Original Article

Protective Effect of Heat-Treated Cucumber (*Cucumis sativus* L.) Juice Against Lead-Induced Detoxification in Rat Model

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ABSTRACT

Background: In recent years, the development of efficient green chemistry approaches for detoxification of heavy metal such as lead (Pb) poisoning has become a major focus of researchers. **Objectives:** This study was aimed to evaluate the effectiveness of heat-treated cucumber juice on the protection of Pb-induced acute liver and kidney damages. **Methods:** Initially, during detoxification of lead, lead acetate (200 ppm dissolved in distilled water) was given to rats in drinking water for 5 weeks. Cucumber juice was orally administrated following three concentrations (1, 10 and 100 mg/kg) once in a day for 5 weeks. Further, the effect of heat-treated cucumber juice was evaluated on body weight, food intake, lead contents of rat tissues, and histopatholgical analysis of liver and kidney of test animals. **Results:** As a result, all treatments of cucumber juice exhibited a significantly higher protective effect on body weight, food intake, lead contents of tissues, count of red blood cell (RBC), and reticulocytes, as compared with Pb-control. Moreover, histology, and histomorphometry analysis of treatment tissue samples of liver and kidney also confirmed protecting effect of cucumber juice by showing normal histology and histomorphy when compared with Pb-control. **Conclusion:** These findings suggested that heat-treated cucumber juice has a significant protective effect on Pb-induced acute liver and kidney damages in experimental rats.

Key words: *Cucumis sativus*, Cucumber juice, Lead-detoxification, RBC, Histology, Histomorphometry.

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrate, protein and fat metabolism, detoxification and secretion of bile. Therefore, the maintenance of healthy liver is vital to overall health and well-being.[1] Unfortunately, the liver is often abused by environmental toxins, poor eating habits and over the counter drug use, which can damage and weaken the liver and eventually leading to hepatitis, cirrhosis and liver disease. Environmental chemistry is an area of increasing interest both to chemists and to the general public. Nowadays, more and more people consider that the magnitude of the pollution problem in our soils and water calls for immediate action.[2] Among toxic substances reaching hazardous levels are heavy metals, including mercury, lead, chromium, arsenic, zinc, cadmium, uranium, selenium, silver, gold and nickel. The danger of heavy metals is aggravated by their almost indefinite persistence in the environment due to their immutable nature.[2] Moreover, a heavy metal pollution of the environment is of major ecological concern due to its impact on human and animal health through the food chain, and its high persistence in the environment.[3] Lead is the most abundant toxic metal in the environment with no beneficial biologi-

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cal roles. Lead (Pb) as an environmental and occupational toxicant has been known to damage vital organs and suppresses cellular processes.[4] Lead is dispersed throughout the environment such as ambient air, many foods, drinking water and dust.[5] The major environmental sources of metallic lead and its salts are paint, auto exhaust, and contaminated foods and water.[6] Lead has many undesired effects, including neurological[6], growth retardation[7], anemia[8], renal[9], hepatic[10] and reproductive dysfunctions.[11]

These anthropogenic activities and vehicular emissions contribute to the entry of toxic metals to humans and other animal's food chains.[12] Chronic exposure to these sulfhydryl reactive metals through various routes results in their higher accumulation in tissues, bones, hair and blood, and monitoring of these toxic metals in biological materials essentially indicates the status of environmental pollution.[13] In addition, lead can interfere with exchangeability or distribution of calcium, and substitute it in physiological processes.[14] Hence, conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis.[1]

Plants and herbs have traditionally been used by herbalists and indigenous healers for the prevention and treatment of several diseases associated with liver injury.[15] Since cucumber (*Cucumis sativus* L.) is easy to cultivate and commonly available world-wide, this study focused on the selection of this green vegetable to evaluate its protective effect against Pb intoxication associated with live injury.[16] In the ancient Korean system of medicine, a number of plants and herbs have been indicated for detoxification of various poisonings. However, cucumber has gained plenty of research subjects being a processed foodstuff such as pickles, whereas, no scientific report has been published so far on cucumber as a functional food supplement. In addition, fresh cucumber is nutritiously a very good source of vitamin C, calcium, potassium, and also provides some dietary fiber, vitamin A, vitamin B6, thiamin, folate, pantothenic acid, magnesium, phosphorus, copper, and manganese, which may further support its role as a hepatoprotective agent.

Therefore, in this study, heat-treated cucumber juice was assessed to evaluate its protective effect on lead (Pb)-induced acute liver and kidney damage in experimental rats.

MATERIALS AND METHODS

Sample preparation

Cucumbers (*Cucumis sativus* L.) were purchased from an agricultural market in Gun-Wi, Gyeongbuk, Republic of Korea, and stored at -20°C until further processing. After thawing for 2-3 h at 4°C, the cucumbers were subjected to crush and filtered with Whatman No. 2 filter paper resulting to gain raw cucumber juice. Further, this cucumber juice was subjected to heat treatment in water bath for 40 min at 80°C, and then heat-treated cucumber juice was prepared by re-filtration, and freeze-drying and stocked at -20°C.

Animals

Male Sprague–Dawley rats used in this study were obtained from Orient Co. Ltd., Republic of Korea. All rats were given with *ad libitum* access to standard laboratory chow and tap water, and were kept under standard conditions (temperature; 24 ± 1°C, relative humidity; 55 ± 3% and 12 h light/dark cycle). All rats were allowed to acclimatize for 1 week prior to experimentation.

Acute toxicity assay

The acute toxicity test was performed on the experimental rats using the oral route. Heat treated cucumber juice was administered at various doses, ranging from (5–500 mg/kg), to different groups of rats. The animals were observed continuously for 1 h and then at half-hourly intervals for 4 h on the first day for clinical signs and symptoms of toxicity and further up to 72 h followed by 14 days for any mortality.

Detoxification of lead (Pb)

Male albino rats of Sprague–Dawley strain (body weight, 230 ± 20 g) were used for this study. The amount of fodder consumed by rats was monitored daily. Lead acetate (200 ppm dissolved in distilled water) was given to rats in drinking water for 5 weeks. Rats were weighed every day. Cucumber juice was orally administrated with following three concentrations (1, 10 and 100 mg/kg) once in a day for 5 weeks. The rats were divided into five groups and each group consisted 10 rats for the statistical analyses as follows:

Group 1: Normal diet as a control (C); Group 2: Lead acetate treatment with normal diet and saline supplement (negative control (CCl_4-con); Group 3: Lead acetate treatment with normal diet 1 mg/kg cucumber juice diet (Pb-1); Group 4: Lead acetate treatment with normal diet 10 mg/kg cucumber juice diet (Pb-10); and Group 5: Lead acetate treatment with normal diet 100 mg/kg cucumber juice diet (Pb-100).

Collection of blood and tissue samples

After lead administration, the rats were sacrificed at the end of the treatment period, blood samples were collected from the descending aorta. Collected blood samples were examined for complete blood cell count immediately using automatic full digital cell counter (MS9-5, France). The residual blood samples were stained with methylene blue for evaluating the changes in red blood cells using the equation below. The observation was made by light microscope (NIKON JP/E-600, Japan), and photographs of cells were taken by digital camera (NIKON COOL PIX5400, Japan). The number of reticulocytes were counted using following formula:

$$\text{Count of reticulocytes } (\times 10^6 \text{cell}/\mu\text{L}) = \text{RBC (cell}/\mu\text{L)} \times \{\text{reticulocytes } (\%)/100)\}$$

The liver, kidney, femur and brain were also cut off and divided into two portions, one of them frozen and stored at −70°C for estimation of lead content, and another was fixed for histopathological observation.

Quantitation of lead (Pb)

All tissue samples were crushed after lyophilizing, and 0.25 g each of the samples was mixed with 6 mL of nitric acid and 2 mL of hydrogen peroxide. Then all samples were digested using microwave oven. Lead concentrations in the digested samples were estimated by atomic absorption spectrophotometer (Varian Spectra AA-200, Australia) at 217 nm wavelength following the instrument instruction manual. An air-acetylene mixture was used as the oxidant gas. The analytical quality was maintained by repeated analysis of the reference standards. The results were expressed as mg/L of the sample. The detoxification potential of the test samples was assessed on the basis of reduced Pb concentration in the tissues of the body.

Histological examination

Tissue samples from liver and kidney were separated, sliced and fixed in Bouin solution (Picric acid: Formalin: Acetic acid, 15:5:1), then embedded in paraffin. Sections of 3~4 μm thickness were made using a microtome and stained with hematoxylin-Eosin (H-E), and afterwards observed under a light microscope (Nikon, Japan), to observe histopathological changes in the liver and kidney.

Histomorphometric examination

Percentage of degenerative regions in liver showing focal acute cellular swelling on hepatic lobules, and the number of acute cellular swelling hepatocytes were calculated as $\%/mm^2$ and N/1000 hepatocytes, respectively. In kidney parenchyma, percentage of degenerative regions showing tubular necrosis and focal inflammatory cell infiltration, and the number of vasodilated atrophic glomerulus and degenerative tubules were calculated as $\%/mm^2$, N/100 glomeruli and N/1000 tubules, respectively. The histomorphometry was conducted by using an automated image analyzer (DMI-300 Image Processing; DMI, Korea) under 100 magnification of microscopy (Nikkon, Japan) at 5 fields (n=5), respectively.

The percentage changes between normal-control, Pb-control and test groups were calculated to evaluate the efficacy of cucumber juice, and detect the severity of Pb-intoxication, respectively, by following equation:

$$\% \text{ Changes } vs \text{ Pb-control } (\%) = [((\text{Data of test groups} - \text{Data of Pb-control})/\text{Data of Pb-control}) \times 100]$$

Statistical analysis

Data were expressed as mean ± S.E.M. and were analyzed with SPSS software, version 11.5. Differences between group means were calculated by a one-way analysis of variance (ANOVA). Results were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity test

There were no symptoms of any toxicity in the experimental rats of any of the groups. The LD_{50} value by oral route could not be determined as no lethality was observed in the animals.

Effect of heat-treated cucumber juice on growth response

In this experiment, we observed the changes in body weight and food intake during the period of lead exposure in experimental rats for 5 weeks. As shown in Table I, the body weight and food intake were increased in normal rats, whereas significant differences ($P<0.05$) were observed in cucumber juice-treated rats compared to the Pb-control. These results confirm that the intoxication by lead affected the growth regulation in rats. As confirmed by others, intoxication by lead may also reflect the influence of lead on the catecholaminergic and dopaminergic transmission.[17] These results clearly indicated that lead caused a significant decrease in the gain of body weight. As reported previously, harmful effect of lead on the body weight gain was elevated paralleled with the increase of lead acetate doses.[18]

Count of red blood cell (RBC) and reticulocytes

The results from Figure 1A and 1B, showed the effect of lead acetate toxicity observed in animal blood sample. As shown in Figure 1A and 1B, there was a slight difference in the count of red blood cells (RBC) for all the treatment groups as compared to normal control group. In case of the Pb treated group, slightly reduced numbers of RBC were observed. Similar findings were

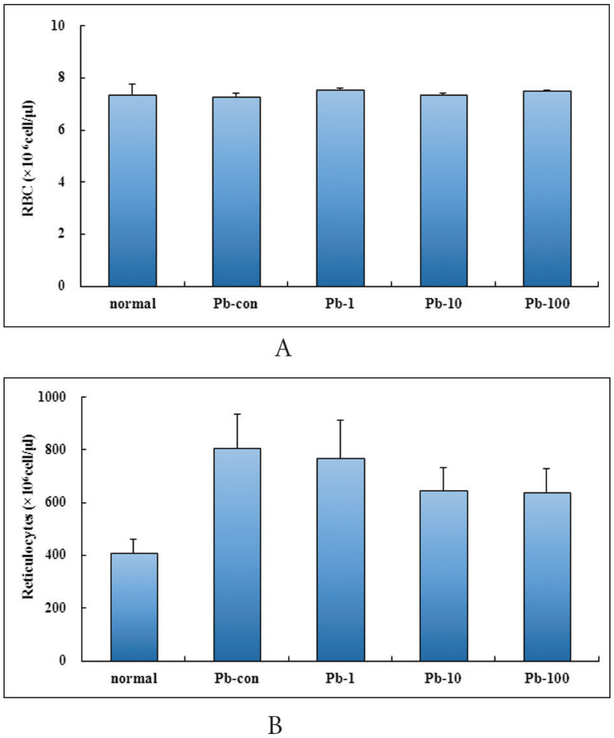

Figure 1: (A) Count of red blood cells (RBC); (B) Count of reticulocytes in rats exposed by Pb and fed with heat-treated cucumber juice for 5 weeks. Normal: No treatment; Pb-con: Treatment of Pb; Pb-1: Treatment of Pb and 1 mg/Kg concentration of cucumber juice; Pb-10: Treatment of Pb and 10 mg/Kg concentration of cucumber juice; Pb-100: Treatment of Pb and 100 mg/Kg concentration of cucumber juice.

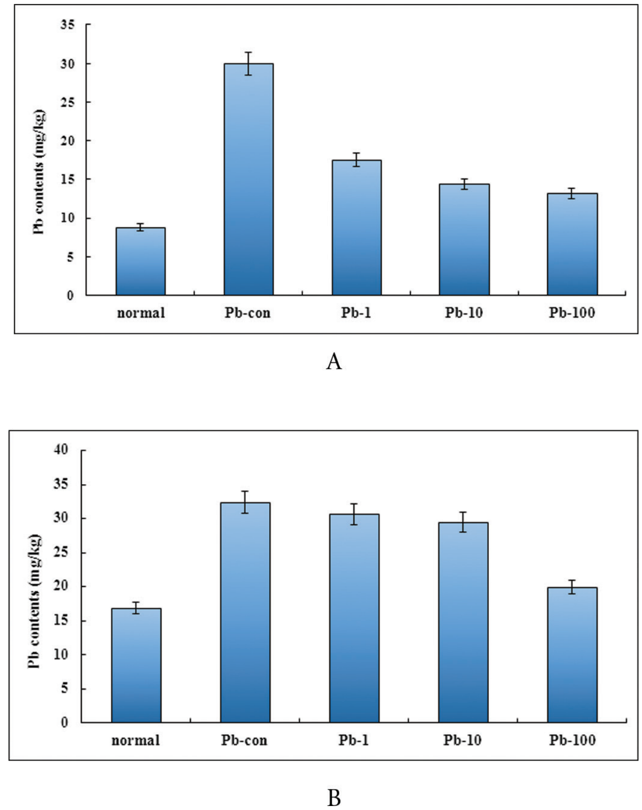

Figure 3: (A) Pb contents of rat liver exposed by Pb and fed with heat-treated cucumber juice for 5 weeks; (B) Pb contents of rat kidney exposed by Pb and fed with heat-treated cucumber juice for 5 weeks. Normal: No treatment; ▪ Pb-con: Treatment of Pb; Pb-1: Treatment of Pb and 1 mg/kg concentration of cucumber juice; Pb-10: Treatment of Pb and 10 mg/kg concentration of cucumber juice; Pb-100: Treatment of Pb and 100 mg/kg concentration of cucumber juice.

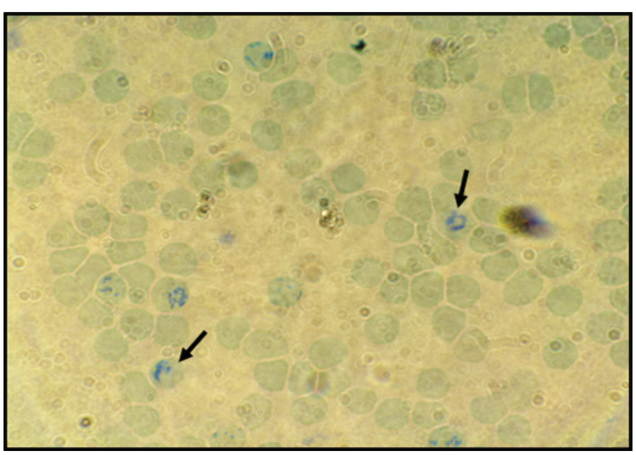

Figure 2: Clinicopathological finding of red blood cells and reticulocytes in Pb-administered rats, which was stained with methylene blue.

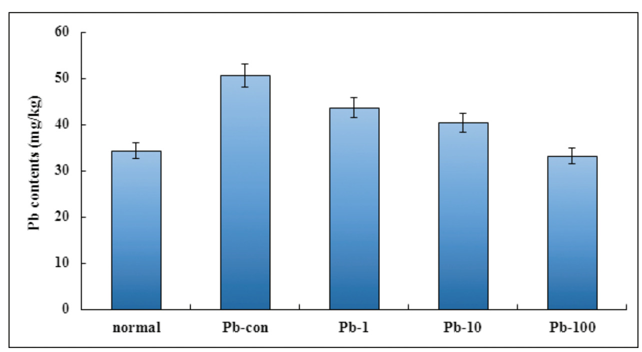

Figure 4: Pb contents of in rat femur exposed by Pb and fed with heat-treated cucumber juice for 5 weeks. Normal: No treatment; ▪ Pb-con: Treatment of Pb; Pb-1: Treatment of Pb and 1 mg/kg concentration of cucumber juice; Pb-10: Treatment of Pb and 10 mg/kg concentration of cucumber juice; Pb-100: Treatment of Pb and 100 mg/kg concentration of cucumber juice.

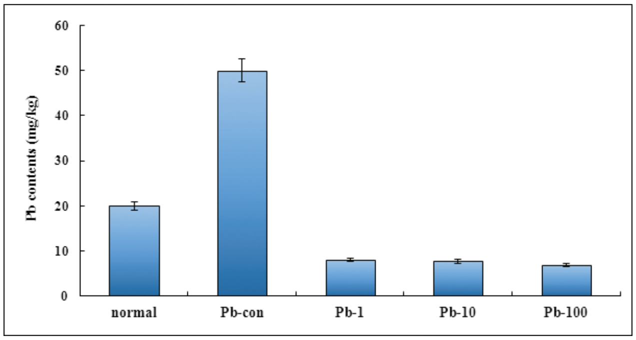

Figure 5: Pb contents of rat brain exposed by Pb and fed with heat-treated cucumber juice for 5 weeks. Normal: No treatment; Pb-con: Treatment of Pb; Pb-1: Treatment of Pb and 1 mg/kg concentration of cucumber juice; Pb-10: Treatment of Pb and 10 mg/kg concentration of cucumber juice; Pb-100: Treatment of Pb and 100 mg/kg concentration of cucumber juice.

observed by Ibrahim *et al.*[18] According to the strategy of Ibrahim *et al.*[18], the reduction of Pb confirmed the decrease in RBCs which may be attributed to the toxic effect of lead acetate. It is in agreement with the elevation of the plasma bilirubin level by Pb^{2+} ingestion which could be due to the induction of heme-oxygenase.[18] In addition to this, treatment of cucumber juice also significantly reduced the number of reticulocytes as also shown in the clinical pathological image (Figure 2). Lead has multiple hematologic effects. In lead-induced anemia, the red blood cells are microcytic and hypochromic as in iron deficiency.[19] Generally, there are increasing numbers of reticulocytes, premature red blood cells, with basophilic stippling in blood sample which result from the inhibition of pyrimidine-5-nucleosidase (Py-5-N). The anemia that occurs in lead poisoning results from two basic defects: (1) shortened erythrocyte lifespan, and (2) impairment of heme-synthesis. Shortened lifespan of the red blood cell is thought to be due to the increased mechanical fragility of the cell membrane. The biochemical basis for this effect is not known, however the effect is accompanied by inhibition of sodium- and potassium-dependent ATPase.[20] Ahmed *et al.*[21] reported significant decrease on the level of GSH and SOD in the liver of lead acetate treated rats in comparison to control, suggesting that lead may increase the level of oxidative stress in the lead treated rats. It is known that lead-induced oxidative stressed-tissue damage could be caused by two mechanisms: such as by increased generation of ROS, and by causing direct depletion of antioxidant reserves.[22] Intense lipid peroxidation caused by lead exposure may also affect the mitochondrial and cytoplasmic membranes causing more severe oxidative damage in the tissues and consequently releasing lipid hydroperoxides into circulation[23], eventually leading to oxidative stress.

Quantification of lead content

There is no such level of lead (Pb) that appears to be necessary or beneficial to the body and no "safe" level of exposure to lead has been found. Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects.[19] Pb accumulation patterns of the tissues in the experimental rats are shown in Figure 3-5. In healthy rats (normal), the mean Pb concentrations in liver, kidney, femur and brain were estimated to be 8.8 ± 0.051, 16.8 ± 0.057, 34.5 ± 0.086, and 20.0 ± 0.07 mg/kg, respectively. Exposure of Pb acetate at the concentration of 200 ppm for 5 weeks led to an increased tissue Pb concentration to 30.0 ± 0.063, 32.4 ± 0.052, 50.75 ± 0.041, and 50.0 ± 0.064 mg/kg in liver, kidney, femur and brain, respectively. Administration of cucumber juice significantly reduced tissue Pb accumulation as compared with Pb-control. The liver Pb concentrations were noted to be as 17.5 ± 0.057, 14.4 ± 0.052 and 13.2 ± 0.028 mg/kg in rats of Pb-1, Pb-10 and Pb-100 groups, respectively (Figure 3A). The mean Pb concentrations in kidney were recorded as 30.67 ± 0.011, 29.5 ± 0.033 and 20.0 ± 0.038 mg/L in rats of Pb-1, Pb-10 and Pb-100 groups, respectively (Figure 3B). The Pb concentrations of the femur were reduced in rats of Pb-1 (43.75 ± 0.06 mg/L), Pb-10 (40.5 ± 0.089 mg/L) and Pb-100 (33.25 ± 0.07 mg/L) (Figure 4). Whereas, the Pb concentrations in the brain dramatically decreased in Pb-1 (8.0 ± 0.079 mg/L), Pb-10 (7.75 ± 0.048 mg/L) and Pb-100 (7.0 ± 0.069 mg/L), that were found lower than no treatment group (Figure 5). Results confirmed that concomitant use of cucumber juice prevented the accumulation of Pb in these organs. Therefore, it can be suggested that the detoxifying potential of cucumber juice was perhaps due to the combined effects on both metal absorption and excretion from the body. Our findings also revealed that cucumber juice had the ability to reduce residues of Pb in soft tissues (liver, kidney and brain) as well as hard tissue (femur).

Cucumber includes high concentrations of metal ions based on its dry weight as nutritional components such as calcium and potassium. Nutritional factors are thought to play an important role in Pb detoxification, because Pb toxicity can be reduced by supplementation of certain metals. Acute toxicity is related to occupational exposure and is quite uncommon. Chronic toxicity on the other hand is much more common and occurs at blood lead levels of about 40–60 ug/dL. It can be much more severe if not treated in time and is charac-

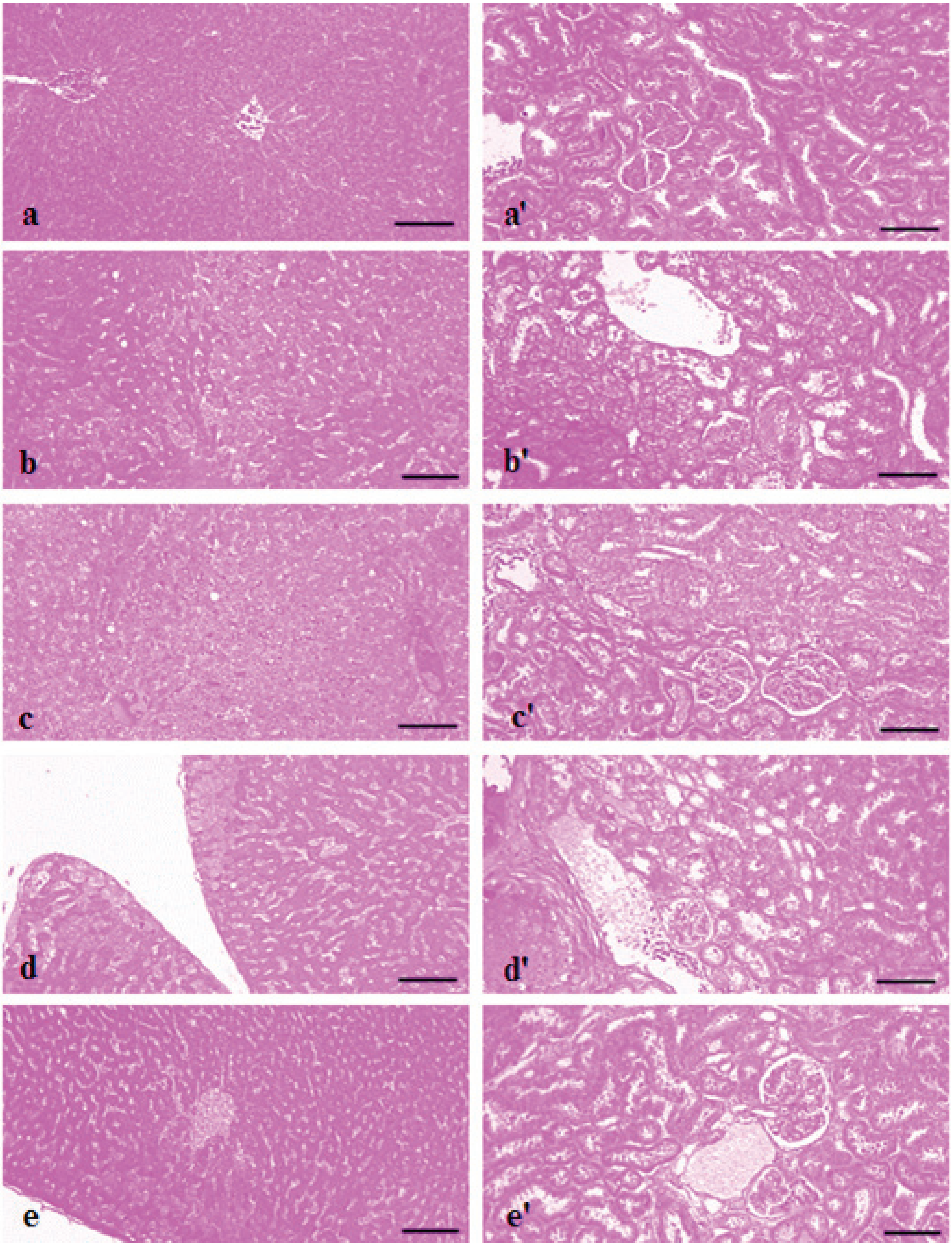

Figure 6: Histological profiles of the liver (A) and kidney (B) in normal rats (a, a'), Pb-con (b, b'), Pb-1 (c, c'), Pb-10 (d, d') and Pb-100 (e, e') groups. Tissue samples were stained H&E. Scale bars = 100 µm. Normal: No treatment; Pb-con: Treatment of Pb; Pb-1: Treatment of Pb and 1 mg/Kg concentration of cucumber juice; Pb-10: Treatment of Pb and 10 mg/Kg.

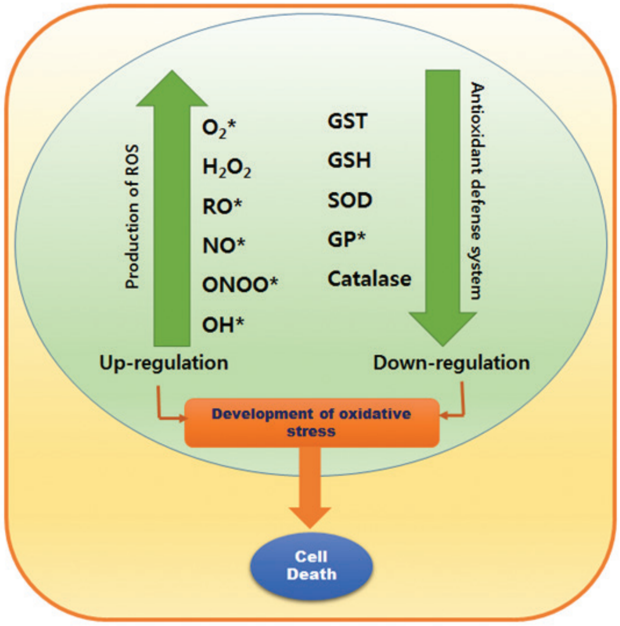

Figure 7: Mechanism of the development of the stress in a cellular system on lead exposure.

terized by persistent vomiting, encephalopathy, lethargy, delirium, convulsions and coma.[19,24]

One of such well-known metals that can affect the absorption of Pb is calcium (Ca). Calcium is an essential element that plays a vital role in many body functions, including bone growth and maintenance, muscle and nerve physiology, blood clotting and blood pressure regulation. Lead is known to exert its neurotoxic effects by competing with Ca for Ca receptors coupled with second messenger functions, and in some cases Pb inhibits the actions of Ca as a regulator of cell function.[19] Animal studies have shown higher retention of Pb in animals fed low-Ca diets, raising the possibility that diets low in Ca might affect the plasma levels of Pb in humans.[25] Besides, cucumber can also influence the diuretic effect in the body system, resulting in reduced Pb concentration. It can also be assumed that potassium also exhausts the waste materials from the body as well as reduces the sodium and heavy metal via urinary excretion.

Histological studies

The protective effect of cucumber juice against lead toxicity was described in liver histopathological changes in all experimental groups. The histological profiles of liver and kidney are shown in Figure 6A and 6B, respectively. This method is generally used as a microscopic observation of parenchymal organs that provided good information about organ morphology. Although the efficacy test for hepatoprotective purpose using Pb-intoxication hepatopathy is also seldom as compared to Pb-intoxicated nephropathy, Pb-intoxicated hepatopathy showed slight necrosis in liver cell, and acute cellular swelling foci were detected as Pb-intoxicated hepatopathies (Figure 6A). It was observed that lead exposure produced pronounced hepatic damage as evident by histological alternations in liver, including focal necrosis with hepatocyte vacuolization and swelling, pyknotic nuclei, as well as dilation of central vein and sinusoids. These findings are in strong support with Sharma *et al.*[26] El-Sokkary *et al.*[27] also showed that liver of lead-treated rats revealed remarkable degenerative alterations. Lead hepatotoxicity led to vacuolization of the cells, polymorphism of the nuclei, and decrease in glycogen content of the hepatocytes.[28] Pb-intoxicated nephropathy including tubular necrosis and vacuolation with dysfunction of glomerular filtration systems has been regarded as the valuable methods for the test of nephroprotective agents. In the present study, quite similar to those of previous reports, severe to moderate kidney tubular necrosis and vasodilated glomerulus were detected as Pb-intoxicated nephropathies (Figure 6B). In the kidney, lead intoxication has been found to causes interstitial fibrosis, as well as both hyperplasia and gradual atrophy of tubules and glomeruli.[26] It is well known that chronic lead exposure also results in glomerular and tubulointerstitial changes that lead to glycosuria, proteinuria, and chronic renal failure and hypertension.[19]

Histomorphometry

The changes in the percentage of degenerative regions in hepatic parenchyma, number of degenerative hepatocytes in liver and degenerative regions in kidney parenchyma, number of degenerative glomerulus and tubules in kidney are listed in Table II and III, respectively. These Pb-related tissue damages were re-confirmed with histomorphometry in this study.

The Pb-related hepatopathies and nephropathies were dramatically decreased in Pb-10 and Pb-100 group as compared to that of Pb-control, respectively. The percentages of degenerative regions in hepatic parenchyma and the number of degenerative hepatocytes were significantly decreased in Pb-10 and Pb-100 group. In addition, the percentage of degenerative regions in kidney, number of degenerative tubules and glomerulus were also dramatically decreased in Pb-10 and Pb-100 as compared to that of Pb-control, respectively. However, no meaningful changes in Pb-1 were detected as compared to that of Pb-control.

The percentage changes of degenerative regions in hepatic parenchyma of Pb-control were found as 528.63%, which were found -3.48, -34.66 and -52.54%

Table 1: Effect of cucumber juice on body weight and food intake in rats exposed to 200 ppm lead (Pb)

Group	Initial body weight (g)	Final body weight (g)	Body weight gain (g/day)	Food intake (g/day)
Normal	238.13 ± 12.12	437.50 ± 29.15	5.69 ± 0.69	51.48 ± 5.28
Pb-con	223.13 ± 12.30	388.75 ± 30.20	4.73 ± 0.71	46.44 ± 5.12
Pb-1	230.00 ± 10.21	406.88 ± 20.34	5.05 ± 0.57	48.24 ± 5.05
Pb-10	233.75 ± 13.70	416.88 ± 24.77	5.23 ± 0.64	49.85 ± 5.81
Pb-100	229.38 ± 9.66	419.38 ± 27.51	5.43 ± 0.75	48.51 ± 5.62

Normal: No treatment; CCl_4-con: Treatment of CCl_4; CCl_4-10: Treatment of CCl_4 and 10 mg/Kg concentration of cucumber juice.

Table 2: Changes in the Pb-induced hepatopathies in Pb-intoxicated rats

Group	Pb-induced hepatopathies	
	Percentage of hepatic degenerative regions	Number of degenerative hepatocytes
Normal	4.17 ± 1.41	17.40 ± 2.11
Pb-con	26.21 ± 2.58	511.80 ± 44.39
Pb-1	25.30 ± 2.98	517.00 ± 52.37
Pb-10	17.13 ± 1.69	248.00 ± 28.68
Pb-100	12.44 ± 2.06	238.20 ± 34.14

Normal: No treatment; Pb-con: Treatment of Pb; Pb-1: Treatment of Pb and 1 mg/Kg concentration of cucumber juice; Pb-10: Treatment of Pb and 10 mg/Kg concentration of cucumber juice; Pb-100: Treatment of Pb and 100 mg/Kg concentration of cucumber juice

Table 3: Changes in the Pb-induced nephropathies in Pb-intoxicated rats

Group	Pb-induced nephropathies		
	Percentage of kidney degenerative regions	Number of degenerative tubules	Number of degenerative glomerulus
Normal	6.30 ± 1.75	48.80 ± 4.15	10.40 ± 0.51
Pb-con	27.29 ± 1.71	359.80 ± 32.61	54.00 ± 6.30
Pb-1	31.59 ± 3.29	331.40 ± 34.59	49.00 ± 7.83
Pb-10	19.12 ± 0.96	191.20 ± 12.95	32.80 ± 4.43
Pb-100	14.29 ± 4.05	177.20 ± 41.11	29.80 ± 3.01

Normal: No treatment; Pb-con: Treatment of Pb; Pb-1: Treatment of Pb and 1 mg/Kg concentration of cucumber juice; Pb-10: Treatment of Pb and 10 mg/Kg concentration of cucumber juice; Pb-100: Treatment of Pb and 100 mg/Kg concentration of cucumber juice.

in 1, 10 and 100 mg/kg of cucumber juice treated groups, respectively as compared to that of Pb-control. Whereas percentage changes in the number of degenerative hepatocytes in Pb-control were 2,841.38%, that were 1.02, -51.54 and -53.46% in 1, 10 and 100 mg/kg of cucumber juice treated groups, respectively as compared to Pb-control. The changes in the percentage of degenerative regions in the kidney parenchyma of Pb-control were found at 333.17%, whereas found as 15.76, -29.95 and -47.62% in 1, 10 and 100 mg/kg of cucumber juice treated groups, respectively as compared to Pb-control.

The percentage changes in the number of degenerative kidney tubules in Pb-control were found as 637.30%, which were -7.89, -46.86 and -50.75% in 1, 10 and 100 mg/kg of cucumber juice-treated groups, respectively as compared to Pb-control. Also, percentage changes in the number of degenerative glomeruli in Pb-control were found as 419.23%, that were found as -9.26, -39.26 and -44.81% in 1, 10 and 100 mg/kg of cucumber juice treated groups, respectively as compared to that of Pb-control. Based on the above findings, it was hypothesized that the biologically active compounds present in cucumber juice might have chelated lead and enhanced its excretion from the body, resulting in reduced lead accumulation in tissues. The mechanism of cucumber juice-mediated chelation of lead nitrate might include formation of ionic bonds between sulfur-containing compounds and lead.[26].

In addition, lead is probably the most extensively studied heavy metal. Studies have reported the presence of various cellular, intracellular and molecular mechanisms behind the toxicological manifestations caused by lead in the body. Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage.[24] It has been reported as a major mechanism of lead induced toxicity. Under the influence of lead, as shown in Figure 7, onset of oxidative stress occurs on account of two different pathways operative simultaneously, which involves the generation of ROS, like hydroperoxides (HO_2), singlet oxygen and hydrogen peroxide (H_2O_2), and depletion of antioxidant reserves.[24].

Preventive measures are preferred over the treatment regimens,[29,30] considering the toxic effects of lead. This is due to the fact that once lead enters the body, it is almost impossible to remove it completely or to reverse its damaging effects on the body. Guidotti and Ragain[31] suggested a three-way measure as a preliminary preventive approach towards lead toxicity. Preventive medicine strategy mainly aims at screening the blood levels of individuals that are at a high risk of lead exposure.[32] Apart from the above mentioned strategies, nutrition also plays an important role in the prevention of lead induced toxicity. Studies have shown that uptake of certain nutrients such as mineral elements, flavonoids and vitamins can provide protection from the environmental lead as well as from the lead present in the body.[33,34] These nutrients play a pivotal role in restoring the imbalanced prooxidant/oxidant ratio that arises due to oxidative stress. Although the mechanism by which these nutrients restore the delicate prooxidant/oxidant ratio is still unclear, significant data are available suggesting a protective role of nutrients against lead poisoning.[19,35]

CONCLUSION

This study showed that cucumber juice possesses protective effects against the injury caused by lead (Pb) in liver and kidney. The cucumber juice treatment partly mitigated lead-induced changes in hepatological parameters due to its antioxidant nature, which combines free radical scavenging with metal chelating properties. The healing effect of cucumber juice was also confirmed by histological observations, which suggested that the cucumber juice was effective in bringing about functional improvement of hepatocytes. These findings reinforce the suggestions that cucumber juice can be given as a dietary supplement to human populations exposed to environmental toxicants and can provide protection against the toxic effects without being appreciably harmful itself. Moreover, efforts are needed in the choice of the appropriate dose, duration of treatment, and possible side-effects on major organs.

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CONFLICT OF INTEREST

None of the authors has a conflict of interest to disclose.

ABBREVIATION USED

Pb: Lead; **ANOVA:** Analysis of variance; **RBC:** Red blood cells; **Py-5-N:** Pyrimidine-5-nucleosidase; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **ROS:** Reactive oxygen species; **Ca:** Calcium.

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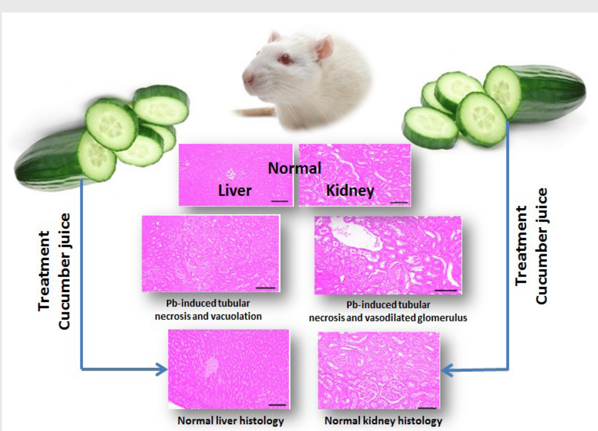
Pictorial Abstract



SUMMARY

- This study reports the efficacy of cucumber (*Cucumis sativus* L.) for heavy metal detoxification in an animal model.
- The cucumber juice significantly reduced level of histopathological parameters in the lead (Pb) induced detoxification in a rat model.
- Also cucumber administration had a significant effect on body weight, food intake and lead content in animal tissues.
- Administration of cucumber juice also exhibited significant effect on count of red blood cell (RBC), and reticulocytes, as compared with Pb-control.
- Eventually cucumber juice evoked a significant protective effect on liver and kidney induced by Pb

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