

Toxic effect of tobacco smoke and nicotine on the Mitral cells of the Rabbits

Rima Gawad Hamshari*, and Huda Abdulwadood O**

*Department of Morphology, Anatomy Unit, Faculty of Medicine, Aden University, Yemen

** Department of Paraclinic, Forensic Medicine & Toxicology Unit, Faculty of Medicine, Aden University, Yemen

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Abstract

Tobacco use creates a tremendous burden on the health care system and is the largest non-communicable source of disease globally. Olfactory bulb was the structure in the ventral surface of the brain which receives olfactory input data and also known to involve in the regulation of basic behaviors.

This study was carried on 75 domestic rabbits with a mean weight of 1500-2000gm from January 2011- August 2011 in Ain Shams university. These rabbits were divided into three groups as follow:

Group I: Included 25 rabbits that received fresh food and water.

Group II: Included 25 rabbits were injected subcutaneously with 1mg/kg body weight of nicotine in a single daily dose.

Group III: Included 25 rabbits that were exposed to two cigarettes smoke three times per day in a closed chamber.

There was a statistically significant difference between the groups as regards the mean longitudinal and transverse diameter of the mitral cells. Treated group III showed lowest mean of longitudinal and transverse diameter of mitral cell. Microscopical examinations showed that there are disruption in the mitral layer and degeneration and disappear of mitral cells in some areas and vacuolated cells in the others, particularly in group III.

It was concluded that nicotine exposure and passive cigarette smoking caused reductions in the longitudinal and transverse diameter of mitral cells of the rabbits as well as several histopathological changes, which lead to loss of smell sensation and change in the behavior.

Key words: Nicotine, Smoke, mitral cells, Tobacco.

Introduction

Tobacco use creates a tremendous burden on the health care system and is the largest non-communicable source of disease globally; annual tobacco-attributable deaths surpassed 5 million in 2010⁽¹²⁾. Cigarette smoking has wide-ranging effects on the user, depending on both extrinsic and intrinsic factors, with a well-described influence on oncogenesis, pulmonary function, vascular health, and immune response⁽¹³⁾.

Cigarette smoking still tops the list as the most preventable cause of death in the United States today, accounting for 480,000 deaths annually. In 2016, more than 15 of every 100 U.S. adults aged 18 years or older (15.5%) currently smoked cigarettes. This means an estimated 37.8 million adults in the United States currently smoke⁽⁴⁾.

Nicotine exposure during periods of developmental vulnerability can impair development of neurons and brain circuits, leading to changes in brain architecture, chemistry, and neurobehavioral function and may impair or dysregulate non-neuronal cellular function⁽¹⁸⁾. It also exerts its physiologic effects by binding nicotinic acetyl choline receptors (nAChRs), which are expressed by both neuronal and non-neuronal cells throughout the body⁽²⁾. The cholinergic system in the central nervous system is associated with cognitive function; including memory, selective attention, and emotional processing⁽¹⁹⁾.

Olfactory bulb is considered as a critical relay step between the olfactory epithelium and olfactory cortex. A marked feature of the bulb is its massive innervation by cholinergic inputs from

the basal forebrain, the activity of which is thought to be correlated with animal behaving states such as attention^(5,16). Olfactory bulb (OB) exhibit high levels of nicotinic acetylcholine receptor (nAChR) in all layers⁽¹⁶⁾, which transmits smell information from the nose to the brain, and is thus necessary for a proper sense of smell^(5,7). Within the olfactory system, information flow from the periphery on to output mitral cells (MCs) of the olfactory bulb (OB) has been thought to be mediated by direct synaptic inputs from olfactory sensory neurons (OSNs)⁽¹⁰⁾.

It has been written that the olfactory system provides “an internal depiction of our external world” through the capture of odorant molecules in the main olfactory epithelium by several large families of G-protein coupled receptors. These receptors transduce the chemosignals into electrical signals that travel via topographically defined projections into the olfactory bulb⁽³⁾.

However, careful consideration of the potential adverse health effects from nicotine itself is often absent from public health debates.

Aim of the Work:

The aim of the present work is to investigate the effect of tobacco smoke and nicotine injection on the mitral cells of the olfactory bulb in rabbits.

Material and Methods

This study is a randomized single blind control trail in which seventy five adult domestic rabbits, weighing 1500-2000 gm were investigated. There were lived in the animal house, Faculty of Medicine, Ain Shams University from January 2011- August 2011. The rabbits were divided into three groups as follows:

Group I (control): Included twenty five domestic rabbits that received fresh food like (lettuce, carrot, dried bread and green grass) and water.

Group II: Included twenty five domestic rabbits that received fresh food & water as group I but were also injected subcutaneously with nicotine 1mg/kg body weight in a single daily dose⁽¹⁾.

Group III: Included twenty five adult domestic rabbits that exposed to cigarettes smoke (each cigarette containing 15 mg cotran and 1mg of nicotine). They were exposed to the smoke of two cigarettes, three times per day, in a closed chamber for 15 minutes each time (total number = 6 cig./day⁽⁸⁾).

Dissection:

The heads of all rabbits were cut by a razor blade. After removal of the skin, a cut was made one cm. posterior to the anterior openings of the nose for rapid fixation of the nose. An opening was made at the bregma (meeting point of coronal suture and mid sagittal suture). A longitudinal incision along the mid sagittal suture was made for rapid fixation of the olfactory bulb.

Twenty five heads of rabbits from each group (I, II & III) were put in Bouin’s solution for two days. Craniotomy was performed by incising through the coronal suture and along the mid sagittal suture to elevate the bones overlying the brain. The whole brain was removed and the olfactory bulbs were extracted carefully. Measurement of the total length and width of the olfactory bulb was performed after careful blotting with filter paper. The specimens were Processed in order to form paraffin blocks:

Specimens of olfactory bulb and mucosa were put in Bouin’s solution for 10 days and then processed to paraffin blocks for histological studies.

In hematoxylin and eosin stained sections, the diameter of mitral cells of the bulb was measured in each of the treated groups and compared to their control. Two orthogonal diameters of the mitral cells were measured: the transverse diameter of the cell body and the longitudinal diameter till the base of the apical dendrite. These measurements were done in sections where the nucleus and nucleolus of the mitral cell were clear. The measurements were performed by using an ocular scale grid calibrated with a micrometer stage slide at 40 fold magnification or 400 fold magnifications.

The means and standard deviation of these measurements were recorded and the results were tabulated and analyzed using SPSS(20).

Results:

Regarding the longitudinal and transverse diameters of the mitral cell, the present study revealed that group II & III showed a very highly significant reduction (p<0.001) in diameters when compared with those of control group. Group III was the most affected group (lowest mean of longitudinal & transverse diameter of mitral cell). (Table 1)

Table 1: Comparison between Longitudinal and Transverse Diameters of the mitral cell in the Three Groups by Summary Statistics

Characteristic	Group I* (n=25)		Group II** (n=25)		Group III** (n=25)	
	L. Diam. [¶] (mm)	T. Diam. [§] (mm)	L. Diam. [¶] (mm)	T. Diam. [§] (mm)	L. Diam. [¶] (mm)	T. Diam. [§] (mm)
Mean ± SD	32.13±15.2	10.81±3.9	17.55±5.7	7.86±2.3	14.69±4.4	6.14±3.1
Median	30.35	10.71	17.85	8.93	14.28	7.14
Maximum	53.55	17.85	32	10.71	21.42	10.71
Minimum	14.28	5.36	8.93	3.57	7.14	1.79
Range	39.27	12.49	23.07	7.14	14.28	8.92

Note: *Group I: Control group; **Group II and III: Study groups Longitudinal Diameter; § Transverse Diameter

This study revealed that microscopical sections which were obtained from study group II showed multiple lesions that include slightly disruption in the arrangement of the mitral cells layer (figs.3, 5A) compared to control group (Fig.1). Normally, mitral cells had large vesicular nuclei with one or more nucleoli (fig.2). Some mitral cells appeared as groups with scanty nissl granules in their apical dendrites in some areas(fig.4A ,5), while in other areas these cells appeared shrunken (figs.4B,5), degenerated with loss of the nuclear membrane or vacuolated cytoplasm. Small dark cells (oligodendroglia) were observed in this layer (Figs.3 and 4).

Histopathological examination of group III showed markedly disruption of mitral cell layer(Fig.9A). Mitral cells appeared different in their distribution within the mitral cell layer(fig.6A) ,with vacuoles in their cytoplasm, and displaced into external plexiform and granular layers (Fig.6B and 8B). In some areas swollen mitral cells, with vacuoles in their cytoplasm and neuropil appeared (figs.7A), in some areas mitral were shrunken, while disappeared in other areas (Figs.7B). Mitral cells in some areas were collected in groups and had no clear cell membrane (Figs.8A and 9A&B). The dendrites of some mitral cells appeared disrupted with multiple small vacuoles (Figs.8A, 9A and B). Some small dark shrunken cells , with vacuoles in their cytoplasm, were observed in this layer (Figs.8A,B and 9A &B).

Group I:

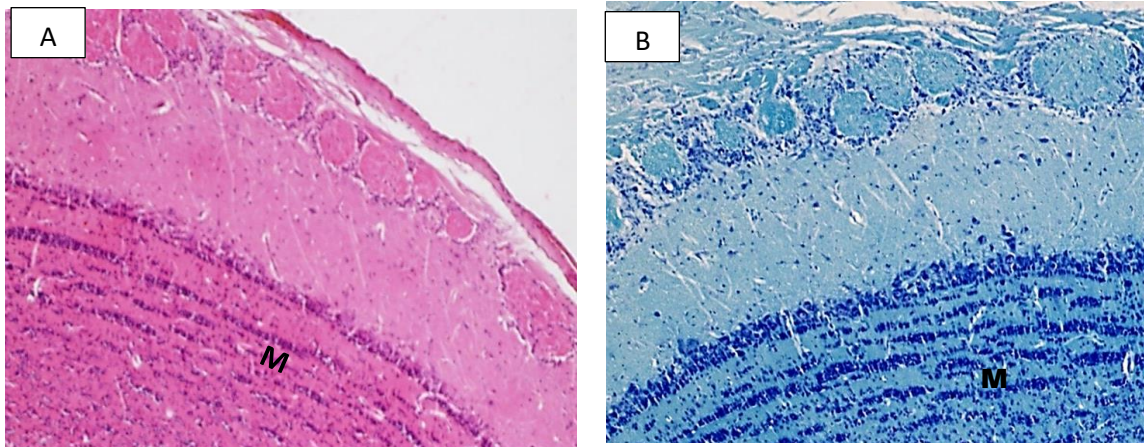
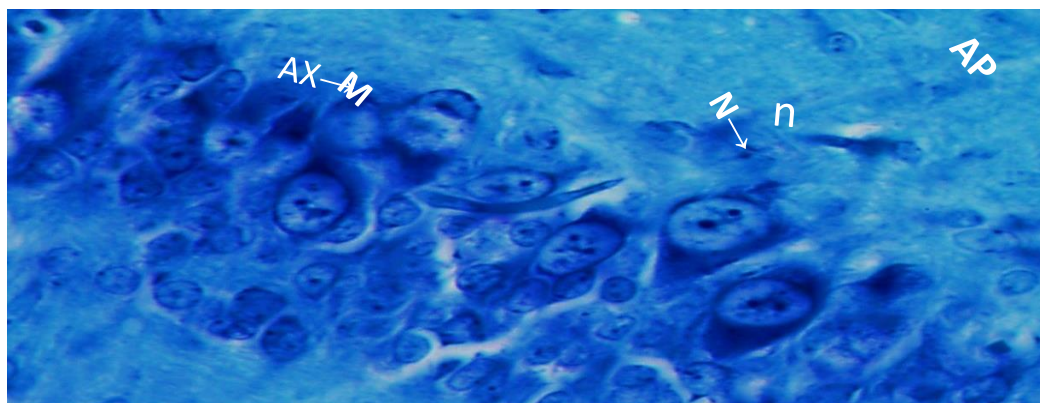


Fig (2): Photomicrograph of a coronal section of the olfactory bulb of adult rabbit of control group I, showing mitral cells with open face nucleus(N) and two nucleoli(n) and apical dendrite (AP) filled with Nissl granules. The axon (AX) is seen passing deeply into the granular layer. (Toluidine blue x1000).

M: mitral cell layer.



Group II:

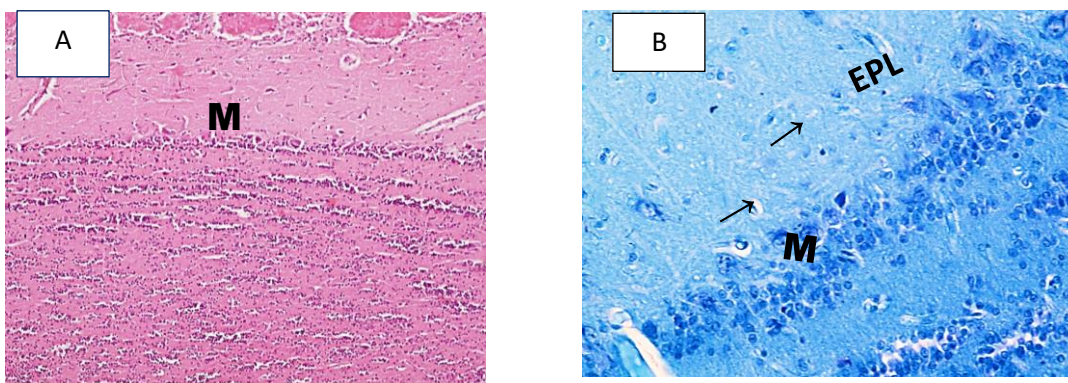


Fig (3): A. Photomicrograph of a coronal section of the olfactory bulb of adult rabbit of group II, showing slight disruption in the arrangement of the neurons in mitral cells layers. (H and Ex100). B. Slight disruption in the arrangement of the mitral cells. Some mitral cells (→)in external plexiform layer (EPL). (toluidine blue x400).

M: mitral cells layer.

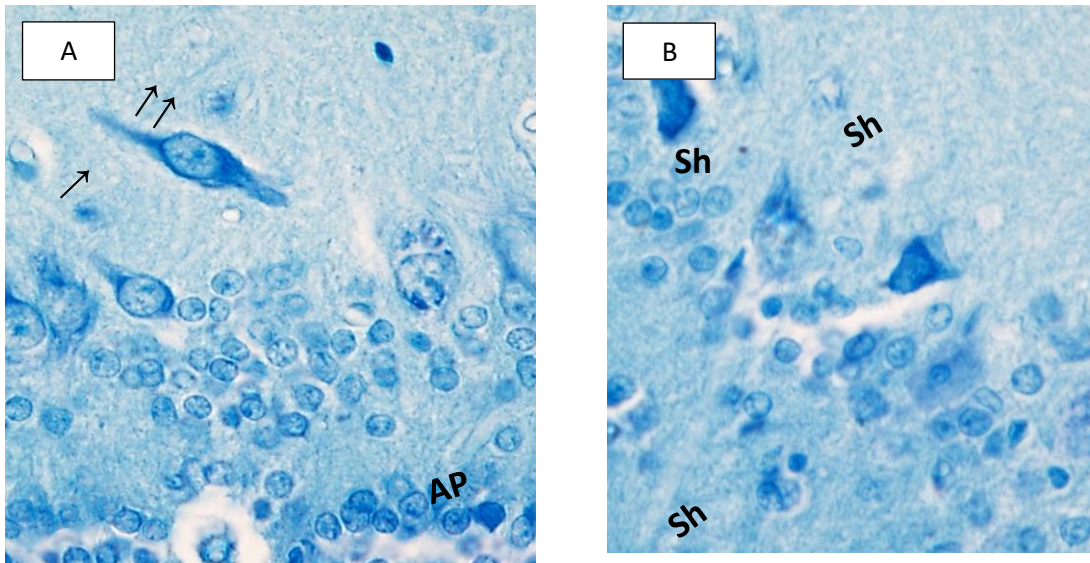


Fig (4): A. Photomicrograph of a coronal section of the olfactory bulb of adult rabbit of group II, showing mitral cells with scanty nissle granules(→) and mitral cell horizontally disposed away of mitral layer(↑↑). (Toluidine blue x400). B. dark shrunken mitral cells (Sh) (toluidine blue x400).

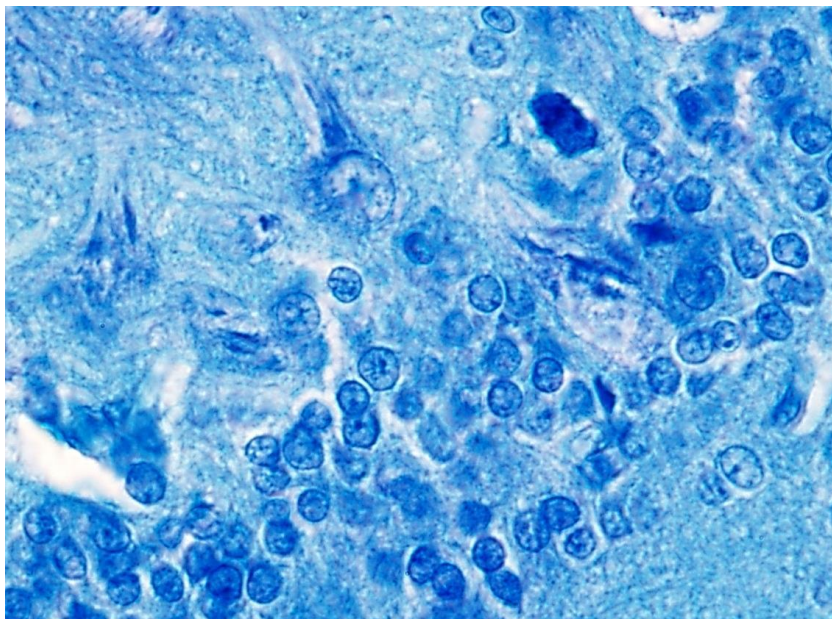


Fig. (5): Photomicrograph of a coronal section of the olfactory bulb of rabbit of treated group II, showing the mild disorganized of mitral cell layer. Note the mitral cells with scanty nissl granules in their apical dendrites(AP) and shrunken dark mitral cells (Sh).(Toluidine blue x 1000)

Group III:

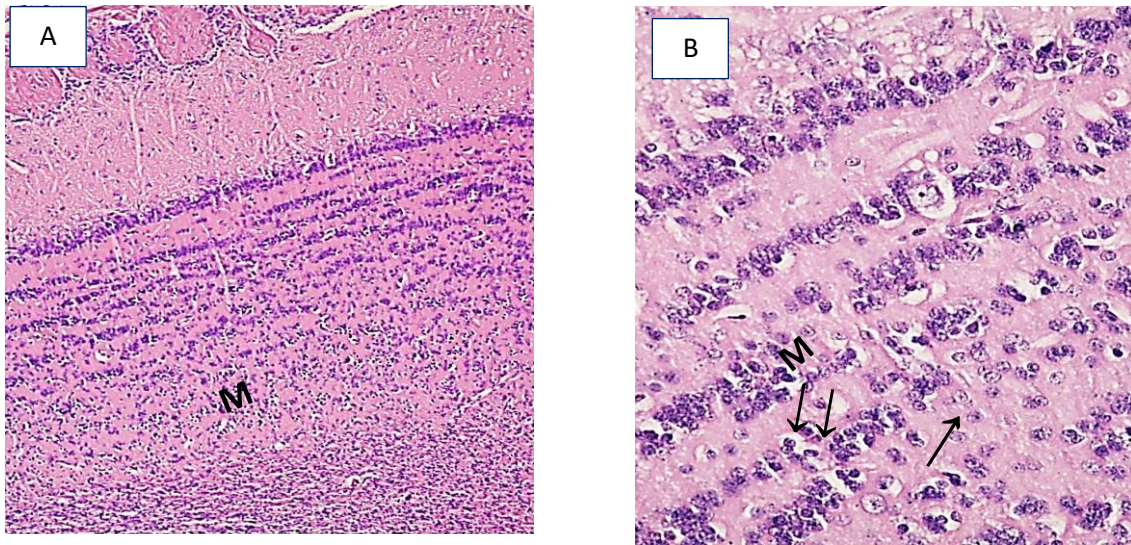


Fig (6): A. Photomicrograph of a coronal section of the olfactory bulb of rabbit g of group III, showing greatly disarrangement of the neurons in mitral cells layer (M). (H and Ex100) B. (→): points to mitral cells with vacuoles in their cytoplasm lying in the granule cells layer. (↑↑) points to the appearance of empty space in the neuropil. (H and E x 400)

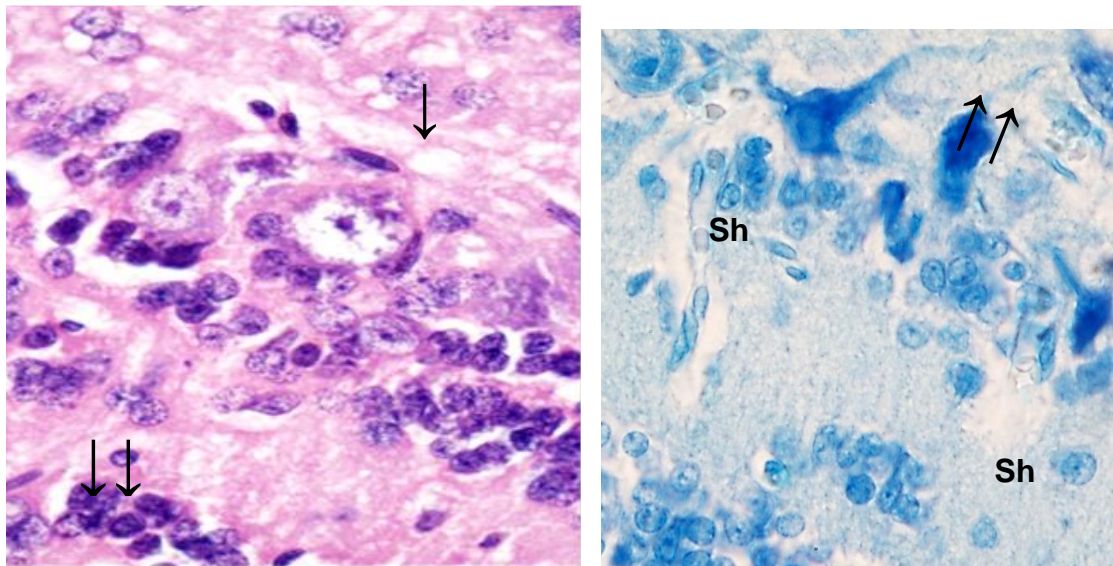


Fig (7A): Photomicrograph of a coronal section of the olfactory bulb of rabbit of group III, showing the some swollen mitral cells bodies (↓) with no clear cell membrane. (↓↓) pointed to the empty space in the neuropil. (H & E x1000) Fig. (7B): showing some obliquely and horizontally disposed shrunken mitral cells bodies (Sh) . (Toluidine blue x1000).

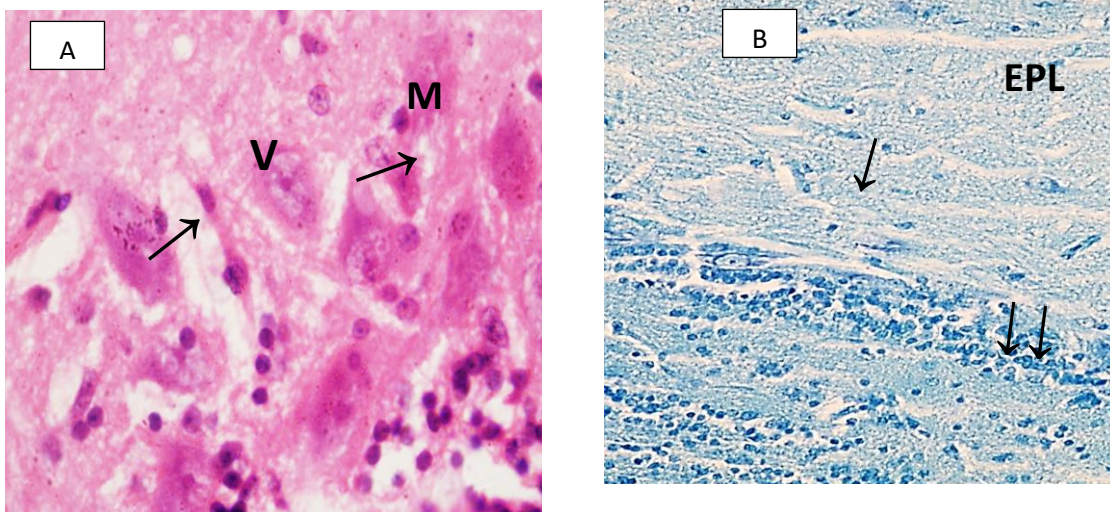


Fig (8): A. Photomicrograph of coronal section in olfactory bulb of group III showing: an arrow (→) points to mitral arranged in group and vacuoles inside their cytoplasm, and vacuoles in the neuropile between the mitral cells (V). (H&E x 1000). B. Mitral cells (↓) horizontally disposed in the mitral cell layer with scanty nissles granules. (↓↓) points to some mitral cells displaced into the external plexiform layer (EPL). (Toluidine blue x 100)

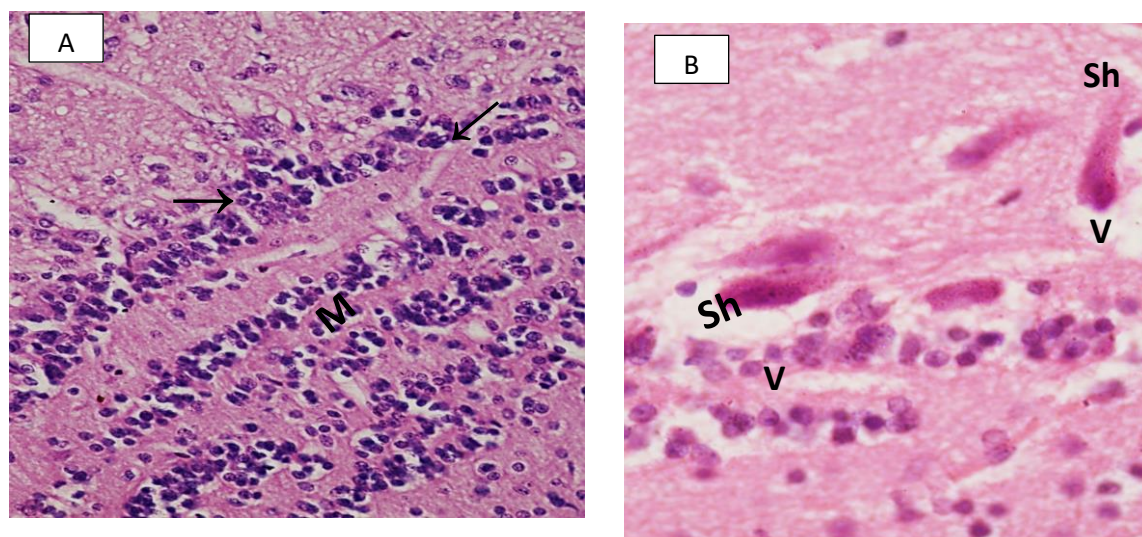


Fig (9): A. Photomicrograph of a coronal section of the olfactory bulb of rabbit of treated group III, showing :Greatly disorganized mitral cell layer (M). (→) points to swollen mitral cells with vacuoles in their cytoplasm (H and E x 400). B. (Sh) shrunken dark mitral cells with no clear cell membrane .(V) vacuoles in the cytoplasm of the shrunken mitral cells . ((H and E x 1000)

Discussion:

The olfactory bulb is divided into multiple layers, with different types of neurons found in each of the layers. Therefore, neurons in the olfactory bulb have conventionally been categorized, based on the layers in which their cell bodies are found, namely, juxtglomerular cells in the glomerular layer, tufted cells in the external plexiform layer, mitral cells in the mitral cell layer, and granule cells in the granule cell layer.(14).

Mitral cells are a neuronal cell type in the mammalian olfactory bulb, distinguished by the position of their somata located in an orderly row in the mitral cell layer of the bulb. They typically have a single primary dendrite, which they project into a single glomerulus in the glomerular layer, and a few lateral dendrites that project laterally in the external plexiform layer. Mitral cells are closely related to the second type of projection neuron in the mammalian bulb, known as the tufted cell.(13)

In our study, the mitral cell diameter of both study groups revealed that both study groups had smaller longitudinal and transverse diameter, compared to the control group. However; the differences between the two groups was relatively less. These were in consistence with Khilji et al. (2010) and Hamshari (2012) which found that there were reduction in the diameter of mitral cells in offspring of mother expose to nicotine and passive tobacco smoke.

The present study demonstrated several histopathological findings among groups II and III that included slightly disruption in the arrangement of the mitral cells layer , shrunken, degenerated with loss of the nuclear membrane or vacuolated cytoplasm, and scanty nissl granules. Hamshari (2012) reported similar changes in offspring of mother expose to nicotine and passive smoke. Kivatkin (2014) observed that, when administered systemically, nicotine first physically activates nicotinic receptors located on the afferents of sensory nerves at the sites of drug administration before reaching the brain and directly interacting with central neurons. Nicotine acting peripherally produces a rapid sensory signal to the CNS that is followed by slower, more prolonged direct drug actions in the brain. Chen (2005) suggested that alcohol and nicotine significantly reduced Purkinje cell numbers. Few references in the medical litterers describe all findings in our results; this may be contributed to the fragile nervous tissue that may be lost during preparation.

Conclusion:

Nicotine exposure and passive cigarette smoking causes a reduction in the longitudinal and transverse diameter of mitral cells as well as several histopathological changes which may lead to loss of smell sensation and change in the behavior.

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التأثير السام للنيكوتين ودخان التبغ على بصلة الشم في الأرانب

ريما جواد همشري* وهدى عبدالودود عمر**

* قسم العلوم المورفولوجية وحدة التشريح، كلية الطب والعلوم الصحية، جامعة عدن.

** قسم الباراكلينيك وحدة الطب الشرعي والسموم، كلية الطب والعلوم الصحية، جامعة عدن.

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الملخص

يعد التنباك من المواد المؤثرة على صحة الانسان واحد المسببات الرئيسية للأمراض حول العالم. و تعد بصلة الشم من الأجزاء الموجودة في السطح الأمامي للدماغ التي تستلم حاسات الشم لتقوم بتنظيمها إلى إحساس سلوكي.

الغرض من الدراسة هو بحث تأثير النيكوتين على بصلة الشم للأرانب. تم في هذه التجربة استعمال خمسة وسبعون أرنب متوسط أوزانها 1500-2000 جم

وتم تقسيم الأرانب كالاتي:

المجموعة الأولى (الضابطة): استعملت خمسة وعشرين أرنب تتغذى على الطعام الطازج والماء طوال فترة الحمل" خس -جزر -عيش جاف وبرسيم . "تم حقن الارانب تحت الجلد بمحلول ملحي مرة يومياً لمدة شهر .

المجموعة الثانية: استخدمت خمسة وعشرين أرنب تتغذى على نفس غذاء المجموعة الأولى و ماء لمدة شهر . وتم حقن جميع الأرانب تحت الجلد بالنيكوتين المخفف بمحلول ملحي ١ جرام/ كيلوجرام مرة يومياً لمدة شهر كاملة .

المجموعة الثالثة: استعملت خمسة وعشرين أرنب تتغذى على نفس غذاء المجموعتين السابقتين وماء .تم تعريض جميع الأرانب للدخان ٦ سجائر (٢سيجارة في ٣ أوقات) يومياً في صندوق مدة شهر .

وجد أنّ هناك نقص ذو دلالة إحصائية في أطوال و سماكة بصلة الشم وفي طول و عرض الخلية التاجية في المجموعة المعالجة الثانية والمعالجة الثالثة بالمقارنة مع المجموعة الأولى. والنقص الشديد ظهر في المجموعة الثالثة. ووجدت تغييرات نسيجية في الخلية التاجية تراوحت بين فقد الترتيب لهذه الطبقة و انحلال الخلية وتغييرات على مستوى الخلية في ظهور الفجوات واختفاء النواة والنوية مما أدى إلى اختفاء الخلية التاجية في بعض الأجزاء.

لوحظ أن التعرض للنيكوتين مباشرة أو عن طريق استنشاق دخان السجائر له تأثير كبير على الخلايا التاجية لبصلة الشم وهذا يعد سبب في فقدان حاسة الشم وتغيير في السلوك.

الكلمات المفتاحية: نيكوتين , تدخين , خلايا تاجية و تبغ.