

Histological evaluation of the effect of nutmeg on parotid salivary glands of adult male albino ratsMoheb, M. 1, El-Sakhawy, M. A.², Sherif, H.³ and Shredah, M. ¹¹Department of Oral Biology, Faculty of Dentistry, Minia University, Egypt.²Department of Cytology & Histology, Faculty of Vet.Med., Cairo University, Egypt.³Department of Oral Biology, Faculty of Dentistry, Al-Azhar University, Egypt.profsakhawy@yahoo.com

Abstract: A total number of 80 adult male albino rats (average weight = 200gm) were used and were classified into two main groups: **1.** The Control group (Group A): comprised 20 animals. These animals received 1ml of distilled water orally on a daily basis for the duration of the experiment (2, 4, 6 and 8 weeks). **2.** The Experimental group (Group B): comprised 60 animals and was subdivided into 4 subgroups corresponding to 4 durations (2, 4, 6 and 8 weeks). Each subgroup in turn was divided into 3 subgroups in which each rat was given 1ml of the prepared nutmeg aqueous extract orally on a daily basis in the following doses (100, 300 and 500 mg/kg b.w.) respectively. On termination of the experiment, the parotid salivary glands were dissected out, cleaned and fixed in 10%buffered formalin solution. Then, paraffin wax sections were obtained and stained with: Haematoxylin and Eosin (H&E) to verify histological details and Masson's trichrome for detection of collagen fibers. The parotid gland of the control group (Group A) was composed of parenchyma which showed normal appearance of secretory acini and ducts; and fine C.T. stroma in between lobes and lobules and comprising rich capillary network. As for the experimental group (Group B), the most noticeable changes were cytoplasmic vacuolizations in acinar cells, dilatation of intercalated and striated ducts, congestion of blood vessels, signs of nuclear changes (pyknosis, karyolysis, karyorrhexis, hyperchromatism), lymphocytic infiltration, hyalinization of C.T. stroma and finally appearance of duct-like structures replacing severely atrophied acini. These changes gradually increased as the duration increased and as the dose administered increased. The experimental group showed more thickening of collagen fibers than in the control group.

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Key words: Nutmeg, Parotid, Histology

1-Introduction:

The nutmeg tree (*Myristica fragrans* Houtt.) is a tropical aromatic evergreen tree that yields an apricot-shaped fruit. Contained in the fruit is a nutmeg seed covered by a protective layer or aril. The seed is processed to make nutmeg and the aril can be processed into a spice called mace (**Kisby 2003**). Nutmeg is a common household spice and is frequently used in cooking and baking. Recently, nutmeg has become popular among individuals seeking legal and natural hallucinogenic and euphoric experiences. Available forms of nutmeg include the whole nut, ground powder, and volatile oils (**Kisby 2003**). Besides nutmeg and mace, a number of other products, namely oleoresin, nutmeg butter and essential oils, are also derived from *M. fragrans*. These products find varied use in the food, medicine and perfume industries (**Leela 2008**). Nutmeg is widely used in a variety of ways and for various purposes. In addition to its common use as a kitchen spice, it is used for several purposes in traditional medicine. Nutmeg is used more commonly in Oriental medicine than in Western medicine (**Leela 2008**). With the recent gain in popularity of herbal

medicine all over the world, it is also possible to abuse the use of *M. fragrans* because of its medicinal properties. It has been reported that the spice can be toxic when ingested in large quantities causing convulsions, hallucinations, and possibly death. The medicinal use of nutmeg and its use as a spice suggest that it contains some constituents which are responsible for the reported biological activities. Some of these active principles may at the same time possess some adverse effects (**Olaleye, et al., 2006**). The principal constituent of nutmeg are fixed oil, volatile oil and starch. The fixed oil contains myristin and myristic acid. The volatile oil contains pinene, sabinene, camphene, myristicin, elemicin, isoelemicin, eugenol, isoeugenol, methoxyeugenol, safrole, dimeric phenylpropanoids, lignans and neolignans (**Malviya et al., 2011**). Phytochemical screening of aqueous extract of the seed of *M. fragrans* revealed the presence of alkaloids, saponins, anthraquinones, cardiac glycosides, flavonoids and phlobatanins while tannins were absent (**Olaleye et al., 2006**). Dietary phytochemicals including flavonoids, polyunsaturated fatty acids, and others have been the subject of increasing amounts of

research for their potential beneficial effects and also because it is becoming increasingly apparent that these compounds have deleterious effects at certain doses. Some researches were done to study the various effects of nutmeg on different organs such as the kidney, liver, spleen, heart, testes, brain, etc. But no studies were done on any oral tissues. That's why our study was done to try to participate in more understanding of the effect of nutmeg administration in different durations and in different doses. Our study was done on the parotid salivary gland.

2-Material and methods

2-1. Animals:

A total number of 80 adult male albino rats (average weight = 200gm) were used in this study. The animals were housed in cages at the Faculty of Dentistry, Minia University, under the optimal experimental conditions. They were kept at room temperature 22-24°C. Animals were fed on ground barley and supplied water *ad-libitum*.

The animals were classified into two main groups:

I. The Control group (Group A): comprised 20 animals. These animals received 1ml of distilled water orally on a daily basis for the duration of the experiment (2, 4, 6 and 8 weeks).

II. The Experimental group (Group B): comprised 60 animals. This group in turn was subdivided into 4 subgroups (15 animals each):

- Subgroup 1: was subdivided into 3 subgroups (5 animals each). These animals were given 1ml of nutmeg extract orally on a daily basis for 2 weeks in doses of 100, 300 and 500 mg/ kg b.w. respectively.

- Subgroup 2: was subdivided into 3 subgroups (5 animals each). These animals were given 1ml of nutmeg extract orally on a daily basis for 4 weeks in doses of 100, 300 and 500mg kg/ b.w. respectively.

- Subgroup 3: was subdivided into 3 subgroups (5 animals each). These animals were given 1ml of nutmeg extract orally on a daily basis for 6 weeks in doses of 100, 300 and 500 mg/ kg b.w. respectively.

- Subgroup 4: was subdivided into 3 subgroups (5 animals each). These animals were given 1ml of nutmeg extract orally on a daily basis for 8 weeks in doses of 100, 300 and 500mg kg/ b.w. respectively.

2-2. Preparation of The Nutmeg Seed's Extract

Nutmeg seeds were obtained from local markets and botanically identified through herbarium staff and a voucher specimen was deposited at the herbarium of Botany and Microbiology Department, Faculty of Science, Minia University, Minia, Egypt.

The dry seeds were washed thoroughly to remove dust, fungal spores and/or other undesired particles, then left to dry under room temperature overnight. The seeds were then macerated into a fine flour-like paste using a mortar and pestle to pass through 0.2 mm mesh. Aliquot weight of nutmeg powder (125 gm) was soaked in 500 ml hot distilled water and left to stand for 72 hrs, then filtered the extract. The extract was kept frozen until used. Doses of 100, 300 and 500 mg nutmeg extract were prepared as outlined by **Olaleye *et al.* (2006)**.

2-3. Histological Procedures

On termination of the experiment, the animals were humanely sacrificed under anaesthesia. Then the parotid salivary glands were dissected out, and fixed immediately in 10% neutral buffered formalin solution. Then, the specimens were washed by tap water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of 6-7 μ m thick were obtained and mounted on clean glass slides and stained with: Haematoxylin and Eosin (H&E) to verify histological details and Masson's trichrome stain for detection of collagen fibers. The aforementioned methods were adopted after **Drury & Wallington (1980)**.

3-Results and Discussion

The parotid gland of the control group was composed of parenchyma and stroma which divided the gland into lobes and lobules. The terminal secretory units were arranged in spherical fashion. The secretory cells within the acini were arranged in pyramidal shape with basophilic cytoplasm and basally situated nucleus with very narrow lumen (Fig. 1). The intercalated ducts were very difficult to be distinguished and appeared compressed between the acini. The striated ducts were easily identified in between the acini. There was a rich capillary network in between the acini and was concentrated around the striated ducts. The connective tissue stroma in between the lobules and the acinar cells was very delicate and fine. In the present study, there were varying changes in the glandular architecture including changes in the acini, ducts, connective tissue stroma and blood vessels. These changes varied from one group to another according to the dose administered and the duration of administration of nutmeg extract.

One of the prominent features of this study is vacuolization. Rats given the lowest dose of nutmeg extract (100 mg/kg b.w.) and those given the intermediate dose (300 mg/kg b.w.), started to show vacuoles in their acinar cells at about 4 weeks. However, in rats taking the highest dose of nutmeg extract (500 mg kg b.w.), vacuoles were formed earlier at the first duration (2 weeks) (Figs. 2&3).

Laslie et al. (1983) stated that the extent of vacuolization was time-dependent which agreed with our results. Vacuolization due to nutmeg administration, agreed with (**Eweka et al., 2010**), who studied the effect of nutmeg on the rat kidneys. The rats in the treatment groups were given 0.1g (500 mg/kg b.w.) and 0.2g (1000 mg/kg b.w.) of nutmeg thoroughly mixed with the feeds respectively on a daily basis for forty-two days (6 weeks). The treated sections of the kidneys showed distortion of the renal cortical structures, cytoplasmic vacuolizations, and some degree of cellular necrosis, with degenerative and atrophic changes when compared to the control group. **Al-Hazmi et al. (2004)** also studied the effect of aqueous extract of nutmeg seeds on the liver of mice and observed similar effects. Liver sections showed cellular changes in the form of hydropic degeneration, cellular vacuoles and some nuclear changes. Moreover, studies performed on rat brain treated with nutmeg also showed vacuolization (**Adjene, 2010**). It had been reported that myristicin obtained from nutmeg had cytotoxic effects on the body (**Adjene, 2010**). The process of vacuolization in response to injury is fully reversible, while in others, continued exposure to certain levels of inducers, is irreversible and leads to cell death. The changes which crucially decide whether a cell recovers rather than engages some active or passive process leading to death remain unknown, but have been loosely attributed to the inability of the cell to maintain its energy status (**Henics et al., 1999**). Dilatation of the ducts was one of the prominent findings in our study. Both the intercalated and striated ducts were dilated in all experimental groups (Fig.4). **Scott et al. (1999)** studied the atrophied rat parotid gland due to duct-ligation and described that the striated ducts appeared to be qualitatively more prominent in the lobules than normally. Intralobular and interlobular ducts appeared dilated. This finding agreed with our study as it was clear that duct dilatation occurred whenever the gland was atrophied or there was impairment in its function as what happened in our study. Moreover, amitriptyline administration adversely affected the duct system of the parotid salivary glands in our study. Both striated and excretory ducts showed dilatation with retaining eosinophilic secretion in their lumen. This finding was attributed to accumulation of the salivary secretion and failure of exocytosis due to glandular injury and dysfunction (**Moubarak, 2012**). We could understand from the previous study that dilatation of the ducts in our study occurred due to glandular damage and dysfunction caused by nutmeg administration.

In addition to vacuolization and ductal dilatation, presence of congested blood vessels was a

very obvious feature in nutmeg treated parotid glands in the present investigation (Fig.5). This came in agreement with **Olaleye et al. (2006)** who studied the effect of nutmeg on several organs. He noticed congestion of blood vessels in the liver and congestion of renal blood vessels in the kidney. **Moubarak (2008)** explained that the dilatation and congestion of the blood vessels might be a part of inflammatory response to bring more blood to the areas of fibrosis or degeneration. This simulates the results of our study.

Presence of inflammatory cells in the C.T. stroma in experimental subgroups 3 and 4 was also detected in the present study (Fig. 6). Sections of mice liver treated with 500 mg/kg of *Myristica fragrans* essential oil for 7 days showed focal area of degeneration and necrosis with infiltration of mononuclear cells and also section of mice liver treated with 1000 mg/kg showed more degenerative changes and necrosis of hepatocytes with focal mononuclear cells infiltration. (**Al-Jumaily et al., 2012**). The nutmeg extract in the dosage range used by **Bamidele et al. (2011)** significantly increased total WBC count in the treated animals.

Masson's trichrome stained sections clarified the presence of extensive collagen fibers in the parotid glands of our experimental groups (Figs.7&8). Light microscopic results carried out by **Moubarak (2008)** revealed widening of the connective tissue septa, extensive fibrosis and vacuolization. Excessive fibrosis might be due to toxic effect of amitriptyline. Besides the previous findings, various signs of degeneration were apparent in our present study and the nuclei of acinar cells showed all signs of nuclear changes (Fig.9). This was confirmed by the results reported by (**Alalwani, 2013**). The results of light microscopy of the kidney of male animals revealed some congested, fragmented glomeruli, obliterated urinary space and necrosis with nuclear pyknosis of tubular cells.

In the present work, with long duration of administration of nutmeg extract (8 weeks) and high dose (500/ mg kg b.w.) there was complete atrophy of some lobules which replaced by thick fibrous tissue and formation of duct-like structures (Fig.10). We believe that these duct-like structures were derived from surviving atrophic acini, as mentioned by **Scott et al. (1999)**. **Fujita-Yoshigaki et al. (2009)** concluded that isolated parotid acinar cells transiently changed to immature duct-like cells during epithelial-mesenchymal transition process. This change might be the retrograde process of development and differentiation of salivary glands. These results suggested that parotid acinar cells retain a plasticity of differentiation.

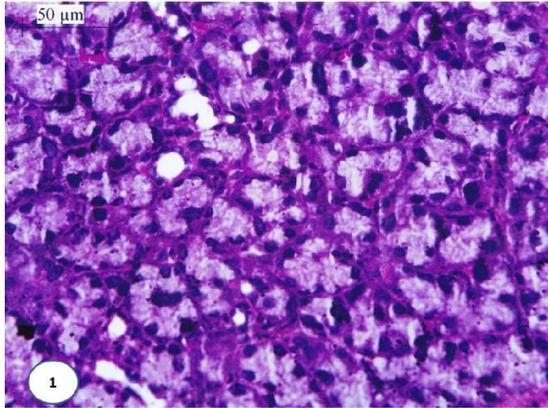


Figure 1: Photomicrograph of parotid salivary gland of control group (A) showing normal structure (H&E x400).

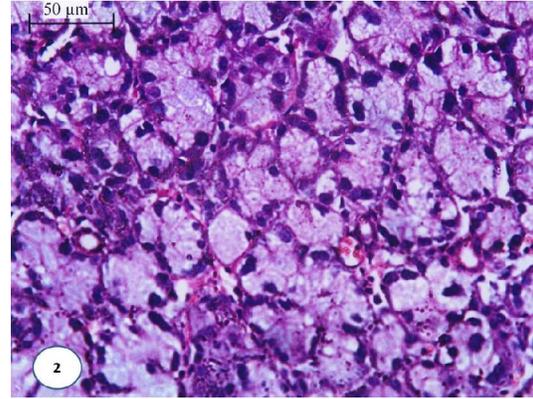


Figure 2: Photomicrograph of parotid salivary gland of experimental sub group 1 (500 mg/ kg b.w.) showing vacuolization of acinar cells and congested blood vessels (H&E x400).

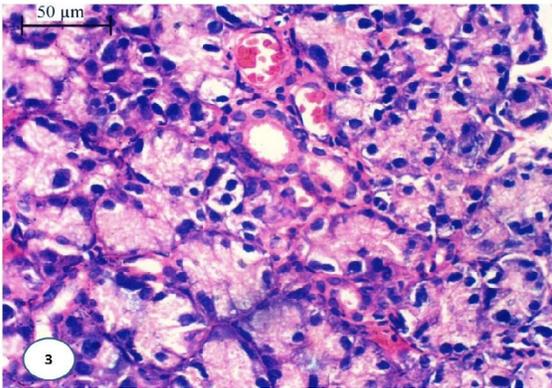


Figure 3: Photomicrograph of parotid salivary gland of experimental sub group 4 (100 mg/ kg b.w.) showing completely atrophied acini, dilatation of the ducts and congested blood vessels (H&E x400).

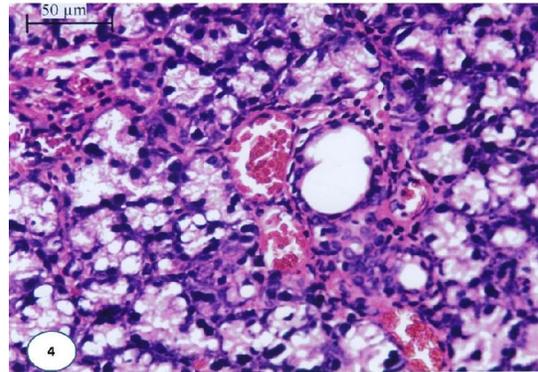


Figure 4: Photomicrograph of parotid salivary gland of experimental sub group 3 (100mg/ kg b.w.) showing congested blood vessels, lymphatic infiltration and hyalinization of connective tissue septa (H&E x400).

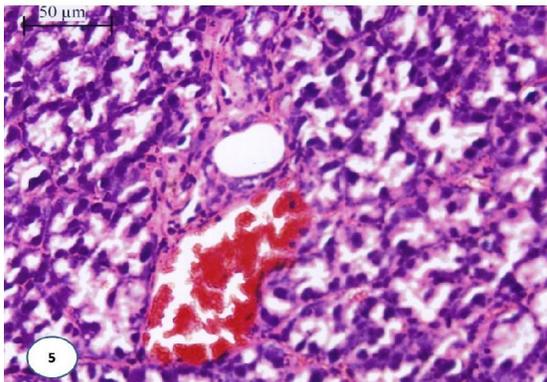


Figure 5: Photomicrograph of parotid salivary gland of experimental sub group 4 (300 mg/ kg b.w.) showing degeneration of acinar cells, congested blood vessels and hyalinization of connective tissue septa (H&E x400).

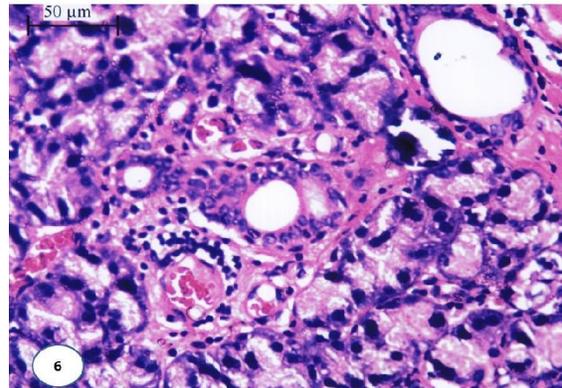


Figure 6: Photomicrograph of parotid salivary gland of experimental sub group 3 (500 mg/ kg b.w.) Showing dilatation and degeneration of epithelium lining the intercalated and striated duct, congested blood vessels and lymphocytic infiltration (H&E x400).

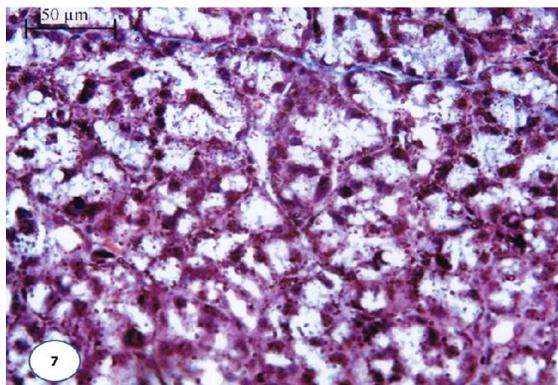


Figure 7: Photomicrograph of parotid salivary gland of control group (A) showing fine connective tissue fibers (Masson's trichrome x400).

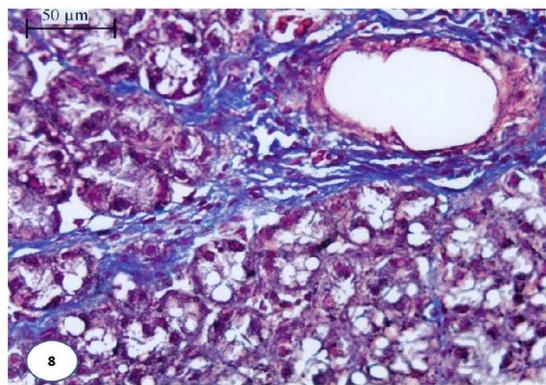


Figure 8: Photomicrograph of parotid salivary gland of experimental sub group 3(500 mg/ kg b.w.) showing thick connective tissue fibers (Masson's trichrome x400).

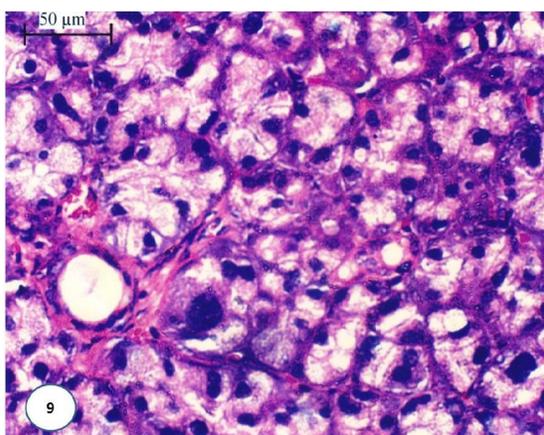


Figure 9: Photomicrograph of parotid salivary gland of experimental sub group 4(500 mg/ kg b.w.) showing completely atrophied acini and different forms of nuclear changes (H&E x400).

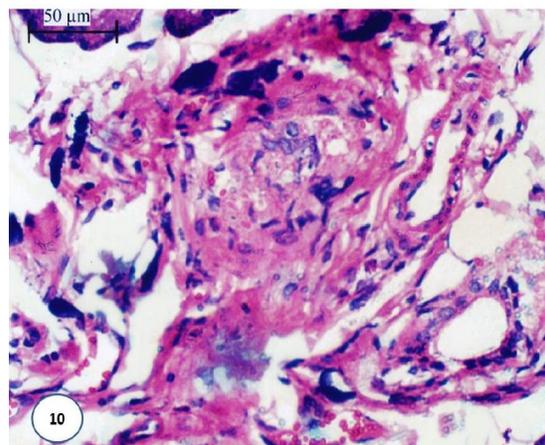


Figure 10: Photomicrograph of parotid salivary gland of experimental sub group 4(500 mg/ kg b.w.) showing fibrosis with some persistent nuclei of acinar cells and duct-like structure (H&E x400).

4-Conclusion:

Administration of nutmeg had damaging effects on the parotid salivary glands especially when taken at high doses and for long durations.

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