Physiological and histological effects of broccoli on lead acetate induced hepatotoxicity in young male albino rats

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Abstract

The aim of this study was to investigate the ameliorative effect of Broccoli against lead acetate (PbA) hepatotoxicity by some physiological and histological indicators. The results showed that intraperitoneal (I.P.) injection by 12 mg/kg body weight of lead acetate once a week for 8 weeks led to an increase in the activity of Glutamate pyruvate transaminase (GPT), Glutamate oxaloacetate transaminase (GOT), Alkaline phosphatase (ALP), Acid phosphatase (ACP), Total serum protein (TP) and Total serum bilirubin (TSB). Liver's histological sections of lead acetate injected rats showed infiltration of inflammatory cells with sinusoid dilation, necrosis, and apoptosis of Kupffer cells. Broccoli has an ameliorative effect, that the physiological parameters and histological examination have been showed an improvement. In conclusion, lead acetate produces hepatic disorder and the potential use of Broccoli as a source of natural antioxidants or nutraceuticals protects against lead acetate hepatic toxicity.

Keywords: Broccoli, Lead, Liver function, Liver histology, Histopathology
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التأثيرات الفسلجية والنسجية للبروكلي على السمية الكبدية المحدثة بخلات الرصاص في ذكور الجرذان البيض اليافعة

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الخلاصة

تهدف الدراسة الحالية إلى معرفة التأثير العلاجي للبروكلي على السمية الكبدية المستحدثة بخلات الرصاص PbA في ذكور الجرذان البيض اليافعة بواسطة بعض المعايير الفسلجية والنسجية. أظهرت النتائج أن حقن ١٢ ملغم/كغم من وزن الجسم من خلات الرصاص داخل الصفاق IP مرة واحدة في الأسبوع ولمدة ٨ أسابيع أدت إلى زيادة في نشاط إنزيم كلوتامات بايروفت ترانساميز GPT، إنزيم كلوتامات أوكزالواسيتت ترانساميز GOT، إنزيم الفوسفات القلوي ALP، إنزيم الفوسفات الحمضي ACP، البروتين الكلي TP والبيليروبين الكلي TSB. أظهرت المقاطع النسجية لكبد الجرذان المعاملة بخلات الرصاص ارتشاح الخلايا الالتهابية مع اتساع الجيبانيات، تنخر في خلايا الكبد والموت المبرمج لخلايا كوفر. أظهر البروكلي تأثيراً ايجابياً على المعايير الفسلجية والفحوصات النسيجية في ذكور الجرذان. ونستنتج من هذه الدراسة ان خلات الرصاص ينتج اضطرابًا كبديًا واستخدام البروكلي كمصدر لمضادات الأكسدة الطبيعية أو المغذيات يحمي الكبد من سمية خلات الرصاص.

Introduction

The use of heavy metals is intimately connected to human history, lead is a toxic heavy metal with global concern, there are large number of studies on its effect and another studies about the root of its toxicity, on the other hand how human being protect or cure themselves of this toxic heavy metal for example in recent study on the effect

of lead on children they found that lead accumulated in liver, kidney and bone by blood circulation and they tried to find solution in order to protect school children from lead toxicity (1). Lead (Pb) has been shown to have a multisystem effect like the nervous, hematopoietic, skeletal, renal, circulatory and endocrine systems (2-4). Long-term exposure to Pb may also result in a nephropathy or renal adenocarcinoma, neuromuscular weakness, behavioral, and neurochemical alterations (5,6). These serious affecting of lead can occur through a derangement of the antioxidant enzymes and neurotransmitter balance (7,8). Lead has affinity for proteins and capacity to simulate iron (Fe) and calcium channels (9). The increase in intracellular calcium together with the increase in reactive oxygen species, can trigger apoptosis through the cytochrome C release and a fall in the mitochondrial potential (9,10). Due to the increased using of lead in industry and agriculture we have to develop special nutritional strategies in order to overcome the negative effects of lead toxicity. Dietary strategies are advantageous, as nutritional ingredients can easily and affordably be added to the daily diet (11).

Epidemiological data indicate that frequent fruit and vegetable consumption cause reduction in morbidity and mortality from several dangerous diseases (12,13) and this is due to their high content in polyphenols, vitamins, minerals and other bioactive molecules. Reports on the increasing role and usefulness of medicinal plant products in maintenance and optimization of health have led to research on the efficacy of these medicinal plants/nutrient products' effects on the amelioration of the metal-induced toxicity (14). These plant products are reported to affect the bioavailability, transport, and toxico-dynamics of this metal in the tissue. Broccoli is an edible plant which is classified under the italic cultivar group of species Brassica oleracea. It is belonging to family Brassicaceae. Broccoli has large green flower head which is arranged like a tree like structure branching out from a thick edible stalk and has very promising future (15). Broccoli also helps in attenuate of many diseases like arthritis, kidney diseases, diabetes, and skin disorders. Various researches done earlier showed that it contains sulforaphane which helps in treating many disease (16). Up- to-date study have demonstrated that eating raw Broccoli has been associated with improvement of fertility of male rats and decreasing lead poisoning effects (17). Also, other studies recommended using the whole vegetable instead of using isolated bioactive chemicals to demonstrated the effects of possible interactions of different molecules within of the food matrix on several chronic disease (18). Raw green Broccoli is chemically defined as a complex mixture of very biological active structures like carotenoids, phenols, vitamins C, E, Fe and glucosinolates (19-21) and used as a source of antioxidant and anti-inflammatory effects (22).

The aim of the study is to evaluating the ability of raw Broccoli that has rank among the top 20 foods in regards to ANDI score (Aggregate Nutrient Density Index), which measures vitamin, mineral and phytonutrient content in relation to caloric content (19) to reduce the hepatotoxic effects of Pb in male rats by estimation levels of important liver enzymes, total serum protein, and bilirubin, with microscopic examination of liver.

Material and methods

Experimental animals

Twenty-eight young male rats (160-180) g aged 8-10 weeks were randomly housed in plastic cages. The cages were cleaned and sterilized weekly with 70% ethanol. Each cage was embedded with wooden shelve and maintained under standard laboratory condition for at least two weeks before initiation of the experiments (23).

Broccoli

Dry Broccoli (*Brassica oleracea*) was grinding with using a grinder (WAHL James MARTIN) into a fine powder. The powder used for the Preparation of Standard diet containing 12% Broccoli.

Experimental Design

Twenty-eight young male albino rats, weight 160-180 g were used for this study. Rats were housed in temperature-controlled rooms at 25°C with constant humidity 40-70%, and a 12 h light/dark cycle. All animals were treated in accordance with the principles of laboratory animal care. The rats that included in this study were divided into four groups of seven rats each as follow. A: the animals served as the control group, rats were kept on normal diet and pure distilled water. B: the animals were fed with 12% Broccoli diet and water *ad libitum* for eight weeks. C: the animals were injected I.P.with 12 mg/Kg body weight of lead acetate, ones each a week for eight weeks (24). D: the animals were injected I.P with 12 mg/Kg body weight of lead acetate ones each a week for eight weeks respectively, and were given 12% Broccoli diet and water *ad libitum* during the period of experiment. At the end of each experiment, animals have been fasted for 12 hrs and sacrificed after chloroform anesthesia. Blood samples have been taken by heart puncture and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min by using Centrifuge and micropipette then serum was stored in the deep freeze at -45C°.

Biochemical study

The activity of GPT, GOT, ALP, ACP, TP and TSB of all groups have been determined by enzymatic colorimetric methods using commercial laboratory kit purchased from (BIOLABO-FRANCE) and by using Cobas instrument (25).

Histopathology

After the animals have been sacrificed, the livers were removed and immediately fixed in 10% formalin, treated with conventional grade of alcohol and xylene, embedded in paraffin, and sectioned at 4-6μ thickness. The sections were stained with Hematoxylin and Eosin (H&E) stains for studying the histopathological changes (26).

Statistical Analysis

Analysis of data was performed by using SPSS version 15, results were expressed as mean ± SE statistical differences were determined by Duncan Post Hoc test for multiple comparisons after ANOVA.

Results and discusssion

Occupational and environmental exposure to lead, a toxic metal pollutant, is of global concern. Lead has been found in drinking water. It can come from plumbing and fixture that may be made of lead (27). Absorbed lead is stored in soft tissues mainly the liver tissues (28). The liver is the first organ exposed to internally absorbed nutrients via the portal vein (29). Four enzymes in addition to the levels of total proteins and bilirubin were used to evaluate the function of the liver as shown in table 1, and proved by microscopical examination of histological structure of liver as shown in figures 1-4. Table 1 has been shown that there are no significant differences between group A the control and group B the one that fed on 12% Broccoli.

Table 1: Effect of Broccoli and Lead acetate on serum Glutamate pyruvate transaminase (GPT), Glutamate oxaloacetate transaminase (GOT), Alkaline phosphatase (ALP), Acid phosphatase (ACP), Total serum bilirubin (TSB), and Total serum P (TP)

Parameters	Mean ± SE			
	A	B	C	D
GPT (IU)	122.4±0.19^{a}	122.1±0.24^{a}	144.4±0.15^{c}	125.9±0.08^{b}
GOT (IU)	41.8±0.1^{a}	40.7±0.1^{a}	62.1±0.14^{c}	45.8±0.1^{b}
ALP (IU)	221.9±0.1^{a}	221.8±0.3^{a}	251.9±0.12^{c}	226.6±0.15^{b}
ACP (IU)	4.2±0.03^{a}	4.2 ±0.04^{a}	6.7±0.04^{c}	4.6±0.04^{b}
TSB (mg/dL)	0.2 ± 0.002^{b}	0.2± 0.003^{b}	0.1±0.003^{a}	0.21±0.004 ^{b}
TP (mg/dL)	6.8±0.04^{c}	6.8±0.03^{c}	0.2±0.002^{a}	4.5±0.05^{b}

Standard Error (SE) of Mean P<0.05 significant, similar small letters refers to nonsignificant differences while the different letters refers to significant differences among the groups.

The histological analysis of rats livers that represented in figure 1 and 2 of group A, as well as liver of group B respectively, showed normal histological structure of active functioning cells, the light microscopic images of sections of liver tissue of both A and B groups showing cords of hepatocytes (HC) central veins (Cv), blood sinusoids(BS), Kupffer cells (Kc), and portal area note bile duct (Bd) Figure (1).

While Lead acetate treated group as shown in table 1 reveled significant increasing of the liver function parameters GPT, GOT, ALK and ACP and serum bilirubin while significantly decreasing in the levels of TP compared by control and Broccoli feed groups due to the damage in liver cell of Pb^{+2} intoxicated animals. These results could be expected to occur associating with pathology involving necrosis (N) and apoptosis Kupffer cells (A), infiltration of inflammatory cells (Inc), sinusoid dilation (Bs) of the liver as shown in figure 3.

In such cases the cellular enzymes escape to the plasma from the injured hepatic cells as these enzymes are present in large quantities in the liver. These observations are in agreement with previous study which reported that lead has hepatotoxic effect (30). Alteration the levels of GPT, GOT, ALP and ACP are of value indicating the existence of liver diseases, as this enzyme is present in large quantities in the liver and this in agreement with the study of (31). One of the liver functions is proteins and bilirubin synthesis (29). So, decreasing the level of serum protein and increasing of serum bilirubin are an excellent indicator of liver dysfunction, that may be due to inhibition of protein biosynthesis by liver as a result of lead effects due to precipitation of soluble plasma protein that used by lead poison as carrier and alter the activity of high number of enzymes with disruption of protein synthesis in hepatocytes (32). Also decrease in total serum protein may be due to both hepatic and renal damage induced by lead (33).

Also the result are in agreement with result of (17,34) were their result shown decrease in serum total protein levels that reflects major functional changes liver functions. One of the main targets of lead poisoning.

Also increase in serum bilirubin in lead acetate toxicated rats are agood indecator of hepatotoxicity of lead that in agreement with result of (35). The table 1 and figure 4 indicates the important histological and physiological

effects of Broccoli on the liver because the levels of GPT, GOT, ALK, ACD and TSB decreased significantly in the group that injected with lead acetate and ate Broccoli while the levels of serum TP increased significantly compare to lead acetate treated group, near to their levels in control groups. These results supported by histological examination of liver as shown in figure 4 that revealed the normal cords of hepatocytes, blood sinusoids, and Kupffer cells.

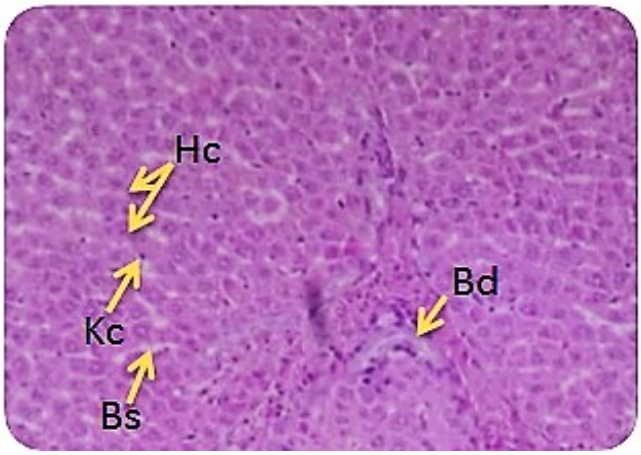


Figure 1: Liver section of group A the control group. The light microscopic images of sections of liver tissue of both A groups showing cords of hepatocytes (HP), blood sinusoids (BS), Kupffer cells (Kc), and portal area note bile duct (Bd). 100x, H&E.

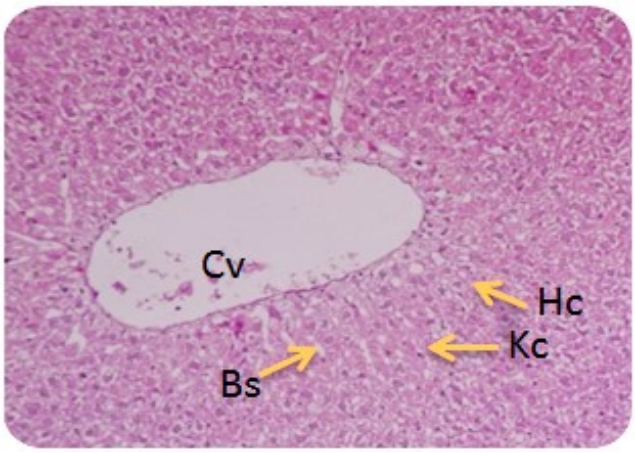


Figure 2: Liver section of group B (that ate Broccoli at 12% in their standard diet). The light microscopic images of sections of liver tissue of both B groups showing cords of hepatocytes (Hc), central veins (Cv), blood sinusoids (BS), and Kupffer cells (Kc). 40x, H&E.

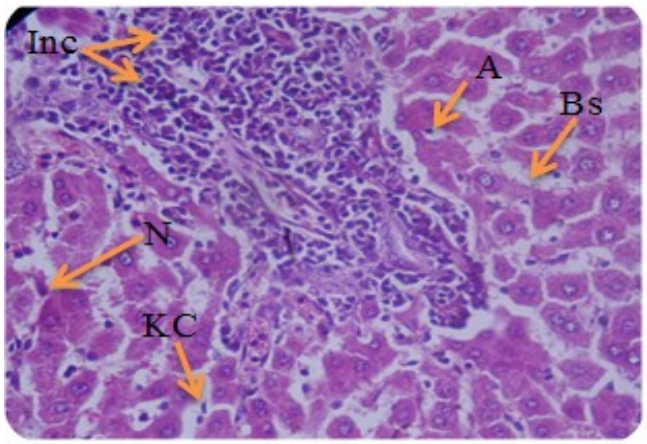
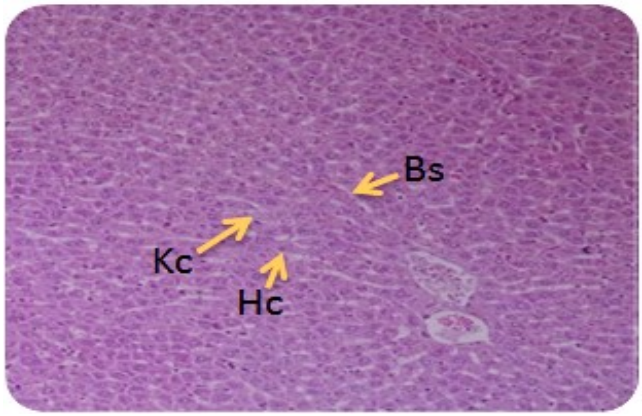


Figure 3: Liver of lead acetate treated rats, that showed great histological changes in the liver tissue of group C that treated by lead acetate. The figure revealed the presence of infiltration of inflammatory cells (Inc) with sinusoid dilation (Bs), necrosis (N), apoptosis of hepatocyte (A), and Kupffer cells (Kc). 400x, H&E.



.Figure 4: Liver section of rat treated with lead acetate and Broccoli. Normal cords of hepatocytes (Hc), blood sinusoids (Bs), and Kupffer cells (Kc). 40x, H&E.

There are several recent studies on the ability of some medical plants like *G. kola* (36) *A. sativum* (14), *L. officinale* (37) and *L. esculentum* (38) to reduce hepatotoxic effect of lead through a hepato-protective role. Protective effects of medicinal plants are varied and depend on the nature of lead exposure (39). Dietary consumption of Brassica vegetables has been associated with a reduction in the incidence of several pathological conditions like cancers and many chronic inflammatory diseases (40). Another recent sudy by (41) they focused on the farmaceutical benefits of Broccoli juices were shown to confer protection to an in vitro model of inflamed human intestinal epithelium based on Caco-2 cells treated with TNF-α under

marginal zinc deprivation. Many reports exist in literature about the efficacy of medicinal plant products in ameliorating or reducing the toxicity and accumulation of lead in tissues due to their metal chelating, antioxidant and scavenging properties (42) so may be Broccoli has chelating activities on lead.

Enhancement of the antioxidant capacity of the liver, reduction of hepatocyte injury and lipid peroxidation, improvement of barrier functions and antioxidant activity, decrease oxidative DNA damage in the liver and increased hepatic detoxification and bile production are some of the ways by which broccolis can offer hepatoprotective ability and reduce toxicity of lead. There are some evidences that vitamin C can inhibit lead uptake at a cellular level as well as lead's cytotoxicity (cellular toxicity). In combination with zinc, glycine and vitamin E, it has been found effective in partially protecting a range of body organs, particularly the liver, from lead-induced damage in animal experiments by acts to protect structures such as cell membranes (43). Broccoli ability to ameliorate hepatotoxicity of lead in rats may be attributed to its high content of vitamins especially C and E and minerals. This study indicates the potential use of broccoli as a source of natural antioxidants or nutraceuticals as protective nutrient from lead hepato- toxicity.

Conclusion

The results of present study showed that the using of lead acetate produces hepatic disorder and the potential use of Broccoli as a source of natural antioxidants or nutraceuticals protects against lead acetate hepatic toxicity.

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