# Can Eruca *Sativa* Oil Ameliorate Nicotine Induced Alteration in Rat Parotid Gland? Histological Study

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**Original Article**

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## ABSTRACT

**Objective:** To investigate the possible protective effect of oral administration of Eruca *sativa* oil against nicotine induced alterations on rat parotid gland using histological ,immunohistochemical and morphometric methods.

**Materials and Methods:** Thirty adult male rats were included in the study. They were randomly divided into to the following equal groups G1, control group which received standard rat pellets and oral saline. In G 2 nicotine tartrate was given daily at a dose of 50 mg/kg for 3 weeks. G3 which received nicotine tarterate orally at a dose of 50 mg/kg plus Eruca *sativa* oil at a dose of 0.5 ml/Kg for 3 weeks. At the end of study, animals were euthanized under deep ether anesthesia and the front of neck was dissected to free and extract the parotid glands in one block. They were fixed in 1% neutral buffered formalin and processed. 5 micron thick sections were stained by Hematoxylin and eosin and Masson trichrome. Immunostaining using specific antibodies for α smooth actin and tumor protein P53 was done, this was followed by morphometric and statistical studies.

**Results:** Histological sections revealed that oral nicotine induced histological alterations in parorid gland compared with control sections manifested as focal atrophy, vacuolation and karyomegaly of serous acini cells. Loss of striated ducts basal striation with degenerated nuclei and vascular congestion along large ducts were observed. Masson trichrome sections showed increased collagen fiber deposition with nicotine intake . Immunohistochemical sections demonstrated increased expression of α smooth actin and P53 in myoeithelial cells. Most of these changes were ameliorated by oral administration of Eruca *sativa* oil.

**Conclusion:** Oral nicotine has exerted degenerative effect on rat parotid gland. Eruca *sativa* oil was found to have protective role and can improve the nicotine-induced degeneration. Thus, it can be advised with antismoking management therapy.

**Received:** 02 January 2022, **Accepted:** 19 February 2022

**Key Words:** Eruca *sativa*; immunohistochemistry; morphometry; nicotine; parotid gland.

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**ISSN:** 1110-0559, Vol. 46, No. 4

## INTRODUCTION

Dental health and normal functions of the mouth greatly depend on normal flow of saliva[1]. Saliva is essential in the partial digestion of carbohydrate and plays a role in buffering the mouth and esophagus .It provides protection against acid-induced dental caries[1,2].

People worldwide are practicing the bad habit of smoking despite its impact on the oral and dental health[3]. Nicotine is the most abundant component in cigarette smoke, which represents one of the most common pharmacologically active substances used by human. It has genotoxic effects on human blood cells[4]. It has been reported to induces DNA damage in the salivary gland of human[5].

Contradictory data was reported regarding the effect of smoking and nicotine on salivary gland functions. Some researchers reported that short-term smoking upsurge the volume of saliva via stimulating taste receptors by nicotine[6]. However, another study has demonstrated that long-term smoking lowers salivation and changes its quality[7]. A previous study demonstrated the chemical stimulation of nicotine on an animal model and explained that nicotine acts as sialogogue via working on nicotinic receptors as agonists thus motivated the secretion of saliva[8].

 *DOI: 10.21608/ejh.2022.111447.1610*

Alves da Silva *et al*. 2018 raised the importance of dentists' awareness about indication of using herbal medication for clinical application and their possible interaction with other medicinal drugs especially used for treatment of chronic diseases[9].

Eruca *sativa* (Mill) is Known as Rocket plant or Aljarjeer, which is a herbal pant belongs to Brassicacae family[10]. It has diverse biological and pharmacological properties and it is commonly used as salad vegetable and spice, especially among Mediterranean people.

Eruca *sativa* components were studied for their biological effects including its anti-bacterial activity[11]. Importantly, it was found to be a potent natural antioxidant, since it is a rich source of vitamins, like vitamin C, polyphenols and carotenoids[12-14].

It has been shown that Eruca *sativa* seeds and leaves influenced a free radical antioxidant effect and protect against oxidative injury by increasing or sustaining the levels of antioxidant enzymes and molecules[15,16]. Studies revealed that Eruca *sativa* seeds have a hepatoprotective and chemopreventive potency against hepatic injury and liver cancer cells[17,18].

The aim of this work was to evaluate the possible ameliorative effect of Eruca *sativa* on the altered structure of the parotid gland induced by oral nicotine administration in rats. Histological ,immuno-histochemical and morphometric techniques were applied to confirm the hypothesis.

## MATERIALS AND METHODS

### *Materials*

### *Animals*

Thirty adult male Wistar albino rats (3 months old) weighing 200–250 gm were obtained from the Animal House King Fahd Medical Research Center (KFMRC) at King Abdulaziz University, Jeddah, Saudi Arabia. The rats were housed in wire-meshed cages at a temperature (22 ± 2 ˚C), humidity (55%) and light-dark cycle (12/12 hours). Animals had free access to Purina chow diet and drinking water ad libitum. In this study, the protocol followed the guidelines of animal care at KFMRC. Rats were left for one week to acclimatize lab conditions. Ethical approval and animal care guidelines were taken before carrying out the experiment.

### *Plant Materials*

Eruca *sativa* oil was obtained from Nature's way company (GNC) from the local market in Jeddah, KSA.

### *Nicotine*

Nicotine tartrate was purchased from Fluka AG, Chemische Fabric, Ch-9470 Buch; Switzerland. Hydrogen tartrate salt of nicotine powder (Sigma N-5260) was dissolved in 0.9% NaCl solution (0.15 mg/ml concentration of nicotine solution). The PH of solution was then adjusted to 7.4 using 0.1 NaOH[19].

### *Methods*

### *Experimental approach*

This experimental study was approved by the local animal care committee of King Abdulaziz University, Jeddah, Saudi Arabia, and carried out in accordance with the international principles of laboratory animal research.

Rats were randomly assigned into three equal groups:

**Group I:** 10 rats served as control and were given saline in a quantity equal to that of nicotine tartrate orally using gastric gavage.

**Group II:** 10 rats were given nicotine tartrate orally daily using gastric gavage tube in a dose of 50 mg/kg BW dissolved in saline for 21 days.

**Group III:** 10 rats were treated with 0.5 ml of Eruca *sativa* oil in addition to the 50 mg /kg BW of nicotine tartrate daily by gavage for 21 days.

### *Histological preparation for routine staining*

By the end of the experiment, all rats were euthanized under deep ether anesthesia, the skin over the front of the neck was opened at midline and salivary glands were exposed and removed completely, unsheathing from surrounding connective tissue, fixed in 10% neutral phosphate buffered formalin and routinely processed for paraffin blocking. Sections (5 micrometer-thick) were stained with Hematoxylin and Eosin (H&E) for studying general histological structure, Masson's trichrome for collagen fibers Slides were visualized using Olympus light microscope (model: BX51TF- Japan) and photographed.

### *Preparation of tissues for routine immunostaining*

The preparation process was performed as descried by Randall and Pearse, 2008. For alpha smooth actin, after blocking, the excess blocking serum was removed, and the slides were incubated with mouse monoclonal antibody SMA-CAT NO-7602601 and P53 CAT NO – 6012332 individually. Each was diluted 1:50 in TBST (Tris Buffered Saline with Tween) for 60 minutes. After washing, the slides were incubated for 30 minutes in mouse-specific EnVision+System–HRP (Dako UK Ltd., Ely, UK), and visualized by incubation in DAB (diaminobenzidine solution) for 10 minutes. The slides were then counterstained by hematoxylin. Staining was negatively controlled by replacing mouse immunoglobulin (Ig) fraction, diluted to the exact Ig concentration, for the primary antibody[20].

### *Morphometric analysis*

The area percentage of collagen fibers in Masson trichrome stained sections, as well as immunostained myoepithelial cells and expression of p53 in myoepithelial and in cells lining the secretory acini were measured for five non-overlapping visual fields (x400) in ten random sections using Image Pro Plus software (version 4.5) connected to Olympus light microscope, (Figure 1).

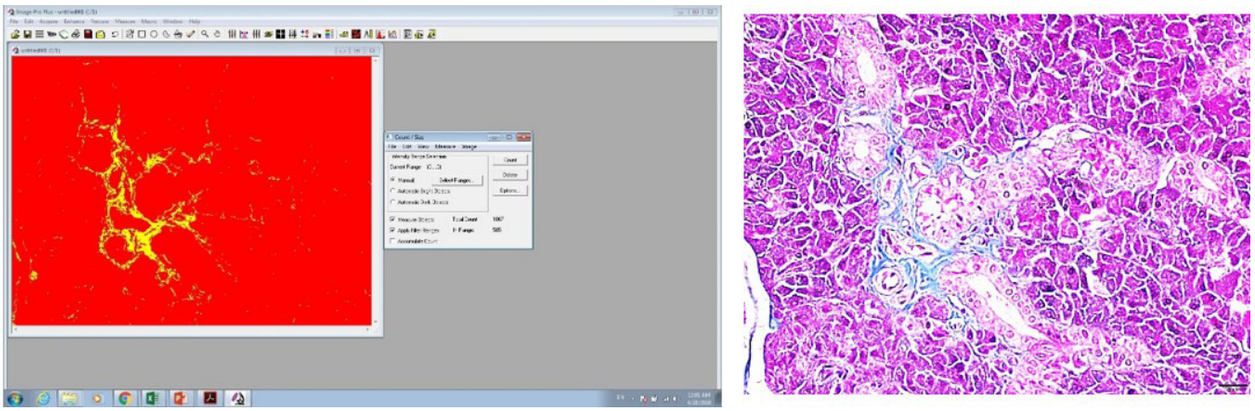

**Fig. 1:** The threshold tools implemented in measuring the percentage of collagen stained area using Image Pro-Plus software.

### *Statistical analysis*

Statistical analyses were done using SPSS program version 16 (IBM-USA). The quantitative data were expressed as averages ± standard deviation. Data were then graphed and statistically analyzed. Statistical differences were investigated using One-way analysis of variance (ANOVA) when equal variance could be assumed, the LSD-t test was applied. $P<0.05$ was considered statistically significant.

## RESULTS

### *Histological results*

H&E staining revealed the histological structure of parotid gland of the control rat (G1) as secretory acini of the serous type. the acinar cells were pyramidal, with basal dense basophilic cytoplasm surrounded the rounded vesicular nuclei. Their apices defined the acinar lumen and showed acidophilic granules. Intercalated ducts were lined by cuboidal epithelium while the striated ducts were lined with high cuboidal epithelium showing well defined basal striations (Figure 2a).

Parotid gland sections of group II (receiving nicotine) showed acini lined by cells with cytoplasmic vacuoles of various sizes, compressing the nuclei to be crescentic. Some acinar cells had large sized nuclei or karyomegaly. Striated duct cells exhibited focal loss of basal striation and some sections revealed mononuclear cell infiltrate. Focal vascular congestion was also observed (Figure 2b ).

Microscopic examination of parotid gland sections of group III (receiving nicotine and Eruca) revealed well organized architecture of both ducts and acini. Focal areas of cellular vacuolization of the striated ducts were still observed (Figure 2c).

Masson trichrome stained sections of the control group revealed minimal amount of collagen fibers in the vicinity of inter lobular ducts (Figure 3a). An increase of collagen fibers deposition was revealed in sections of group 2 (Figure 3b). Collagen fibers decreased in group 3 administered Eruca *sativa* with nicotine (Figure 3c). The decrease in mean area percentage of the collagen stained area was statistically significant compared to nicotine group (Figure 3d)

### *Immunohistochemical results*

### *Expression of Alpha smooth actin*

Immunohistochemistry for alpha smooth actin showed uneven moderate positive reaction (brown cytoplasmic staining ) in the myoepithelial cells located at the basal parts of acini in the control group (Figure 4a).

More increase in alpha smooth actin expression was observed in sections from G2 (Nicotine group) (Figure 4b)than in sections from G3 (rats receiving Eruca *sativa*) (Figure 4c) compared to control group This was confirmed by morphometric study where the mean area percentage of Alpha smooth actin immune expression was significantly increased in Gr 2 sections in comparison with group I ($P < 0.01$) .The increase in Gr 3 sections was insignificant (Figure 4d).

### *Expression of P53*

Focal or mild positive reaction was observed in some acinar cells in control sections (Figure 5a). An increase in apoptotic cells stained by P53 was detected in some cells of the secertory acini from nicotine group (Figure 5b). In GIII, expression of p53 was much similar to control with few scattered myoepithelial cells showed positive reaction (Figure 5c). Statistical analysis of the mean area percentage of p53 stained cells was represented in (Figure 5d).

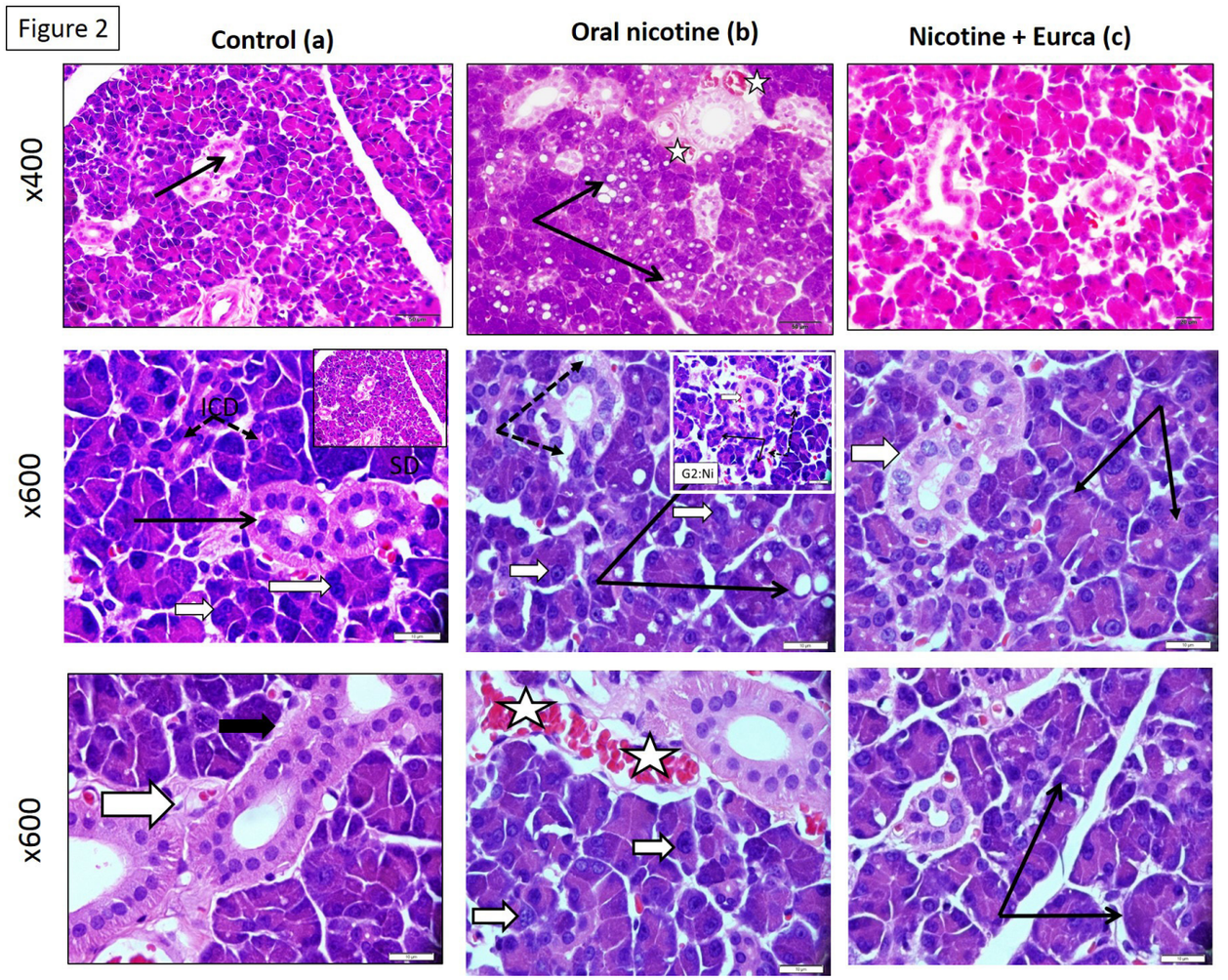


**Fig. 2:** Photomicrographs of sections in rat parotid gland stained by H&E :
a. G1- Control: showing well organized acini lined with pyramidal cells that have basal basophilic cytoplasm with vesicular nuclei (white arrows). The apical part appears acidophilic in staining. Intercalated duct (ICD, dotted arrows) have cuboidal cell lining and narrow lumina. Striated ducts (SD) show typical basal acidophilic striation and apical rounded vesicular nuclei. Blood capillaries between the acini and near ducts appear normal.
b. G2- Nicotine: showing acini lined by cells with cytoplasmic vacuoles of various sizes, compressing the nuclei to be crescentic. Some acinar cells showed large size nuclei or karyomegaly. Striated duct cells showed focal loss of basal striation (dotted arrows) Inset: showing mononuclear cell infiltrate (arrows) Notice focal vascular congestion
c. G3- Nicotine and Eruca: gland section showing marked decrease in nicotine induced changes. Most of the serous acini are similar to the control group. Note few cytoplasmic vacuolation in the acini and striated ducts

Figure 3

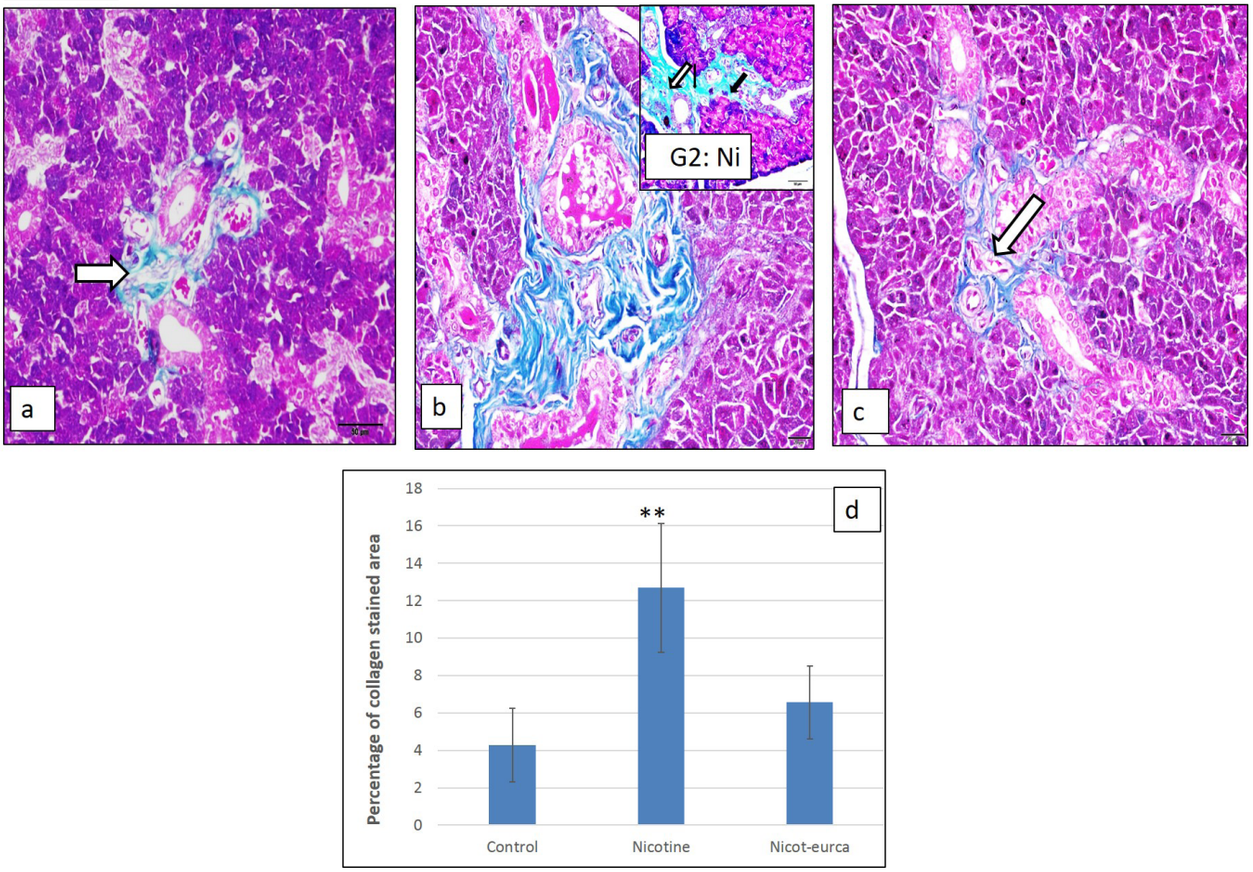


**Fig. 3:** Photomicrographs of sections from rat parotid gland stained with Masson trichrome for collagen fibers:
a. G1- Control: showing collagen fibers distribution (white arrow) in the vicinity of inter- lobular ducts.
b. G2- Nicotine: showing apparent increase in collagen deposited around the ducts (white arrows).
c. G3- Nicotine and Eruca: showing preservation of normal distribution of collagen.
d. Statistical graph: showing the mean area percentage of collagen in the rat parotid gland sections of control, Nicotine and Nicotine + Eruca groups. One-way analysis of variance (ANOVA) test.
Values are expressed as mean ± SD. ** = Highly significant ($P < 0.01$).

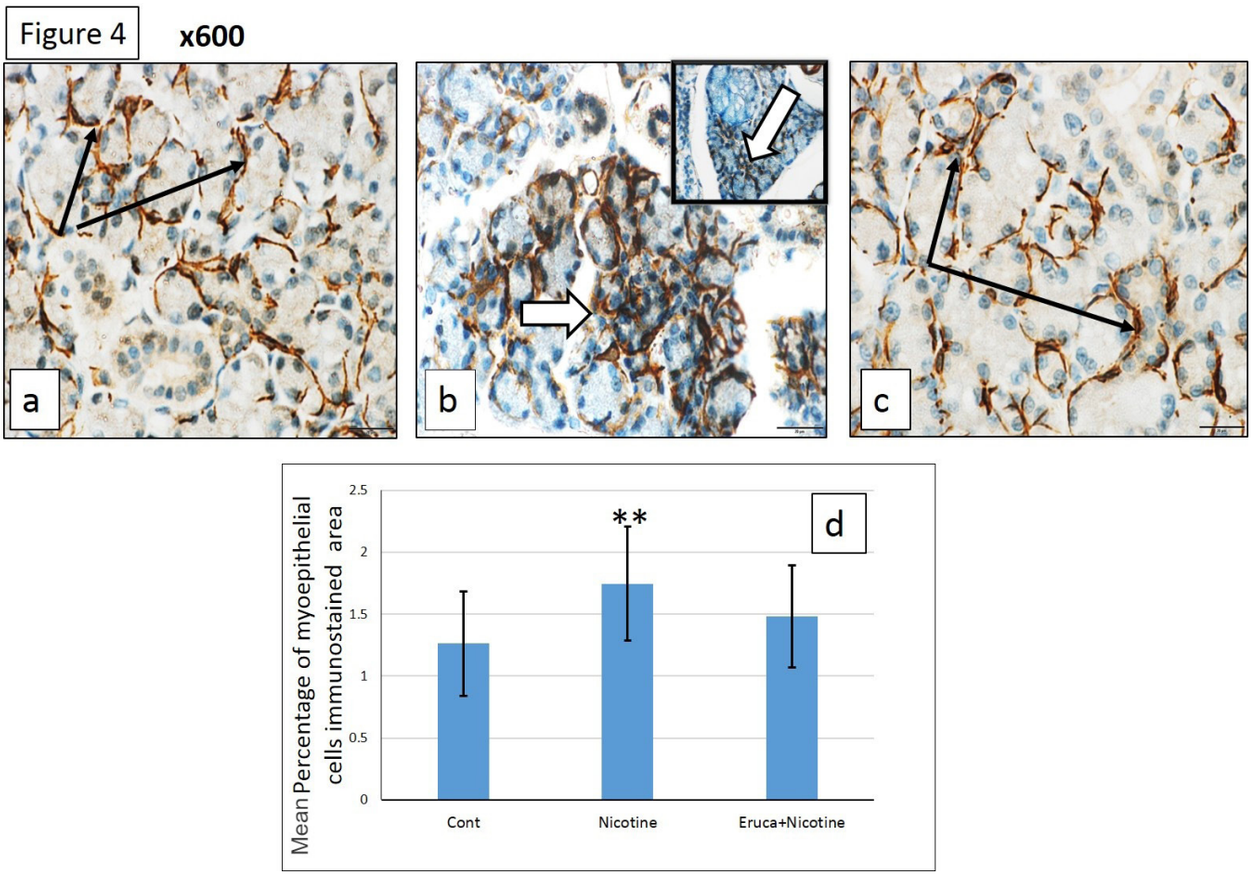


**Fig. 4:** Photomicrographs of sections from rat parotid gland immunohistochemically stained for α- Smooth actin (in myoepithelial cells):
a. G1- Control: showing positive expression at the basal parts of most acini (black arrows).
b. G2- Nicotine: showing focal increase in expression around degenerated acini (white arrow).
c. G3- Nicotine and Eruca: showing positive expression nearly similar to control (arrows).
d. Statistical graph: showing statistical analysis mean area percentage of α- Smooth actin in different groups. One-way analysis of variance (ANOVA) test.
Values are expressed as mean ± SD. ** = Highly significant ($P < 0.01$).

Figure 5

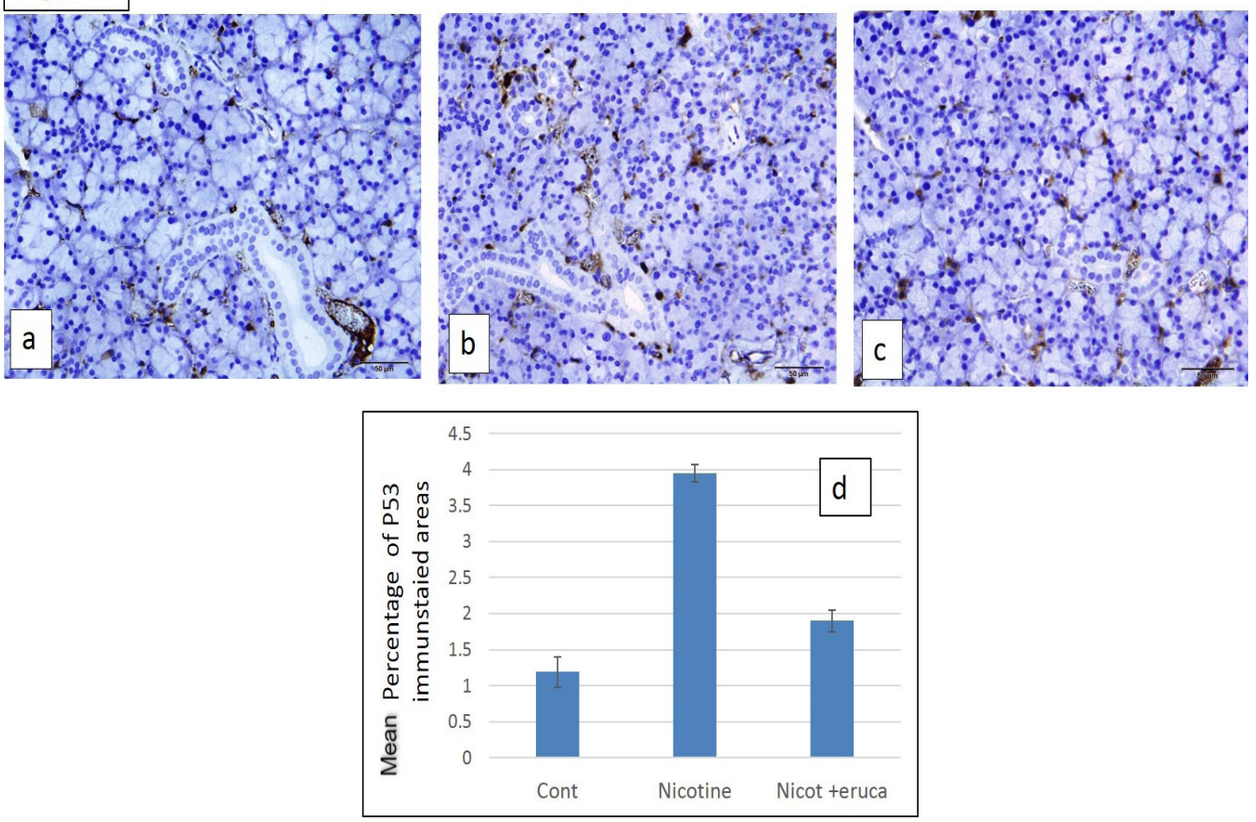


**Fig. 5:** Photomicrographs of sections from rat parotid gland immunohistochemically stained for p53 (brown nuclear staining ):
a. G1- Control: showing scanty reaction of the cells for P53.
b. G2- Nicotine: showing apparent increase of the apoptotic cells.
c. G3- Nicotine and Eruca: showing expression of p53 in few scattered myoepithelial cells
d. Statistical graph: showing statistical analysis of mean area percentage of P53 immunostained cells in different groups. One-way analysis of variance (ANOVA) test.
Values are expressed as mean ± SD. ** = Highly significant ($P < 0.01$)

## DISCUSSION

In the present study, Hematoxylin and Eosin stained sections of the parotid gland of rats of GI: control revealed well organized secretory portion and intercalated ducts. The acinar cells were pyramidal in shape with basophilic basal cytoplasm surrounded the rounded vesicular nuclei. Their apices define the acinar lumen and contain acidophilic finely granular cytoplasm. Intercalated ducts are lined with low cuboidal cells while striated ducts are lined with high cuboidal epithelium that shows basal acidophilic striations. Such normal structure did not differ much from what was reported in previous literature[21,22]. Basal striations of striated ducts are due to membrane invagination which are known to be involved in transport of sodium and potassium across their basal membranes[23].

Parotid gland of G II: receiving oral nicotine for 21 days showed congestion of the blood vessels, focal atrophy of acinar components or vacuolization of their lining cells. Large sized nuclei (Karyomegaly) were also observed in some hypertrophied vacuolated acinar cells. Striated ducts also showed damages of basal striation and alteration of nuclear appearance. Focal increase in vascular dilation and congestion was observed in parotid glands of G II receiving oral nicotine. The present results go in hand with what was reported by Mavropoulos, *et al*. 2001 and Argacha *et al*. 2008[24,25] that tobacco smoke and nicotine causes dilatation in the peripheral vessels with increased blood flow in gingival mucosa upon exposure to tobacco smoke. A previous study reported that in smokeless tobacco users there is a reduction in the total cell numbers and metabolic activity of acinar salivary lactoferrin and lysozyme compared to nonusers[26,27].

The effect of nicotine on acinar components was studied by many authors.. Jung, *et al*., 2014 reported that nicotine administration did not alter volumetric size of acinar components[28]. He attributed such differences could most properly related to rat species or type of nicotine, dose and route of administration.

Khoso *et al*., 2016 described that smokeless tobacco result in atrophy of parotid gland in albino rat with lymphocytic infiltration, which was similar to focal atrophy observed in the present study. Vacuolation observed in acinar cells here were described to be as a sign of cellular injury and damage to salivary gland acinar cells[29,30]. The authors reported that failure of the cell to maintain normal homeostasis and regulate the excretion of fluid result in cell enlargement and accumulation of fluid within the cells and formation of translucent vacuoles. Miletich *et al*. 2010[31] observed that nicotine caused alterations in secretory cells of the parotid glands. Nicotine was well known to stimulate sympathetic nervous system. Increased sympathetic nerve activity through the stimulation of sympathetic nerve upon exposure to free radicles was reported after tobacco smoke exposure[32]. Proctor and Carpenter, 2007 previously reported that nicotine receptor agonist increased intracellular $Ca^{2+}$ concentration in acinar cells with intact nerve terminals resulting in increased salivation which is partly blocked by an adrenergic receptor antagonist. Vacuolation was also reported in rat submandibular glands of rats exposed to chronic mild stress[34].

Karyomegaly or enlarged nuclei is uncommon feature and may be a part of cell hypertrophy. Karyomegaly was described in metabolic disorders, virus infection and exposure to various toxicant substances[35-37]. Focal increase in vascular dilation and congestion was observed in parotid glands of GII receiving oral nicotine. The present results go in hand with what was reported by Argacha *et al* that nicotine dilate the peripheral vessel[24].

In the present study, Masson trichrome stain revealed that collagen fibers of moderate amount were found mainly around ducts and blood vessels in the inter-lobular and inter-lobar connective tissue of control rat parotid. Administration of oral nicotine results in increased deposition of collagen in those areas compared to control. Such increased was prevented by Eruca *sativa* oil administration. Nicotine was reported to be cytotoxic and increased pro-fibrotic molecule and type I collagen production with subsequent periodontal fibrosis[38]. This was explained by Ferragut *et al*., 2011, where the connective tissue alterations is triggered by passive smoking and may provide a favorable environment for destructing the glandular cells.

Immunohistochemistry showed that in the glands of nicotine group there was an increase expression of alpha smooth actin expression as well as apoptotic marker P53 in myoepithelial cells. In G III, administration of Eruca *sativa* seed oil via oral route was found to potentially decreased such alteration. It was reported that nicotine has both pro-apoptotic and apoptotic effects on different body cells[40]. Thus, it is expected to affect salivary glands structures in a similar way. Ginzkey, *et al*, 2009 found that nicotine result in DNA damage in mini organ cultures of human parotid gland. A decrease in immunohistochemical expressions of Ki76; a marker of cell proliferation was observed in mice submandibular gland after nicotine exposure which pointed to decreased activity and this may explain the increased in P53 expression in the present study. The interesting finding is that apoptosis based on P53 expression was encountered in myoepithelial cells sparing secretory units. Myoepithelial cells are linked to the basal lamina of secretory units with characteristic contractile function to regulate the rate of secretion flow[21]. Its damage or interference of their function may lead to disordered secretory functions[41]. Overstimulation of contractile function of myoepithelial cells due to nicotine induced sympathetic activation may result in their exhaustion that ultimately end in their apoptosis[33].

The potential protective effect of Eruca *sativa* observed in this work and decreased P53 expression may be linked to previously mentioned antioxidant protective effects of this herb against toxic insults reported in many organs[42,43]. Cytoprotective activity was also proved previously by Taviano *et al*., 2017 against oxidative insult induced in

human peripheral blood mononuclear cells (PBMCs). Further work is needed to investigate the anti-oxidant activity of Eruca *sativa* on salivary gland tissue and could clearly prove this suggestion.

## CONCLUSION

Nicotine taken orally exerted histological alteration in rat parotid gland. Eruca *sativa* oil was found to ameliorate these effects . It thus appears to have potential protective role against nicotine. However, performing clinical trials on human is still mandatory.

## CONFLIC OF INTERESTS

There are no conflicts of interest.

## REFERENCES


1. Humphrey, S.P. and R.T. Williamson, A review of saliva: normal composition, flow, and function. J Prosthet Dent, 2001. 85(2): p. 162-9.
2. Animireddy, D., *et al*., Evaluation of pH, buffering capacity, viscosity and flow rate levels of saliva in caries-free, minimal caries and nursing caries children: An in *vivo* study. Contemp Clin Dent, 2014. 5(3): p. 324-8.
3. Rad, M., *et al*., Effect of Long-term Smoking on Whole-mouth Salivary Flow Rate and Oral Health. J Dent Res Dent Clin Dent Prospects, 2010. 4(4): p. 110-4.
4. Sobkowiak, R., J. Musidlak, and A. Lesicki, In *vitro* genoprotective and genotoxic effect of nicotine on human leukocytes evaluated by the comet assay. Drug Chem Toxicol, 2014. 37(3): p. 322-8.
5. Ginzkey, C., *et al*., Nicotine induces DNA damage in human salivary glands. Toxicol Lett, 2009. 184(1): p. 1-4.
6. Khan, G.J., *et al*., Effects of long-term use of tobacco on taste receptors and salivary secretion. J Ayub Med Coll Abbottabad, 2003. 15(4): p. 37-9.
7. Petrusic, N., *et al*., The Effect of Tobacco Smoking on Salivation. Acta Stomatol Croat, 2015. 49(4): p. 309-15.
8. Iida, T., *et al*., Nicotinic receptor agonist-induced salivation and its cellular mechanism in parotid acini of rats. Auton Neurosci, 2011. 161(1-2): p. 81-6.
9. da Silva, E.M.A., *et al*., Medicinal Plants and Herbal Medicines for Oral Health Promotion: Analysis of Students and Professionals Knowledge. European Journal of Medicinal Plants, 2018: p. 1-8.
10. Lynn, A., *et al*., Cruciferous vegetables and colo-rectal cancer. Proc Nutr Soc, 2006. 65(1): p. 135-44.
11. Al_Ameri, K.H., *et al*., Study of biological activity of Eruca *sativa* as antimicrobial agent. AL-Qadisiyah Journal of pure Science, 2018. 23(2): p. 190-197.
12. Zeb, A. and L. Rahman, Eruca *sativa* seed oil: Characterization for potential beneficial Properties. Pak J Pharm Sci, 2018. 31(4): p. 1251-1258.
13. Martínez-Sánchez, A., *et al*., Identification of new flavonoid glycosides and flavonoid profiles to characterize rocket leafy salads (Eruca vesicaria and Diplotaxis tenuifolia). Journal of agricultural and food chemistry, 2007. 55(4): p. 1356-1363.
14. Michael, H.N., R.E. Shafik, and G.E. Rasmy, Studies on the chemical constituents of fresh leaf of Eruca *sativa* extract and its biological activity as anticancer agent in *vitro*. Journal of Medicinal Plants Research, 2011. 5(7): p. 1184-1191.
15. Kim, S.J., S. Jin, and G. Ishii, Isolation and structural elucidation of 4-(beta-D-glucopyranosyldisulfanyl) butyl glucosinolate from leaves of rocket salad (Eruca *sativa* L.) and its antioxidative activity. Biosci Biotechnol Biochem, 2004. 68(12): p. 2444-50.
16. Sarwar Alam, M., *et al*., Eruca *sativa* seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. Food Chem Toxicol, 2007. 45(6): p. 910-20.
17. Lamy, E., *et al*., Antigenotoxic properties of Eruca *sativa* (rocket plant), erucin and erysolin in human hepatoma (HepG2) cells towards benzo(a)pyrene and their mode of action. Food Chem Toxicol, 2008. 46(7): p. 2415-21.
18. Alqasoumi, S., Carbon tetrachloride-induced hepatotoxicity: Protective effect of 'Rocket' Eruca *sativa* L. in rats. Am J Chin Med, 2010. 38(1): p. 75-88.
19. Suleyman, H., *et al*., Beneficial effects of Hippophae rhamnoides L. on nicotine induced oxidative stress in rat blood compared with vitamin E. Biol Pharm Bull, 2002. 25(9): p. 1133-6.
20. Randall, K.J. and G. Pearse, A dual-label technique for the immunohistochemical demonstration of T-lymphocyte subsets in formalin-fixed, paraffin-embedded rat lymphoid tissue. Toxicol Pathol, 2008. 36(6): p. 795-804.
21. Amano, O., *et al*., Anatomy and histology of rodent and human major salivary glands—Overview of the japan salivary gland society-sponsored workshop—. Acta histochemica et cytochemica, 2012. 45(5): p. 241-250.
22. Khoso, S.M., *et al*., Histological Features of Parotid Gland of Albino Rats Exposed to Smokeless Tobacco. Journal of Northeast Agricultural University (English Edition), 2016. 23(3): p. 54-60.
23. Holsinger, F.C. and D.T. Bui, Anatomy, function, and evaluation of the salivary glands, in Salivary gland disorders. 2007, Springer. p. 1-16.

24. Argacha, J.F., *et al*., Acute effects of passive smoking on peripheral vascular function. Hypertension, 2008. 51(6): p. 1506-11.
25. Mavropoulos, A., H. Aars, and P. Brodin, The acute effects of smokeless tobacco (snuff) on gingival blood flow in man. J Periodontal Res, 2001. 36(4): p. 221-6.
26. Ho, P.S., *et al*., Consumption of areca quid, cigarettes, and alcohol related to the comorbidity of oral submucous fibrosis and oral cancer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2007. 104(5): p. 647-52.
27. Gregory, R.L., *et al*., Effect of smokeless tobacco use in humans on mucosal immune factors. Arch Oral Biol, 1991. 36(1): p. 25-31.
28. Jung, J.-W., J.-K. Choi, and J.-S. Byun, Effects of Chronic Nicotine Administration on Salivary Gland in Mice. Journal of Oral Medicine and Pain, 2014. 39(3): p. 83-89.
29. Henics, T. and D.N. Wheatley, Cytoplasmic vacuolation, adaptation and cell death: a view on new perspectives and features. Biol Cell, 1999. 91(7): p. 485-98.
30. Myers, R., M. McGavin, and J. Zachary, Pathologic basis of Veterinary disease. Cellular and tissue responses to injury. Mosby Elsevier, 2007: p. 1-62.
31. Miletich, I., Introduction to salivary glands: structure, function and embryonic development, in Salivary Glands. 2010, Karger Publishers. p. 1-20.
32. Middlekauff, H.R., J. Park, and R.S. Moheimani, Adverse effects of cigarette and noncigarette smoke exposure on the autonomic nervous system: mechanisms and implications for cardiovascular risk. J Am Coll Cardiol, 2014. 64(16): p. 1740-50.
33. Proctor, G.B. and G.H. Carpenter, Regulation of salivary gland function by autonomic nerves. Auton Neurosci, 2007. 133(1): p. 3-18.
34. Ayuob, N.N., S.S. Ali, and M.G.A. El Wahab, Can Ocimum basilicum (Basil) relief the impact of chronic unpredictable mild stress on the mice salivary glands? African Journal of Traditional, Complementary and Alternative Medicines, 2018. 15(2): p. 1-11.
35. Baze, W.B., *et al*., Karyomegaly and intranuclear inclusions in the renal tubules of sentinel ICR mice (mus musculus). Comp Med, 2006. 56(5): p. 435-8.
36. Bokhari, F. and S.S. Ali, Effects of ochratoxin A-contaminated Arabian coffee seeds on liver and kidney functions and structure in mice: Protective role of roasting and vitamin C. Adv. Biol. Res, 2008. 2: p. 17-25.
37. Taniai, E., *et al*., Ochratoxin A induces karyomegaly and cell cycle aberrations in renal tubular cells without relation to induction of oxidative stress responses in rats. Toxicol Lett, 2014. 224(1): p. 64-72.
38. Takeuchi, H., *et al*., Nicotine-induced CCN2: from smoking to periodontal fibrosis. J Dent Res, 2010. 89(1): p. 34-9.
39. Ferragut, J.M., *et al*., Epithelial-stromal interactions in salivary glands of rats exposed to chronic passive smoking. Arch Oral Biol, 2011. 56(6): p. 580-7.
40. Zeidler, R., K. Albermann, and S. Lang, Nicotine and apoptosis. Apoptosis, 2007. 12(11): p. 1927-43.
41. Kim, J.M., *et al*., Protective effects of curcumin on radioiodine-induced salivary gland dysfunction in mice. J Tissue Eng Regen Med, 2019.
42. Abd El-Aziz, G.S., M.O. El-Fark, and R.M. Hamdy, Protective effect of Eruca *sativa* seed oil against oral nicotine induced testicular damage in rats. Tissue & Cell, 2016. 48(4): p. 340-348.
43. Sultan, K., *et al*., Biofunctional properties of Eruca *sativa* Miller (rocket salad) hydroalcoholic extract. Nat Prod Res, 2016. 30(10): p. 1202-4.
44. Taviano, M.F., *et al*., Contribution of the glucosinolate fraction to the overall antioxidant potential, cytoprotection against oxidative insult and antimicrobial activity of Eruca *sativa* Mill. leaves extract. Pharmacognosy magazine, 2017. 13(52): p. 738.

**الملخص العربى**

# هل يحسن زيت الكتأ التغيرات الناجمه عن النيكوتين في الغده النكفيه للجرذ؟ دراسه هستولوجيه

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**الهدف:** هدفت الدراسه الي التحقيق من التأثير الوقائي المحتمل لتناول زيت الكتأ (الجرجير) عن طريق الفم ضد التغيرات الناجمه عن النيكوتين في الغده النكفيه للجرذ وذلك باستخدام طرق هستولوجيه وهستو كيميائيه مناعيه وقياسيه **المواد والتجربة:** شملت الدراسه ثلاثون جرذابالغا تم تقسيمهم بصوره عشوائيه الي ثلاث مجموعات متساويه . استخدمت المجموعه الاول كمجموعه ضابطه تم اعطاؤها الحبيبات الاساسيه للجرذ ومحلول ملحي بالفم بينما اعطيت المجموعه الثانيه تارترات النيكوتين بالفم بجرعه مقدارها ٥٠ ملجم لكل كجم من الجسم يوميا لمده ثلاثه اسابيع في حين اعطيت المجموعه الثالثه نفس جرعه النيكوتين كالمجموعه الثانيه بالاضافه الي زيت الكتأ بجرعه٠,٥ ملل لكل ١ كجم من الجسم يوميا لمده ٣ اسابيع وبعد انتهاء التجربه تم ذبح الحيوانات تحت التأثير العميق لمخدر الايثر واستخراج الغده النكفيه بعد تشريح مقدمه العنق. حفظت العينات في فورمالين ١٪ ثم تحضيرشرائح سمكها ٥ ميكرون من قطاعات برافينيه بالطرق المعتاده . تم الصبغ بالهيماتوكسلين والايوسين وكذلك بالماسون ثلاثي الالوان وايضا بالصبغ الهستوكيميائي المناعي باستخدام الاجسام المضاده للالفا اكتين وكذلك p٥٣بروتين الورم واتبع ذلك دراسه قياسيه واحصائية.

**النتائج:** أظهر الفحص الميكروسكوبي وجود تغيرات هستولوجيه في الغده النكفيه ناجمه عن النيكوتين مقارنه بالمجموعه الضابطه تمثلت في ضمور بؤري وتفجي سيتوبلازمي وتضخم نوي في خلايا العنيبات المصليه بالاضافه الي فقد التخطط القاعدي في القنوات المخططه مع وجود انويه متنكسه واحتقان دموي اظهر صبغ الماسون زياده في الياف الكولاجين مع تناول النيكوتين .وكذلك دلل الصبغ المناعي علي وجود زياده في الفا اكتين وبروتين الورم p٥٣ وقد تحسنت معظم هذه التغيرات مع تناول زيت الكتأ

**الاستنتاج:** ومما سبق فقد خلصت الدراسه الي ان النيكوتين قد تسبب في تأثير تنكسي علي الغده النكفيه للجرذ وكان لزيت الكتأ تأثيرا وقائيافقد احدث تحسنا في التنكس الناجم عن النيكوتين وعليه فمن الموصي به استخدامه في العلاج ضد التدخين