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STUDY ON THE HISTOPATHOLOGICAL CHANGES DUE TO ALUMINUM CHLORIDE ($ALCL_3$) AND AMELIORATE EFFECTS OF VITAMIN A ON THE SALIVARY GLANDS OF THE MOUSE

Nuha Shaker Ali

Department of Basic Sciences, College of Dentistry, University of Al-Qadisiyah, Iraq.
e-mail: nuha.albadry@qu.edu.iq

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ABSTRACT : Aims of the current study investigate toxic effecting of aluminum chloride ($AlCl_3$) on tissues of the salivary glands of the mouse and determining the histological and pathological changes that occur due to aluminum chloride toxicity, also studying the Vitamin A effects as an antioxidant factor or ameliorate effect. The total used animals in our study are (40) mice, the mice are divided into four groups, each group is consist of (10) mice. The first group is administrated aluminum chloride (18.5 mg/kg/DW) only by using gastric lavage for (30) day, the second group is administrated Vitamin A (4500 IU/ kg/ DW) only by using gastric lavage for (30) day, the third group is administered aluminum chloride (18.5 mg/kg/DW) at morning for (30) day intragastric with Vitamin A (4500 IU/ kg/DW) at evening for same the (30) day intragastric by using gastric lavage, while the fourth group (control group) administrated distal water. Our findings are included the histopathological changes of the tissues of the salivary glands of the mouse in the first group (G1) are included changes in the tissues of the submandibular gland with decreased parenchyma and increased stroma, decrease in size of the acini with an increase in the area of ducts as compared with the control group and there are no changes in the parotid glands tissues. The third group and second group don't show differences significant as compared with the control group. The fourth group (control group) is included normal tissues of salivary glands of the mouse, normal acini, normal striated ducts, and normal granular ducts. Our conclusions are the aluminum chloride has negative effects on the salivary glands of the mouse, while vitamin A has a positive, ameliorate, and protective effect and leading to the return of the toxic tissues to the normal status.

Key words : Salivary glands, submandibular, parotid, aluminum chloride, vitamin A, mice.

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INTRODUCTION

Aluminum chloride is a toxic chemical compound; it is formed from the reaction one atom of an Aluminum element with three atoms of the alachlor. It has a yellow color (Mandriota *et al*, 2016). Aluminum chloride ($AlCl_3$), also known as aluminum trichloride, describes compounds with the formula $AlCl_3$, it consists of aluminum and chlorine atoms at (1:3) ratio (Exley, 2013). Aluminum chloride has a low boiling and melting point and is used in the chemical industry. It is an inorganic compound (Banasik *et al*, 2005; Woolery-Lloyd and Valins, 2009).

Aluminum chloride has negative effects on all the body tissues and its function in animals and humans, such as the digestive system, nervous system, cardiovascular system and excretory system such as salivary glands (Sappino *et al*, 2012).

The mouse has three paired salivary glands (sublingual gland, parotid, and submaxillary gland) (Maruyama *et al*, 2019). The submandibular gland is the largest and lobulated, and it has a single excretory duct that opens on the floor of the oral cavity (Amano *et al*, 2012).

Many studies have handled the effecting of aluminum chloride on salivary glands of mice, wherever, in the study, aluminum salts causes several histological and physiological disorders. Aluminum chloride can cause oxidative stress in submandibular and parotid glands of mice and also, results in morphological and histological impairment. Aluminum chloride causes reduced glutathione and showed some histological changes in the parenchyma, ducts, stroma, and acini. And it causes great changes in salivary glands structure and causes decreased parenchyma and increased stroma area (de Souza-

Monteiro *et al*, 2020).

Aluminum absorption deposits in several tissues, and causes many cellular changes in the parotid and submandibular glands. The study showed decreased activity of the parotid gland with no changes in the submandibular gland. Aluminum citrate accumulated in the parotid and submandibular glands and causes damages to the cytoskeleton of the myoepithelial cells in both glands (da Costa *et al*, 2014).

The aims of our study are an investigation of the toxic effects of Aluminum chloride on salivary glands of mice and studying the ameliorated effecting of vitamin A as a therapy factor for removing the pathological changes and treatment of the damaged tissues of the glands.

MATERIALS AND METHODS

Animals

Forty albino mice (21 days old) were provided by the local market in Baghdad. The animals were kept in cages (30×20×13) cm. The animals were provided with water and healthy food and were housed at room temperature with (12) hour dark with (12) light cycle. The experiments were provided by the Ethics Committee on science College, Baghdad University.

Experimental design

1. The first group (10) mice: administrating aluminum chloride (18.5 mg/kg/DW) dissolved with distal water directly inside the stomach for 30 days by gastric lavage.
2. The second group (10) mice: administrating vitamin A (4500 IU/ kg/DW) directly inside the stomach for 30 day by gastric lavage
3. The third group (10) mice: administrating aluminum chloride (18.5 mg/kg) directly inside the stomach for 30 day by gastric lavage in the morning and administrating vitamin A (4500 IU/ kg/DW) in the evening directly inside the stomach for (30) day by gastric lavage.
4. The fourth group (10) mice (control group): administrating distal water directly inside the stomach only by gastric lavage.

Euthanasia and killing

Each group was euthanized and killed by neck region vertebrate dislocation, salivary glands were separated and washed in normal saline then frozen at -80°C and should then thawed for make histopathological sections.

The animals were killed after anesthetized by the administration of ketamine HCL and xylazine HCl and then put in saline heparinized solution and paraformaldehyde.

Histopathological examination

The area of the tissues and their structures was evaluated in this study. Submandibular and parotid glands were keeping in formaldehyde (4%) for processing, the tissues are dehydrated in different concentration of ethanol (70, 80, 90%) (absolute 1 and absolute 2), diaphanized in xylol then embedded in Paraplast then make sections at 5 μm in thickness by using a microtome.

Finally, staining of the sections is done by hematoxylin and eosin to make histopathological analyses by the color digital camera (Sony, Japan) with a microscope (Nikon, Tokyo, Japan at ×40). The total area of the acinar, duct, parenchyma and stroma are adopted as parameters for evaluation.

RESULTS

According to our results, the histopathological examination of the salivary glands of the mice of the first group which administrated aluminum chloride intragastric showed are marked histopathological changes of the submandibular glands including decreased parenchyma and increased stroma as compared with the control group. Furthermore, no there marked differences in the parenchyma and the stroma of the parotid glands as compared with the control group as demonstrated in Fig. 1 (a) and (b).

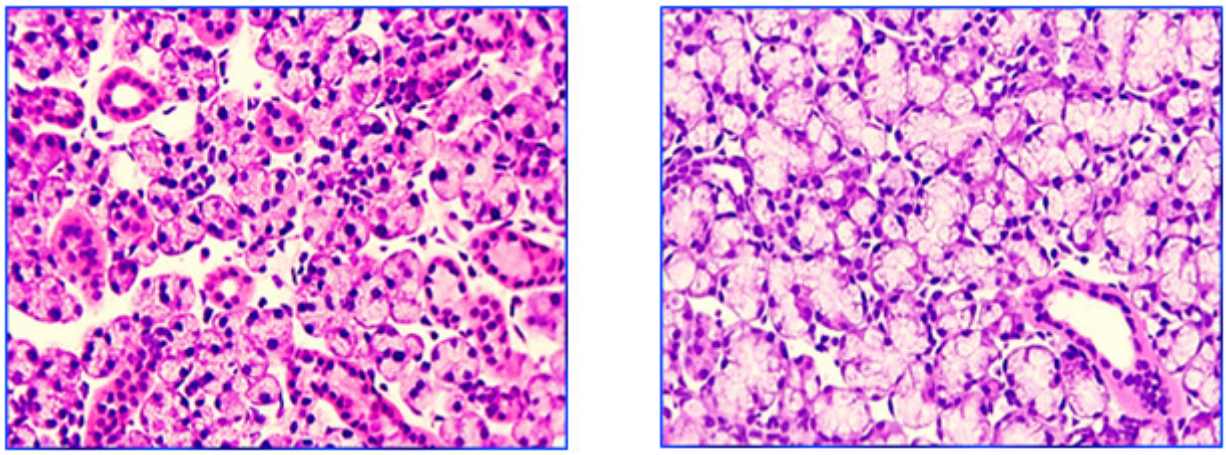
The study found that aluminum chloride causes changes in the submandibular gland structures as compared with the control group; the changes are including a decrease in the acini with an increase in the ducts. Also, there are no histological changes in the parotid glands look at Fig. 1 (a) and (b).

Our finding showed the histopathological changes of salivary glands in the second group which administrated vitamin A only directly intragastric for 30 days as shown in Fig. 2 (a) and (b).

The study found that the second group showed normal tissues and have normal structures of the submandibular gland and the parotid gland as compared with the control group; as shown in Fig. 2 (a) and (b).

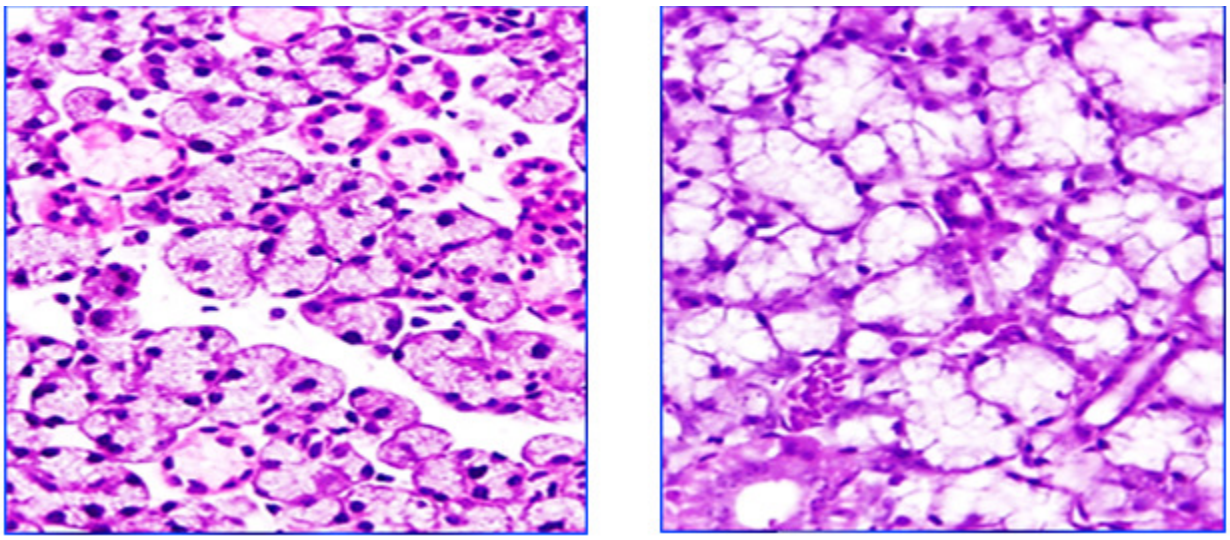
While the third group showed the histopathological changes of the salivary glands in the third group which administrated aluminum chloride intragastric directly for 30 days with vitamin A directly intragastric directly for 30 days as shown in Fig. 3 (a) and (b).

The study found that the third group showed normal tissues and have normal structures of the submandibular gland and the parotid gland as compared with the control group; as shown in Fig. 2 (a) and (b).



(a) (b)

Fig. 1 : showed histopathological changes of the salivary glands in the mouse which treated with aluminum chloride for (30) day a and B (the first group), wherever (a) is exposed parotid salivary gland for aluminum chloride while (b) is exposed submandibular salivary gland for aluminum chloride.



(a) (b)

Fig. 2 : showed histopathological changes of the salivary glands in the mouse which treated with vitamin A for (30) day (a) and (B) (the second group), wherever (a) is exposed parotid salivary gland for vitamin A while (b) is exposed submandibular salivary gland for vitamin A.

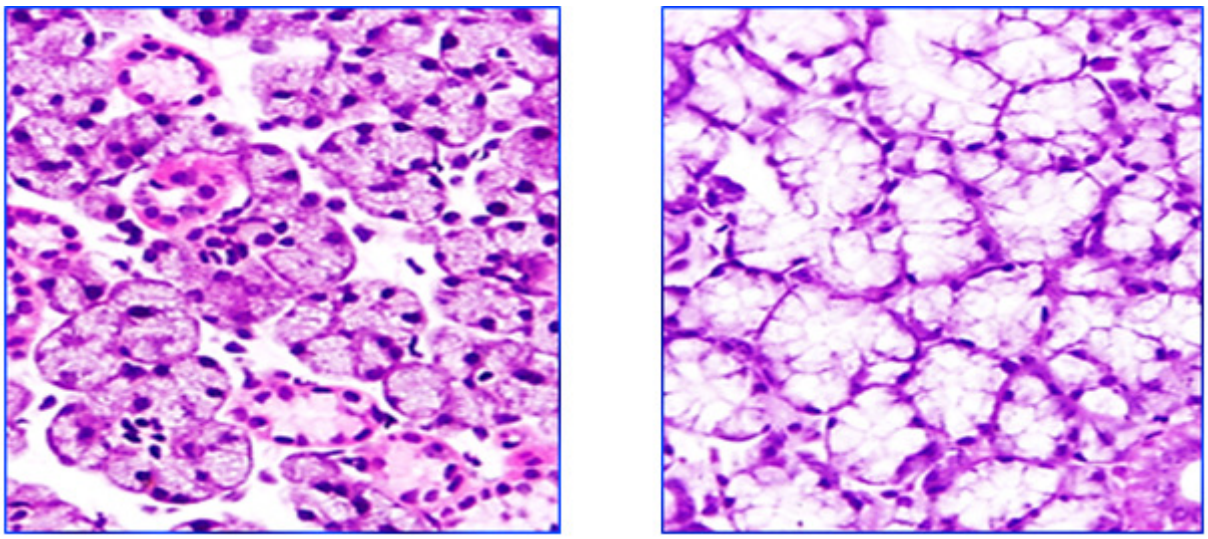
While, Fig. 4 (a) and (b) included a histological examination of normal salivary glands of the mouse in the fourth group (control group), which administrated distal water intragastric directly as showed in Fig. 4 (a) and (b).

Our findings are included that histopathological changes of the fourth group (control group) showed that the salivary glands of the mouse are (control group) is included normal tissues, normal acini, normal striated ducts and normal granular ducts as showed in Fig. 4 (a) and (b).

DISCUSSION

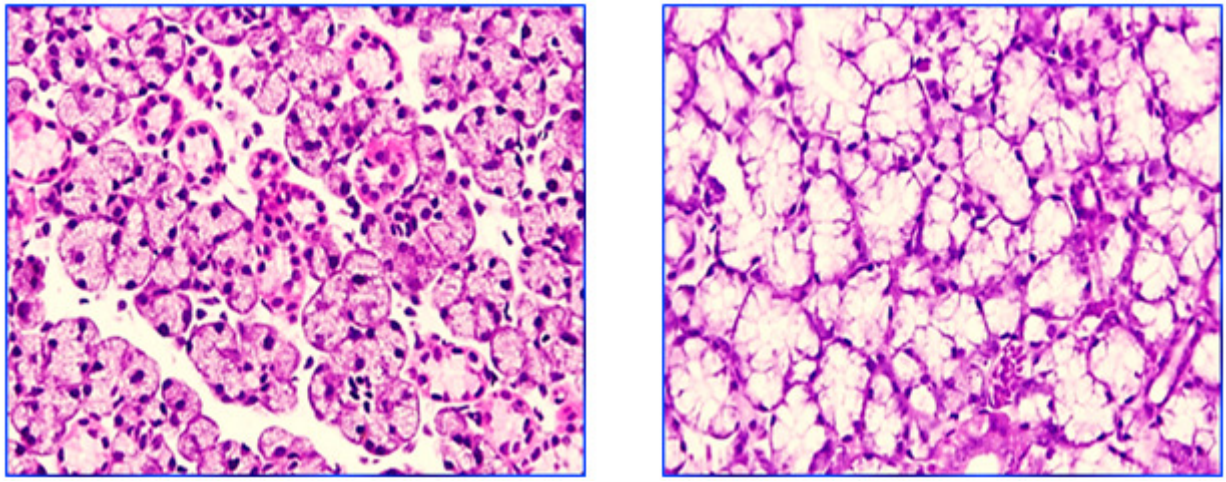
Based on our study, the used dose is a long-term exposure dose to aluminum chloride that could cause histopathological changes in the salivary glands of mice (parotid gland and submandibular gland). The used dose in the current study was (18.5 mg/kg/DW) for 30 days for induction histopathological changes in the salivary gland. The main function of the salivary gland is to produce saliva; the saliva is fluid products from the salivary glands (Proctor and Carpenter, 2007).

Also, the study selects the parotid and submandibular glands only because they were the largest glands in mice and it produces more (50%) of the saliva and left rests the salivary glands because its size is small and difficult removing and sectioning (Sas and Dawes, 1997). Aluminum chloride is absorbed by the stomach and intestine and deposit in all the tissue types, after transferring by the blood and circulating to different



(a) **(b)**

Fig. 3 : showed histopathological changes of the salivary glands in the mouse which treated with aluminum chloride with vitamin A for (30) day a and B (the third group), wherever (a) is exposed parotid salivary gland for aluminum chloride with vitamin A while (b) is exposed submandibular salivary gland for aluminum chloride with vitamin A.



(a) **(b)**

Fig. 4 : showed histology sections of the normal salivary glands in mouse (a) and (b) (the fourth group), wherever (a) is normal parotid salivary gland while (b) is normal submandibular salivary gland.

organs, Aluminum chloride causes tissue damages and histopathological changes (Nagaoka and Maitani, 2005).

The excretory system (kidney, sweat glands and salivary glands) try to secret and flush out the toxic substances outside the body, therefore, leading to the accumulation of aluminum chloride inside the excretory organs such as the salivary gland and results in several histopathological changes (Higuchi *et al*, 1994).

The mechanism of Aluminum chloride toxicity is not fully understood, but new reports suggested that Aluminum chloride inhibit cell activity because Aluminum chloride reacts with pro-oxidants metals such as iron, leading to formation the of oxidative materials Aluminum chloride participates in the Fenton reaction, which results in formation reactive oxygen species (ROC) (Kurutas, 2016; Ruiperez *et al*, 2012).

Many studies (Costa *et al*, 2014) found the exposure to aluminum citrate orally will increase aluminum concentration in the salivary glands such as parotid and submandibular glands. Deposition of the aluminum compounds is diagnosed in many studies in the salivary glands in the exposed animals (Krewski *et al*, 2007). Moreover, the study mentioned aluminum causes histological damages in the acinar and myoepithelial cells; and leading pathological changes in the histological structure of salivary glands, and that agreement with our results, as our results found that exposure to Aluminum chloride causes changes in the stroma and parenchyma of the submandibular glands (Kwak *et al*, 2016; Porcheri and Mitsiadis, 2019). Damage of acinar related to

Aluminum chloride toxicity and that similar to our results (Dodds *et al*, 2005).

Vitamin A is a nutritional organic compound, has multiple functions and is important for the growth and development of the immune cell, the epithelial cell and has antioxidant effects (McCullough *et al*, 1999; Huang *et al*, 2018).

Aluminum chloride is a toxic substance and helps to form oxidant material distributed in all tissue and cause negative effects on tissue functions, the body needs antioxidant substances to remove all oxidant substances, vitamin E, vitamin C (Huimin Tong *et al*, 2020) and vitamin A, wherever vitamin A is good substances could use for this purpose (Vailati-Riboni *et al*, 2017).

Our results don't show any pathological changes in the parotid gland, which may occur due to the large diameter of its duct as compared with the submandibular gland, therefore prevent the accumulation of Aluminum chloride inside the duct and that agrees with de Souza-Monteiro *et al* (2020).

Based on our results, the second group (take vitamin A only) and the third group (take vitamin A with Aluminum chloride) showed normal tissue and don't show any difference with histological sections of the fourth group (control group), many studies found results agree with our results and support it, wherever (Mario *et al*, 1981) found vitamin A was very important to the general health of the rat, and have great effect on growth and development of the salivary glands, thus secretion normal saliva.

Vitamin A is essential for mouse embryo growth and development such as heart and blood vessels and all the epithelial cells. The study found the mouse embryo's growth becomes impaired and loses of growth if exposed to vitamin A deficiency. Also, the study found vitamin A has a great effect on the formation of the epithelium that lining all body system such as salivary glands and that support our finding (Buenger *et al*, 2015).

A study by Buenger *et al* (2015) found that the salivary gland of the rat needs vitamin A for growth, however, vitamin A deficiency causes acinar atrophy, so, vitamin A has an important role in the excretory activity and development of salivary glands and that compatible with our results.

Based on our results and results of Mario *et al* (1981) and Buenger *et al* (2015) vitamin A could use for as an ameliorated factor for intoxication of Aluminum chloride, and that explains why the third group is showed normal tissue same to the control group.

CONCLUSION

According to our findings, the results showed that chronic exposure to aluminum chloride causes morphological changes in the salivary glands of mice and leading to tissue dysfunction and loss of function or low efficiency of the salivary glands low saliva flow leading to many problems.

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