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Prominent nephroprotective effect of methanolic Broccoli (*Brassica olaracea* var. *italica*) extract on oxidative lead damage in mice kidney: Biochemical parameters and pathological changes

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Exposure to Lead causes oxidative stress and thereby reduces the antioxidant capacity of the cells. Broccoli is a well-known important source of antioxidant and mineral compounds. Here, we investigated the antioxidant potency of methanolic extract of broccoli in preventing renal toxicity. NMRI mice in the control group were given normal drinking water and the T1 experimental group received 500 ppm lead acetate in drinking water. The T2, T3, and T4 experimental groups received 300, 400 and 500 mg/kg of broccoli methanolic extract, respectively. On the last day, biochemical parameters and oxidative stress were measured in the kidney. Kidney tissue was used for preparation of sections and pathological studies. The highest concentrations of biochemical parameters in T1 showed a significant difference in comparison with the control and T4 groups (P <0.01). TAC, CAT, and SOD in T1 showed the lowest level, whereas the highest concentration of TAC and SOD were in T4 (P=0.032). MDA in T1 was 20.85 ± 0.270 nmol/mg and the difference with T4 was remarkable (P <0.001). The highest score of pathological damage to the renal tissue was in T1 and the lowest damage in T4. There was a significant difference between T4 compared to the control group (P=0.034). Over all, the methanolic extract of Broccoli *(Brassica olaracea, var. italica)* at 500 mg/kg exhibited significant nephroprotective effects against injuries caused by lead acetate in drinking water.

Keywords: Heavy metal pollution, Kidney, Oxidative stress, Reactive oxygen spices (ROS), Renal damage

Lead (Pb) is one of the most important environmental pollutants which inflicts considerable destructive effects on human health[1,2]. The highest level of lead accumulation is in the kidneys[1,3]. People are often exposed to acute and chronic lead poisoning, through breathing air, food and contaminated water. Among the body tissues, lead, at its highest level, is accumulated in the kidney and causes histopathological changes[4]. Several studies have explored lead toxicity mechanisms including production of reactive oxygen species (ROS) and oxidative damage to cells. Excessive production of ROS induces oxidative stress reduces the antioxidant protection in the body and result in damage to cellular macromolecules[5,6].

Vitamins, minerals and phytonutrients in natural and herbal compounds with antioxidant potentials contribute to the body health[7]. Broccoli contains all three types of antioxidants *viz*., phytochemicals, enzymes and vitamins[8]. This medicinal herb is a good source for vitamins C, E, and A (carotenoids) and a great source of manganese, calcium, and selenium[9]. Moreover, the centralization of broccoli is on flavonoids, such as Kaempferol, sulforaphane and Quercetin, and Carotenoids. Carotenoids include Lutein, Zaxentin, and Betacarotene which all are important as potent antioxidants such as promising in the control of diabetes by reducing blood glucose and oxidative stress[7-9]. Sulforaphane in broccoli extract prevents neurodegeneration and thereby has its effect on Alzheimer's disease and Parkinson's disease. Other characteristics include inflammation, neuronal loss, and oxidative stress[10].

In this context, we have earlier reported (data not shown) the effect of hydroalcoholic extract of broccoli (*Brassica olaracea* var. *italica*) on lead acetate induced renal toxicity mice. In the current study, we investigated the same aspect with various concentrations of methanolic extract of broccoli.

Materials and Methods

Animals and Experimental designs

This work was an experimental interventional study on 50 adult male mice NMRI with a normal appearance from the Pasteur Institute of Iran. Mice were kept under the standard conditions of 12 h of light/ darkness, and free access to standard concentrate and water. This study was done at the faculty of veterinary medicine of Sanandaj Branch and approved by the Ethics Committee of Kurdistan University of Medical Sciences (IR.MUK.REC.1397/ 5001). Mice were divided into 5 groups of 10 each[11], and they were under treatment for 4 weeks. The

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control group received a normal diet with drinking water while every group received 500 ppm lead acetate salt in drinking water. Then, using intraperitoneal injection daily, the second, third, and fourth experimental groups received 300, 400 and 500 mg/kg body wt. of broccoli methanolic extract, respectively.

Analysis of serum biochemical parameters

At the end of the period, on the 29th day, rats were weighed in each group. Then blood samples were taken from the mice's heart. Serum was isolated from blood at 3000 rpm for 10 min. The kidney biochemical parameters were measured, including urea by diacetylmonoxime method, creatinine by Jaffe method, uric acid by Rapid test, and potassium and sodium levels by Flame photometric method[12].

Sodium and chlorine concentrations were evaluated using the flame photometric method, chlorine levels were measured by titrimetric method[13] and total antioxidant capacity was measured by the ferric reducing ability of plasma (FRAP) method. In this method, the ability of serum to reduce iron (III) to iron (II) ions was measured. Thus, 3 mL of FRAP solution containing 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was added to the serum sample, the maximum blue light absorption for the TPTZ + Fe complex at 539 nm wavelength was read by spectrophotometer (USA, Rad-Bio, Spec S).

Measurement of renal oxidative stress parameters

After the experimental period, the mice's left and right kidneys were cut. The weight of the kidneys was measured with the use of a digital scale with 0.001 resolution. The kidney tissue was washed in saline and ice in the bath and homogenized in the ratio of 1:10 (w:v) with ice-cold 150 mM KC1. The amount of protein and malondialdehyde (MDA) in the recent homogenized tissue was measured immediately. To measure the catalase (CAT) and superoxide dismutase (SOD) enzymes, the homogenized tissue was stored at –70°C.

Protein, CAT and SOD measurements

Protein levels were measured by Lowry *et al*.[14] method with bovine albumin as the standard. Catalase activity was measured by Claiborn method[15] as described by Haque *et al*.[16] (2003). The sample was mixed with 0.09 M H_2O_2, 0.1 M Phosphate buffer, and PMS (10%) and volume up to 3 mL. After 30 s, absorption was read with a spectrophotometer at 240 nm wavelength. The activity of the superoxide dismutase enzyme was based on the superoxide radicals produced by xanthine and xanthine oxidase after reaction with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride, and the red colour formation. The level of superoxide dismutase enzyme activity was determined by the amount of inhibition of this reaction[17].

MDA Measurement

The level of malondialdehyde was measured by Wright *et al*.[18] method. In this method, the amount of added TAC 10% was two times the volume of homogenized kidney tissue. After an intense vortex, it was centrifuged at 2500 rpm for 10 min, and TBA 0.67% was added with the same volume of supernatant solution. It was placed in a water bath at 100 degrees for one hour, and the absorption was read at 532 nm.

Methanolic broccoli extract

The broccoli (*Brassica olaracea* var. *Italica*) was collected from agricultural field in Hamadan in April 2018 and was confirmed by the Herbarium Center of Kurdistan University (No: 30072). After removing the leaves and hard stems, the broccoli heads were cut into small florets. Two grams of dried florets were mixed with 200 mL 80% methanol and kept overnight at 25°C. After filtering with (Germany-Whatman) paper, methanol was removed from the solution by a vacuum rotary machine (IKA-RV 8). Then, it was dried in an oven and lyophilized to obtain a powder. The powder was dissolved in sterile distilled water (0.1g/mL concentration) and was injected, in needed doses, into mice (intraperitoneally). Using previous studies and with the help of a pilot project, the tolerable dose range of 300, 400 and 500 were considered

Histopathological study of the kidney tissue

The kidneys were fixed in 10% formaldehyde buffer and evaluated pathologically after staining with H&E stain. Indicators of necrosis, tubular degeneration, and the presence of inflammatory cells were evaluated and graded according to the observed amount between 0 and 100% (0% absence, less than 25%, between 25-50%, between 75-50%, and more than 75%).

Statistical analysis

The results of the study were analyzed using SPSS 24, mean and standard deviation. The data were checked for homogeneity of variances using Leven's test with an alpha level of 0.05. It was further checked for normality using the Kolmogorov–Smirnov test. After that, One-way ANOVA and Tukey test were

used to compare the results between groups. The significance level was considered at (P <0.001), (P <0.01) and (P <0.05).

Results and Discussion

On the last day, the T1 group had the highest mean weight 30.40±0.360 g and the lowest weight was in the control group 29.83 ± 0.400 g. There was a significant difference between the mice's weight in T1 and T2 groups and the control group (P =0.045).

The control group had lowest mean weight of the right kidney 0.164±0.300, and T1 had the highest weight 0.189±0.369 g. There was a significant difference in the right kidney weight between the control and T1 group (P <0.001) and T2 with the control group (P =0.042). The mean weight of the left kidney in the control group was 0.168±0.231 g and in T1 was 0.190±0.023 g. There was a significant difference between the left kidney weight in T1 with the control (P =0.002) and T2 with the control group (P =0.038) (Table 1).

As shown in Table 2, the highest level of changes in potassium concentration was in T2 4.56±0.109 mmol/L and the lowest was in T4 group with an average of 4.11±0.084 mmol/L. There was no significant difference in potassium concentration among different groups (P=0.48). Concerning chlorine concentration, the lowest serum levels were observed in the T4 and the highest in T1 with an average of 113.00±2.90 mmol/L. Significant differences were observed between serum chlorine levels between T1 and the control group (P =0.003), T2 and the control group (P =0.028). The highest concentrations of sodium in the blood serum were in T1 with 149.03±8.84 mmol/L and the control group had the lowest concentration 132.22±14.868 mmol/L. There was a significant difference in the sodium levels between T1 and the control group (P =0.002). Also, there was a significant difference between sodium concentration in T2, T3 with T1 at the level of (P =0.032) and T4 with T1 (P =0.002).

With respect to changes in urea concentration in different groups, the highest serum level was in T1 81.88±8.70 mg/dL, and the lowest was in T4 39.44±0.728 mg/dL. There was a significant difference between urea concentrations in T1 with the control group (P <0.001) and there was a significant difference in urea concentrations in T2, T3 and T4 groups with the T1 group. The lowest creatinine concentration was in the T4 group 0.37±0.083 mg/dL, and the highest in T1 1.97±0.098 mg/dL. Creatinine concentration in T1, T2 and T3 was significant compared to the control group (P =0.003). Considering the concentration of uric acid, the highest level was in T4 2.71±0.064 mg/dL, and the lowest concentration was in the control group 2.13±0.061 mg/dL. There was no significant difference in the uric acid levels among different groups (P=0.41) (Table 2).

Table 3 refers to the evaluation of oxidative stress parameters. The highest concentration of malon-dialdehyde was in T1 20.85±0.270 nmol/mg protein, and the lowest concentration was in the control group 8.96±0.634 nmol/mg protein (P <0.001). There was no significant difference between T4 and the control group (P = 0.45). The total serum antioxidant capacity highest levels were in T4 0.47±0.023 mmol/g protein, and the lowest in T1 0.04±0.008 (P = 0.032). There was a significant difference between the TAC concentration of the T1 and the control group (P =0.004). The highest concentration of catalase

Table1 — Measurements of mice's body weight and kidney weight in the experimental and control groups

Groups	C	T_1	T_2	T_3	T_4
Last day body weight	29.83±0.400	$30.40±0.360^{b*}$	$30.23±0.300^{b*}$	29.33±0.124	29.00±0.687
Right kidney weight	0.164±0.300	$0.189±0.369^{b***}$	$0.175±0.348^{b*}$	0.170±0.256	$0.169±0.436^{a*}$
Left kidney weight	0.168±0.231	$0.190±0.023^{b***}$	$0.184±0.678^{b**}$	$0.185±0.431^{b*}$	$^{*b}0.187±0.238^{b*}$

[Results are expressed as means±SEM. C, Control; T1, Lead treated; T2-T4, Lead treated+Broccoli at 300, 400 and 500 mg/kg, respectively. * P <0.05, ** P <0.01, *** P <0.001. ^{b}: significant difference with the control group; and ^{a}: significant difference with T1]

Table 2 — Measurement of biochemical parameters of kidney damage by lead in the different groups

Groups	C	T_1	T_2	T_3	T_4
Potassium concentration (mmol/L)	4.19±0.810	4.55±0.166	4.56±0.109	4.35±0.137	4.11±0.084
Chlorine concentration (mmol/L)	98.48±1.459	$113.00±2.90^{b**}$	$104.96±2.71^{b*}$	96.63±4.87	$94.49±3.73^{a*}$
Sodium concentration (mmol/L)	132.22±1.868	$149.03±8.084^{b**}$	$140.60±5.408^{a*}$	$140.53±2.422^{a*}$	$136.00±3.807^{a**}$
Urea concentration (mgm/dL)	44.70±2.76	$81.58±8.70^{b***}$	$45.66±6.57^{a**}$	$46.16±1.19^{a**}$	$39.44±0.728^{a***}$
Creatinine concentration (mgm/dL)	0.40±0.038	$1.97±0.098^{b**}$	$1.56±0.028^{b**}$	$1.42±0.064^{b**}$	$0.37±0.083^{a**}$
Uric Acid concentration (mgm/dL)	2.13±0.061	2.52±0.113	2.54±0.056	2.57±0.055	2.71±0.064

[Results are expressed as means±SEM. C, Control; T1, Lead treated; T2-T4, Lead treated+Broccoli at 300, 400 and 500 mg/kg, respectively. *P <0.05, ** P <0.01, *** P <0.001. ^{b}: significant difference with the control group; and ^{a}: significant difference with T1]

enzyme was 123.45±12.230 µmol/g in the control group and the lowest was 54.124 ± 14.602 µmol/g of protein in T1 which was statistically significant (P <0.001). The concentration of superoxide dismutase in the T1 and T2 groups had a significant difference with the control group (P <0.001) and T3 with the control group (P = 0.004). Also, the difference in superoxide dismutase concentration of T4 was significant with T1 (P = 0.032) (Table 3).

Histopathological results

For pathological changes in the kidney, indicators including inflammatory cell permeation, urinary tubules degeneration, urinary casts, urinary tubules necrosis were evaluated and graded. The highest score of damaged tissue was in the T1 group with grades of 7.21±0.531 and then T2, T3, T4, and control groups with grades of 5.56±0.65, 4.60±0.580, 2.50±0.580, and 0.01±0.021, respectively. Using Kruskal–Wallis one way analysis of variance, there was a significant difference between the changes in the pathological indicators of T1 with the control group (P <0.001), T2 and T3 with the control group (P = 0.005), and T4 with the control group (P =0.034) (Fig. 1).

In this study, the highest left and right kidney weight were in T1 group which was treated with lead acetate. The biochemical parameters indicated that chlorine, sodium, urea, and creatinine increased in the T1 group more than the control group and other treated groups with broccoli methanolic extract. However, the amount of uric acid in the group which received the extract in maximum dose was higher than the other groups probably because of the presence of purine in broccoli and its conversion to uric acid.

On the other hand, regarding oxidative stress parameters, the highest damage was observed in T1 and the lowest in the control group. In the treatment groups with broccoli, the extract was reduced by dose-response dependent malondialdehyde concentration, increased antioxidant capacity serum, and antioxidant enzymes concentration. The most effective dose was obtained at 500 mg/kg of broccoli extract in controlling oxidative lead damage in the kidney.

In confirmation of this effect at 500 mg/kg dose of broccoli, pathological lesions including, necrosis, renal tubular degeneration, and inflammatory cell were significantly reduced in kidney damage of the lead and the microscopic profile of the tissue was similar to that of the control group .The degree of injury between the T1 group and the T4 showed a significant difference from 7 to 1.

In the previous studies, body weight gain due to DNA hypermethylation as a result of lead and also kidney weight gain because of slight inflammation and fibrosis had been studied in mice[19,20]. Lead acetate is a peroxidation factor, and the membrane lipids peroxidation damage leads to the renal tubular tissue destruction[4]. Free radicals that have an effect on cell membrane lipids peroxidation and renal tissue atrophy prevent the kidney's proper function and thus

Table 3 — Evaluation of oxidative stress parameters in the experimental and control groups

Groups	C	T_1	T_2	T_3	T_4
MDA (nmol/mg)	9.96±0.634	$20.85±0.270^{b***}$	$15.50±0.445^{b**}$	$13.32±0.112^{b**}$	9.94±0.430
TAC (mmol/mg)	0.20±0.018	$0.04±0.008^{b**}$	$0.26±0.034^{a***}$	$0.33±0.076^{a**}$	$0.47±0.023^{a*}$
CAT (µmol/mg)	123.45±12.230	$^{b}54.124±14.602^{b**}$	$61.47±18.236^{b**}$	$73.27±21.650^{b*}$	$113.59±19.450^{a**}$
SOD (µgm/gm)	21.69±3.040	$14.01±4.2b^{**}$	$15.19±1.79^{b***}$	$17.32±4.112^{b*}$	$28.93±5.36^{a**}$

[Results are expressed as means±SEM. C, Control; T1, Lead treated; T2-T4, Lead treated+Broccoli at 300, 400 and 500 mg/kg, respectively. MDA = malondialdehyde; TAC = total capacity antioxidant; CAT = catalase; and SOD = superoxide dismutase. * P <0.05, ** P <0.01, *** P <0.001. [b]: significant difference with the control group; and [a]: significant difference with T1]

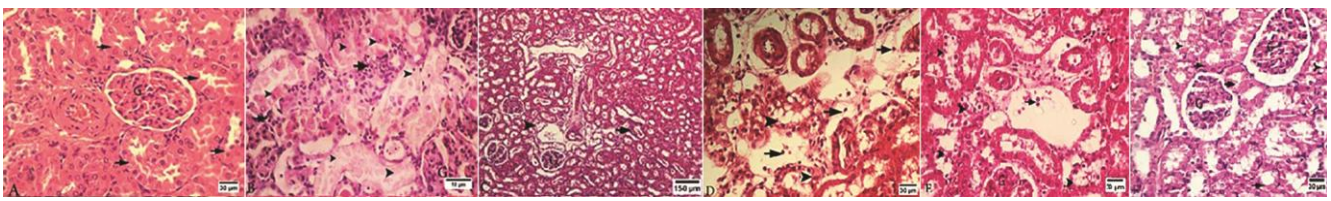

Fig. 1 — (A), Control group, the normal structure of glumeruli (G) and renal tubules (arrow) is seen; **(**B) T1 group, necrosis in renal tubule epithelium as nuclear piknosis and cytoplasm eosionophilia(arrowhead) and infiltration of mononuclear inflammatory cells(arrow) are seen, glumeruli (G) has a normal structure; **(**C) T2 group, mild infiltration of inflammatory cells in the interstitium (arrow), and tubular degeneration(arrowhead) with normal glomerular structure(G) are seen; (D) T2 high magnification slide of T1, that tubular degeneration(arrowhead) and infiltration of mononuclear inflammatory cells(arrow) is seen; (E) T3 group, that mild tubular degeneration(arrow head) and infiltration of mononuclear inflammatory cells(arrow) are seen. Glumerular structure(G) is normal; and (F) T4 group that mild tubular degeneration (arrow head) with many normal renal tubules(arrow) and normal glumerui(G) is seen. (H&E, X400).

the concentration of excretive factors in the kidney will increase in serum[1]. On renal effects of lead accumulation in the body, Sisombath *et al*.[21] has revealed the significant difference between the lead concentration, creatinine, urea, sodium, and chlorine in those exposed to lead with the control group but there was no significant difference in potassium and bicarbonate concentrations.

The main mechanism of lead toxicity is the induction of reactive oxygen spices (ROS) and oxidative stress. Increasing ROS produces a chain reaction that can cause lipid peroxidation, DNA, RNA and proteins oxidation, resulting in serious damage to the cell structure[22]. Lead can inhibit the δ-aminolevulinic acid dehydrasase enzyme (ALAD) and cause δ-aminololucinic acid (ALA) accumulation. Auto oxidation of accumulated ALA produces ROS[23]. Lead can forward oxidative stress by inhibiting antioxidant enzymes and also decrease these sources in cells.

Catalase is the first active enzyme in terms of oxidative stress which shows efficacy[24]. ROS production causes deactivation and a significant reduction in catalase tissue concentration[24]. In the present study, catalase had a significant reduction in the group which had received lead (T1 group) compared to the control group and the other treatment groups. SOD and TAC had a significant decrease at the time of lead intake and increased in the group that received 500 mg/kg body wt. of extract. Hence, the lead oxidative mechanism, injections of antioxidants to reduce the antioxidant capacity of the cell is considered as a reliable solution[24].

Several studies have demonstrated the harmful effects of heavy metals and controlling these injuries with natural antioxidants[25-27]. Curcumin is reported to ameliorate pathological damages caused by sodium arsenite in mice kidney by increasing total antioxidant capacity and reducing lipids peroxidation[28]. Broccoli is a good source of polyphenols, vitamins A, C, E, flavonoids, glycosides, beta-carotene and xanthine compounds whichhave a potent antioxidant effect[29]. Broccoli also has 1% sulforaphane which reduces 8-hydroxy-2 deoxy-guanosine which inhibits the oxidative damage of DNA and lipids[30]. Dharmender & Gurinder reported about improved CAT, SOD and GPx stress biomarkers along with reduced lipid peroxidation (LPO) levels and enhanced the total antioxidant activity (AOA) suggesting that the exposure of female albino rats to triazophos (TZ) could be reversed by the antioxidant potential of broccoli extract[31]. Researchers have also reported the antioxidant properties of thiamine, vitamin B6, E and C in removal of toxic metals, such as lead[32]. Broccoli extract has also been shown to contain high amount of vitamin E and C suggesting its antitoxicity potential[33]. Shah *et al*.[34] administered of broccoli extract at 300, 2000 and 4000 mg/kg for 28 days showed no signs of poisoning in hematological, biochemical and liver parameters. Broccoli can be considered as one of the herbal compounds with potent antioxidant effects and also have high safety and healthy[34]. Moreover, the antioxidant properties in the various methods of processing can be increased[35], this can be considered as a unique feature of the broccoli. Basha *et al*.[32] have demonstrated reversal of lead induced alterations in cholinergic system (ACh and AChE) of hippocampus and the aminergic system (epinephrine, dopamine and MAO) of cerebellum in Wistar rats by calcium. Broccoli, with its high calcium content, could be effective in reducing the lead induced toxicity.

Overall, our results have also demonstrated the protective effects of the methanolic extract of broccoli, particularly in in healing of lesions and reversal of alterations of biochemical parameters caused by lead induced oxidative stress in the kidney.

Conclusion

According to the results of the study, broccoli methanolic extract with high antioxidant potency and significant amounts of calcium at the dose 500 mg/kg body wt. could prevent kidney damage caused by 500 ppm lead acetate in drinking water in a valuable amount. It indicates possible use of broccoli in controlling oxidative damage in humans and promote good health for the human society. However, other oxidative stress and measurement of antioxidant and minerals compounds in the extract are required to validate this study.

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Conflict of Interest

Authors declare no competing interests.

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