Effect of aflatoxin contaminated diet on major salivary glands and the protective role of Ozone application

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Abstract: Saliva is produced by the submandibular (SMG), sublingual (SLG) and parotid (PTG) glands in addition to multiple minor salivary glands present throughout the mouth. Saliva and salivary glands are important because of their role in oral mucosal health. Food contaminants entering the body through the oral route are directly exposed to the action of saliva. The aim of the current study was to evaluate the histopathological and histochemical changes in SMG, SLG, and PTG glands in rats fed aflatoxin (AFB_1) contaminated peanuts and evaluation of the decontamination effect with Ozone (O_3). Thirty male Sprague-Dawley rats were divided into control and four tested Groups treated for 4 weeks as follow: Control Group fed regular basal diet, Group 1 fed control peanuts; Group 2 fed peanuts treated with O_3 (40 mg) for 10 min; Group 3 fed AFB_1 contaminated peanuts (1.5 mg/ kg) and Group (4) fed AFB_1 contaminated peanuts treated with O_3 using the same dose and period as in Group 2. Tissue specimens of SLG, SMG and PTG were taken at the end of experiment and fixed for histological and histochemical examinations. The results indicated that AFB_1 increased the incidence of histopathological alterations in SMG, SLG and PTG. These alterations were manifested by pleomorphic serous acinar and vacuolar changes as well as fatty degeneration of acinar cells. The results also revealed a marked dilatation in the stromal spaces which resulted in a significant increase in fibrous tissues mainly collagen fibers especially in SMG and PTG. Some acinar nuclei were replaced by irregular, hypertrophy chromatin condensed mass and lost their nuclear envelop. The histochemical results showed that AFB_1 also induced a significant decrease in total protein and acid glycoprotein. All these alterations were improved significantly in the group fed with the Ozone treated AFB_1 contaminated diet. It could be concluded that AFB_1 induced severe toxic effects on the studied salivary glands and these alterations could be prevented or reduced when AFB_1 contaminated food was treated with Ozone. Moreover, Ozone itself was safe and did not show any detrimental effects.

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1. Introduction

Aflatoxins (AFs) are naturally occurring toxic fungal metabolites produced by certain fungi mainly *Aspergillus flavus* and *Aspergillus parasiticus* and are known to cause immune system suppression, growth retardation, liver disease, and even death in humans. These natural mycotoxins under favorable conditions on a wide range of foods such as maize and groundnuts/peanuts constitute a worldwide problem than that of the synthetic toxins (Yan *et al.,* 2014). Several types of aflatoxin (B1, B2, G1, and G2) are produced by these fungi. The aflatoxin B1 (AFB1) form is recognized by the International Agency for Research on Cancer as one of the most naturally occurring toxic and carcinogenic substances found in nature (Hussaini *et al.,* 2012). AFB1 induce reactive oxygen species (ROS) generation which causes oxidative stress, leading to impairment of DNA, RNA, proteins and lipids (Mary *et al.,* 2012). The Food and Agriculture Organization (FAO) estimated that 25 percent of world food crops are affected while the USA Center for Disease Control (USCDC) estimated that more than 4.5 billion people in the developing world are exposed to aflatoxin. Children below five years remain the most vulnerable, with exposure causing stunted growth and damaging their immunity (ACMPP, 2013). Globally, about $1.2 billion in commerce is lost annually due to aflatoxin contamination, with African economies losing $450 million each year (Lopez *et al.,* 2013). Due to the concern for the potential carcinogenic effects of AFs on human health, most countries have legislation that restricts marketing of aflatoxin contaminated grains and foods as diet is the major way through which humans are exposed to aflatoxin. In July 2005, the United States Center for Disease Control and Prevention (USCDC) and the World Health Organization (WHO) hosted a workshop to create an integrated plan intended to generate culturally appropriate, long term, public health strategies to reduce aflatoxin exposure in developing countries (Hussaini *et al.,* 2012).

Saliva represents the first barrier to the entry of bacteria, fungi, viruses and other pathogens into the body and thus secretions are important in the statement and progression of oral infectious processes (Giuca *et al.,* 2014). The salivary gland secretions lubricate ingested food for ease of swallowing, protect the mucous membrane of the upper digestive tract, and provide an effective barrier against desiccation and enzymatic acidic elements in contact with the buccal mucosal surface (Terézhalmy *et al.,* 2013). The numerous biologic functions of saliva resulting in maintenance of mucosal integrity make it a necessary instrument in oral health. Salivary gland function is impaired by autoimmune diseases and inflammations (Reeves, 2013). The adverse effects of the AFs (and other mycotoxins) on human and animals health have led to the investigation and development of practical detoxification or inactivation strategies in food grains and livestock feeds (McKenzie *et al.,* 1998). Detoxification of AFs appears to be an attractive approach. Several strategies for the detoxification of AFs by physical, chemical and biological means have been reported (Yan *et al.,* 2014). However, each treatment has its own limitations, since the treated product should be safe and unaffected by the chemicals used; moreover the nutritive values of the treated product should not be altered. Therefore, the food industry is in search of disinfectants that are effective against common and emerging pathogens and safe to use in many specific applications of food processing. One of such compounds is Ozone (O_3) that has been utilized as a sanitizer in many European water treatment plants since the beginning of the seventeenth century (Yan *et al.,* 2014). There are many advantages of using Ozone as a potent oxidizing agent in food and other industries. It is potentially useful in decreasing the microbial load (Pascual *et al.,* 2007). The high oxidizing power and spontaneous decomposition of Ozone make it a viable disinfectant for ensuring the microbiological safety and quality of food products (Tiwari *et al.,* 2010). Ozone is also reported to be effective in the detoxification and degradation of commonly occurring mycotoxins such as aflatoxin, patulin, cyclopiazonic acid, secalonic acid D, ochratoxin A, and zearalenone (McKenzie *et al.,* 1998). Although Ozone has been produced artificially and used in Europe for several decades, the FDA only approved ozonation for use in the US for food processing industry in 1997. There is no available literature concerning the effect of AFs on salivary glands, consequently; this suggested the need for studies regarding the association between AFs contamination and cellular effects on different tissues including salivary glands. Thus, this work was conducted to study the effect of AFB_1 on tissue architecture of the major salivary glands and to evaluate the safety of Ozone in AFB_1 detoxification.

2. Material and Methods:

Chemicals:

Aflatoxin (AFB_1) standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ozone gas was produced from air using Ozone generator unit (OzoMAX Ltd, Shefford, Quebec, Canada). One batch (5kg) of aflatoxin-contaminated or control peanuts were placed in a stainless steel chamber and Ozone was allowed to distribute, mix and flow throughout the peanuts seeds for 10 min. at doses of 40 mg. The peanuts seeds were then removed and placed into flat pans for 24 hours. The concentration of AFB_1 after ozonation was determined using HPLC (High Performance Liquid Chromatography). Three-month old male Sprague Dawley rats (170-190 g) were obtained from the Animal House Colony, National Research Centre, Cairo, Egypt. Animals were maintained on standard laboratory diet (protein: 160.4; fat: 36.3; fibers: 41 g/kg and metabolisable energy 12.08 MJ). After an acclimatization period of 1 week, animals were divided into control and four tested groups (10 rats/Group) and treated with their respective treatments for 4 weeks as follow: Control Group fed regular basal diet; Group 1 fed peanuts; Group 2 fed peanuts treated with Ozone (40 mg) for 10 min; Group 3 fed AFB_1 contaminated peanuts (1.5 mg/ kg) and Group 4 fed AFB_1 contaminated peanuts treated with Ozone like the same dose and period as Group 3. At the end of the experimental period, all animals were sacrificed and the sublingual, submandibular and parotid glands were removed and fixed in 10% neutral buffered formalin. The fixed samples were then thoroughly washed in running water, processed routinely for paraffin embedding, sectioned at 4-5 µm thickness and stained with Hematoxylin and Eosin and Masson Trichrome stains for histopathological and histomorphometric examination. For histochemical studies, acid glycoprotein was detected by Alcian Blue stain (AB) at pH 2.5 and total protein was determined by Mercuric bromophenol blue (Mazia *et al.,* 1953). The optical density of total protein as well as the area and percentage of collagen fibers were expressed in sublingual, submandibular and parotid glands. However, the optical density of acid glycoprotein was measured in sublingual gland. The intensity of color and the percentage of positive reaction of stains were measured in ten fields of view (FOV=25mm²) using Image Analyzer Leica Q DM L25 program at the Pathological Research Laboratory, College of Dentistry, Misr International University.

3. Results:

1-Histopathological results:

Histopathological observations of major salivary glands of the control rats or those fed normal peanuts (Figs. 1a, b, c) or those fed normal peanut exposed to Ozone (Figs. 2a, b, c) revealed that sublingual, submandibular and parotid glands showed normal architecture of acini and ducts. The microscopic examination showed that sublingual gland is composed of mucous acini with a flattened darkly staining nuclei and clear cytoplasm (Fig. 1a). The submandibular gland consists of acini and tubules; the acinar portion is composed of serous cells with oval darkly staining nuclei and granular cytoplasm. The tubules are lined with columnar epithelium with flattened nuclei at the base and form an extensive ductal system of the glands (Fig. 1b). The parotid gland in the control rat consists of serous acini, intercalated and striated ducts. The serous cells present a darkly staining nucleus and granular cytoplasm separated by thin layer of connective tissue (Fig. 1c). Glands of rats fed normal peanut or those fed normal peanut exposed to Ozone revealed significant increase in stromal spaces involved connective tissues and hemorrhage (Fig. 2a) and no significant histopathological changes were presented (Figs. 2 a, b, c).

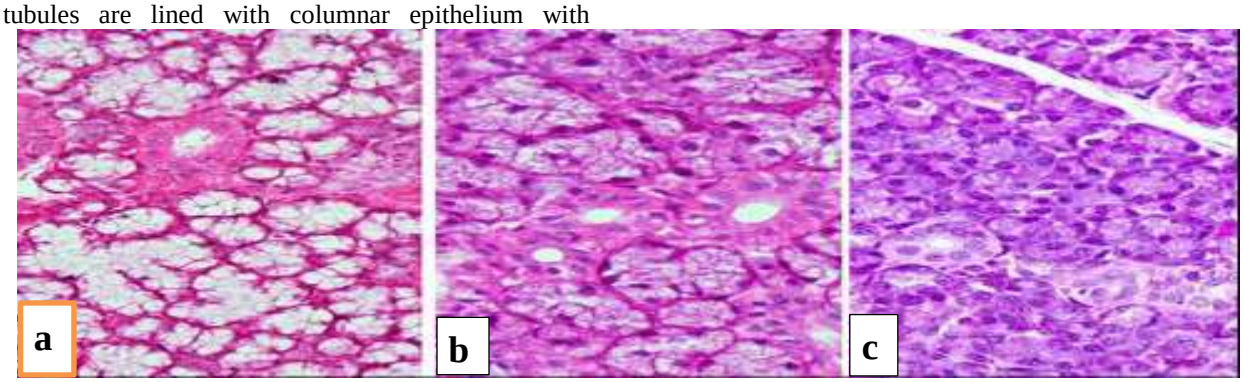


Fig. 1: Photomicrographs of (a) Sublingual, (b) Submandibular and (c) Parotid salivary glands from control rats fed normal diet showing their normal architecture of acini and ducts (H&E X 200)

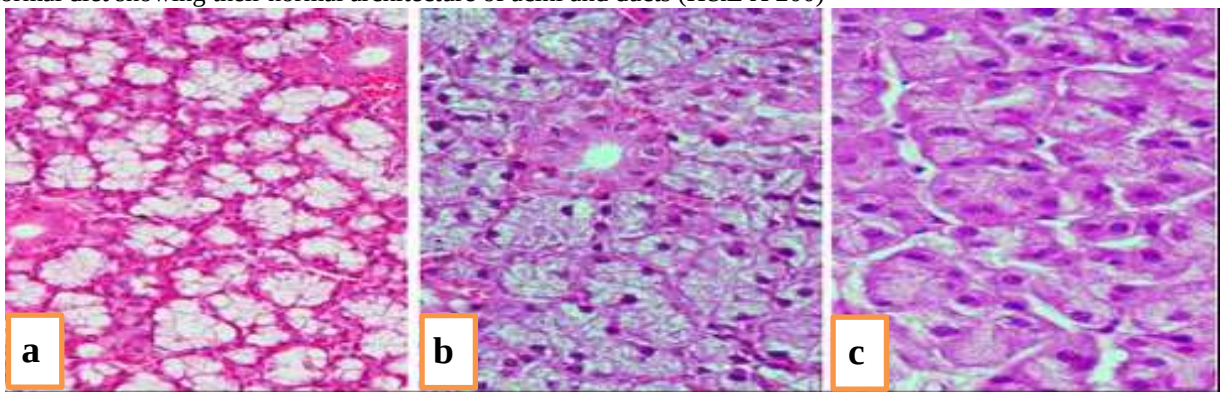


Fig. 2: Photomicrographs of (a) Sublingual, (b) Submandibular and (c) Parotid salivary glands from rats fed normal peanut exposed to Ozone showing nearly normal structure of mucous and serous acini, same picture of granulated cytoplasm and dark stain nuclei. Considerable increase in the interstitial spaces (H &E X200)

The salivary glands of rats that were fed AFB_1 contaminated diet showed abundant tissue parenchymal alterations mainly in submandibular and parotid glands; they presented nearly a similar structural damage. Moreover, the sublingual gland showed prominent vacuolization of the glandular acini, the inter acinar spaces was enlarged and stroma presented accumulation of connective tissues mainly collagen fibers around the ducts and blood vessels (Fig. 3a, b). On the other hand, the most prominent changes concerned with the submandibular gland in this group were the severe vacuolar degeneration that appeared in secreting acinar cells and the epithelial cells of convoluted ducts were swollen or vacuolated. The affected nuclei were shrunken, pyknotic and hyper stained, a finding characterizing the typical chromatin condensation seen in apoptotic processes in glandular acini and ducts. There was marked increase in collagen fibers in acinar septa, around the ducts and blood vessels (Figs. 3c, d) compared to control in which acini were separated by a linear connective tissue septa indicated the collagen fibers in SMG of control rats (Fig. 3e). The parotid gland revealed prominent parenchyma alterations. Pleomorphic serous acini characterized by intense basophilia and atrophied or distorted, most of them showed severe vacuolar and fatty degeneration. The parotid acinar nuclei showed pyknosis or apoptosis. In addition, some acinar nuclei replaced by

irregular, hypertrophy, chromatin condensed mass and lost their nuclear envelope. Stromal spaces between acini were enlarged and abnormal accumulation of connective tissues and mononuclear cellular infiltration (Fig. 4). Abundant collagen fibers were seen in between the acini and around the congested blood vessels (Fig. 3f). On the other hand, animals fed AFB_1 decontaminated using Ozone showed less pronounced structural alterations in the sublingual, submandibular and parotid glands (Figs. 5a, b, c,).

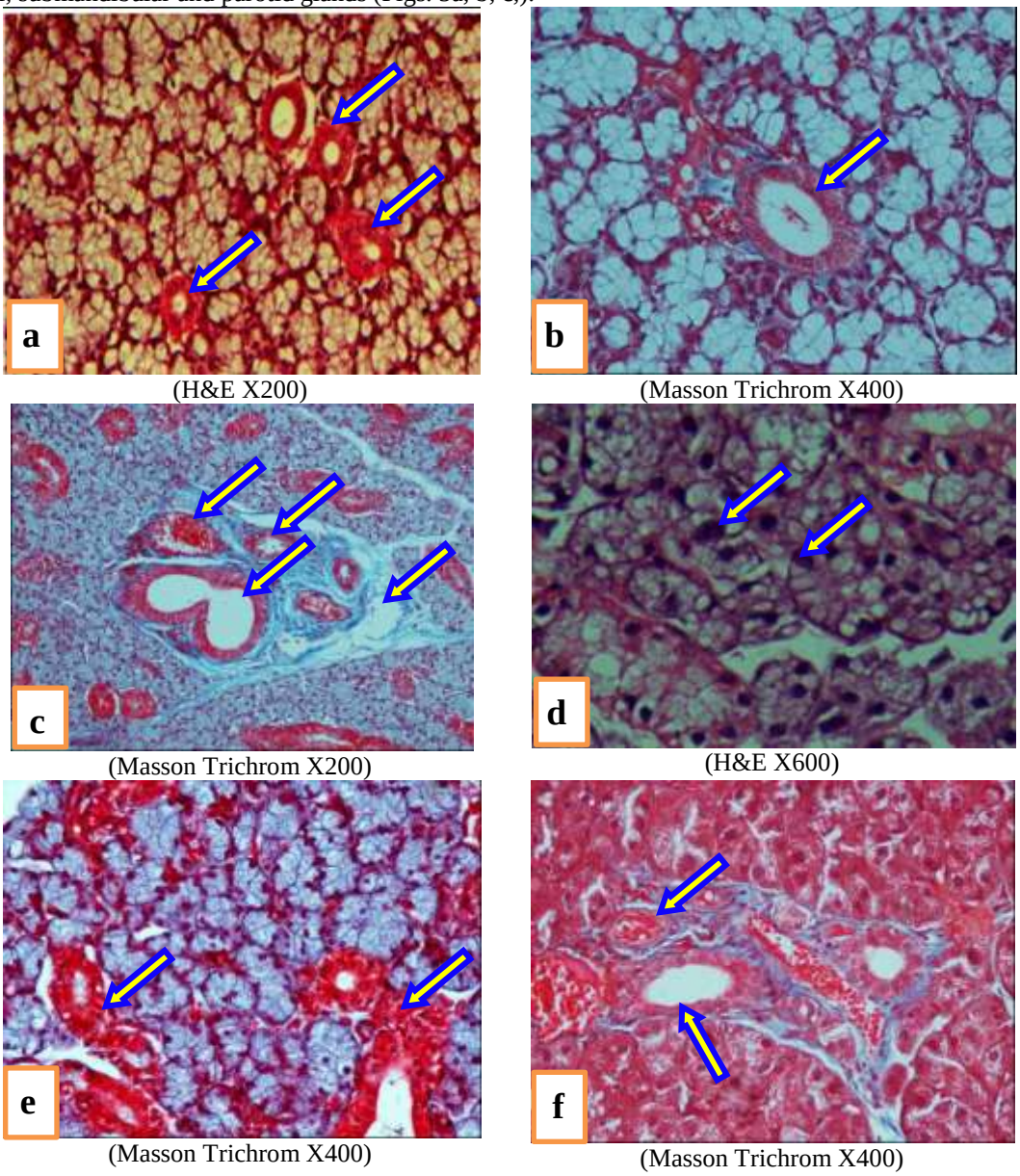


Fig. 3: Photomicrographs of (a, b) Sublingual gland from rats fed AFB_1 contaminated diet showing vacuolization of the glandular acini (arrow), the inter acinar spaces were enlarged and stroma presented accumulation of connective tissues mainly collagen fibers around the ducts and blood vessels, (c, d) Submandibular gland from rats fed AFB_1 contaminated diet showing severe vacuolar and fatty degeneration appeared in secreting acinar cells and the epithelial cells of convoluted ducts are swollen or vacuolated. The affected nuclei were shrunken, pyknotic and hyper stained (arrow), there is a marked increase in collagen fibers in acinar septa and around the ducts and blood vessels, (e) SMG of control rats showing the acini are separated by linear connective tissue septa indicated the collagen fibers, (f) Parotid gland from AFB_1 fed rats showing the ducts are surrounded by collagen fibers in which congested blood vessels and striated ducts were observed.

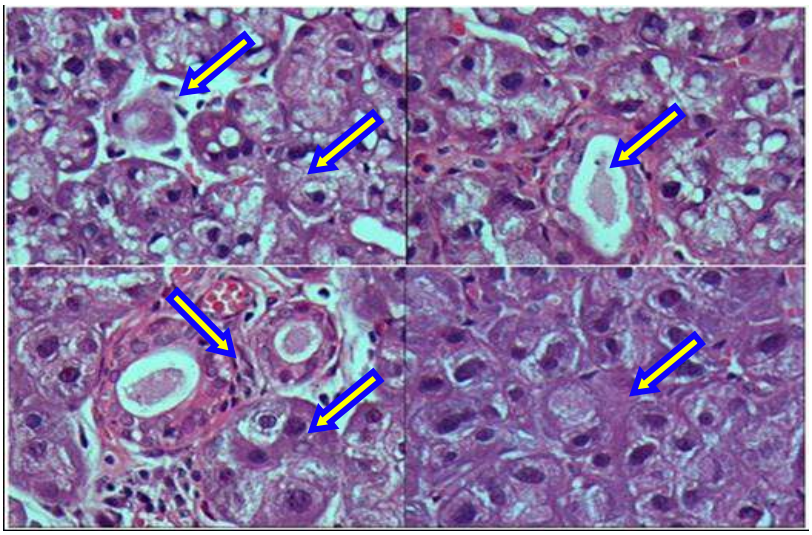


Fig. 4: A photomicrographs of parotid gland from rats fed AFB_1 contaminated showing prominent alteration in parenchymal architecture, characterized by pleomorphic acini, focal necrosis, acinar vacuolar and fatty degeneration (arrow head). Nuclear pleomorphism, acinar nuclei are apoptotic or pyknotic or replaced by irregular, hypertrophy, chromatin condensed mass which lost their nuclear envelops. The stroma spaces are enlarged in between acini characterized by the presence of connective tissues and mononuclear infiltration (H&E X600)

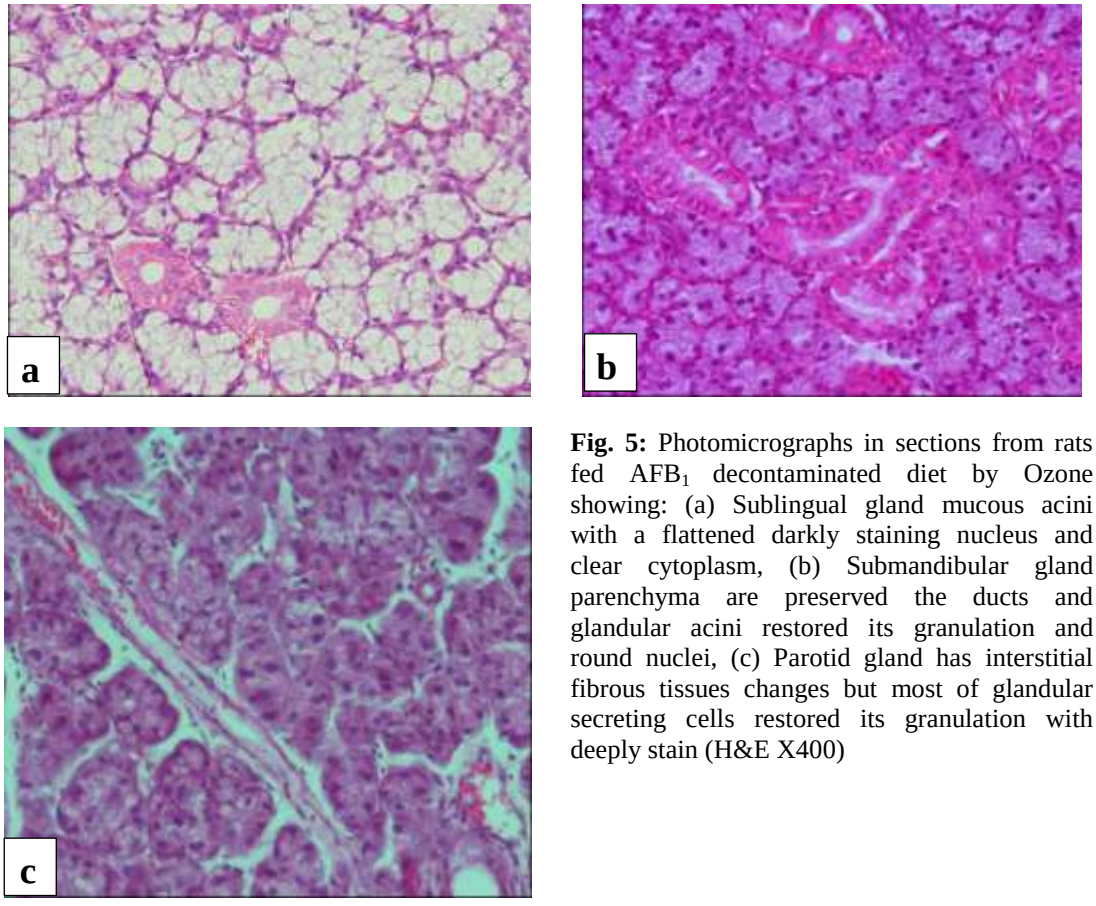


Fig. 5: Photomicrographs in sections from rats fed AFB_1 decontaminated diet by Ozone showing: (a) Sublingual gland mucous acini with a flattened darkly staining nucleus and clear cytoplasm, (b) Submandibular gland parenchyma are preserved the ducts and glandular acini restored its granulation and round nuclei, (c) Parotid gland has interstitial fibrous tissues changes but most of glandular secreting cells restored its granulation with deeply stain (H&E X400)

2. Morphometric and Histochemical Results:

2.1- Area percentage of the interstitial tissues (Stroma):

The mean area percentage of SMG and SLG stromal spaces revealed that animals fed AFB_1 contaminated diet showed a significant increase in interstitial tissue spaces in both glands compared to the control and the others groups. Animals fed AFB_1 decontaminated diet showed a significant improvement in the mean area percentage of the interstitial tissues towards the Control Group (Diagram 1).

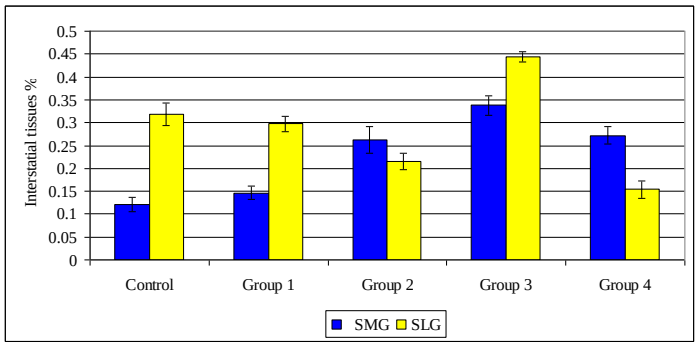
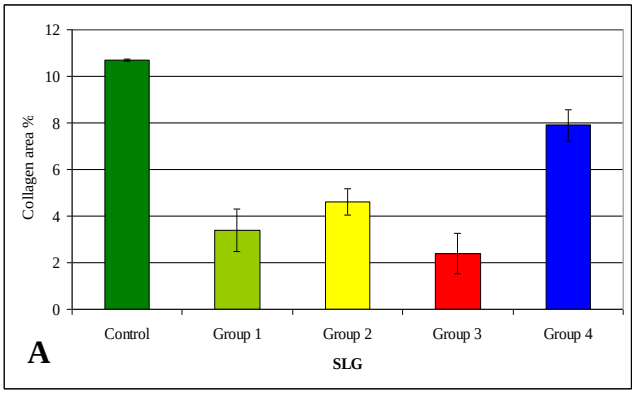
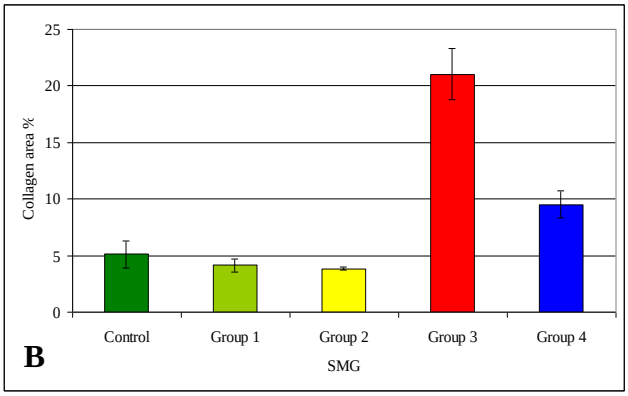


Diagram (1). The area percentage of the glandular stroma in submandibular and sublingual glands stained by Masson Trichrom staining

2.2- Area percentage of collagen fibers:

The area percentage of collagen fibers in sublingual gland showed a significant decrease in collagen fibers in all Groups. This decrease was pronounced in Group 3 followed by a significant increase in Group 4 compared to Control Group (Diagram 2A). In submandibular glands the area percentage of collagen fibers of Group 3 and 4 significantly increased compared to other groups. Area percentage of collagen fibers of Group 4 significantly decreased compared to Group 3 (Diagram 2B). In parotid glands the area percentage of collagen fibers significantly decreased in Group 4 compared to all groups and restored in Group 4 which significantly increased compared to Group 3 (Diagram 2C).





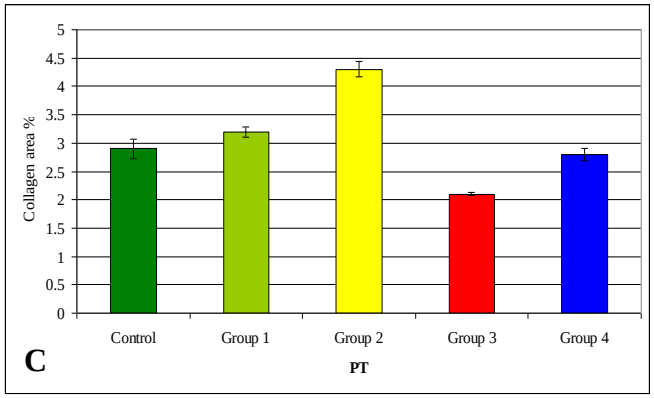


Diagram (2): The area percentage of collagen fibers in septa between acini and around the ducts among studied Groups: (A) Sublingual glands, (B) Submandibular gland (C) Parotid gland

2.3 -The optical density of total protein:

The histochemical staining of total protein resulting in blue color stain within deeply protein secreting cells (Fig. 6a, b). In SMG and SLG bromophenol blue reaction in ductal epithelial cells was strong than in acini granular secreting cells so that, the optical density in the acini and ducts was measured per gland. The reaction in parotid glands was homogenous in all parenchyma (ducts and acini). The results also revealed that; in SMG no significant differences in total protein contents in different groups however, significant decrease in total protein in ductal epithelial cells of rats in Group 4 was noticed (Diagram 3A, B). SLG serous demilune secreting cells and its simple ductal cells showed a significant decrease in animals fed AFB_1 contaminated peanuts while animals fed the decontaminated diet showed an increase in normal secreting cells manifested by a significant increase in total protein compared to AFB_1 group and appeared nearly similar to the group fed Ozone-treated peanuts alone (Diagram 3C, D).

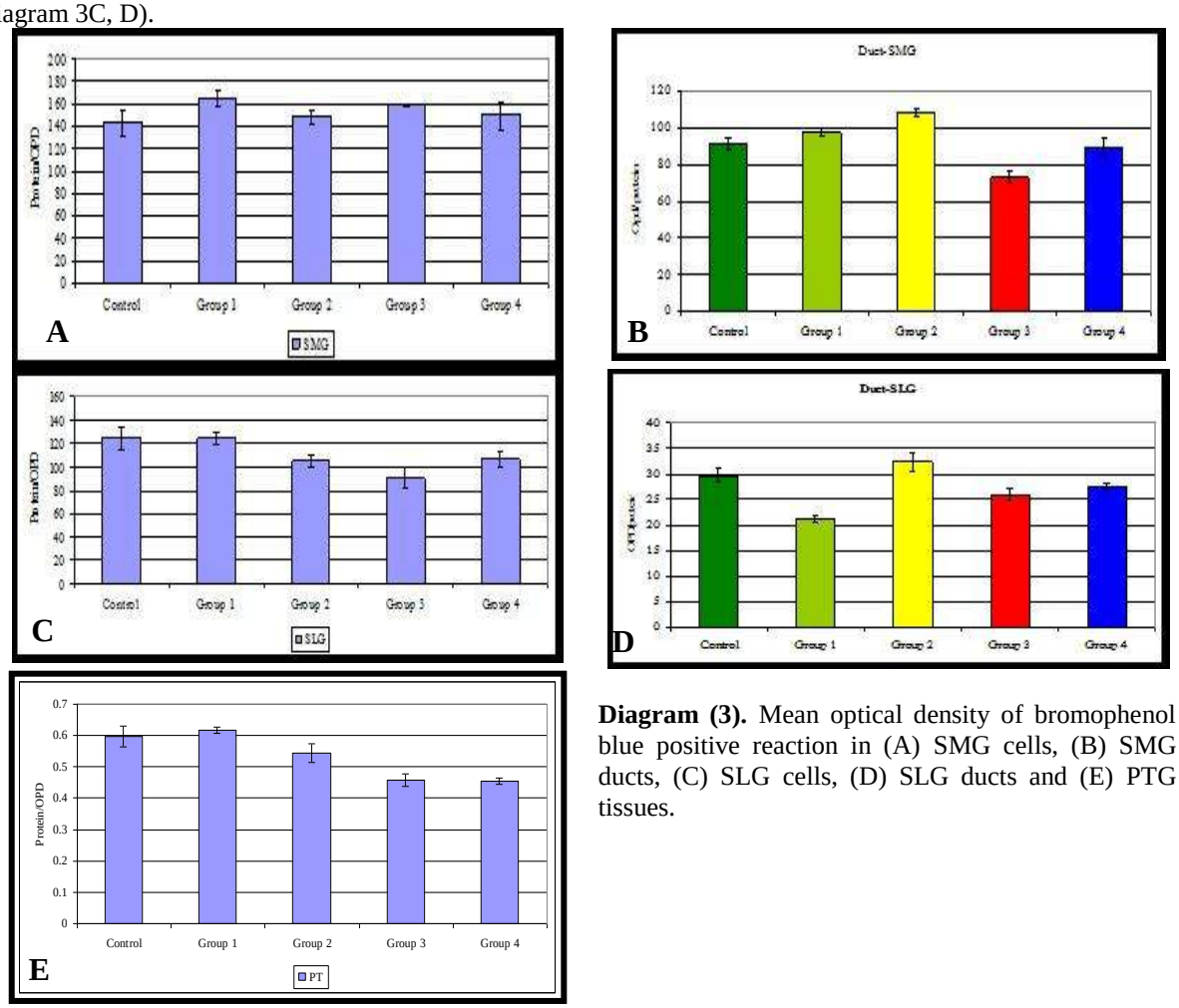


Diagram (3). Mean optical density of bromophenol blue positive reaction in (A) SMG cells, (B) SMG ducts, (C) SLG cells, (D) SLG ducts and (E) PTG tissues.

On the other hand, the parotid gland showed a significant decrease in total protein contents in rats fed AFB_1 contaminated diet (Group 3) followed by improvement in total protein contents in Group 4 whereas; restoration in acinar secreting cells structure resulting in significant increase in total protein content were noticed. No significant differences were noticed between the Control Group and the group fed normal or ozonated peanuts (Diagram 3E).

The acid mucous glycoprotein content was manifested by the blue color of Alcian Blue (AB) reaction (Figs.7a, b). In sublingual gland tissues, weak, moderate and strong reaction of alcian blue was accumulated and the optical density was measured. Furthermore, the mean area % of the strong positive (AB) reaction compared to the mean area % of weak reaction per group was calculated (Diagram 4). The results in this study also revealed that the amount of positive reaction of acid glycoprotein in Control Group that fed normal diet revealed 30 % weak and 22% strong positive reaction. Rats fed control peanut (Group 1) revealed significant decrease in mucin contents compared to the Control Group, 39% of this amount was weak and 20 % was strong effective.

Animals fed Ozone-treated control peanuts (Group 2) showed a significant increase in the content of acid glycoprotein compared to the other treated groups but 41% of them were weak reaction and 25% have strong reaction. Animals fed AFB_1 alone (Group 3) showed a significant decrease in acid glycoprotein contents compared to Group 2 or the Control Group and have 21 % weak positive reaction and 26 % was strong. However, animals fed Ozone treated contaminated diet (Group 4) showed a significant decrease in acid glycoprotein contents compared to Group 2 or the Control Group. About 28% of this amount was weak and 31% was strong positive reaction (Diagrams 4A, B).

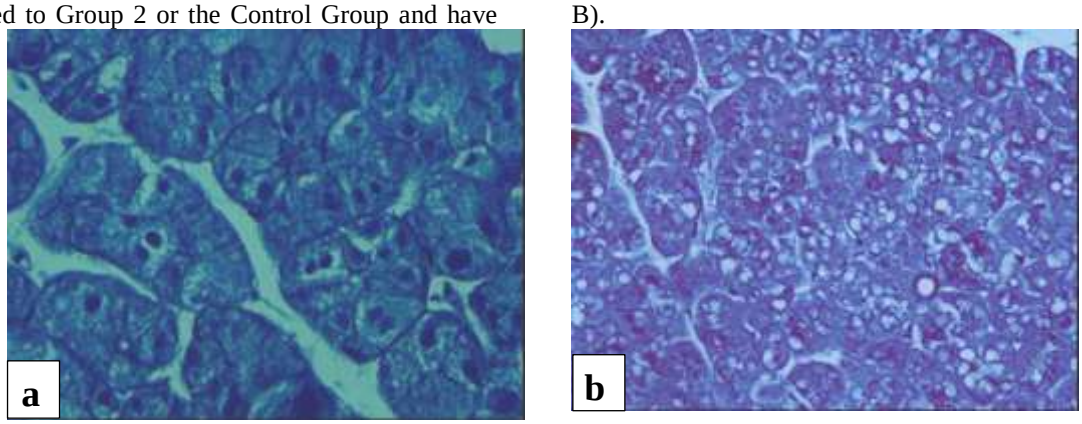


Fig. 6: Photomicrographs of parotid gland from (a) Control rats showing strong positive bromophenol blue reaction among normal secreting granules, (b) Rats fed AFB_1 contaminated diet showing weak reaction in the affected areas (Bromophenol blue reaction X 600, 400)

2.4-Evaluation of acid mucous glycoprotein:

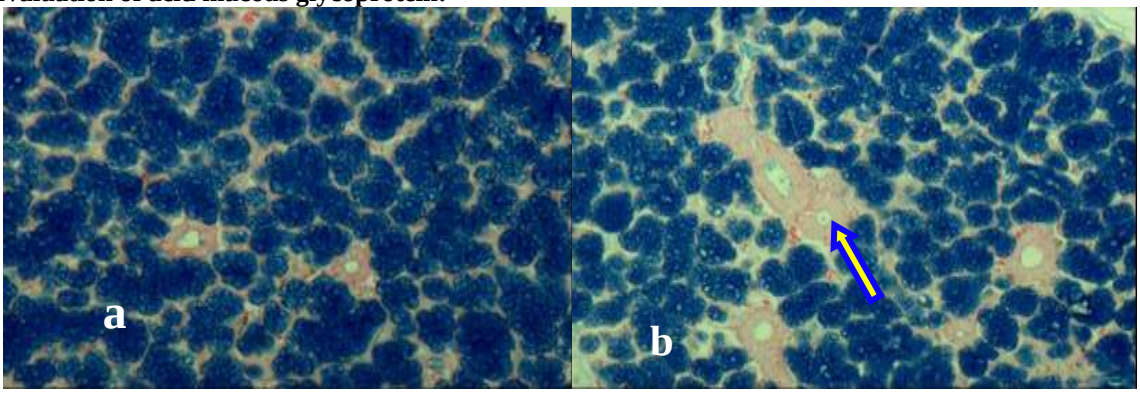


Fig. (7). (a) A section in the sublingual gland of control rat stained with AB (pH 2.5). The blue color indicates the site of acid mucosubstances. (b) A section in the sublingual glands from a rat fed AFB_1 contaminated diet stained with AB showing bluish cytoplasm coloration of the glandular cells. The damaged acini showing weak reaction and loss of mucin (AB reaction X150).

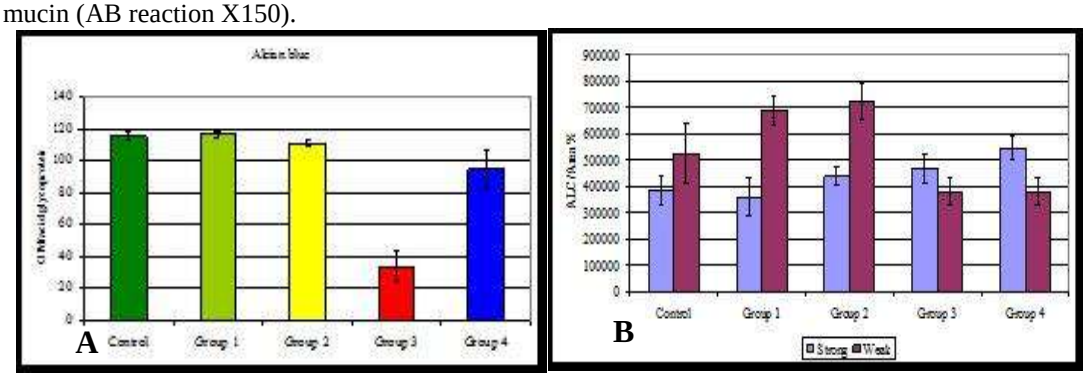


Diagram 4: (A) Mean optical density of acid glycoprotein positive reaction in sublingual gland. (B) Comparison of area percentage of weak or strong acid glycoprotein reaction in sublingual glands of the studied groups

4. Discussion:

Aflatoxins are highly toxic chemical poisons produced mainly by the fungus *Aspergillus flavus* in certain food crops. Previous studies have shown that acute aflatoxicosis can pose a considerable threat to productivity, tissue development and biochemical parameters (Miazzo *et al.,* 2005 and Mary *et al.,* 2012). Chronic exposure to low levels of contamination in crops consumed regularly increases liver cancer risk and can suppress the immune system, particularly for populations that test positive for the hepatitis B virus (HBV). Aflatoxins can also enter the human diet through livestock products if the livestock are given contaminated feed. High levels of aflatoxin contamination in consumed crops by human causes poisoning (aflatoxicosis) and even death. Children, women, and the poor are particularly vulnerable (Yan *et al.,* 2014). Children can be affected through breast milk, direct consumption of weaning foods or eating contaminated food. There is also some evidence to suggest that aflatoxin exposure can affect child stunting and lead to greater susceptibility or exacerbation of symptoms associated with human immunodeficiency virus (HIV), tuberculosis, and malaria (Bandyopadhyay *et al.,* 2007). Moreover, salivary gland secretions are of importance to oral mucosal health. Food contaminants entering the body through the oral route are directly exposed to the action of saliva. In the present study, the SLG, SMG and PTG of animals fed AFB_1 contaminated diet were characterized by alterations in their acinar cells. Previous study suggested that there were age related parenchymal changes in SLG of healthy subjects including acinar atrophy with concomitant ductal hyperplasia, and stromal changes, which demonstrate usually an increase in the connective tissue, blood and lymph vessels, adipose tissue and inflammatory infiltrate (Terézhalmy *et al.,* 2013). In the current study SLG alterations evidenced by increase in the mean area percentage of stromal tissues involved the inflammatory cells, edema, congestion and blood vessels accompanied by low mean area percentage of collagen fibers. The acinar mucous cells appeared vacuolated, shrunken or atrophy. Previous work indicated that AFB_1 induce fatty degeneration and increased collagen fibers (Abdel Wahhab *et al.,* 2010). The salivary glands produce peroxidase, an enzyme that protect against toxic agents including carcinogenic and mutagenic compounds (Miletich, 2010), however, glandular hypofunction can expose tissue to these agents and cause morphological alteration including malignant transformation (Jouzdani *et al.,* 2010). Former studies reported that biochemical and histochemical analysis of saliva revealed the presence of mucin as the main component, which showed sugar moieties and amino acids. The histochemical properties of mucins showed the presence of glycoproteins. According to the present study SLG represented positive reaction of bromophenol blue stain in their ducts and serous demilune cells, resulted in significant decrease in their total protein. The mucous cells produce and secrete mucous glycoproteins while the serous demilune cells secrete common salivary protein 1(csp-1), meanwhile 70% of salivary mucins are secreted from major SLG and numerous minor mucous glands (Terézhalmy *et al.,* 2013). Furthermore, salivary mucins possess properties (low solubility, high viscosity and adhesiveness) which enable them to concentrate on buccal mucosal surfaces, where they provide an effective barrier against desiccation, while glycoproteins may exert a protective role against enzymatic acidic elements in contact with the mucosa (Wang *et al.*, 2013).

In this study alcian blue stain at pH 2.5 was used to demonstrate the acid glycoprotein in SLG mucous cells, there was a marked reaction in all examined groups resulted in a significant decrease in acid glycoprotein contents in rats fed with AFB_1 contaminated diet compared to other treated groups. AFB_1 induced its toxicity and carcinogenicity through different mechanisms including DNA and the production of reactive oxygen species (ROS) resulting in oxidative damage (Abdel Wahhab *et al.,* 2010). Oxidative damage induced by ROS cause tissue damage by a variety of mechanisms including DNA damage, lipid peroxidation, protein oxidation and depletion of thiols. It is widely accepted that the cytotoxic effect of aflatoxin on normal differentiated cells is due to the production of ROS at high levels (Towner *et al.,* 2003). Additionally, the present results showed that SMG of rats fed AFB_1 contaminated diet revealed fatty and vacuolar degeneration in their acinar cells with significant increase in the mean area percentage of interstitial stromal tissues, this accompanied by a significant increase in the mean percentage of collagen fibers. The important component of saliva, which provide the salivary glands functions are proteins and glycoproteins (Wang *et al.,* 2013). In human 30% of mucin are secreted by SMG which are mixed glands composed of both serous and mucous acinar structure. The current study on SMG showed positive reaction of bromophenol blue stain in their ducts and acinar serous cells, and insignificant differences were noticed among the studied groups, however, significant decrease in total protein in ductal epithelial cells in rats fed AFB_1 contaminated diet was noticed. Because acinar cells contribute to a majority of saliva constituents including amylase and water, loss of these cells would impair normal saliva composition and lead to reduction in saliva flow (Wang *et al.,* 2013). The

findings in this study indicates increased incidence of histopathological alterations in parotid gland concerning rats fed with AFB_1 contaminated diet manifested by severe acinar atrophy, variable size and severe vacuolization of acinar cells. Marked dilatation in the stromal spaces resulted in significant increase in fibrous tissues namely collagen fibers and inflammatory cells infiltrate. In addition, some acinar nuclei replaced by irregular, hypertrophy, chromatin condensed mass and lost their nuclear envelops. These alterations may be attributed to ROS produced from AFs which may affect the cuboidal epithelial cells of the salivary glands (Sree *et al.*, 2008). The key step in oxidative stress is the production of reactive oxygen species (ROS) which initiate a variety of auto oxidative chain reactions on membrane unsaturated fatty acids and proteins, producing lipid peroxides and protein carbonyls respectively resulting in a cascade of reactions ultimately leading to destruction of organelles and macromolecules (Joanisse *et al.*, 1996). Hydroxyl radical $[OH]^{\cdot}$, hydrogen peroxide $[H_2O_2]^{\cdot}$ and superoxide radical $[O_2]^{\cdot}$, the ubiquitous products of single electron reductions of dioxygen, are among the most reactive compounds known to be produced during oxidative stress (Dietz *et al.*, 1999). Removal of ROS and cellular homeostasis is regulated by antioxidant enzymes such as superoxide dismutase, catalase and peroxidase along with various other endogenous antioxidants like ascorbate, thiols and glutathione. The glutathione cycle enzymes and the glutathione metabolism enzymes operate for the maintenance of glutathione levels and prevention of free radical induced cellular damage (Joanisse *et al.*, 1996). Moreover, it has already been reported that the antioxidant genes have the lowest expression in salivary glands when compared with mid gut and body fat (Mittapalli *et al.*, 2007) and hence the salivary glands are more prone to oxidative stress induced by AFs. On the other hand, AFs has been found to affect the calcium balance and protein phosphorylation in the cell (Abdel Wahhab *et al.*, 2006). All these together play a vital role in adversely damaging the salivary glands as shown in AFs contaminated feed. The current study also revealed that animals fed AFB_1 decontaminated diet (Group 4) showed a more or less normal histological and histochemical picture of SLG, SMG and PTG. These results indicated that treatment of the contaminated diet with O_3 succeeded to eliminate the AFB_1 content and consequently reduce the ROS production resulting in the prevention of its dangerous effects on salivary glands. This is coincide with the work of Tiwari *et al.*, 2010 which indicated that O_3 gas being a powerful oxidant capable of reacting with numerous chemical groups, though it has an affinity for double bonds.

Conclusion:

The current study revealed that AFB_1 induced alterations in salivary glands in rats. These alterations are mainly attributed to the oxidative stress induced by AFB_1. Detoxification of AFB_1 by Ozone succeeded to avoid these effects. Moreover, Ozone itself was safe and has no side effects on salivary glands.

Recommendation:

1-Place special focus on monitoring foods especially those used for pregnant women and infants/children (cereals, complementary foods).

2-Conduct multi sectorial behavioral change campaigns for food safety against aflatoxin, especially among mothers, pregnant and lactating women, caregivers of infants, and immune compromised individuals.

3-Information is needed concerning base line levels of chronic exposure to aflatoxin in different areas of Egypt for better quantification of the health risks. Such knowledge will enable the public health community to better understand health effects associated with chronic exposure and allow for the evaluation of future public health and agricultural interventions.

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