The Scientific Journal of University of Benghazi

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Effects of Interaction between Cadmium and Selenium on Parotid Glands in Rats (Histopathological and Histochemical Studies)

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Received: 26 / 04 / 2021; Accepted: 05 / 06 / 2021

الملخص:

الكادميوم (Cd) هو أحد الملوثات الكيميائية الرئيسية الموجودة في بيئة البلدان المتقدمة. تعتبر السجائر مصدرًا كبيرًا للمعدن، مما يجعلها مهمة من حيث صحة تجويف الفم. الكادميوم كائن حيوي نشط بشكل خاص، يدمر التمثيل الغذائي الخلوي على مستوى الأنظمة الأنزيمية المختلفة للخلية، مما قد يزعج عمل الغدد اللعابية .

استغرقت التجربة 3 أشهر وأجريت على 50 جرذًا بالغًا من الذكور. تم اختيار الجرذان بشكل عشوائي إلى 5 مجموعات، المجموعة 1 (المجموعة الضابطة) تلقت المجموعة الثانية 50/1 سي دي وحدها، المجموعة الثالثة تلقت 100/1 سي دي، المجموعة الرابعة تلقت سيلينوم (سي) +50/1 سي دي والمجموعة الخامسة سي دي + 100/1 سي دي. تمت إضافة المكملات أثناء التعرض لـ Cd و Se في مياه الشرب .

تظهر التغيرات النسيجية المرضية والكيميائية النسيجية لخلايا الغدة النكفية بشكل أكثر وضوحًا عند تركيز الكادميوم بينما تؤكد بيانات النتائج التأثير الوقائي لزيادة تناول السيلينيوم على أنسجة الغدة النكفية في التعرض المزمن للكادميوم .

في الختام، تؤكد بيانات النتائج التأثير الوقائي لتناول Se على أنسجة الغدة النكفية في التعرض المزمن للـ CD.

الكلمات المفتاحية:

الكادميوم ، السيلينيوم ،الغدد اللعابية.

Abstract

Purpose: Cadmium (Cd) is one of the main chemical pollutants found in the environment of developed countries. Cigarettes are a significant source of the metal, which makes them notable in terms of oral cavity health. Cadmium, a particularly active xenobiotic, damages cellular metabolism at the level of various enzymatic systems of the cell, which may disturb the functioning of salivary glands.

Material and Methods: The experiment presented in this paper was conducted over the period of three months and was conducted on 50 adult male rats. The rats were randomized into five groups. Group 1 (control), Group II received 1/50 Cd alone, Group III received 1/100 Cd, Group IV received Selinum (Se) +1/50 Cd and Group V received Se+ 1/100 Cd. Supplementation during exposure to Cd and Se was administrated in drinking water.

Results: The histopathological and histochemical changes of the cells of the parotid gland are most pronounced at Cd concentration while the outcome data confirmed the protective effect of increased Se intake on the parotid gland tissue in chronic Cd exposure.

Conclusion: The outcome data confirmed the protective effect of Se intake on the parotid gland tissue in chronic Cd exposure.

Keywords: Cadmium, Selenium, salivary gland.

1. INTRODUCTION

The more advanced the anthropogenic processes, the greater the impact of heavy metals on the organism. Industrial waste contains cadmium, which is commonly found in polluted areas but is absolutely dispensable and toxic to human organisms. Several studies on cadmium metabolism have revealed that it is almost exclusively toxic in its effects [(1)]. Cadmium exerts multi-organ toxic effects in mammals and the kidney is the critical target organ for cadmium toxicity; the nephrotoxicity of cadmium has been extensively studied and widely reported in literature [(2)]. There is growing evidence that oxidative stress via reactive oxygen species (ROS) generation and mitochondrial damage are among the fundamental molecular mechanisms of cadmium nephrotoxicity [(3,4)]. Cadmium toxicity is reported as one of the factors responsible for many disorders and diseases in humans. It has been reported that Cd induces cardiovascular diseases and arterial hypertension[(5)]. Moreover, cadmium is particularly xenobiotic and damages cellular metabolism at the level of various enzymatic systems of the cells, which may disturb the functioning of the salivary glands [(6)].

The mutual interaction of cadmium and zinc suggests a protective role from zinc through the induction of metallothione which inactivates cadmium[(6)]. Cadmium accumulates in various tissues and organs and may have serious consequences for the general population's health[(2)]. Chronic exposure to cadmium may damage the kidneys, bones, liver, lungs, and other organs, including sublingual glands [(7, 8, 9)]. Accordingly, intensive research has been

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conducted on how to neutralize its harmful effects. On the other hand, previous studies indicated that selenium was toxic but only at high concentrations [10]. It seems to suppress the deleterious effects of cadmium at lower doses [10]. Selenium is an indispensable element participating in the reduction of free radicals in cells of the organs of the body [11]. Selenium is like a factor inducing Cd redistribution from metallothionein proteins and Se binds to multi-molecular proteins via sulfur bonds. Dietary deficiency in Se enhances the toxic effects of cadmium [12 , 13]. Cadmium is absorbed from the gastrointestinal tract and the lungs and is mainly accumulated in the liver and kidneys where it is bound to metallothionein (MT). When the amount of Cd in the body exceeds the binding capability of MT, the non-MT-bound Cd ions cause hepato- and nephrotoxicity [14]. The antioxidant defence system is in turn affected by alteration of trace element balance since several trace elements, such as Se, Cu, iron (Fe) and Zn are an integral part of various antioxidant enzymes. Previous studies in rats revealed that essential trace elements like Zn and Se may modify health risks from exposure to Cd [15,16]. Cadmium is toxic to the cellular processes by dis¬rupting the mitochondrial function [15] and can interfere with the transportation and metabolism of many essential metals, such as iron, copper and zinc [5]. Adequate availability of both zinc and copper is essential for normal growth and development. In¬sufficient zinc availability in fetal or early postna¬tal life retards growth [17].

The aim of present study is to investigate the effects of cadmium, selenium and cadmium- interaction on parotid gland in rats.

2. Material and Methods

The experiment took three months and was conducted on 50 adult male rats with an initial body weight of 220 g. Throughout the experiment period, the animals were kept in normal conditions. They were housed in stainless-steel cages (five mice/cage) in a room maintained at 18-20° C on a 12-h alternating light-dark cycle. The animals were classified into five groups each with 10 rats/group.

Group I (C) Controls were fed a standard diet and given tap water ad libitium.

Group II (Cd). Experimental animals received a standard diet and tap water supplemented daily with 1.76 mg of medication /kg (mgm/kg) 1/50 (Cd as cadmium acetate)

Group III. Experimental animals received a standard diet and tap water supplemented daily with 1/100 (Cd as cadmium acetate0.88 mgm/kg)

Group IV. Experimental animals of group II received a standard diet and tap water supplemented daily with 5.0 mg Se/kg (Se as acid sodium selenite). The selenium dose was specially prepared and daily weighed.

Group V. Experimental animals of group III received a standard diet and tap water supplemented daily with 5.0 mg Se/kg Se as acid sodium selenite. The selenium dose was specially prepared and daily weighed.

Cd and Se were given orally using syringes three times weekly for three months.

The animals were scarified and parotid glands were fixed in 10% neutral formalin, embedded in paraffin and prepared sections (5 µm thick) were stained with:

1-Harris haematoxyline and Eosin (H&E) for histopathological examination.

2-Azo dye reaction and stain for alkaline phosphatase activity study

3. Results

Histopathological study:

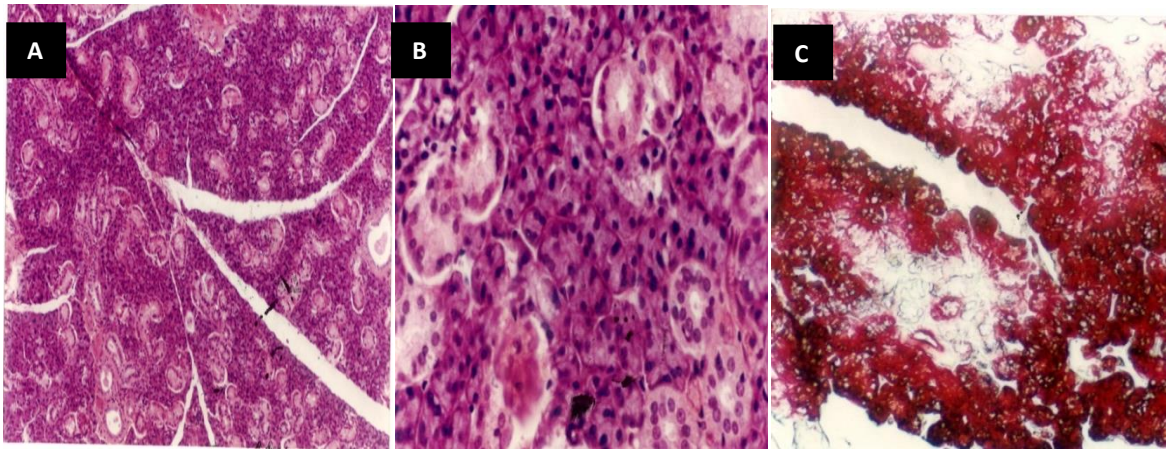

Figure.1: A representative micrograph of Group I (control group) showing the normal histological structure of rat parotid gland .A) H&E ×200. B) H&E ×400. C) showing the normal distribution of alkaline phosphatase reactivity with Azo dye in Rat parotid gland (Azo dye X200).

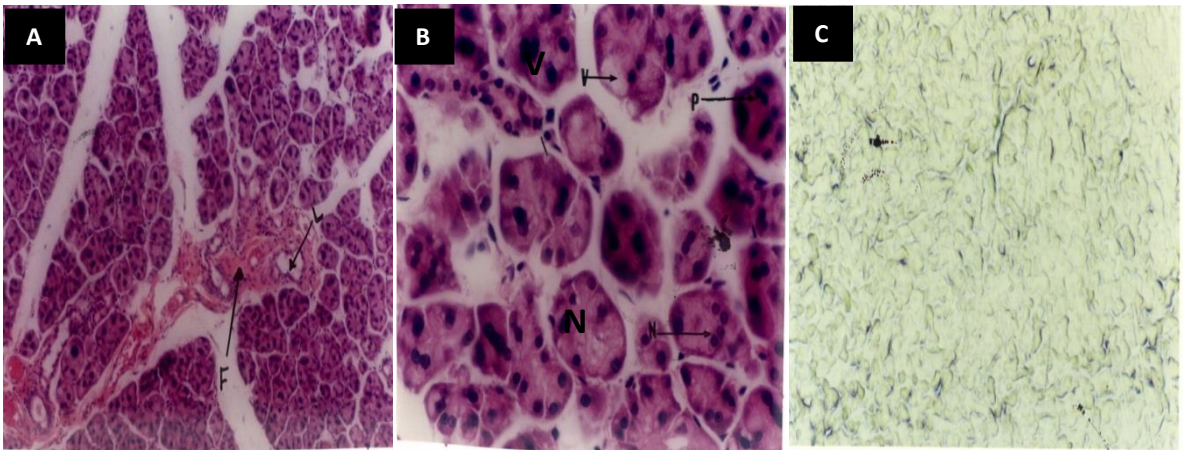

Figure.2: A representative micrograph of Group II showing shrinkage of acini and edema between them. A) showing areas of fibrosis F, dilated lymphatic drainage space L, (H&E ×200). B) showing vaculation in some acini V, pyknotic nuclei P, prominent nucleoli N (H&E ×400). C) showing nil alkaline phosphatase reactivity as compared to the control group (Azo dye X200).

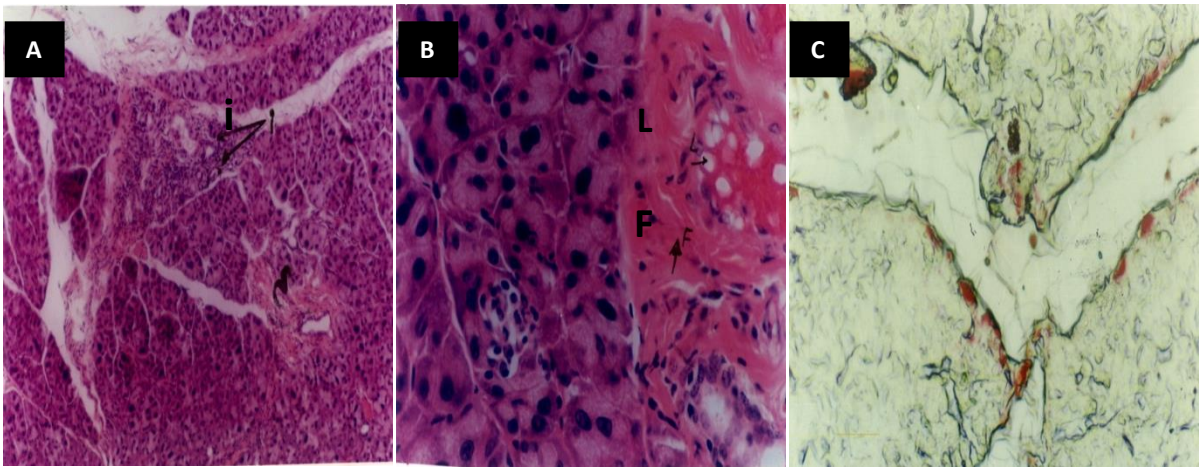

Figure.3: A representative micrograph of Group III showing. A) marked inflammatory cell infiltrate connective tissue stroma i, (H&E staining magnification ×200). B) marked fibrosis F, dilated lymphatics drainage space L (H&E ×400). C) showing weak alkaline phosphatase reactivity on the boundaries of C.T stroma (Azo dye X200).

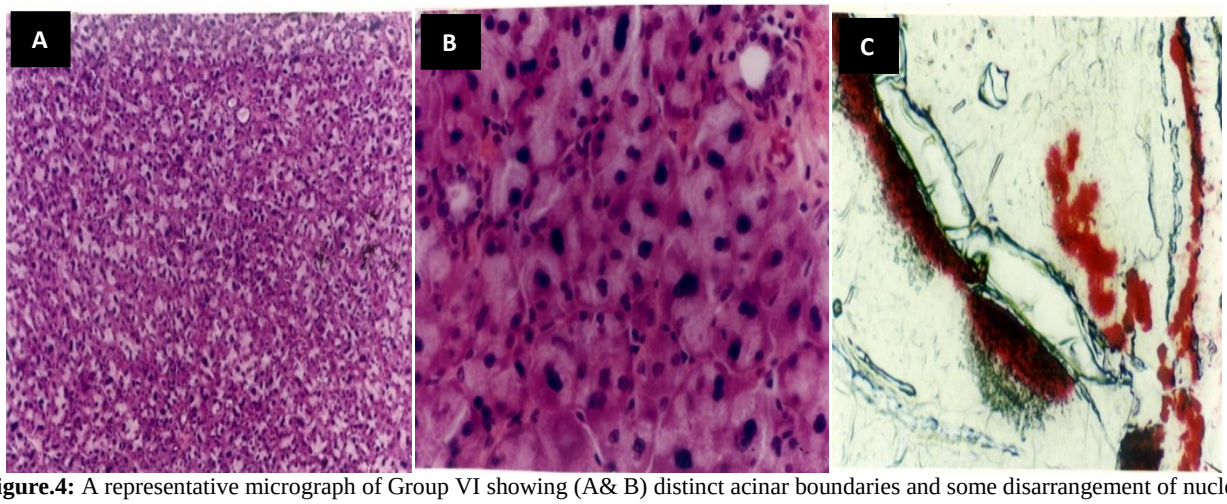

Figure.4: A representative micrograph of Group VI showing (A& B) distinct acinar boundaries and some disarrangement of nuclei (H&E ×200 & x400). C) showing increase areas of alkaline phosphatase reactivity as compared to previous groups but still lesser than the control group (Azo dye X200).

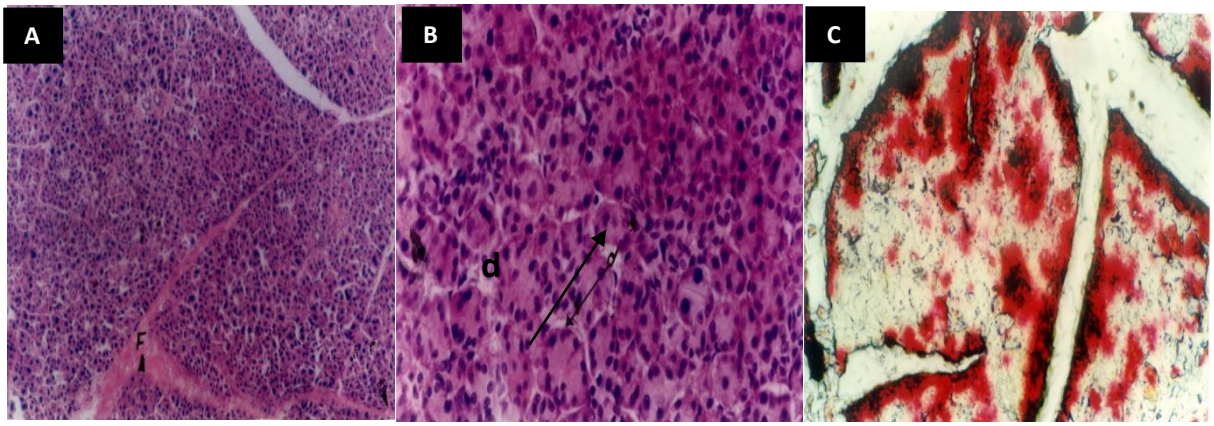

Figure 5: A representative micrograph of Group V showing A) less fibrosis in C.T stroma F (H&E ×200).B) minimal degenerative areas between the acini d (H&E ×400) .C) showing marked alkaline phosphatase reactivity similar to great extend to that in the control group (Azo dye X200).

1. Histopathological study

Group I (control group) showed normal histological structure of rat parotid gland (Fig 1A and 1B). Group II (1/50 Cd) showed obvious Cd toxic effect in the form of shrinkage of the acini with edema, areas of fibrosis and dilated lymphatic space (Fig 2A arrows F&L). Higher magnification showed deformity of acinar shape with loss of cell boundaries and lumen with vaculation in some acini (Fig 2B arrow V). Irregularity in nuclear size and position, hyperplasia and hypertrophy were also seen with some pyknotic nuclei and prominent nucleoi (Fig 2B arrow PN). Group III (1/100 Cd) showed the same histopathological findings of Group II but with a mild degree and less degenerative changes. Lesser acinar deformity, degree of nuclear atypism and number of nuclei as compared to the previous group. The connective tissue stroma showed marked inflammatory cell infiltrations (Fig 3A arrow i). Marked fibrosis and dilated lymphatic drainage space in some areas (Fig 3B, arrow F&L). Group IV (1/50 Cd + Se) showed lesser changes as compared to the previous groups in the form of ill distinct acinar boundaries and some disarrangement of nuclei (Fig 4A & B) while Group V (1/100 Cd +Se) appeared as normal since it showed minimal changes in the form of some smaller acini than normal, less fibrosis in the C.T stroma (Fig 5A arrow f) and minimal degenerative areas between the acini (Fig 5B arrow d).

2. Alkaline phosphatas activity study:

The activity of alkaline phosphatase in Group I (control group) showed positive enzymatic reaction around the acini and on the peripheries and boundaries in C.T stroma (Fig 1C) and the reaction in Group II (experimental group 1/50 Cd) showed a marked reduction in the activity of alkaline phosphatase with subsequent functional impairment of the acinar cells with negative reaction around the acini as compared to the control group (Fig 2C). The reaction in Group III (experimental group 1/100 Cd) showed some enzymatic reactivity on the peripheries of lobules in the C.T stroma but still negative reaction around the acini as the Group II (Fig 3C). Group IV (experimental group 1/50 Cd+ Se) showed a moderate degree of enzymatic reaction as compared to previous groups with greater precipitation of the Azo dye in the reactive areas (Fig 4C). Group V (experimental group 1/100 Cd + Se) showed minimal affection of the enzyme activity and there is a nearly normal distribution of the enzymatic reaction similar to a great extent to the normal group (Fig 5C).

4. DISCUSSION

Cadmium is a toxic metal commonly found in the daily environment. Due to the increasing number of reports on the harmful impact of low exposure to toxic elements published worldwide, researchers are focused on finding methods to reduce dietary cadmium intake and mitigate its impact on the organism (18,19). Particular attention is paid to using certain nutrients to overcome the effect of Cd such as zinc, selenium, calcium and magnesium. Studies on animals revealed that selenium and zinc reduce the absorption of cadmium from the gastrointestinal tract and its accumulation in the liver and kidneys and prevents oxidative medicine and cellular longevity (20). Similar to other heavy metals, cadmium can accumulate in living organisms. The largest accumulation of cadmium occurs in organs rich in metallothionein (MT), that is, the liver and kidneys (19). However, in chronic exposure to cadmium, the concentration levels of this metal in various tissues and body fluids increased including those in which the accumulation is much lower than in shielding organs, for example, the salivary gland tissue (21). The present study showed that the chronic effects of Cd toxicity were clear in Groups II and III as the results emphasized the dramatic effect of Cd on the acini causing marked shrinkage with oedema and areas of inflammatory cell infiltration. Cd toxicity was also clear on the nuclear level and obviously in the direction of neoplastic changes. This result was in agreement with the results reached by Hurana et al (22). Selenium decreases the adverse effect of Cd toxicity, possibly by forming non-toxic Cd selenite or by the ability of Cd to induce metallothionein (metal-binding protein) that is thought to confer tolerance to Cd toxicity (23). Selenium conferred protection against toxicity of Cd and mercury (24), its effect with glutathione (GSH), ∞-tocopherol, and Se restricted the uptake and distribution of Cd in liver and kidney of rats (25). The protective effect of Selenium against Cd toxicity was clear in this study in Groups IV and V where the addition of selenium minimized the destructive effect of Cd on rat parotid glands which is apparent in Cd groups. Our findings generally agreed with those reported in the literature, i.e. that Cd's toxic effect is markedly inhibited by selenium which was observed in histopathological and alkaline phosphatase results.

5. CONCLUSION

In conclusion, the present study shows several correlations between essential, probably essential and toxic metals indicating

the importance of balance between pro-oxidants and antioxidants. Future studies on Cd toxicity may reveal the appropriate inclusion level of Se in the diet and its form (i.e inorganic or organic sources) and the possible synergistic effects of Se with other antioxidants.

6. ACKNOWLEDGMENT

Special thanks to Prof. Gamal Zulheema for his estimable support and help in the laboratory work.

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