The potential ameliorative effects of broccoli against biochemical alterations and hepatorenal oxidative stress induced by lead intoxication in adult rats

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Abstract:

Lead is one of the top ten most dangerous substances for public health. Oxidative stress is an implicated mechanism in lead toxicity. The antioxidant activities of broccoli floret have been extensively confirmed. This study was conducted to evaluate the potentially ameliorative effects of broccoli powder (BP) against lead-hepatorenal toxicity. Thirty adult male albino rats were randomly assigned into five groups of six rats each: G1: negative control, fed on the basal diet (BD); G2: positive control, received lead acetate PbA (25 mg/kg BW) daily and was fed on BD; and G (3-5), received PbA and were fed BD containing (3.0, 6.0 and 9.0%) BP, respectively. At the end of the experiment (8 weeks), the rats were slaughtered, blood was drawn, and the liver and kidney were excised and processed for the biochemical and oxidative stress markers assays. Administration of PbA caused significant biochemical alterations that involved increased serum, liver, and kidney lead concentrations; dyslipidemia; and hepatic and renal dysfunction. Oxidative stress biomarkers in liver and kidney tissues of PbA-treated rats showed a significant ($P{\leq}0.05$) drop in the levels of both non-enzymatic antioxidants (reduced glutathione) and antioxidant enzymes (superoxide dismutase and catalase); and a marked elevation of lipid peroxidation (malonaldehyde) content. Feeding lead-intoxicated rats on BP diets showed significant amelioration in the aforementioned biochemical parameters and oxidant/antioxidant status by recovering the values toward their normal rate. The ameliorative effect was concentration-dependent and increased by increasing BP concentration, which may be attributed to its antioxidant activity. In conclusion, BP is a promising natural source for preventing and mitigating lead toxicity. Further investigations may be needed to identify the exact mechanism of action.

Keywords: *broccoli, lead toxicity, oxidative stress, antioxidants enzymes, liver function, kidney function, lipid profile*

Graphical Abstract
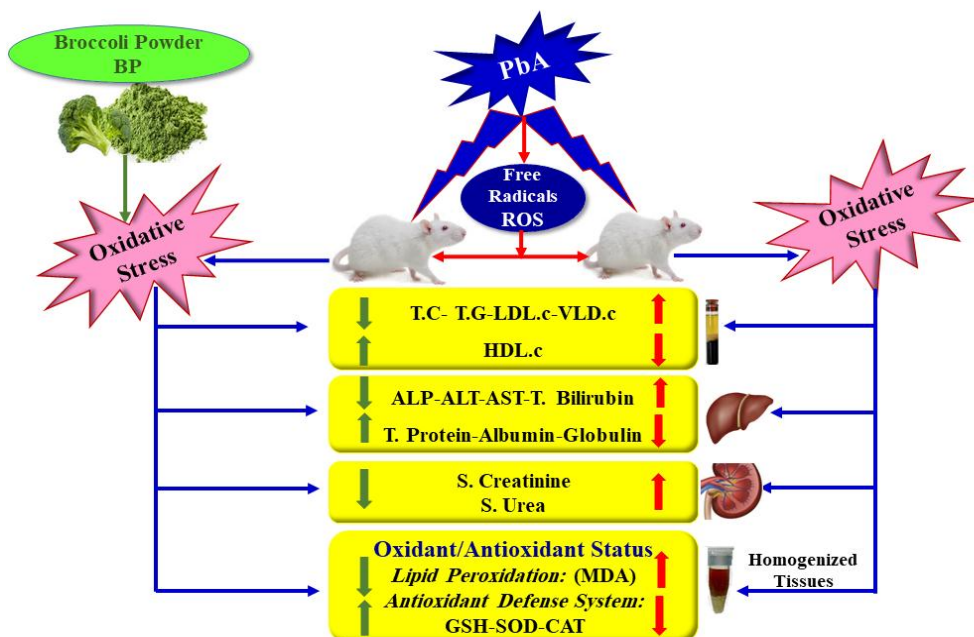
Introduction

Lead (Pb) is one of the top ten most dangerous pollutants that provoke public health concerns (Irawati *et al*., 2022). Despite the dramatic decline in environmental lead sources over the last few decades, low-level lead exposure continues to be a global public health issue in many nations (Al Osman *et al*., 2019 and Rezaee *et al*., 2022). Lead accounts for 1.5% of annual deaths worldwide. According to IHME, lead exposure is responsible for around 902,000 deaths and 21.7 million Disability-Adjusted Life Years (DALYs) globally (IHME, 2019). Lead is a highly toxic heavy metal whose widespread use has adversely affected human health, including behavioral, biochemical and physiological consequences, therefore attracting worldwide attention (Abdelhamid *et al*., 2020 and Gundacker *et al*., 2021). Lead toxicity has severe clinical consequences for practically all organs, including the central nervous system and brain, hematological, neurological, reproductive, gastrointestinal, immune, kidneys and cardiovascular systems (Kabeer *et al*., 2019 and WHO, 2022). Accumulated lead in the tissues induces cellular oxidative damage by generating reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals, hydroxyl radicals and lipid peroxides, which cause oxidative stress, in addition to the reduction of cellular antioxidant activity that activates inflammatory signaling cascades (Ericson *et al*., 2017

and Mohamed *et al*., 2020). This inflammatory process plays an important role in the negative health consequences caused by lead (Kurabi *et al*., 2016).

      Medicinal plants with antioxidant properties have attracted increasing attention in recent years to protect against heavy metal toxicity and eliminate the adverse effects of environmental and food contaminants (Darwesh *et al*., 2018). Broccoli (*Brassica oleracea var. italica*) is an annual plant known as a "vegetable crown" has a high concentration of health-promoting ingredients and is referred to as a functional food (Villarreal-García *et al*., 2016). Broccoli is abundant in bioactive constituents like quercetin, kaempferol, glycosides, phenolic compounds, and various glucosinolates such as glucobrassicin and glucoraphanin, as well as vitamins C and E (Wagner *et al*., 2013; Khedr *et al*., 2020; Yao *et al*., 2021 and Yao *et al*., 2022). Phenolic compounds are known to be powerful antioxidants. Similarly, glucosinolates prevent oxidative stress caused by reactive types of electrophiles. One of the most frequent isothiocyanates found in broccoli, sulforaphane, has been demonstrated to decrease chronic inflammation (Villarreal-García *et al*., 2016). Conventional chelation therapies such as Calcium Disodium EDTA and other antidotes used to treat lead toxicity have a variety of adverse effects and are unable to regulate lead-induced oxidative stress (Kosnett *et al*., 2010 and Lamidi and Akefe, 2017). Thereby, Antioxidant protection agents capable of avoiding lead adverse effects are urgently needed. So, the present study was designed to assess the effect of broccoli against lead toxicity-induced hepatorenal oxidative damage in adult rats.

Materials and Methods

Materials

Chemicals and reagents

      Lead acetate trihydrate [$Pb(CH_3COO)_2$].$3H_2O$ was purchased from Sigma-Aldrich (St. Louis, MO, USA). The biochemical assay Kits were obtained from Alkan Medical Company, St. El Doky, Giza, Egypt. All other chemicals and reagents utilized in this investigation were secured from El-Gomhoreya Company, Cairo, Egypt, of analytical grade or of the highest commercially available purity.

Experimental animals

      Thirty male albino rats eight-week-old of Sprague Dawley strain weighing (160 ± 10 g), were sourced from Medical Insects Research Institute, El-Doky, Giza, Egypt. They were housed in well-ventilated cages under standard laboratory conditions (22 ± 2 °C; 12 h light/dark cycles) and relative humidity (50%−60%). The rats were allowed free access to the basal diet and water before dietary manipulation. The rats were acclimatized for 7 days prior to the experiment in the Animal Laboratory at the Faculty of Home

Economics, Shibin El-kom, Menoufia, Egypt. The experiments were conducted following the standards of care and use of animals for scientific purposes. This work was ethically approved by the Institutional Animal Care and Use Committee (IACUC), Menoufia University. (Reg. No, MUFHE /F/NFS/8/23).

Plant material

Broccoli florets (*Brassica oleracea var. italica*) were obtained from the local market of Shiben El-Kom City, Menoufia, Egypt.

Methods

Broccoli powder (BP)

Fresh broccoli florets were carefully washed with running water, cut into small pieces, and was dried at 50 °C in an Alab Tech oven under vacuum (Model No. Lvo-2040-Korea). The mixture was then crushed in an electric mill and sieved through 80 mesh screens (British Standard Sieve). The fine powder was used to prepare the experimental diets containing BP at concentrations of (3.0, 6.0 and 9.0 %, w/w) of the basal diet. The selection of broccoli concentrations was pursuant to the results of prior investigations.

Basal diet (BD)

The basal diet was prepared from fine ingredients in accordance with Reeves *et al.*, (1993) as follows: protein (casein) 12%, sunflower oil 10%, cellulose 5%, choline chloride 0.2%, and DL-methionine 0.3%, salt mixture 4% following Hegsted *et al.*, (1941), vitamin mixture 1% as described by Campbell, (1963), and cornstarch up to 100 g.

Lead acetate (PbA) solution

A dose (25 mg/kg B.W) of lead acetate was used to induce lead intoxication in rats according to Grosicki and Kowalski, (2002). The PbA solution was prepared by dissolving the required quantity of lead acetate in distilled water and used immediately. The lead acetate solution was freshly prepared daily.

Experimental design

Thirty adult male albino rats were randomly grouped into five groups of six rats each and were subjected to the following treatments: *Group 1*) the negative control group fed on the basal diet (BD); *Group 2*) the positive control group, received PbA (25 mg/kg B.W) by oral gavage daily and was fed on the BD; and *Groups 3-5*) were treated with PbA (25 mg/kg B.W) and were fed on BD containing (3.0, 6.0 and 9.0%) of broccoli powder (BP) respectively. The treatment lasted 8 weeks. The rats were subjected to lead acetate for eight weeks to simulate subchronic lead exposure and adequate induction of lead intoxication according to Obafemi *et al.*, (2019).

Blood and tissue samples collection

After 24 hours of the last lead acetate administration dose, the rats were fasted overnight before being slaughtered; blood samples were obtained; and serum was isolated to estimate some biochemical markers. The liver and kidney tissue samples were removed after blood collection and immediately processed for biochemical analysis. All samples were kept at -20°C until performing assays as described by Sharma *et al*., (2014).

Preparation of tissue homogenate

Cooled saline solution was used to wash tissue samples (0.5 g) from the liver and kidney. Specimens were chopped and homogenized in (PBS) phosphate-buffered saline (10% w/v; pH7.4) followed by centrifugation for 15 minutes at 3000 rpm as described by Joseph *et al*., (2015). The supernatant was frozen (−30°C) to determine the antioxidant and lipid peroxidation indicators.

Biochemical assays
Lead intoxication markers

The lead levels in the serum, liver, and kidneys were estimated by atomic absorption spectrophotometer (Perkin–Elmer instrument Model 2380) as described by Yeager *et al*., (1971).

Serum lipid profile

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL.c) were measured using the procedures outlined by Allain *et al*., (1974), Fossati and Prencipe, (1982), and Demacker *et al*., (1980), respectively. According to Lee and Nieman (1996), very low-density lipoprotein cholesterol (VLDL.c) and low-density lipoprotein cholesterol (LDL.c) were computed as follows:

$$VLDL.c = TG/5$$
$$LDL.c = Total\ cholesterol - (HDL.c + VLDL.c.)$$

Liver function markers

Enzymes activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using the method of Moss and Henderson, (1999). The alkaline phosphatase (ALP) and total bilirubin (TB) were determined using the methods of Bergmeyer and Harder (1986); and Pearlman and Lee, (1974). The Doumas, (1975) method was used to estimate total protein. According to Tietz, (1994) albumin and globulin concentrations were measured spectrophotometrically utilizing commercial kits.

Kidney function markers

Serum creatinine and urea levels were determined in accordance with Houot, (1985).

Hepatorenal oxidative stress markers and antioxidants status

The concentration of malondialdehyde (MDA), a biomarker of lipid peroxidation (LPO), was measured in the form of thiobarbituric acid-reacting substances according to Sharma and Krishnamurti, (1968). *Enzymatic antioxidant status:* superoxide dismutase (SOD) activity was determined using the Misra and Fridovich, (1972) technique, while catalase (CAT) activity was assessed spectrophotometrically using the method of Aebi, (1972). *Non-enzymatic antioxidant status:* the reduced glutathione (GSH) level was determined following the technique described by Brehe and Burch, (1976).

Statistical analysis

The Statistical Package for Social Sciences was used to analyze the collected data (SPSS, version 22). All the results were provided as mean ± standard deviation (SD). The differences between groups were examined using a one-way analysis of variance (ANOVA) followed by Duncan's test. Differences were judged statistically significant at the value of ($P \leq 0.0$) according to Artimage and Berry, (1987).

Results and Discussion

Effect of BP on lead levels in the serum and liver and kidney tissues

Data in Table (1) indicated the mean lead concentrations in the serum and liver and kidney tissues in the negative control, positive control and lead-intoxicated groups treated with BP. Based on such data, it was shown that exposure to lead acetate (25 mg/kg B.W) daily for 8 weeks in the positive control group resulted in a significant ($P \leq 0.05$) increase in lead concentration in the serum and hepato-renal tissues comparing to those in the negative control group. These findings are consistent with Abd El-Ghffar and Abd El-Aal, (2018) and Andjelkovic *et al*., (2019). The lead acetate ingested through the gastrointestinal tract is transferred to the bloodstream and disseminated to all vascular organs. More than 90% of blood lead is carried as a lead phosphate molecule by erythrocytes (Jackie *et al*., 2011). Also, the same table showed that the lead level in the liver tissue was higher than kidney after lead administration. Herman and Geraldine, (2009) reported that the liver is one of the major organs impacted by lead intoxication due to lead accumulating in the liver following lead exposure. In addition, the liver is the primary organ engaged in storage, detoxification and biotransformation of toxic chemical relevant to heavy metals. Moreover, the basic diagnostic of lead toxicity is a high blood lead level, which causes a variety of biochemical, physiological, and behavioural disorders. The liver, spleen, and kidneys are the key target sites for lead toxicity (Assi *et al*., 2016). This confirms the induction of acute lead toxicity in the rats in the current study.

Regarding our results, lead-intoxicated rats treated with BP diets (3.0, 6.0 and 9.0%, w/w of BD) revealed dramatically decreased alterations

recorded in lead levels in the serum and soft tissues (liver and kidney) significantly (P ≤ 0.05) in comparison to the positive control group, however, failed to normalize it. The amelioration rate increased by increasing BP concentration in the rats' diets. The best result was obtained in the group treated with the BP diet of 9.0%, w/w. Several studies have exhaustively screened the bioactive compounds of broccoli, and its antioxidant properties have been well clarified (Ares *et al*., 2013; Gaafar *et al*., 2013; Villarreal-García *et al*., 2016; Khedr *et al*., 2020 and Yao *et a*l., 2022). The antioxidant agents may have had a substantial role in scavenging the reactive oxygen species caused by lead acetate (Jackie *et al*., 2011). In the same context, Jeffery and Araya, (2008) reported that sulforaphane (a hydrolysis product of glucoraphanin) in broccoli could upregulate various detoxification enzymes that contribute to the clearance of chemical carcinogens and reactive oxygen species. This evidence may support our results regarding lead levels reduction in the blood and tissues in lead-intoxicated rats treated with BP diets, indicating the potential chelating effect of broccoli however, this property needs further investigation.

Table (1): Effect of BP on lead levels in the serum and liver and kidney tissues

Parameters	Control (-)	Lead-intoxicated rats		
		Control (+)	(BP, w/w)	
			3.0%	6.0%	9.0%
Serum (µg/dl)	2.97[e]±0.19	60.74[a]±1.52	38.89[b]±1.48	20.67[c]±1.85	9.1[d]±1.18
Liver (µg/g)	3.25[e]±0.57	25.39[a]±1.23	19.25[b]±0.56	13.18[c]±0.69	9.34[d]±0.47
Kidney (µg/g)	1.51[e]±0.39	23.41[a]±0.67	17.62[b]±1.08	11.01[c]±1.14	7.48[d]±0.56

Results were expressed as means±SD. Means in the same row with different letters are significantly different (p≤0.05)

Effect of BP against lead-induced alterations in serum lipid levels

Lead intoxication caused aggravated alterations in serum lipid levels. Table (2) demonstrated that lead intoxication resulted in a significant (P ≤ 0.05) elevation of the mean values of serum T.C, T.G, LDL.c, and VLDL.c and a significant decline in HDL.c in the control (+) group compared to the corresponding values in the control (-) group. These results are compatible with Abdou and Hassan, (2014) and Offor *et al*., (2017), who noted that administration of lead acetate caused increasing of total cholesterol, triglycerides, LDL and VLDL in rats. Moreover, lead has also been shown to be associated with increased low-density lipoprotein, cholesterol, and systolic and diastolic blood pressure, which affects cardiovascular health in lead-exposed adults (Obeng-Gyasi *et al*., 2018). It has also shown that lead inhibits the activity of hepatic lipoprotein lipase, which can lead to decreased removal

by altering cell-surface receptors for lipoprotein in addition to inhibiting cytochrome P450, which can hinder the production of bile acids, which plays a critical role in eliminating cholesterol from the body (Offor *et al*., 2017). Additionally Ademuyiwa *et al*.; (2009) cleared that lead toxicity may cause dyslipidemia because lead binds directly to phosphatidylcholine in the body, triggering phospholipidosis and cholesterogenesis.

In the present work, it is worth mentioning that consumption of BP diets (6.0 and 9.0%, w/w) by the lead-intoxicated rats repressed significantly ($P \leq 0.05$) either the elevation in the values of each T.C, T.G, LDL.c and VLDL.c or the reduction in the HDL.c values. The same trend was noticed regarding the LDL.c and HDL.c in lead-intoxicated rats treated with BP 3.0%, w/w. Additionally, the BP diet 9.0%, w/w recorded the best ameliorative effects for the mentioned parameters compared to the positive control group. According to Armah *et al*., (2015), broccoli ingestion dramatically reduces plasma LDL-c levels, which is likely due to the combination of dietary components in broccoli, including glucoraphanin, fiber, and SMCSO (S-methyl cysteine sulphoxide). This reduction's mechanism is compatible with the suppression of cholesterol production as opposed to the suppression of bile acids or cholesterol absorption. Broccoli contains a powerful health-promoting compound called sulforaphane (SFN), which forms by hydrolyzing glucoraphanin by the myrosinase enzyme (González *et al*., 2021). The process by which glucoraphanin lowers LDL-c is by the stimulation of nuclear factor [erythroid-derived 2]-like 2, an element of the nrf2-antioxidant response, through transcribed by glucoraphane that reduces the oxidative stress; and suggested that nrf2 expression is closely related to the modulation of mitochondrial fatty acid oxidation, lipid synthesis, and steroid biosynthesis (Choi *et al*., 2014).

Table (2): Effect of BP against lead-induced alterations in serum lipid levels

Parameters (mg/dl)	Control (-)	Lead-intoxicated rats		
		Control (+)	(BP, w/w)		
			3.0%	6.0%	9.0%
T.C	155.87[c]±2.44	175.06[a]±4.7	171.33[a]±2.41	165.74[b]±1.39	159.82[c]±1.40
T.G	152.06[d]±2.54	190.36[a]±1.96	187.31[a]±1.75	167.61[b]±2.1	158.61[c]±2.29
HDL.c	85.68[a]±0.95	41.37[e]±1.38	49.51[d]±0.48	63.63[c]±1.59	71.77[b]±1.28
LDL.c	39.78[e]±0.99	95.62[a]±5.24	84.36[b]±1.69	68.59[c]±0.54	56.33[d]±0.74
VLDLc	30.41[d]±0.51	38.07[a]±0.39	37.46[a]±0.35	33.52[b]±0.5	31.72[c]±0.46

Results were expressed as means±SD. Means in the same row with different letters are significantly different ($p \leq 0.05$)
T.C: Total cholesterol, T.G: Triglyceride, HDL.c: High density lipoprotein cholesterol, VLDL.c: Very low density lipoprotein cholesterol, LDL.c: Low density lipoprotein cholesterol.

Effect of BP against lead-induced alterations in liver function markers

The liver function parameters of lead-intoxicated rats treated with BP diets are shown in Table (3). It was noted that treating rats with lead acetate induced a significant ($P \leq 0.05$) elevation in the liver function parameters, including ALP, ALT, AST and total bilirubin, while the total protein, albumin and globulin levels significantly declined in comparison to the negative control group, which is an indicator of hepatic dysfunction caused by liver cell destruction in lead-intoxicated rats. These results align with Alwaleedi, (2015); Mohammed *et al*., (2019); Mohamed *et al.,* (2020) and Khamphaya *et al.,* (2022), who reported that the treatment of experimental animals with lead acetate caused hepatotoxicity and elevated liver enzymes activities. Elevation levels of ALP suggest biliary injury or blockage of the biliary tree, which disrupts blood flow to the liver (Farida *et al*., 2012). Also, AST and ALT are essential indicators for detecting lead hepatotoxicity (Herman and Geraldine, 2009). The liver's biosynthetic capacity is also measured by AST and ALT, which are used to assess hepatocyte damage, with ALT being a specific indication of hepatocyte necrosis. AST and ALT levels increase in the presence of liver necrosis caused by drugs and toxins (Roy and Bhattacharya, 2006). Exposure to lead induces serious damage in liver cells, which causes necrosis and apoptosis of the Kupffer cells and infiltrating inflammatory cells. Because these enzymes are found in significant amounts in the liver, when these injured hepatic cells are damaged, they escape into the plasma (Mohammed *et al*., 2019).

The liver is responsible for the production of total protein, albumin, and globulin, thereby any condition causing hepatocellular damage alters the synthesis of these compounds and reduces their levels (Tripathi and Kumar, 2011). The reduction in serum total protein level and the elevation in serum total bilirubin indicated liver dysfunction. It may be due to the inhibition of protein biosynthesis by the liver caused by lead poison's effects, such as the precipitation of soluble plasma protein that is used as a carrier for lead poison and alters the activity of numerous enzymes. The reduction in blood total protein and increase in total bilirubin levels suggested hepatic impairment. That might be because lead poison's effects, such as the precipitation of soluble plasma protein, which is utilized as a carrier for lead poison and alters the action of multiple enzymes, disrupting protein synthesis in the hepatocytes (Lowry *et al*., 2012). Also, the reduction of total protein may be due to lead-induced renal and hepatic destruction (Yuan *et al*., 2014). Furthermore, lead toxicity is primarily caused by an increase in the generation of free radicals, including ROS reactive oxygen species and RNS reactive nitrogen species (Singh *et al*., 2018). Lead ions substitute other divalent cations like Fe^{2+}, Mg^{2+}, Ca^{2+}, as well as monovalent cations like Na^+. As a result, the cellular hemostasis is disturbed and several biological processes are affected, such as

cell adhesion, cell signaling, apoptosis, maturation, ionic transportation, oxidant-antioxidant equilibrium, enzymes regulation and inflammation (Jaishankar *et al.*, 2014).

Table (3): Effect of BP against lead-induced alterations in liver function markers

Parameters	Control (-)	Lead-intoxicated rats			
		Control (+)	(BP, w/w)		
			3.0%	6.0%	9.0%
ALP (U/L)	$169.75^d \pm 2.24$	$197.59^a \pm 1.29$	$195.42^a \pm 1.1$	$181.23^b \pm 1.4$	$175.93^c \pm 1.17$
ALT (U/L)	$79.63^d \pm 2.52$	$121.8^a \pm 2.25$	$118.53^a \pm 1.6$	$95.57^b \pm 3.26$	$83.25^c \pm 0.89$
AST (U/L)	$67.0^d \pm 2.16$	$90.57^a \pm 1.41$	$86.83^b \pm 1.68$	$77.4^c \pm 2.57$	$69.07^d \pm 1.17$
Total bilirubin (mg/dl)	$0.53^e \pm 0.25$	$0.89^a \pm 0.24$	$0.80^b \pm 0.25$	$0.71^c \pm 0.24$	$0.60^d \pm 0.13$
Total protein (mg/dl)	$8.46^a \pm 0.64$	$3.31^e \pm 0.41$	$4.37^d \pm 0.39$	$6.05^c \pm 0.21$	$7.15^b \pm 0.23$
Albumin (mg/dl)	$5.57^a \pm 0.98$	$2.19^e \pm 0.4$	$2.98^d \pm 0.93$	$3.78^c \pm 0.9$	$4.79^b \pm 0.16$
Globulin (mg/dl)	$3.29^a \pm 0.19$	$1.59^d \pm 0.27$	$2.18^c \pm 0.25$	$2.38^c \pm 0.17$	$2.61^b \pm 0.08$

Results were expressed as means + SD. Different letters in the same row were significantly different (p ≤ 0.05).
ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Concerning our results, mixing diets with BP at concentrations of (6.0 and 9.0%, w/w) for the lead-intoxicated rats resulted in a significant (P ≤ 0.05) decrease in ALP, ALT, AST and total bilirubin levels; while the levels of serum total protein, albumin and globulin markedly increased as compared to the positive control group. Also, the BP group (3.0%, w/w) exhibited a partial improvement for all mentioned markers except for ALP and ALT. While the BP diet (9.0%, w/w) afforded a marked amelioration by recouping the values toward the normal levels. These results propose that BP may protect against liver damage caused by the toxic effects of exposure to lead acetate and mitigate its adverse effects. Broccoli has an enhancing hepatoprotective and reduces hepatocyte damage and lipid peroxidation, which improves the liver's antioxidant capacity. In addition to providing hepatoprotective properties and reducing lead toxicity, broccoli reduces oxidative damage to DNA in the liver and increases liver detoxification. Furthermore, broccoli's high vitamin content especially E and C, and minerals have been associated with the amelioration of lead hepatotoxicity in rats (Mohammed *et al.*, 2019). Also, Sulforaphane, an isothiocyanate derivative of glucoraphanin, exhibits antioxidant and anti-inflammatory properties which may contribute to liver function improvement (Satomi *et al.*, 2022).

Effect of BP against lead-induced alterations in kidney function markers

The effect of BP against lead-induced alterations in serum urea and creatinine levels were presented in Table (4). It was clear that treating rats with lead acetate resulted in a higher significant ($P \leq 0.05$) elevation in the mean values of serum urea and creatinine more than those in normal rats. These findings are in accordance with those found by Alwaleedi, (2015) and Mohamed *et al*., (2020), who reported that lead acetate administration in rats increased significantly ($P \leq 0.05$) blood urea nitrogen BUN, creatinine, lactate dehydrogenase (LDH) and absolute kidney weight compared to the non-treated rats. On the other hand, Khamphaya *et al*., (2022) found that the BUN levels were significantly elevated in animals exposed to lead acetate compared to the normal group, while creatinine levels showed no significant differences and reported that the BUN changes were more responsive than creatinine changes to the early phases of lead-induced renal impairment. Khalil-Manesh *et al*., (1992) indicated that lead exposure might cause nephropathy due to its effects on kidney function and pathology. The absorbed lead combines with certain proteins in the proximal tubular cells (PCTs) of the glomeruli, and these combinations of lead proteins are implicated as characteristic intracellular inclusions in acute lead nephrotoxicity. In addition, lead accumulates in the kidney's mitochondria, leading alterations in both structure and function (Carocci *et al*., 2016). Furthermore, Reddy *et al*. (2014) suggested a possible mechanism of lead toxicity by altering the balance between pro-oxidants and antioxidants through the generation of reactive oxygen species ROS. As a result of oxidative stress, protein degradation increases, resulting in an increase in ammonia and urea concentrations in serum. Free radicals can also damage renal cells, disrupting their brush border epithelial linings, and rendering the cells impermeable to urea and creatinine (Yuan *et al*., 2014).

Table (4): Effect of BP against lead-induced alterations in kidney function markers

Parameters (mg/dl)	Control (-)	Lead-intoxicated rats			
		Control (+)	(BP, w/w)		
			3.0%	6.0%	9.0%
Urea	$44.08^{d}\pm1.71$	$71.7^{a}\pm1.98$	$66.94^{b}\pm4.07$	$52.66^{c}\pm2.13$	$49.71^{c}\pm1.16$
Creatinine	$0.68^{d}\pm0.26$	$1.71^{a}\pm0.25$	$1.68^{a}\pm0.22$	$1.21^{b}\pm030$	$1.14^{c}\pm0.24$

Results were expressed as means±SD. Means in the same row with different letters are significantly different ($p \leq 0.05$)

In terms of our findings, the experimental diets that contained BP (6.0 and 9.0%, w/w) demonstrated a significant ($P \leq 0.05$) reduction in blood urea and creatinine levels for all lead-intoxicated treated groups compared to the positive group. The same direction was noticed in the BP diet of 3.0%, w/w, except for serum creatinine. Since serum urea and creatinine are typical

indicators for renal dysfunction, the decrease in these parameters by the experimental BP diets indicates the potential ameliorative effects of BP against lead nephrotoxicity. In addition, the BP diet (9.0%, w/w) was far superior to the other diets in neutralizing kidney functions compared to positive control. Broccoli contains a variety of phytochemicals that could promote oxidative defenses, including glucosinolates (β-thioglucoside-N-hydroxy sulphates), which include glucoraphanin (Fahey *et al*., 2001). Glucoraphanin (4-methyl sulfinyl butyl glucosinolate) is metabolized *in vivo* to biologically active sulforaphane SNF (James *et al*., 2012). SNF is an isothiocyanate, present in cruciferous vegetables such as cauliflower and cabbage, as well as in high amounts in broccoli, in stored form like glucoraphanin (Vanduchova *et al*., 2019). SFN treatment of arsenic-induced nephropathy in albino rats improved kidney function by lowering oxidative stress and cell death, suppressing the inflammatory cytokines interleukin-1 and interleukin-6, and suppressing a variety of pro-apoptotic markers (Thangapandiyan *et al*., 2019).

Effect of BP against lead-induced oxidative stress and antioxidant status in hepatic tissue

Oxidative stress has been proposed as a potential mechanism implicated in lead intoxication. Oxidative stress is caused by an imbalance between free radical production and antioxidant generation which detoxifies reactive intermediates or repairs resulting damage in living cells. Table (5) illustrated the effect of BP on antioxidant status and oxidative stress markers in hepatic homogenates in lead-intoxicated rats. It was clear that lead toxicity induced a significant ($P \leq 0.05$) decrease in hepatic SOD and CAT activities and GSH levels, indicating that lead toxicity affects the intracellular enzymatic antioxidant defense system; In contrast, the MDA (a biomarker of oxidative stress) level significantly elevated in the positive control group when compared to their perspective in the negative control. Our results are consistent with the findings of Wang *et al*., (2013); El-Tantawy, (2016), and Mohamed *et al*., (2020), who showed that lead exposure activated the oxidative stress mechanism by increasing biochemical markers such as lipid peroxide and decreasing GSH, SOD, CAT, and GPx (glutathione peroxidase) levels in liver tissue. As a consequence of lead toxicity, free radical damage occurs through two distinct but related pathways: (a) the production of reactive oxygen species (ROS), such as singlet oxygen, hydrogen peroxide, and hydroperoxide, as assessed by MDA levels as lipid peroxidation end products, and (b) the direct depletion of antioxidant reserves (El-Nekeety *et al*., 2009). El-Tantawy, (2016) reported that the increased level of MDA in lead acetate-treated rats was attributed to declined SOD activity, which is an indicator of oxidative stress. In normal conditions, glutathione is mainly found at 90% in the reduced form GSH and at 10% in the oxidized form GSSG. In order to stabilize ROS, the reduced form of glutathione (GSH) provides its reducing equivalents ($H^+ + e^-$)

through its cysteine thiol groups. Hence, GSH plays an important role in the active excretion of lead in bile via attaching to its thiol group and subsequently excreting it. Decreased levels of GSH may increase oxidative stress and MDA accordingly (Jaishankar *et al*., 2014).

Table (5): Effect of BP against lead-induced oxidative stress and antioxidant status in hepatic tissue

Parameters	Control (-)	Lead intoxicated rats		
		Control (+)	(BP, w/w)	
			3.0%	6.0%	9.0%
MDA (mmol/g tissue)	$19.59^c \pm 1.23$	$41.16^a \pm 1.23$	$38.57^a \pm 2.13$	$29.57^b \pm 1.86$	$21.95^c \pm 2.3$
SOD (u/g tissue)	$57.58^a \pm 3.59$	$31.84^d \pm 2.91$	$35.04^d \pm 1.91$	$45.29^c \pm 1.89$	$53.11^b \pm 1.36$
GSH (mg/g tissue)	$9.73^a \pm 0.65$	$3.42^c \pm 0.58$	$4.06^c \pm 0.34$	$6.57^b \pm 0.37$	$7.35^b \pm 0.57$
CAT (mmol/g tissue)	$24.01^a \pm 1.45$	$12.65^d \pm 1.08$	$13.96^d \pm 0.8$	$17.63^c \pm 0.47$	$21.58^b \pm 0.53$

Results were expressed as means±SD. Means in the same row with different letters are significantly different (p ≤ 0.05)
MDA: malonaldehyde, GSH: reduced glutathione, SOD: Superoxide dismutase CAT: catalase

Feeding lead acetate-treated rats on the BP diets (6.0 and 9.0%, w/w) of the basal diet revealed a significant (P ≤ 0.05) increase in both enzymatic antioxidant activities (SOD and CAT); and non-enzymatic antioxidant levels (GSH). While the MDA concentration was significantly decreased when compared to the corresponding values in the positive control group. On the other hand, the BP diet of 3.0%, w/w didn't show any significant differences. Moreover, as compared to the control group, the BP diet of 9.0% w/w markedly improved the antioxidant status over the other diets. Broccoli has powerful antioxidant activities due to two kinds of phytochemicals that exist in broccoli which involved in defenses against oxidative stress: (a) Direct antioxidants: phenolic compounds, carotenoids, vitamin E and vitamin C are the main direct antioxidants in broccoli. They are involved in redox reactions and scavenge oxidation products (Jane *et al*., 2007 and Paulina *et al*., 2014). (b) Indirect antioxidants: consist of a broad range of chemical structures that may trigger cytoprotective (Phase II) response. Endogenous myrosinase hydrolyzes glucosinolates to produce isothiocyanate, the most potent indirect antioxidant in broccoli (Dinkova-Kostova and Kostov, 2012). Glucoraphanin is the most abundant glucosinolate in broccoli, and its hydrolysate is sulforaphane (Yao *et al*., 2021).

Effect of BP against lead-induced oxidative stress and antioxidant status in renal tissue

Table (6) demonstrated the effect of BP diets on antioxidant status and oxidative stress biomarkers in the kidney homogenates of lead-intoxicated rats. It was obvious that treatment of rats with lead acetate resulted in severe oxidative stress, as evidenced by a decrease in the following antioxidant defense systems: the activities of SOD and CAT, as well as levels of GSH; and an increase in the concentration of lipid peroxidation biomarker (MDA) as compared to their corresponding in the negative control group. These differences reached the significant ($P \leq 0.05$) level. These finding aligned with Amin *et al*., (2020), who observed that treated rats with lead acetate showed a significant ($P<0.001$) reduction in the SOD, CAT and GPx activities in kidney tissue in comparison to the normal control animals. Flora *et al*., (2012) and Liu *et al*., (2017) emphasized that oxidative stress is the major proposed mechanism for lead intoxication. Oxidative stress is caused by two concurrent pathways: the first is the formation of reactive oxygen species ROS, and the second is the depletion of antioxidant stores. ROS attacks cell membranes, inducing lipid bilayer oxidation and the formation of malondialdehyde (MDA) as an end product (Amin *et al*., 2020). Lead may also substitute zinc ions, which serve as cofactors to antioxidant enzymes, inactivating them as well as attacking the sulfhydryl groups. Glutathione (a cysteine-based molecule) is one of those affected by lead intoxication. The glutathione sulfhydryl group immediately attaches to toxic elements with a high affinity for sulfhydryl groups, and therefore lead can efficiently inactivate the glutathione molecule, rendering it ineffective as an antioxidant (Khan *et al*., 2008).

Table (6): Effect of BP against lead-induced oxidative stress and antioxidant status in renal tissue

Parameters	Control (-)	Lead-intoxicated rats		
		Control (+)	(BP, w/w)		
			3.0%	6.0%	9.0%
MDA (mmol/g tissue)	13.48^c±1.96	28.22^a±2.23	26.33^a±0.85	19.71^b±1.08	17.9^b±1.97
SOD (u/g tissue)	45.88^a±0.94	24.18^d±1.27	26.1^d±2.48	35.81^c±0.95	40.45^b±0.63
GSH (mg/g tissue)	18.09^a±1.01	11.77^c±0.97	12.51^c±0.47	16.21^b±0.55	17.93^a±0.79
CAT (mmol/g tissue)	19.28^a±1.42	9.59^d±1.47	15.65^d±0.64	14.39^c±1.04	16.41^b±1.01

Results were expressed as means±SD. Means in the same row with different letters are significantly different (p ≤ 0.05)
MDA: malonaldehyde, GSH: reduced glutathione, SOD: Superoxide dismutase CAT: catalase

From the same table, it was clear that feeding lead intoxication rats on the BP diets (6.0% and 9.0%, w/w) suppressed significantly (p ≤ 0.05) either

the elevation of MDA or the reduction of SOD, CAT and GSH values compared to the positive control. While the BP diet of 3.0%, w/w showed no significant changes. The ameliorative effect was concentration-dependent and increased by increasing the concentration of BP in the rats' diets. These results can be supported by Raeeszadeh *et al.*, (2021), who clarified that broccoli methanolic extract at a dosage of (500 mg/kg B.W) reduced the concentration of MDA; enhanced serum antioxidant capacity; raised the concentration of antioxidant enzymes and prevented kidney damages induced by lead acetate (500 ppm in drinking water) in male mice. Also, Raeeszadeh and Mortazavi (2018) concluded that a 300 mg/kg dosage of hydroalcoholic broccoli extract attenuated the consequences of lead acetate-induced oxidative stress in mice kidneys. Additionally, broccoli has been demonstrated to mitigate the toxic effects of certain heavy metals, including arsenic. In experimental arsenic-induced poisoning in male Wistar rats, broccoli was highly effective in reducing kidney and liver damage; improving biochemical markers including SOD, GPx and total antioxidant capacity; and decreasing the concentration of MDA (Raeeszadeh *et al.*, 2022). Broccoli is abundant in all three forms of antioxidants: phytochemicals, vitamins and enzymes (Bongoni *et al.*, 2014). Broccoli is enriche with manganese, calcium, and selenium, as well as vitamins C, E and β-carotene (Thomas *et al.*, 2018), in addition to naturally occurring phytochemicals (e.g. flavonoids, phytosterols, carotenoids, chlorophyll, alkaloids, phenols and glucosinolates (Liu *et al.*, 2018). Broccoli is primarily known for its flavonoids, such as kaempferol, isothiocyanates (sulforaphane, most important knowns for its chemopreventive properties) and quercetin. Carotenoids, however, include lutein, zaxentin and beta-carotene, all of which act as powerful antioxidants (Herr and Büchler, 2010 and Mahn and Reyes, 2012).

Vitamin E is an antioxidant that inhibits lipid peroxidation by inhibiting the free radical chain reaction. Vitamin E deactivates ROS generated by chelating the free radicals chain reaction, inhibits ROS production and keeps the lead (Pb) ion in a redox state (Rendon-Ramirez *et al.*, 2007). A collaboration between vitamin E and other antioxidants has shown to have a more efficient preventive effect against lead toxicity. Vitamin E and C work together to prevent lipid peroxidation in lipid structures. Vitamin C can alleviate the oxidative stress generated by lead by converting free radicals into non-free radicals. Ascorbic acid's paired hydrogen atoms create a complex with the unpaired electrons of free radicals, rendering them non-free radicals (Das and Saha, 2010). Finally, Amin *et al.*, (2020) illustrated that natural products containing flavonoids, polyphenols, vitamins, and other bioactive components have shown to reduce the negative consequences of lead toxicity due to their ability to reduce oxidative stress. These natural products play an important role in reducing the lead-related toxicities via suppressing pro-oxidative factors and enhancing antioxidant levels inside damaged cells.

Consequently, the potential protective/curative properties of broccoli against lead intoxication can be attributed to its scavenging properties and antioxidant components such as vitamins E and C, polyphenols, glucosinolates, and flavonoids which counteract free radicals that may trigger oxidative stress.

Conclusion

Lead toxicity is associated with various health hazards that result in a variety of body disorders. Synthetic chelating medications used to treat lead toxicity have many side effects and cannot mitigate some adverse effects of lead, such as oxidative stress. In the current study, lead intoxication in rats resulted in excessive biochemical disturbances of renal function, liver function, serum lipid profile, as well as hepatorenal antioxidant status. Our results indicated that broccoli powder had attenuated lead-induced hepatorenal toxicity and exhibited ameliorative effects against oxidative stress, as well as restoring antioxidant status and biochemical alterations in lead-intoxicated rats toward normal levels. The ameliorative effects of broccoli may be stemmed from its antioxidant activities. Therefore, broccoli is a natural antioxidant source with the potential capacity for prophylactic treatment of lead toxicity. However, the molecular mechanisms underlying broccoli-induced protection against lead toxicity remain to be explored.

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التأثيرات التحسينية المحتملة للبروكلي ضد التغيرات البيوكيميائية والإجهاد التأكسدي الكبدي الكلوي الناجم عن التسمم بالرصاص في الفئران البالغة

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الملخص

الرصاص هو أحد المواد الكيميائية العشرة الخطرة التي تشكل مصدر قلق كبير للصحة العامة. الإجهاد التأكسدي هو آلية متورطة في سمية الرصاص. تم تأكيد الأنشطة المضادة للأكسدة لزهرة البروكلي على نطاق واسع. أجريت هذه الدراسة لتقييم التأثيرات التحسينية المحتملة لمسحوق البروكلي ضد التسمم الكبدي الكلوي بالرصاص. تم تقسيم ثلاثين من ذكور الفئران البيضاء إلى خمسة مجموعات من ستة فئران لكل منها على النحو التالي: المجموعة (1): المجموعة الضابطة السالبة، تغذت على الوجبة الأساسية؛ المجموعة (2): الضابطة الموجبة، تلقت خلات الرصاص (25 مجم/كجم من وزن الجسم) يوميًا وتم تغذيتها على الوجبة الأساسية؛ والمجموعات (3-5): تلقت خلات الرصاص وتم تغذيتها على الوجبة الأساسية المحتوية على (3.0 ، 6.0 و 9.0٪) من مسحوق البروكلي، على التوالي. في نهاية التجربة (8 أسابيع) تم ذبح الفئران وجمع عينات الدم وإزالة الكبد والكلى ومعالجتهما لقياس مؤشرات التغيرات البيوكيميائية والاجهاد التأكسدي. تسبب معاملة الفئران بخلات الرصاص في احداث تغيرات بيوكيميائية معنوية تضمنت زيادة في تركيز الرصاص في الدم والكبد والكلى؛ اضطراب في مستويات دهون الدم؛ خلل في وظائف الكبد والكلى. أظهرت المؤشرات الحيوية للإجهاد التأكسدي في أنسجة الكبد والكلى للفئران المعالجة بخلات الرصاص انخفاضًا معنويا (P≤0.05) في مستويات كلا من مضادات الأكسدة غير الانزيمية (الجلوتاثيون في الصورة المختزلة) والإنزيمات المضادة للأكسدة (سوبر أكسيد ديسميوتاز والكاتاليز)؛ وزيادة معنوية في تركيز بيروكسيد الدهون (مالونالدهيد). أظهرت تغذية الفئران المسممة بالرصاص بوجبات مسحوق البروكلي تحسنا معنويا في الموشرات البيوكيميائية وحالة مضادات الأكسدة من خلال استعادة القيم نحو المعدل الطبيعي، وكان التأثير التحسني معتمدًا على التركيز وزاد بزيادة تركيز مسحوق البروكلي، والذي قد يعزى إلى نشاطه المضاد للأكسدة. في الختام، يعتبرالبروكلي مصدرا طبيعيا واعدا للوقاية من سمية الرصاص والتخفيف من حدتها. قد تكون هناك حاجة لمزيد من الدراسات لتحديد آلية العمل بدقة.

الكلمات المفتاحية : البروكلي، سمية الرصاص، الاجهاد التأكسدي، الإنزيمات المضادة للأكسدة، وظائف الكبد، وظائف الكلى، دهون الدم