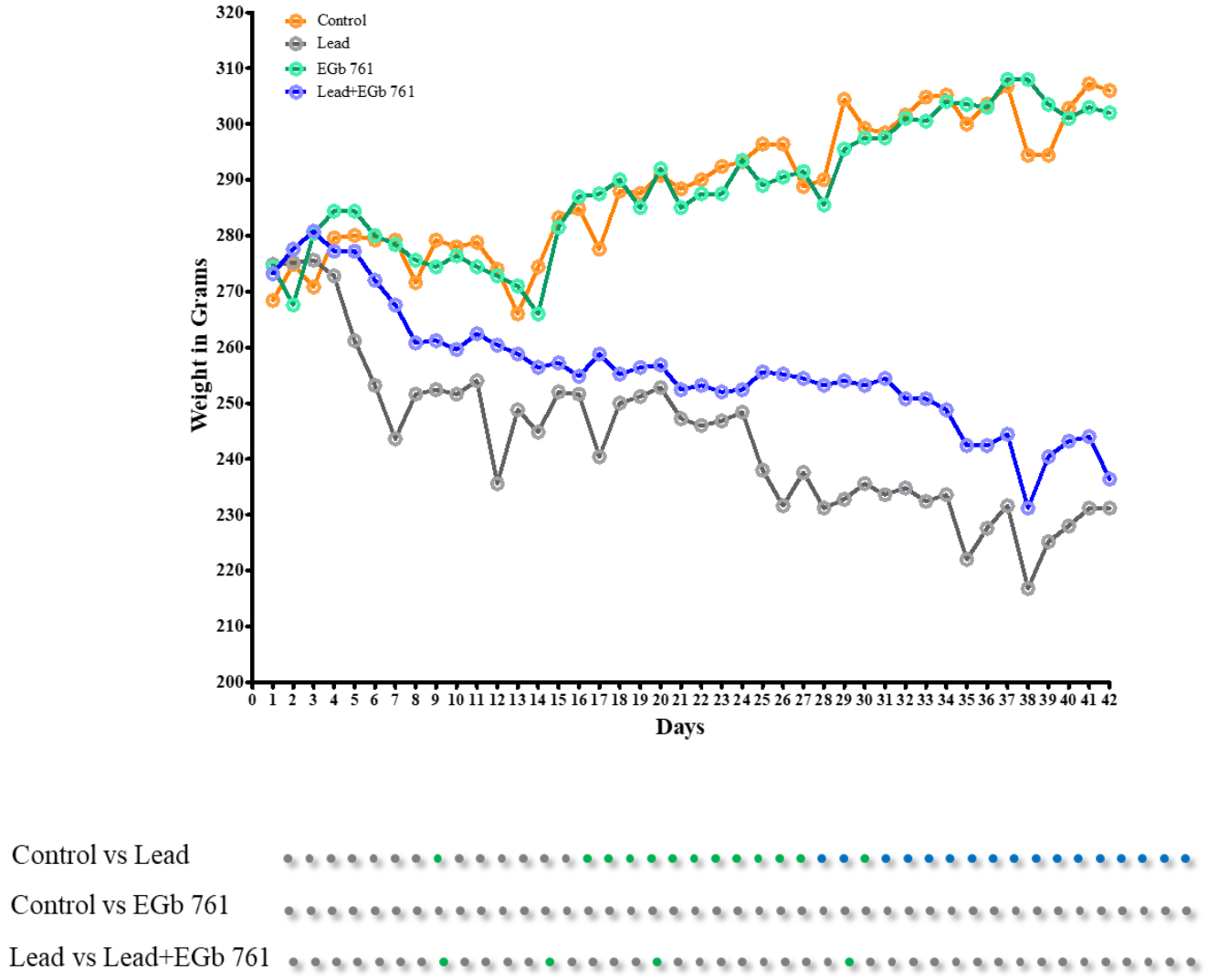

Figure 1: Body weight Distribution.

The graphs showing mean variations in the body weight of all groups. The data points are colored differently for each group (see inset). The moderate and high statistical significance in the difference of the body weight between different groups is denoted by green and blue filled circles respectively at the corresponding day.

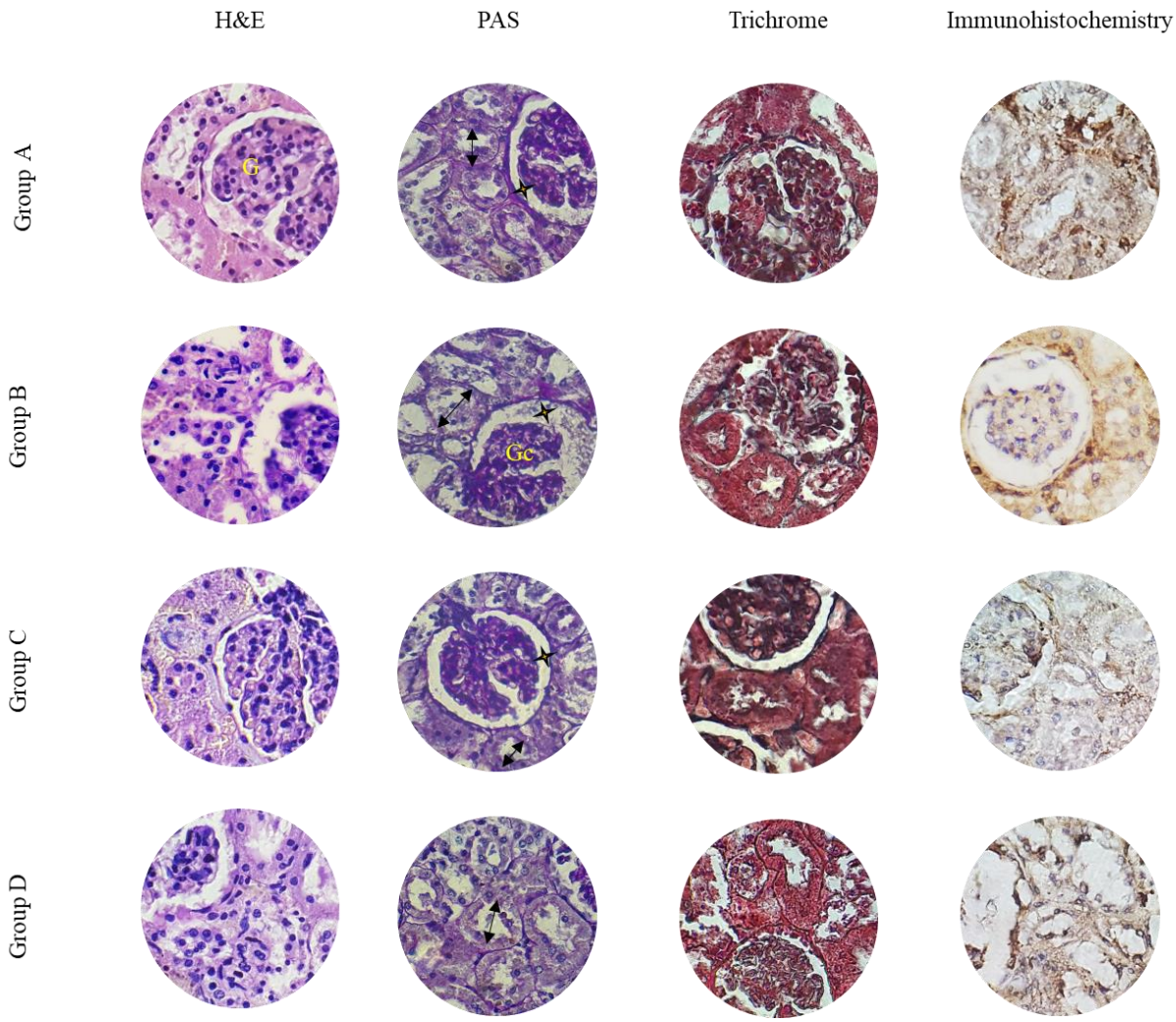

Figure 2: Microanatomy of Kidney.

Histomicrographs of the four experimental groups stained by H&E (first column), PAS (second column), trichrome (third column) and probed with laminin antibodies (fourth column). Tubular diameter and urinary space are marked with double-headed arrow and asterisk respectively. The expression of laminin is indicated by brown coloration. All micrographs were taken at 1600x.

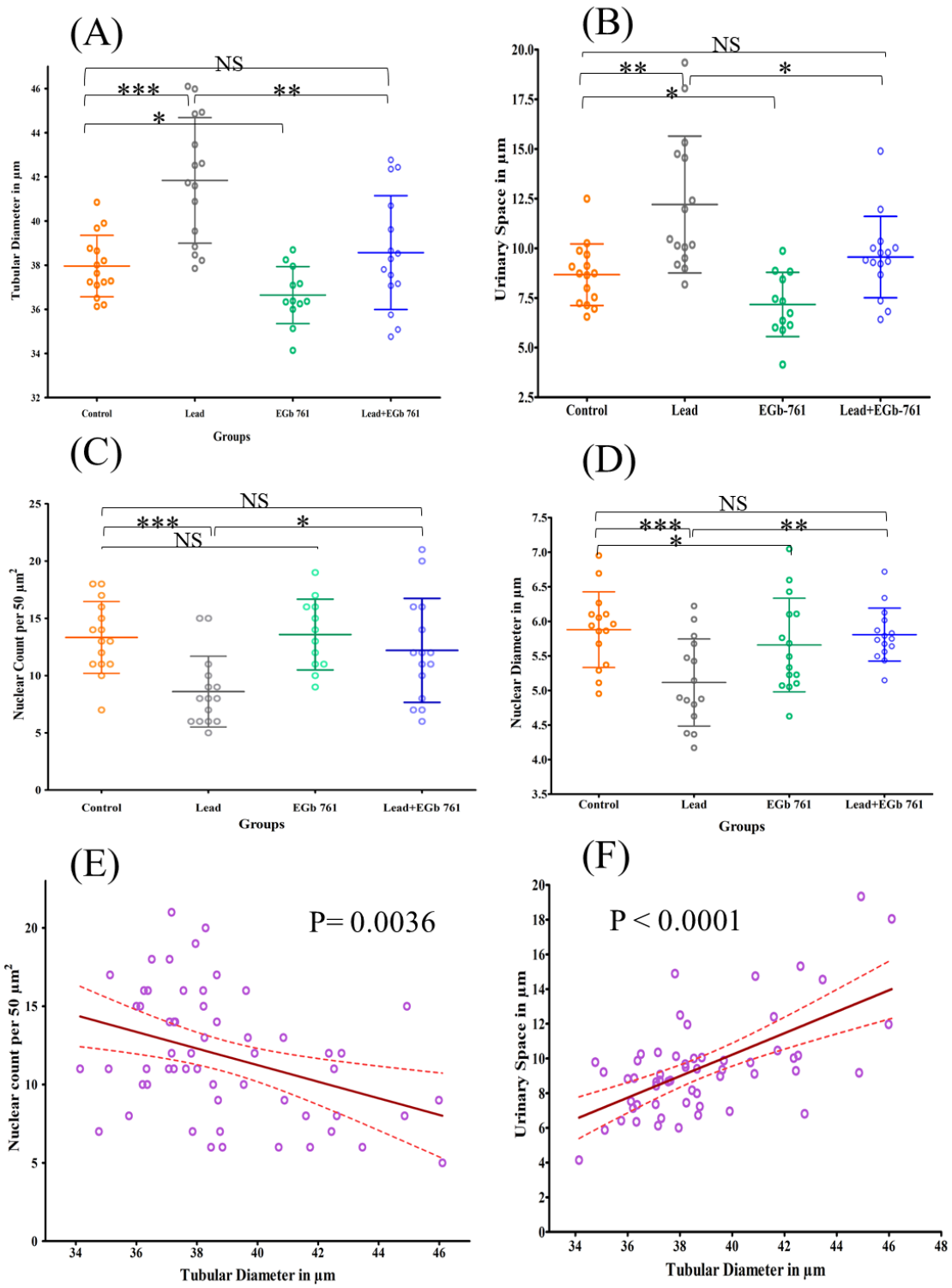

Figure 3: Anatomical Changes in Kidney.

Graphs show comparison of (A) tubular diameter (B) urinary space (C) nuclear count (D) nuclear diameter, correlation between tubular diameter and (E) nuclear count and (F) urinary space. The large and small horizontal lines in A-D, showing mean and standard deviation values with statistical significance between groups are also marked. In E-F, trend line of regression and confidence interval (95 %) band are indicated by solid and dotted lines, respectively.

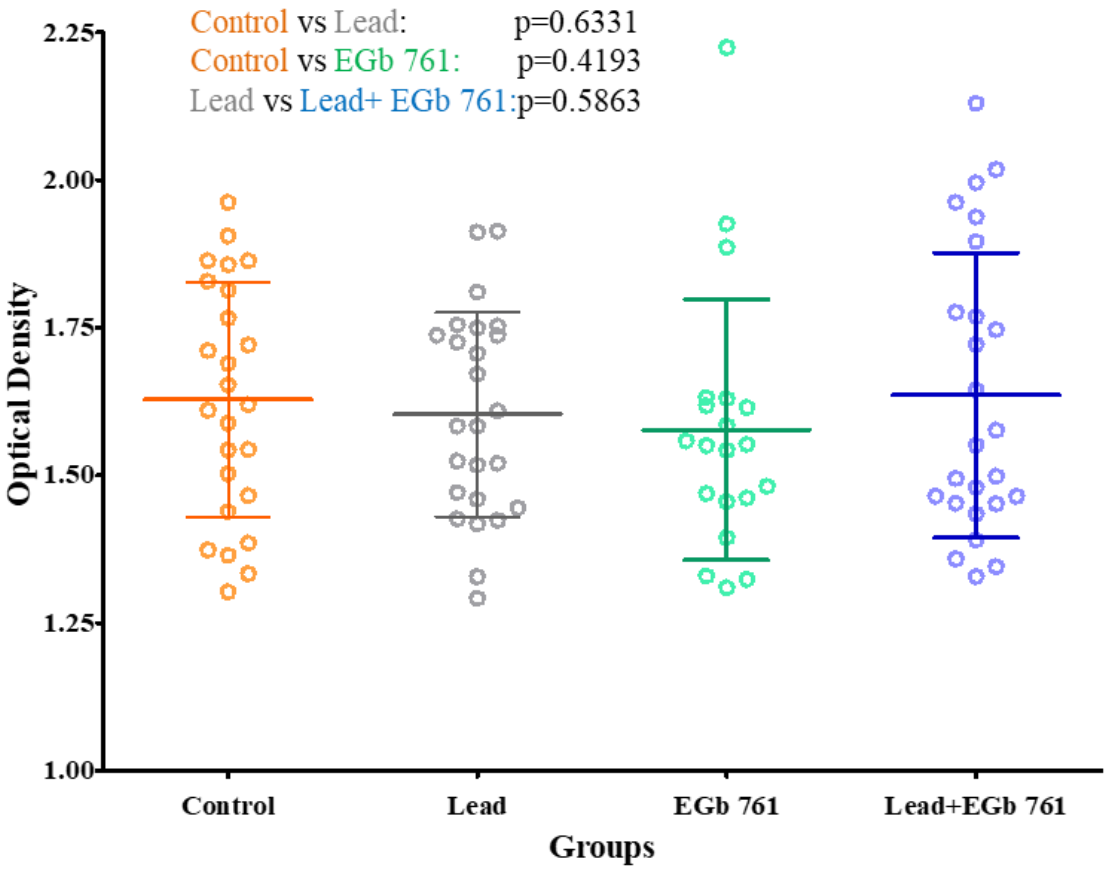

Figure 4: Laminin Expression.

Optical density representing the expression of laminin protein showing insignificant difference between all four experimental groups. The means and standard deviations were represented by large and small horizontal bars, respectively.

Discussion

Nephrotoxicity is a common sequel to exposure to a lot of chemicals, including therapeutic as well as environmental substances. Lead is one of such toxic materials that act as environmental pollutants, and kidneys being the major route of lead excretion, can adversely be affected by lead exposure[26]. Nephrotoxic effects of lead have been widely studied both in animals as well as human beings[27-31]. In our previous study, we have demonstrated a drop in body weight of rats at the endpoint of the experiment after lead exposure[23]. Consistently in the present investigation, the gradual drop in body weight due to lead exposure is also in line with studies undertaken earlier[32,33]. this may be due to anorexia in the animals as a result of heavy metal intoxication. In addition, dysregulation of different enzymes due to the presence of lead may also alter animal metabolism and, in turn, adversely affect the weight. More precisely, the replacement of calcium with lead has been demonstrated in the enzymes, which may affect overall body metabolism[34].

Oxidative stress is suggested to be a major factor rendering histopathological and biochemical changes due to lead toxicity[8,35,36]. The use of antioxidants usually reverts the changes produced by oxidative stress. Surprisingly, in the present study, EGb 761 failed to effectively prevent weight loss in lead-treated rats. It is possible that EGb 761

may not be effective against the lead-induced loss of body weight; rather, there are studies in which EGb 761 has been used as an anti-obesity agent by virtue of its effect of enhanced insulin sensitivity[37]. However, it can also be plausibly conceived that the effect of EGb 761 is concentration dependant, and its manifestation is largely dependant on the exposure concentration of lead and treatment concentration of EGb 761. To further verify this, another set of experiments is warranted.

An increase in the diameter of PCTs is believed to be a function of injury to the cells, the most probable mechanism being the oxidative stress induced by lead. Distortion and damage to the brush border are a prominent cause of the impression of dilated tubules. The current study is consistent with the findings of Muhammad and others who demonstrated dilated PCTs along with a mixture of swollen and atrophied glomeruli in lead-induced nephrotoxicity, which was ameliorated by quercetin, a flavonoid found in EGb 761[38]. Increase in urinary space in the Group B rats is because of the shrunken and atrophic glomeruli. In Group D rats, there was overall less shrinkage of the glomeruli and less widening of the urinary spaces as compared to the Group B rats. Similar observations were made by other investigators who found that widened urinary spaces in lead-induced nephrotoxicity models were reverted back to near normal when treated with curcumin and coriander[36,39]. The possible reason for the beneficial effects on all the histologic parameters in Group B rats was the anti-oxidative effect of these natural substances.

We observed marginal downregulation of laminin in the kidneys of lead treated rats, which is in agreement with another research, in which oral administration of lead showed decreased expression of laminin in kidney lysate of the rats as determined with western blot[40]. Non-significant changes in the expression of laminin in our study maybe because of the fact that different paralogues of laminin are expressed in different organs. Studying the expression of all major paralogues of laminin may throw further light on the role of lead on the specific paralogue of laminin in kidneys. A non-significant decrease in laminin expression in Group C rats may reflect the protective effect of EGb 761 against developing fibrosis and glomerulosclerosis as suggest by other researchers[41, 42].

Conclusion

Lead acetate causes changes in the kidneys of albino rats both at the macro as well as micro levels. The microanatomical changes can partially be ameliorated by the concomitant use of EGb 761. However, more benefits may be achieved if EGb 761 administration is supplemented with chelation therapy. Moreover, quantifying the oxidative stress by immunohistochemistry as well as ultra-structural studying of the tissues is also needed further to enhance the scope and translational application of the research.

Conflicts of Interest

The authors declare no competing interest.

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