Histopathological study on the effect of antioxidants (vitamin E and selenium) in hepatotoxicity induced by lead acetate in rats

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الخلاصة

اجريت الدراسة للتعرف على تاثير الجرع السمية لمحلول خلات الرصاص من خلال دراسة التغيرات النسجية المرضية في كبد الجرذان المجرعة تجريبيا والتأثير العلاجي المشترك لفيتامين E والسلينيوم في التقليل من التسمم الحاصل للكبد. استخدم في الدراسة 32 من ذكور الجرذان البيضاء البالغة حيث قسمت عشوائيا الى اربع مجاميع (كل مجموعة تألفت من 8 حيوانات). اعطيت المجموعة الاولى ماء شرب فقط وعدت مجموعة سيطرة gI اما بقية المجاميع gII, gIII, gIV فقد اعطيت ماء شرب يحتوي على خلات الرصاص بتراكيز (0.5, 1.0, 1.5%) على التوالي لمدة عشرة اسابيع وتمت التضحية بحيوانين من كل مجموعة وشرحت بعد انتهاء خمسة اسابيع وعشرة اسابيع من المعاملة بخلات الرصاص و بعد اسبوع جرعت الحيوانات يوميا فيتامين E والسلينيوم معا عن طريق الفم لمدة اسبوع واحد اذ جرعت 100 ملغم من فيتامين E و0.25 ملغم من السلينيوم لكل كيلو غرام من وزن الجسم وفي الاسبوع الثاني جرعت 200 ملغم من فيتامين E و 0.5 ملغم من السلينيوم. لكل كيلو غرام من وزن الجسم وتمت التضحية ايضا بحيوانين من كل مجموعة في نهاية كل فترة تجريع بمضادات الاكسدة. اظهرت النتائج ان التسمم بالرصاص سبب تغيرات نسجية مرضية واضحة تدرجت مع الزيادة في مدة التعرض و مع الزيادة في الجرع السمية لخلات الرصاص الا ان المعاملة بمضادات الاكسدة (فيتامينE والسلينيوم) قللت من الاضرار التي اصابت الخلايا الكبدية . يستنتج من الدراسة الحالية ان فيتامين E والسلينيوم يمكن استخدامهما سوية كمواد علاجية للتقليل من سمية الكبد .

Abstract

The study was carried out to evaluate histopathological changes in rats liver induced by different doses and durations of lead acetate toxicity and to investigate the therapeutic effects of vitamin E and Selenium together against lead poisoning.Thirty two adult male rats (*Rattus rattus*), randomized into four groups (n=8) were used for this study. In group I rats received only drinking water as control while in group II , III and IV rats were given o.5%,1%,1.5% lead acetate respectively in drinking water for ten weeks.

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Two animals from each group were sacrificed and and dissected after five weeks and ten weeks of treatment with lead acetate.One week later animal groups were treated orally with vitamin E and selenium as a daily dose for two weeks(100mg Vit.E and 0.25mg Se/kg B.W) for first week and (200mg Vit.E and 0.5mg Se/kg B.W) for second week respectively. Also two animals from each group were sacrificed at the end of each period.The obtained results showed that lead acetate caused progressive histopathological changes depending upon the duration of exposure and concentration of lead acetate in drinking water but the treatment with antioxidants (vitamin E and Selenium together) decrease the damage to liver cells . It can be concluded that those antioxidants seem to have a benefit effects to recovery the liver tissue lead burden and had a therapeutic effects against hepatoxicity.

Introduction

Lead (Pb) had modest early uses in ancient medicines and cosmetics. Today, it has industrial uses in, for example, building materials, paints, and gasoline. Also, lead has many agricultural uses. [1] Health problems has been widely reported due long-term ingestion of contaminated drinking water with heavy metals like lead and arsenic.Among heavy meatals, lead is the most ubiquitous common pervasive environmental pollutant having diverse and deleterious effects on man and animals health. Lead induces a wide range of physiological,biochemical and behavioral dysfunctions.[2,3] The extent to which orally administered lead is absorbed is small. However, due to its slow rate of elimination , harmful levels of lead can accumulate in tissues after prolonged exposure to low quantities . [4]

Lead is a non-threshold multi-targeted toxicant that causes alterations in different organs of the body, including the liver . [5] The absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and interferes with their functions.[6]

Antioxidants are substances ,which inhibit or delay oxidation of a substrate while present in minute amounts.The most important source of antioxidants is provided by nutrition. [7] A number of components in food have been found to have antioxidant properties. These components

include vitamin E and selenium. [8] Vitamin E consists of eight closely related fat soluble compounds ,the most active of which is alpha-tocopherol. There are many signs of vitamin E deficiency in animals ,most of which are related to damage to cell membranes and liver necrosis.[9] Selenium is a metalloid element which is an essential constituent of the enzyme glutathione peroxidase which protects membrane lipids and other cell constituents from oxidative damage by free radicals.Selenium deficiency has been observed in different situations include Keshan disease and cardiomyopathy.[10] Also selenium deficiency has resulted in depressed cellular immunity.[11]

All previous studies has been used either one or more antioxidants in treatment lead toxicity and also in conjunction with chelating agents . Little information is available in the literature regarding the antioxidant activity of vitamin E and selenium together with the exception of the study of Sarhan on the combined effect of vitamin E and selenium on myocardium in rats exposed to lead poisoning.[12] Therefore, the aim of the present study was designed to investigate the therapeutic effects of vitamin E and selenium together on the histopathological changes in rats liver exposed to different doses of lead acetate .

Materials and Methods

Experimental animals

Thirty two adult male rats (*Rattus rattus)* were used for this study .They were obtained from the animal house of college of Medicine ,Alkufa university .The rats were housed in standard cages at room temperature 25-28 C and 12/12 hours light /dark cycle. The rats were fed diet and water *ad libitum* and prior to the treatment they have been acclimatized for one week .

Treatment with lead acetate

The rats were allocated randomly into four groups . Animals in group I served as the control group and were drinking distilled water . Animals in group II , III and IV were drinking respectively o.5%,1%,1.5% lead acetate .Two animals from each group were sacrificed and and dissected after five weeks and ten weeks of treatment (i.e, day 35 and on day 70).

Treatment with antioxidants

One week after the end of treatment with lead acetate ,rats were received drinking water containing vitamin E as dl-alpha-tocopheryl acetate and selenium as sodium selenite as a daily dose for two weeks(100mg Vit.E and 0.25mg Se/kg B.W) for first week and (200mg Vit.E and 0.5mg Se/kg B.W) for second week respectively.Two animals from each group were sacrificed at the end of each period.

Histological preparations

The specimens of liver tissues were immediately fixed in 10% formalin, then treated with conventional grade of alcohol and xylol, embedded in paraffin and sectioned at 4-6 micrometer thickness.The sections were stained with Haematoxylin and Eosin (H & E) stain for studying histopathological changes .[13]

Results

Control group:-

The histological sections of liver tissues demonstrated normal histological structure (Fig 1.A).

Lead poisoning :-

In the present investigation , histological analysis of the liver sections revealed the pathological condition subjected to various doses of lead acetate administration in two different periods.

First period (5 weeks):-

In group II , the central veins and sinusoids were dilated and congested with blood . furthermore,the hepatocytes cells mildly degenerated (Fig 1.B) .

In group III , the hepatocytes were necrosed in association with the dilation and congestion of sinusoids (Fig 1.C). The investigation of hepatocytes in group IV showed degeneration of hepatocytes starting in sever form (Fig 1.D).

Second period (10 weeks):-

In this period , the liver sections exhibited marked histological changes in the treated animal groups . These changes include distortion of hepatic cords and necrosis of hepatocytes that revealed in group II (Fig 1.E).The lesions in group III still chtion alizracterized by sever sinusoids dilation with more necrosis of hepatocytes (Fig 1.F).In group

IV abnormal localization of hepatocytes nuclei were appreciated (karyorrhexis) and atrophy of hepatic cords appeared smaller than congested sinusoids (Fig 1.G). Furthermore the liver tissue was disintegrated and the damage was typified by large open spaces (Fig 1.H).

Treatment with antioxidants :

First period (First week) :-

Liver sections of rats treated with antioxidants (100 mg vit.E and 0.25 mg se/kg B.W) for one week revealed that most of the histological alterations induced in lead acetate treated groups were markedly reduced in which the histological analysis manifested that the histological changes observed after lead acetate treatment were attenuated from sever to moderate alterations after treatment with antioxidants. The histological diagnosis of liver sections in group II showed the begining of regeneration by division of some hepatocytes in which there were marked mitotic figures and clear activity of kupffer cells (Fig 2.A) . Group III showed more regeneration with numerous mitotic figures. Sinusoids became narrowand and there were kupffer cells also can be observed (Fig 2.B), while in group IV no clear lesion can be observed ,there were more regeneration with highly mitotic division .The hepatic cords became larger than sinusoids and no congestion can be observed (Fig 2.C).

Second period (Second week):-

The diagnosis of the present results after two weeks of treated groups with antioxidants (100 mg vit.E and 0.25 mg se/kg B.W for first week and 200 mg vit. E and o.5 mg se/kg B.W for second week) showed no appreciable lesions were seen in the liver tissues . The congestion was disappeared and the hepatic sinusoids became narrow irregular blood vessels with infiltration of eosinophils. Mitotic figures and kupffer cells also observed (Fig 2 D, E). Enlargement of hepatic cords were observed and became larger than sinusoids with increasing of eosinophils (Fig 2 F)

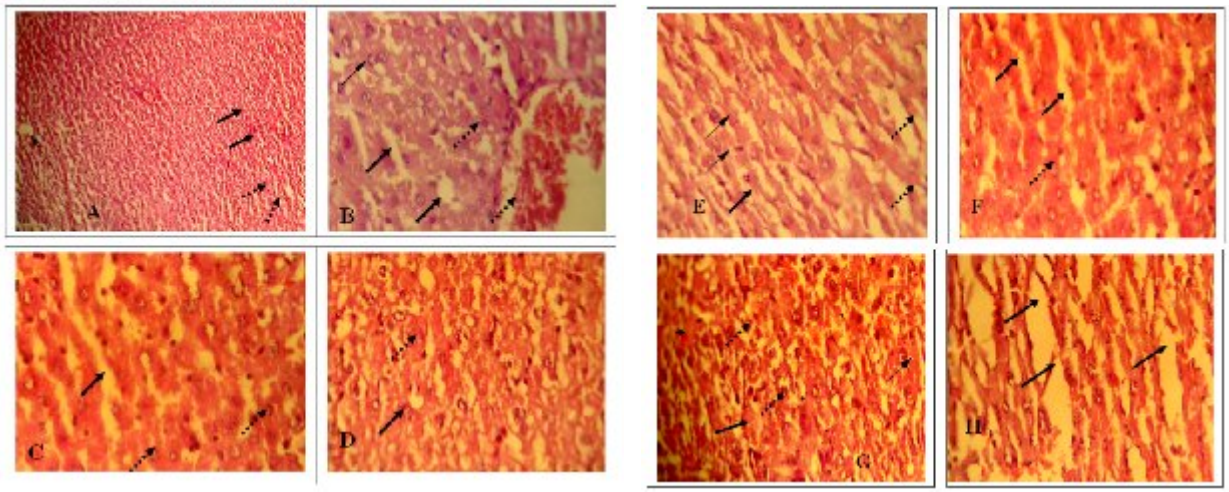

Figure 1: light micrograph sections of liver showing:
A : normal structure of the liver (H&E 100X).
B : beginning of sinusoids dilation (⟶), congestion of central vein (⇢) and degeneration of some hepatocytes (⟶) (H&E 400X).
C : sinusoid dilation (⟶) with necrotic of hepatocytes (⇢) (H&E 400X).
D: degeneration of hepatocytes starts in severe forms (⟶) with congestion (⇢) (H&E 400X).
E: the hepatic cords (⟶) are smaller than sinusoids (⇢) with necrosis of hepatocytes (⟶) (H&E 400X).
F: sinusoids dilation (⟶) with severe necrosis of hepatocytes (⇢) (H&E 400X).
G: congestion of sinusoids (⟶) and necrotic of most of hepatocytes (⇢) with atrophy of hepatic cords (⟶) (H&E 400X).
H: tissue disintegration and damage is typified by large open spaces (H&E 400X).

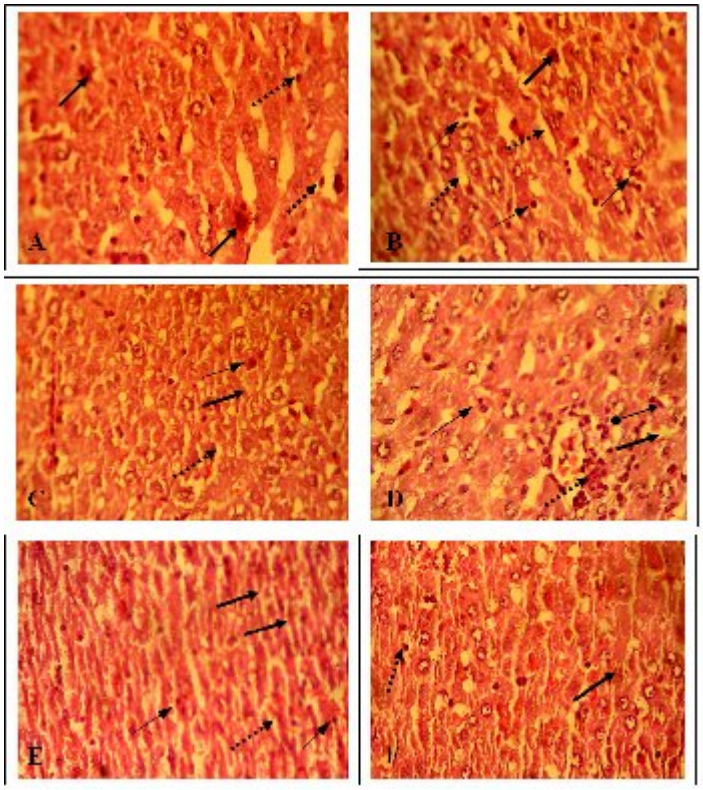

Figure 2: light micrograph sections of rat's liver showing:

A**:** beginning of regeneration by division of some hepatocytes (mitosis) or mitotic figures (⟶) with clear action of kupffer cells (⇢) (H&E 400X).

B: more mitotic figures (⟶), sinusoids become narrow (⇢) with numerous of kupffer cells (⟶) (H&E 400X).

C: more regeneration and the hepatic cords (⟶) become larger than sinusoids (⇢) , no congestion, more mitotic figures (⟶) (H&E 400X).

D: disappearance of congestion , the sinusoids become narrow (⟶) , infiltration of esinophils in sinusoids (⟶) with mitotic figures (⟶) and kupffer cells (⇢) **(**H&E 400X).

E: the sinusoids (⟶) become narrower than hepatic cords (⇢) , with increase in number of esinophils in sinusoids (⟶) **(**H&E 400X).

F: enlargement of hepatic cords (⟶) with increase in number of esinophils (⇢) **(**H&E 400X).

Discussion

Lead poisoning:-

Absorbed lead is stored in soft tissues mainly the liver tissues. [14]The liver, via the portal vein , is the first organ exposed to internally absorbed nutrients in which the histological analysis can be used to examine the morphological changes to reflect possible effect of lead on the hepatocytes .[15]

The results of present investigation showed that subtoxic chronic lead exposure resulted in progressive alterations , some of these were the begining of sinusoids dilation and congestion of central veins with degeneration and necrosis of some hepatocytes and then developed to sever forms according to extent of exposure duration .

All the above results appeared after the first period (five weeks) of the treatment with different concentrations of lead acetate and this completely agree with [15] who reported that lead poisoning via drinking water caused dilation of hepatic vein and congestion of blood vessels with necrosis. Similar hepatotoxicity lesions were also reported by [3] who indicated that the histopathological changes in liver exposed to lead acetate include enlargement of blood vessels along with sinusoids hemorrhage and dilation of central veins.

In second period (ten weeks) of treatment with lead acetate,the severity of lesion by lead poisoning was more pronounced in comparing to first period (five weeks) of treatment which characterized by marked congestion and dilation of blood vessels and necrosis of hepatocytes with distortion of hepatic cords . These findings are in agreement with those mentioned by [3,14] about the histological changes in liver tissue of rats by exposure of lead acetate.

Furthermore ,the present observations of this period showed disintegration of the hepatocytes of treated groups when compared with control group.The disintegration evidenced by the presence of large open spaces .This further supports the fact that lead portends serious damaging effects on the hepatocytes . Also variable degree of nuclear alterations (like karyorrhexis) in which they were represented the early histological alterations developed due to long-term intoxication. This confirms that the nucleus is the key factor in most cell disturbances to

injurious agents and undergoes morphological and functional alterations due to lead poisoning. [16]

It can be said that the varieties in the histological alterations of the liver tissues due to lead intoxication obtained by different investigators could be due to the variations in the level of exposure , duration , route of administration and animal species used in the experiments.

Treatment with antioxidants:-

The histological examination of liver tissue in rats groups treated with daily doses of vitamin E and selenium for two periods (one and two weeks) revealed that most of the histological alterations induced in lead acetate treated groups were markedly reduced .The micrographs of these rats (Fig.2) showed minimal
alterations as compared to the lead acetate treated rats (Fig.1).These findings confirmed the protective effect of vitamin E and selenium against the histological changes in lead acetate hepatotoxicity.

In liver and other soft tissues, Lead-induced oxidative stress has been suggested to be one of the possible mechanisms of lead-induced toxic effects.[2,17-20]Disruption of pro-oxidant/antioxidant balance might lead to the tissue injury. It was reported that lead increased the level of lipid peroxidation [21] and brain thiobarbituric acid-reactive substances and altered the antioxidant defense system .[22] A number of previous studies confirmed the possible involvement of reactive oxygen species(R0S) in lead-induced toxicity. [23-26] Several antioxidants have been used to evaluate lead-induced oxidative damage in animal and human studies. Mudipalli [27] and Madiha *et al* [28] stated that, supplementation with antioxidant agents was explored in experimental laboratory animals to evaluate the protective effects of these agents in reducing overall toxicity, particularly hepatotoxicity.

The modifications in superoxide dismutase activity are the most frequently used markers in tissues.[29] It has been postulated that supplementation of antioxidants might be an alternative method for chelation therapy on the basis of the observation that free radical was generated during the pathogenesis processes induced by lead exposure [30-33]
.

On the other hand, the obtained results indicated that the treatment with vitamin E and selenium caused increase in the number of the

mononuclear cells which are associated with the increase in body defense mechanisms in which vitamin E converts arachiodonic acid into prostaglandin that plays an important role in enhancement of immune response [34] and Lead can bind to selenium and form highly bonded selenium-lead complexes, which have been proposed as a mechanism for selenium's protective effect in lead toxicity. [27,35]

The present results are in agreement with various findings strongly suggested the enhancement of immune response due to vitamin E and selenium treatment in both human and animal models. In this direction [34,36,37] stated that the supplementation of vitamin E alone enhances the humoral immune response in chickens. Similar findings in rodents including rats were reported by. [27,38-40]

On the other hand ,several studies suggested a specific essential role for selenium supplementation alone in improving the immune system function by enhancement the cellular and humoral immunity in mice [41] and human [42,43] in which it appears to be associated with affecting the metabolism of carcinogens and inhibits tumor cell growth[44] while it's deficit causes a decrease of immunologic functions. [45]

It can be said that the present findings lead to the conclusion that vitamin E and selenium together decreased the adverse harmful effect of lead acetate treatment at different doses and durations and they had a therapeutic effects against hepatoxicity.

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