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THE PROTECTIVE ROLE OF SESAME OIL ON RAT PAROTID SALIVARY GLANDS SUBJECTED TO LEAD EXPOSURE

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ABSTRACT

INTRODUCTION: Lead poisoning is a threat to public health due to its permanent health consequences. Since antioxidant integration is considered as the most effective therapy sesame oil has been recommended for preventing lead-induced poisoning. The purpose of this research is to examine the ultrastructural changes that may develop in the acini and duct system of the parotid salivary glands of adult albino rats after exposure to lead acetate, and to identify the possible protective effect of sesame oil.
MATERIALS AND METHODS: This research included 24 adult male albino rats weighing between 180-200g. Randomly, the rats were separated into three equal groups: Group I (Control), Group II (Lead acetate), and Group III (Lead acetate + Sesame oil). After five weeks of experimentation, blood was drawn and tested using atomic absorption spectroscopy (AAS) to determine the blood lead level. After euthanization, glands were dissected for transmission electron microscopy analysis.
RESULTS: Group (II) blood results demonstrated a rise in blood lead levels, but group (III) blood lead levels were midway between groups (I) and (II), demonstrating the therapeutic impact of sesame oil. Group (II) revealed acinar cells with evidence of cytotoxicity, including degenerated mitochondria, apoptotic nuclei, whereas the ducts had lumens with stagnant secretion. The ultrastructure of their acinar cells and duct system improved significantly in group III.
CONCLUSIONS: Sesame oil reduced the lead acetate toxicity in rats, which may be utilized as a prophylactic strategy in order to avoid the permanent negative effects of lead acetate exposure.
KEY WORDS: Lead acetate, salivary glands, parotid gland, sesame oil, sesaminol, antioxidant.
RUNNING TITLE: Effect of lead and sesame on parotid glands.

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INTRODUCTION

In recent years, rising ecological and worldwide public health concerns have been related with heavy metal poisoning of the environment. This is due to a drastic increase in human exposure as a consequence of an exponential rise in their usage in a variety of industrial, agricultural, and residential applications (1).

Lead is a naturally occurring, bluish-gray metal found in trace quantities in the crust of the planet. Due to its remarkable physicochemical characteristics, its usage dates back to ancient times. Although lead is naturally present in the environment, human activities such as the combustion of fossil fuels, mining, and manufacturing contribute to the release of high amounts. It is now used in the manufacture of lead–acid batteries, ammunition, metal goods (solder and pipes), and X-ray shielding devices (1).

Due to its non-biodegradable nature and continued usage, its concentration increases in the environment, posing a growing risk to human health (2). It is the most dangerous heavy metal present in the environment. The United States Agency for Toxic Compounds and Disease Registry (ATSDR) ranked lead as the second on the priority list of toxic substances (3).

Lead is absorbed by inhalation, ingestion, and contact with the skin. Nonetheless, the bulk of lead enters the body via the digestive system through contaminated food and drink (4). It affects almost every organ in the body. From overt clinical signs

of toxicity to concealed biochemical consequences, the hazards constitute a continuum. Lead poisoning often affects the neurological system the most in both newborns and adults (5). Long-term lead exposure has been related to anaemia and a rise in blood pressure, especially in elderly and middle-aged individuals (6).

Although the scientific processes behind the association between lead exposure and dental illness are unknown, it has been hypothesised that lead impairs salivary gland function, hence increasing the incidence of dental caries. Rats exposed to lead throughout the prenatal and perinatal periods exhibited an increase in dental caries and a substantial decrease in salivary flow (7).

There are three pairs of main salivary glands situated outside the oral cavity, the parotid, submandibular, and sublingual, with extensive duct networks that transport gland secretions to the mouth. The parotid glands produce 25% of total saliva, making them the biggest glands. They are glands that secrete a watery saliva abundant in enzymes like amylase, proteins like proline-rich proteins, and glycoproteins (8).

Lead accumulation in the submandibular and parotid glands of rats and its release into saliva have been documented earlier (9). Rats treated with lead acetate were shown to have altered secretions of protein, calcium, and the lysosomal enzyme N-acetyl-/-D-glucosaminidase (NAG) (10).

In addition to lower salivary calcium and protein concentrations, increased lipid peroxidation, and decreased total antioxidant capacity in salivary gland tissue, adult rats exposed to lead through drinking water showed lower salivary calcium and protein concentrations. This suggested the presence of lead-induced oxidative damage. (7).

Oxidative stress is an imbalance between the creation of free radicals and the cell's capacity to detoxify or repair the very reactive intermediates. The formation of reactive oxygen species (ROS), such as hydroperoxides (HO2), singlet oxygen and hydrogen peroxide (H2O2), and the direct depletion of antioxidant reserves both contribute to oxidative stress. In each biological system where the generation of reactive oxygen species (ROS) occurs, antioxidant reserves are reduced (11).

Lead has permanent detrimental effects on the body. Early identification and removal of the lead source to avoid additional exposure is the most effective method for treating lead poisoning (12). Majority of chelators have been used for the treatment of acute poisoning; However, many of them revealed variety of adverse effects and were unsuitable for preventing chronic heavy metal toxicity. Thus, the investigation of natural antioxidants became a necessity(13).

Sesamol is one of the sesame oil's non-fat antioxidants. Effective against drug- and chemical-induced organ damage (14,15). Sesamol is antiphotooxidative due to the fact that it consumes singlet oxygen (16). It suppresses lipid peroxidation, deoxyribose degradation mediated by hydroxyl radicals, and DNA breakage (17). It also inhibits glial astrocytes' synthesis of nitric oxide, hydrogen peroxide, and monoamine oxidase (18). Moreover, sesame oil as a dietary supplement and nutraceutical alleviates certain diseases (19). Oil pulling demonstrates that sesame oil is readily and promptly absorbed by the gastrointestinal tract, beginning in the mouth cavity (20).

In the long history of sesame as a crop and oil, there have been no reports of sesame seed extracts causing damage. Sesame oil and sesamol had no discernible side effects when used to treat lead poisoning in rats (21-24). Because metal chelating treatment is linked with negative side effects, sesame oil and sesamol may be superior options for treating heavy metal poisoning. So, this study was conducted to evaluate the preventive effect of sesame oil on parotid salivary glands of rats subjected to lead acetate.

MATERIALS AND METHODS

Ethical considerations

The study was performed after gaining the approval of the Research Ethics Committee, Faculty of Dentistry, Alexandria University. The animals were housed in specially designed wire mesh bottom cages and received water and diet throughout the experimental period. Besides, the research facilities had procedures that minimize pain and stress to the animals.(Approval number:0217-01/2021)

Study sample

In this investigation, 24 albino male rats weighing between 180 and 200 g and varying in age from 3 to 6 months were included. Randomly, the rats were split into three groups of eight rats each (25).

Chemicals:

1. Lead acetate: Inorganic lead acetate trihydrate powder $Pb(C_2H_3O_2)_2.3(H_2O)$ was purchased from Riedel De Haen, Germany and incorporated in the rats diet.
2. Pure sesame oil was purchased from Imtinan health shop.

Random allocation

- **Group I (control group):** 8 rats were housed under normal conditions and received basal diet.
- **Group II (study group 1) (Lead acetate group):** 8 rats received 500 mg lead acetate/kg diet once daily for 5 weeks.
- **Group III (study group 2) (Lead acetate + sesame oil):** 8 rats were given 500 mg lead acetate/kg diet daily concurrently with sesame oil (5 ml/kg body wt/day) orally by gavage for 5 weeks.

Blood test

By the conclusion of the experiment and before euthanization, blood was collected through sinus puncture to be processed by atomic absorption spectrometry (AAS) to detect the blood lead concentration. (26)

Transmission electron microscope

To prepare for transmission electron microscopy, parotid salivary glands were dissected out and immersed in 2.5 percent glutaraldehyde in phosphate buffer. (27)

Statistical Analysis

Kolmogorov-Smirnov test of normality revealed no significance in the distribution of the variables, so the parametric statistics was adopted. Comparisons were carried out using one-way ANalysis Of VAriance (ANOVA) test. Post-hoc multiple comparisons was done using Games-Howell method. During sample size calculation, beta error accepted up to 20% with a power of study of 80%. An alpha level was set to 5% with a significance level of 95%. Statistical significance was tested at p value <.05.

RESULTS

1- Laboratory investigations: Effect of lead acetate and sesame oil on blood:

At the end of the experimental period (5 weeks), the results showed the comparison among the three groups regarding the concentrations of the blood lead level in micro grams/ml. No statistic significant difference was noted between the (group I) control group and (group III) the group receiving lead acetate and sesame oil. While significant difference was noted between the lead group (group II) and control group (group I), (Fig. 1) (Table 1).

Table 1: Comparison between the different studied groups according to blood lead level.

Concentration (µg/ml)	Group		
	Control (n=8)	**Lead acetate (n=8)**	**Lead acetate + sesame oil (n=8)**
- n - Min. – Max. - Mean ± SD - 95% CI of the mean - 25th Percentile –75th Percentile - Interquartile Range - KS test of normality	8 0.169-0.464 0.330±0.102 0.245-0.416 0.260-0.411 0.166 D=0.210, *p*=.200 NS	8 0.256-1.993 1.241±0.623 0.720-1.762 0.719-1.751 1.150 D=0.153, *p*=.200 NS	8 0.567-0.970 0.745±0.135 0.632-0.858 0.654-0.855 0.217 D=0.184, *p*=.200 NS
Test of significance *p* value	$F_{(BF)\ df=2}$ =11.965 *p*<.001*		
Post hoc multiple comparison (Games-Howell test)			
	Control	Lead acetate	Lead acetate + sesame oil
Control		diff=-0.910875 *p*=.010*	diff=-0.414750 *p*<.001*
Lead acetate			diff=-0.496125 *p*=.133 NS
Lead acetate + sesame oil			

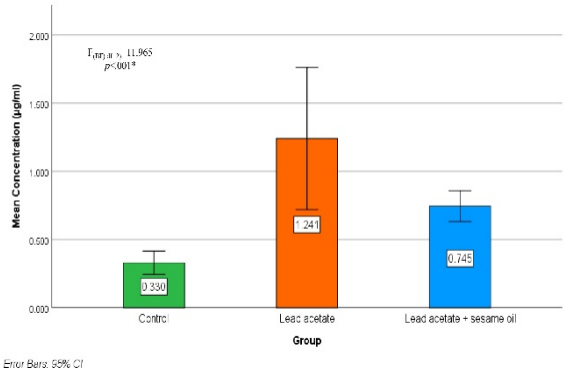

Figure 1: Simple Bar of Mean of concentration (µg/ml) (± 95% CI) in the studied groups.

2-Ultrastructural results: Observations of the parotid salivary glands using a transmission electron microscope showed variations among the 3 groups. Nonetheless, all specimens from the same group had identical findings.

Group I (control)

The serous acini of the parotid gland showed normal euchromatic nucleus with a substantial number of regular rough endoplasmic reticulum in the cytoplasm. In addition, there were many, well-organized Golgi complexes and several mitochondrial figures. Multiple electron dense secretory granules were clearly seen (Fig.2a). The duct exhibited a lumen of uniform shape and size. It revealed typical epithelial lining with nuclei of regular shape, normal parallel rER and multiple secretory granules. (Fig.2b)

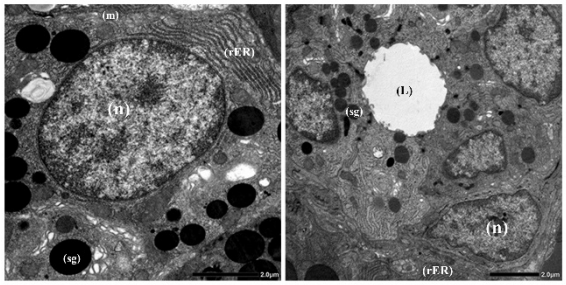

Figure 2: (a) TEM, group (I), showing: large regular euchromatic nucleus (n), regular parallel cisternae of rough endoplasmic reticulum (rER), numerous electron dense secretory granules (sg), well developed multiple mitochondria (m), well developed Golgi apparatus (g). (X4000).
(b) TEM of group (I), showing: intercalated duct with: Regular shaped lumen (L), normal epithelial lining with regular shaped nuclei (n), well developed mitochondria (m), normal well-developed rough endoplasmic reticulum (rER), multiple secretory granules (sg), golgi apparatus (g), myoepithelial cell (X3000).

Group II (Lead acetate group)

In the serous acinar cells, there were many dilated rough endoplasmic reticulum, lysosomes, and cytoplasmic vacuolations. Additionally, the mitochondria were degraded, uneven, and swollen. (Fig.3a) The intercalated duct cellular lining exhibited degenerative alterations including smaller and irregular nuclei and altered mitochondria. The lumen was small in size with obvious stagnant secretion (Fig.3b).

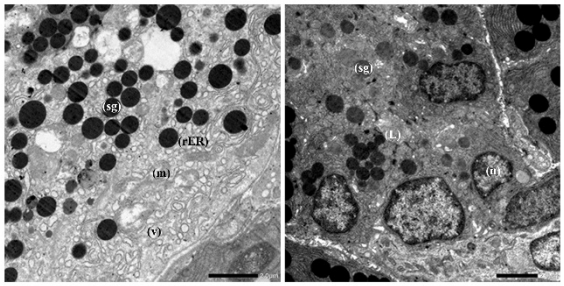

Figure 3: (a) TEM of group II (lead) showing acinar cells with: irregularly dilated rough endoplasmic reticulum cisternae (rER), multiple secretory granules (sg), numerous degenerated mitochondria (m), multiple cytoplasmic vacuolations (v). (X3000).
(b) TEM of group II (lead) showing intercalated duct with: irregular dilated endoplasmic reticulum epithelial lining showing shrunken nuclei (n), Lumen with stagnated secretion (L) (X2500).

Group III (study group 2) (Lead acetate + sesame oil)

Normal nuclei with normal chromatin distribution and many dense secretory granules were seen in serous acini cells. Moreover, the cytoplasm appeared normal with regular mitochondria and a properly oriented rER (Fig.4a) The intercalated duct's epithelial lining was normal, with regular euchromatic nuclei, mitochondria, and a circular lumen (Fig.4b).

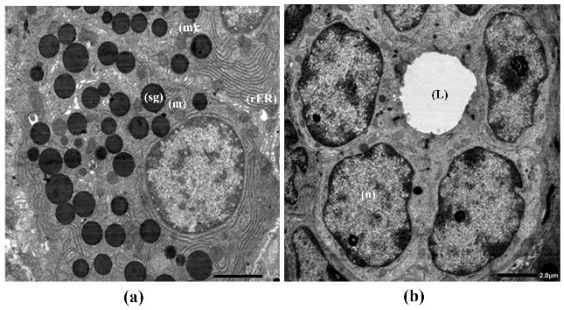

Figure 4: (a) TEM of group III (lead + SO) showing acinar cells with: normal nucleus (n), regular rough endoplasmic reticulum (rER), multiple mitochondria (m), numerous secretory granules (sg), well developed golgi apparatus (g), and microtubules (mt) (X3000).
(b) TEM of group III (lead + SO) showing intercalated duct with: normal epithelial lining showing regular euchromatic nuclei (n), mitochondria (m), regular circular lumen (L), and regular rough endoplasmic reticulum(rER) (X2500).

DISCUSSION

It is thought that lead is quickly absorbed into the circulation and has detrimental effects on almost all body organs. Recent interest in the use of natural medicines to guard against heavy metal toxicity is high owing to their anti-oxidative qualities and clear safety, as well as the fact that metal chelating treatment is linked with side consequences (26). This study was done due to the paucity of research on the effect of lead poisoning on parotid salivary glands and the possible preventive role of antioxidants such as sesame oil.

Through food and drink adults absorb 7–15% of all Pb whereas children absorb 30–50%. (4). In prior research, lead acetate was administered through oral intubation; however, as the majority of lead exposure occurs via food, 500 mg lead acetate/kg was added to the experimental rats' base meals (28). The length of the current study was based on prior research that demonstrated a substantial increase in blood alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) levels after 6 weeks of chronic lead exposure (28).

Sesame oil was administered orally by gavages at a volume of 5 ml/kg body weight based in prior research in which it significantly reduced the oxidative stress of cypermethrin-induced brain damage (29).

In the current research, blood tests indicated a considerable rise in group II compared to group III. Detecting lead through saliva is promising because of its accessibility but a weak association between blood lead level and saliva lead level (30), reported a correlation coefficient of 0.156. Demonstrating that PbS is not a surrogate for PbB but is an important biomarker in its own right and this explains why blood lead level was used in this study.

Electron microscopy of group II's parotid glands demonstrated that lead is capable of causing degenerative effects on the acinar cells and duct system.

In a study of the oxidative impact of lead poisoning on the submandibular gland and saliva of rats, impaired secretary function and changed salivary composition were related to increased oxidative stress (10). The disruption of enzymes and structural proteins is largely responsible for the toxicity of lead. This was corroborated by a biochemical investigation in which rats were administered 100 mg/L of lead in their drinking water for fifteen weeks. (31). By interfering with oxidative metabolism and boosting lipid peroxidation, lead exposure exacerbated stomach

ulcers. It was revealed that lead has a dual impact, increasing the generation of free radicals while simultaneously diminishing endogenous antioxidant enzymes, which are free radical scavengers. (32-34). Therefore, the concentration of free radicals resulted in inflammation and enhanced cell damage, which may explain the observed degeneration of parotid acinar cells in the present research.

In contrast, in the current work, electron microscopic studies of the acinar cells and duct system revealed that group III rats had a nearly identical ultrastructural appearance to group I control rats. These results support the use of sesame as a protective antioxidant agent.

In line with these findings, co-administration of sesame oil and lead acetate in a separate trial dramatically restored the normal organisation and architecture of the hepatocytes, and the central vein seemed normal and biochemical profiles in serum hepatic functioning markers were restored. (26). In another research, it was revealed that the livers of rats given sesame oil and Fenvalerate had normal histology, indicating that sesame oil provides significant protection (35). In rats with acetaminophen-induced acute liver damage, sesame oil improved antioxidant status and prevented lipid peroxidation (36).

Although the exact mechanism by which dietary sesame oil reduces oxidative damage is unclear, it is believed that the antioxidant components (sesamol, sesamolinol, and sesaminol) may be substantially responsible for this protective effect (26).

CONCLUSIONS

The present research demonstrated the dietary toxicity of lead acetate on the parotid salivary glands of rat models. In addition, it verified the preventive function of sesame oil against these toxicological effects. Therefore, sesame oil might be utilised as a precaution against lead acetate risks.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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