COMBINED THERAPEUTIC POTENTIAL OF EDTA DISODIUM SALT AND (GARLIC) ALLIUM SATIVUM L. IN THE MANAGEMENT OF EXPERIMENTAL LEAD INTOXICATION OF THE RAT ALVEOLAR PROCESS

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ABSTRACT

Objectives: The aim of the present study was to compare between two treatments in prevention of lead-induced toxicity in alveolar bone. EDTA disodium salt was used individually or as a combined therapy with Garlic extract.

Methods: Forty adult male Wistar rats with an average weight 150-200 g were randomized into 4 groups, control (C), lead administration (GI), and lead administration followed by administration of EDTA disodium salt (GII), both Garlic and EDTA disodium salt were administrated in (GIII) after lead administration. After 6 weeks, the rats were sacrificed. The mandibles were examined histologically, histomorphometrically and ultrastructurally.

Results: Histopathological and ultrastructural results revealed disorganized wide marrow cavities in GI. The marrow cavities of GII were narrower than those in G I. However, group III showed interdigitating bone trabeculae with narrow medullary cavities almost similar to that of control group. Histomorphometric analysis showed significant reduction in area percentage of bone in the lead group ($p= 0.042$). Groups II, III showed insignificant reduction in the area percentage of bone compared to the control ($p= 0.423$, $p= 0.863$) respectively.

Conclusions: Lead produced remarkable defect in the alveolar process of the rats. EDTA disodium salt proved to improve the architecture of the alveolar process; however, the supplementation of garlic along with EDTA disodium salt as a combination therapy ameliorated the treatment regimen than monotherapy with chelating agent alone.

INTRODUCTION

Lead is one of the most ubiquitous toxic materials encountered in everyday life. Lead is commonly used for commercial purposes and many household products. Lead poisoning is one of the oldest occupational and environmental diseases in the world. Despite its recognized hazards, lead continues to have widespread commercial application. The highest level of exposures of lead occurs principally among people working in lead smelters. In the general population, the major hazard is for young children who chew and swallow objects contaminated with lead containing

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paint on walls and woodwork. Lead is a common cause of poisoning of domestic animals throughout the world. Lead poisoning can occur in all domestic animals including horses, birds/poultry and dogs [1].

Lead is a heavy metal that is present in petrol and octane has been known for many years to produce toxic effects on the central nervous systems. Lead may be deposited in the red blood corpuscles, soft tissues of children mainly in the kidney region, but the greater concerning matter is that 70 to 90% of this lead is deposited in bones. This is the most hazardous because the half-life of lead in the bones is 28 years, whereas lead in the blood and kidneys remains only up to two to four weeks [2].

Monir et al showed that Pb increase bone turnover resulting in weaker cortical bone in adult female mice and suggest that Pb may exacerbate bone loss and osteoporosis in the elderly [3]. It also delays fracture healing at environmentally relevant doses and induces fibrous non unions at higher doses by inhibiting the progression of endochondrial ossification [4]. Researchers showed that Pb hibernate within bone for decades. Although Pb within bone is of uncertain toxicity to bone tissue, conditions of bone resorption, such as osteoporosis, can cause bone Pb to reenter the blood stream where it can then re-expose the soft tissue and potentially, exert delayed deleterious effects [5].

Several studies demonstrate that acute and chronic human intoxications with a wide range of metals can be treated with considerable efficiency by the administration of a relevant chelating agent. The chelating agent formed a stable complex with a toxic metal that shield the metal ion from biological targets, thereby reducing the toxicity [6].

Fresh garlic is an important dietary source of antioxidant phytochemical products that prevent oxidative damage by scavenging free radicals [7].It suppresses the generation of reactive oxygen species and also attenuated caspase-3 activation and DNA fragmentation, associated with apoptosis [8]. Many researchers showed and examined that the effect of treatment with garlic not only confer protection against lead toxicity but it can also perform therapeutical role against toxicity [9].

The objective of the present study is conducted to investigate the effect of EDTA disodium salt alone or associated with Garlic as a combined therapy in lead induced toxicity in rat's alveolar process.

MATERIAL AND METHODS:

Experimental procedure

Forty adult male Wistar rats with an average weight of 150-200 g were used in this study. They were randomly assigned to four treatment groups, 10 rats each. Control group was intraperitoneally injected with 9% saline. Group (I) was intraperitoneally injected with lead acetate* 5 mg/kg body weight daily for 6 weeks [10]. Group (II) received lead acetate 5 mg/kg body weight for 3 weeks followed by concomitant administration of 50 mg/kg body weight /day [11] Ethylene Diamine Tetra Acetic acid disodium salt EDTA** for next 3 weeks. Group (III) received lead acetate 5 mg/kg body weight with concomitant oral administration of garlic powder *** at 40g/kg diet [12].

At the end of 6 weeks, animals were sacrificed by cervical translocation. The mandibles were dissected. The right side of each mandible was used for the light microscopic examination and the histomorphometric analysis, forming a total of 40 bone segments (10 specimens from each group).

* Powder 500g, produced by ADWIC, El Nasr Pharmaceutical Chemicals Co.Egypt.
** Powder 500 g, produced by Oxford Laboratory Reagent, India.
*** Powder 130 g produced by Spicy Trade Co., Egypt.

The other 40 bone segments (10 specimens from each group) of the left sides of the mandibles were used for scanning electron microscopic study.

Light microscopic examination

Specimens were immediately fixed in 10% neutral formalin for 48 h, washed and soaked in 10% EDTA for decalcification for 4 weeks, and then rinsed in distilled water. Specimens were dehydrated in ascending grades of alcohol and embedded in paraffin. From each mandible, 40 to 50 bucco-lingual serial sections of 5 μm thickness were cut from the predilection site: the alveolar bone apical to the first molar. The sections were subjected to haematoxylin and eosin stain according to the conventional method. Histopathologic examination was performed using light microscopy.

Histomorphometric analyses

The data were obtained using Leica Qwin 500 image analyzer computer system (England). The image analyzer consisted of a coloured video camera, coloured monitor, hard disc of IBM personal computer connected to the microscope, and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measument units (pixels) produced by the image analyzer program into actual micrometer units.

The area & area % of bone trabecule of alveolar bone were measured using an objective lens of magnification 20, i.e of a total magnification of 200. Ten fields were measured for each specimen (Fig. 1a). Using the colour detect areas were masked by a blue binary colour. The area % was calculated in relation to a standard measuring frame of area 118476.6 micrometer square (Fig. 1b). The terminology and units used are those recommended by the Histomorphometry Nomenclature committee of the American Society for Bone and Mineral Research [13].

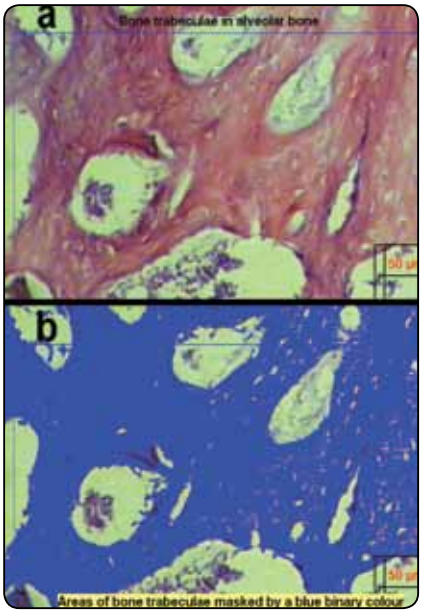

FIG. (1) (a) Bone trabeculae of the alveolar bone process. (b) Areas of the alveolar bone trabeculae masked by a blue binary color.

The data obtained were statistically described in terms of range, mean ± standard deviation (± SD), and median. Comparison between the study groups was done using Kruskal Wallis analysis of variance (ANOVA) test with Conover-Inman test for independent samples as posthoc multiple 2-group comparisons. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and Stats Direct statistical software version 2.7.2 for MS Windows, StatsDirect Ltd., Cheshire, UK.

Scanning electron microscopy (SEM)

The specimens were mounted on SEM stubs. Using SEM Model Quanta FEG 250 (Field Emission Gun) attached with EDX Unit, with accelerating voltage 30 K.V. Samples were not coated with gold or carbon.

RESULTS

Histological results

The control group

The spongiosa of the alveolar process revealed normal architecture. It consisted of dense interconnecting bone trabeculae enclosing medullary cavities containing the bone marrow (fig.2a).

The lead group I

The spongiosa of group I appeared thinner than that of the control group. Irregular bone trabeculae with wide marrow spaces in-between were observed. Thin bone spicules with abundant marrow cavities were also revealed. Howship's lacunae were evident along the lining of the socket denoting osteoclastic activities; osteoclasts were occasionally observed in the resorptive bays (fig.2b).

The EDTA group II:

The bone trabeculae of group II were thicker and denser; the medullary cavities on the other hand, were smaller and fewer than that revealed in group I. osteoclastic activities were still evident along the socket lining, with the osteoclasts seen in the Howship's lacunae (fig.2c).

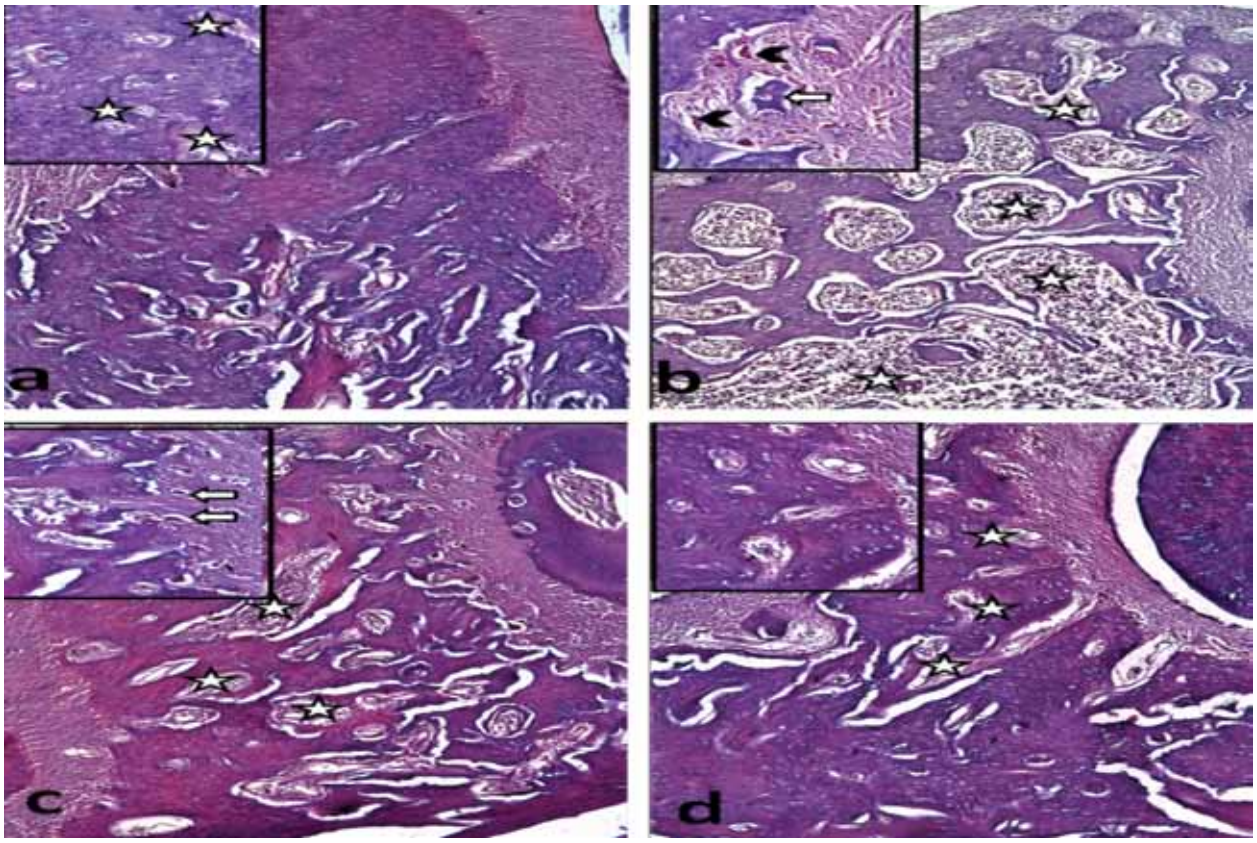

FIG. 2 (a): Control group showing the normal architecture of the alveolar process. Inset: revealing bone trabeculae enclosing marrow spaces in-between (asterisks). (b) Group I with thin bone trabeculae enclosing wide medullary spaces (asterisks). Inset: magnified Howship's lacunae enclosing osteoclasts (arrowheads) and sequestrated bone fragment (arrow). (c) group II showing thick bone trabeculae with resorptive bays enclosing narrower marrow spaces than those in group I (asterisks). Inset: showing high magnification of the resorptive bays and osteoclasts (arrows).(d) group III showing an almost normal architecture of the alveolar process with thick bone trabeculae, and marrow cavities in between (asterisks). Inset: showing magnified bone trabeculae with no resorptive bays . Figs. a, b, c and d (H&E X100), Insets (H&E, 200 X).

TABLE (1) Mean values and standard deviation of the area % of alveolar bone trabeculae in the mandible of the control and experimental groups.

Variables	Area %-C	Area %-GI	Area % -GII	Area % -GIII
Number of samples	10	10	10	10
Mean	69.40	38.53	52.89	60.78
Standard deviation	15.123	6.548	11.061	5.038
Maximum	88.21	47.33	62.132	67.022
Median	73.23	37.42	55.41	62.21
Minimum	53.20	31.44	34.203	54.064

The EDTA_Garlic group III

The architecture of the spongiosa of the alveolar process was almost similar to that of the control group. Dense and thick bone trabeculae were seen interdigitating with each other, with medullary cavities enclosed in between. A smooth socket lining was observed lacking any resorptive bays (fig.2d).

Histomorphometric analyses

The area % occupied by alveolar bone trabeculae in the mandible of the control and experimental groups is summarized by means, standard deviation and median in Table 1.

A decrease in the area percentage of bone trabeculae was evident in the jaws of the three experimental groups. This decrease was statistically significant only in the mandible of group I (p= 0.042), and insignificant in the other experimental groups II, III when compared to the control group (p= 0.423, p=0.863) respectively.

Scanning electron microscopy

On examining the alveolar bone apical to the 1st molar of control animals, thick interdigitating alveolar bone trabeculae enclosing medullary cavities were quite visible at low magnification. Group I revealed thin interconnecting bone trabecular with wide medullary cavities in-between. The bone trabeculae of group II were relatively thicker than that in group I, with wide medullary cavities in-between. Group III was almost similar to that of the control group. Thick bone trabeculae were seen interdigitating with each other, with narrow and numerous medullary cavities enclosed in between (fig.3).

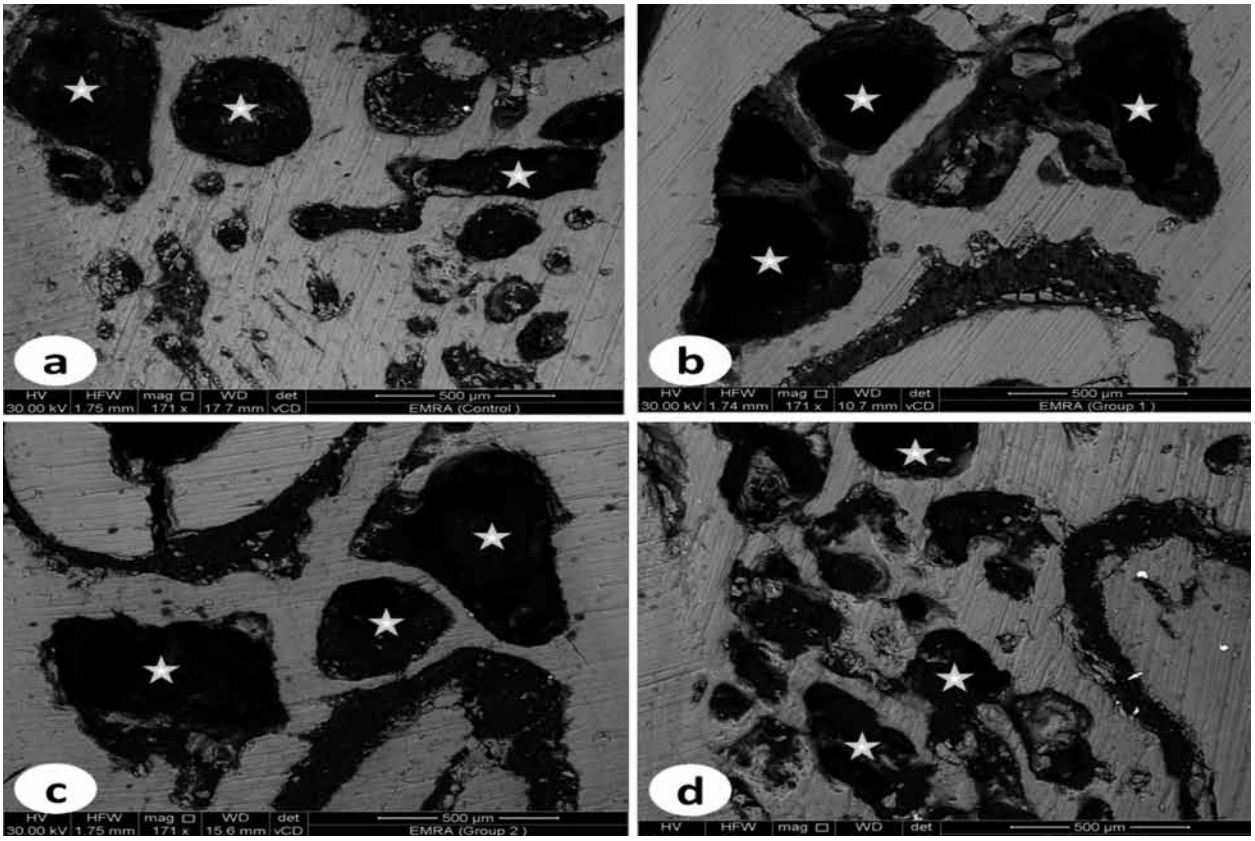

FIG. (3) Scanning electron micrograph of the alveolar bone apical to 1st molar showing; (a) control group with thick interdigitating bone trabeculae enclosing marrow cavities (asterisks). (b) group I with thin bone trabeculae and wide marrow cavities (asterisks) (c) group II revealed relatively thick bone trabeculae with wide marrow cavities enclosed in between (asterisks) (d) group III showed alveolar bone almost similar to that in control group, marrow cavities (asterisks) (171x).

DISCUSSION

Our study demonstrate that lead intoxication produces a remarkably deleterious effect on the alveolar process as revealed by the histopathological and ultrastructural results of group I. Thinning of the bone trabeculae, widening of the marrow spaces, as well as noticeable osteoclastic resorption along the socket lining with small bone spicules even entirely sequestered off the alveolar process. To confirm our histological results, comparing the area percentage between the control group and group I was estimated in H&E sections using the image analyser system. Histomorphometric results showed a noticeable decrease in the area percentage of bone in group II compared to the control group, the decrease was statistically significant (p=.042).

It was documented that in female adult mice lead significantly decreased the bone mineral density in the cortical and cancellous bone, and increased the marrow area in the cortical bone when examined with micro C.T. lead also significantly decreased the mineral/ matrix ratio, collagen maturity in trabecular bone, and increased the bone formation resorption markers [3]. Radiographic and histological analysis carried out by Jonathan et al.[14] demonstrated that in mice exposed to low lead concentration, healing was characterized by delaying bridging cartilage formation, decreased collagen type II and X expression as well as delayed maturation and calcification.

There are several conceptual and technical intricacies to establishing the relationship of lead exposure to skeletal toxicity and to elucidating the

cellular and molecular mechanisms of lead toxicity in bone. Lead changes the levels of the circulating hormones which modulate the bone cell functions [15-21]. Lead may also directly alter the bone cell function by perturbing the ability of bone cells to respond to hormonal regulation [22-24].

Extensive experience demonstrates that acute and chronic human intoxications with wide range of metals can be treated with considerable efficiency by the administration of a relevant chelating agent [25]. The important end point of chelation should be reduction of metal toxicity through forming a complex with the toxic metal thus shielding the metal ion from biological targets, thereby reducing toxicity. It has been reported that chelation therapy with $CaNa_2EDTA$ is medically accepted for treatment of lead toxicity in bone [26]

One of the reasons for the deleterious effects of lead is its ability to strongly bind to sulfhydryl groups of proteins and to mimic or compete with calcium [27], inhibit calcium entry into the cells, disorder mitochondrial calcium homeostasis, ATP production, and apoptogenic factors [28-30]. Lead toxicity is also associated with increased ROS levels, which in turn cause an imbalance in calcium regulation. Chelation therapy is thought to not only remove contaminating metals but also to decrease free radical production [25,31].

The ongoing histopathological and SEM results of group II are in accordance with the concept that chelation therapy is considered to be one of the best known treatments against metal poisoning. Thicker bone trabeculae and narrower marrow spaces were revealed in the studied group II compared to those in the lead group I. An increase in the area percentage in group II was also present compared to that in group I, however the increase was statistically insignificant (p=.235).

In the present research, supplementation of garlic powder (Allium sativum L) along with Na_2EDTA proves to be a better treatment regimen than monotherapy with chelating agent. Histopathological and ultrastructural results of group III in the herein study was remarkably improved. Thick bone trabeculae enclosing narrow marrow spaces have been revealed. An increase in the bone area percentage of group III compared to that in group I was also established, the increase was statistically significant (p=.002).

Garlic administration proved to prevent the accumulation of lead in bone, lung, heart, liver, kidneys and skeletal muscles of goats [32,33]. The prophylactic efficacy of garlic extract to reduce tissue lead concentration was evaluated experimentally in rats, where the mean lead concentration in liver, brain and bone was considerably decreased [34]. It has been suggested that the efficiency of garlic is perhaps due to the presence of the sulfur containing amino acids and compounds having free carboxyl (C=0) and amino (NH_2) groups in their structures. These biologically active compounds might chelate lead and reduce its accumulation in the tissues [33]. Other published results also proved the ability of garlic in preventing lead absorption from the gastrointestinal tract, and it is suggested that the beneficial effect of garlic is perhaps due to the combined effect on metal absorption and its excretion from the body [35].

In conclusion the current study proved that lead produced remarkable defect in the alveolar process of the rats. Na_2EDTA proved to improve the architecture of the alveolar process; however, the supplementation of garlic along with Na_2EDTA as a combination therapy ameliorated the treatment regimen than monotherapy with chelating agent alone.

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