

# Histological Study on the Effect of Methotrexate on the Oral Tissues of Adult Male Rabbits

**Alhan D Al-Moula**  
BDS, MSc (Lec.)

**Mareb H Ahmed**  
MBChB, MSc (Asst. Lec.)

**Makarem H Ahmed**  
BDS, MSc (Asst. Lec.)

**Department of Dental Basic Science**  
College of Dentistry, University of Mosul

**Department of Anatomy and Histology**  
College of Medicine, University of Mosul

**Department of Dental Basic Science**  
College of Dentistry, University of Mosul

## الخلاصة

**الأهداف:** تقييم آثار الميثوتريكسيت عن طريق الفم على الغشاء المخاطي للفم عند الأرانب. **المواد وطرائق العمل:** استخدمت في العمل الحالي ٩ ذكور أرانب، أعمارهم ٣ أشهر، تم تقسيمهم إلى ثلاث مجموعات رئيسية (٣ أرانب لكل مجموعة). المجموعة الأولى: الضابطة بقيت بدون علاج، كمجموعة السيطرة وجرعت بمحلول ملحي متعادل. المجموعة الثانية: أعطيت جرعة واحدة ٠.٥ ملغم/كغم من الميثوتريكسيت أسبوعياً، فمويًا بواسطة إبرة *cavage* لمدة ٨ أسابيع. المجموعة الثالثة: أعطيت نفس الجرعة المذكورة أعلاه فمويًا مرتين / في الأسبوع، ولمدة ٨ أسابيع. تم تشريح الحيوانات وإزالة أرضية الفم، والظهارة، واللسان، وتجهيزها للدراسة النسيجية. **النتائج:** أظهرت الدراسة للمجاميع المعاملة بعقار الميثوتريكسيت أن الغشاء المخاطي للفم هو أقل سمكا من الطبيعي مع وجود فقاعات في الساييتوبلازم، والخليعات كانت أقل وأقصر مع احتقان في الأوعية الدموية و تسلل الخلايا الدهنية وتشويه النظام النسيجي بين الغشاء المخاطي و العضلات. أظهرت الغدد اللعابية تغيرات نسيجية وفقدان النظام النسيجي، وظهور الفجوات في الخلايا العنيفة. وتسلل خلايا الالتهابات، بينها. أظهر السطح الظهري من اللسان ضمور طلائي، مع فقدان الخليعات اللسانية. والخليعات قصيرة واحتقان الأوعية الدموية، مع تسلل خلايا الالتهابات. وتشويه كبير في ترتيب عضلات اللسان. **الاستنتاجات:** الاستعمال الأسبوعي لعقار الميثوتريكسيت عند الأرانب يسبب تغيرات حادة في أنسجة الغشاء المخاطي للفم.

## ABSTRACT

**Aims:** The objective of this study is to evaluate the effects of oral methotrexate on the oral tissues of rabbits. **Materials and Methods:** In the present study nine adults' male rabbits, aged 3 months were used, and divided into three main groups. Group I: served as a control and received normal saline only. Group II: given a single dose of 0.5mg/kg per week methotrexate orally by *cavage* needle for 8 weeks. Group III: given 0.5mg/kg of methotrexate orally twice/weekly for 8 weeks. The animals were sacrificed and the floor of the mouth, cheek and tongue, were excised and processed for histological study. **Results:** The epithelium of oral mucosa in the treated groups is thinner than that of the control group with vacuolation in the cytoplasm, fewer and shorter slender papillae, congestion of the blood vessels in the lamina propria, fatty infiltration and distortion of attachments of mucosa to underlying muscle. The minor salivary glands show loss of normal architecture, inflammatory cellular infiltration, disarrangements of acini, and vacuolation in the acinar cells. The dorsal surface of the tongue showed an epithelial atrophy, with loss of the lingual papillae. The lamina propria showed short papillae, inflammatory cellular infiltration and congestion of blood vessels, as well as considerable distortion of arrangements in the musculature of the tongue. **Conclusion:** weekly administrations of methotrexate produce dramatize histological changes in the oral tissues.

**Keywords:** Methotrexate, oral mucosa, rabbits.

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## INTRODUCTION

Methotrexate is an antimetabolite and immune modulating drug when taken at high dose. Its used as chemotherapeutic agents in the treatment of malignant diseases including Leukemia, non-Hodgkin's lymphoma and a number of solid tu-

mors.<sup>(1)</sup> Methotrexate has another role when taken at lower doses in the control of chronic inflammatory disorders such as rheumatoid arthritis and psoriasis.<sup>(1, 2)</sup> Methotrexate as any cancer chemotherapeutic agent tend to produce damage of normal cells as well causing permanent

damage to normal tissues during killing the cancer cells. The amount of this damage and its severity is usually based on the type, dose, and duration of drug used to treat the disease.<sup>(3)</sup> Another effect of methotrexate treated rats has been found to reduce the salivary gland weight, Ribonucleic Acid, and amylase content.<sup>(4)</sup> The aim of this study is to evaluate the effect of low dose of methotrexate on the oral tissues of rabbits.

### MATERIALS AND METHODS

In this study 9 adult male rabbits aged about 3 months weighing 1.265-2.025 Kg, were used and kept in a standardized animal house condition with room temperature  $25 \pm 2C^0$  and freely fed. The animals were divided into three groups.

Group I: 3 rabbits were served as a control group and received normal saline.

Group II: 3 rabbits given 0.5 mg /kg of methotrexate in a single dose per week orally.

Group III: 3 rabbits given 0.5 mg /kg of methotrexate orally twice weekly.

The drug was administrated orally by cavage needle. Then all animals were sacrificed by ether inhalation anesthesia. Face and neck dissection was done for all animals , the tongue, check and floor of the mouth were collected , fixed in 10% for-

malin fixative for 24 hours ,then dehydrated in ascending grades of alcohol, using 50% -70% -90% and 2 changes of absolute alcohol respectively with a period of one hour for each, procedure for preparing the paraffin section slides and staining with Hematoxyline and Eosin (H&E) was performed to obtain stained histological sections for light microscopic examinations.<sup>(5)</sup>

### RESULTS

Physical and clinical observations :

Side effects like oral ulceration ,vomiting ,white patches on the mouth and lips was not observed. Loss of weight and diarrhea were noticed in most animals.

Control group

Group I:

The histological appearance of the normal buccal mucosa shows the covering epithelium as a very thick non keratinized stratified squamous epithelium. The lamina propria have long slender papillae ,a dense fibrous connective tissue containing collagen and some elastic fibers ,rich vascular supply giving off anastomosing capillary loops into papillae. The mucosa is firmly attached to underlying muscle by dense collagenous connective tissue with fat and minor salivary glands Figure (1).

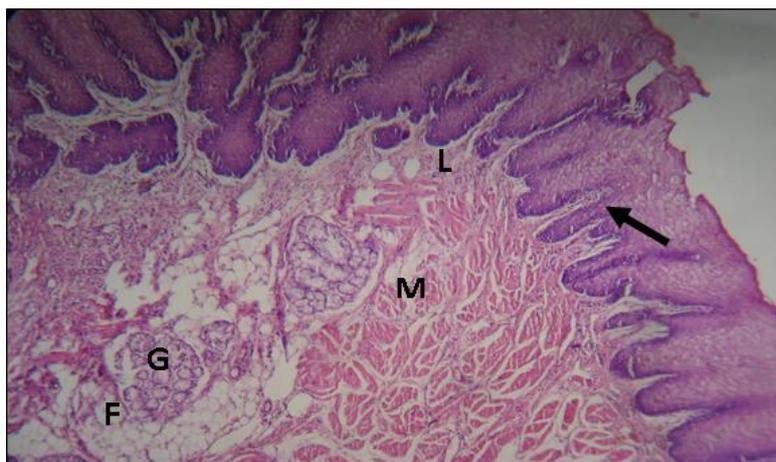


Figure (1): light micrograph of the normal buccal mucosa of rabbit in group I showing very thick non keratinized stratified squamous (black arrow) of the covering epithelium .The lamina propria (L) have long slender papillae . The mucosa is firmly attached to underlying muscle(M) by dense collagenous connective tissue with fat(F) and minor salivary glands (G). H&E.[X-40].

The histological appearance of the floor of the mouth shows that the covering epithelium as a very thin non keratinized stratified squamous epithelium. The lami-

na propria has short papillae, extensive vascular supply .In the submucosa there is loose connective tissue containing fat and minor salivary glands Figure (2).

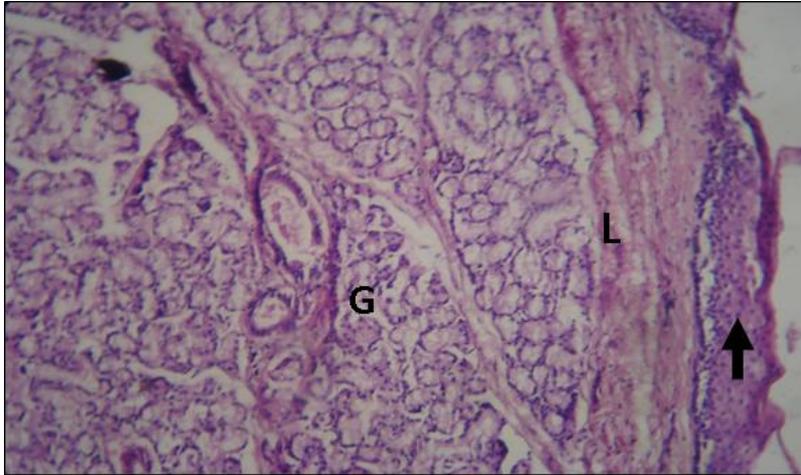


Figure (2): light micrograph of the floor of mouth of rabbit in group I , showing thin non keratinized stratified squamous (black arrow) of the covering epithelium . The lamina propria(L).Minor salivary glands(G) in the submucosa. H&E.[X-75].

The histological appearance of dorsal surface of tongue shows a thick keratinized stratified squamous epithelium forming the lingual papillae. The lamina pro-

pria have long papillae. The mucosa is bound to connective tissue surrounding the musculature of tongue Figure (3).

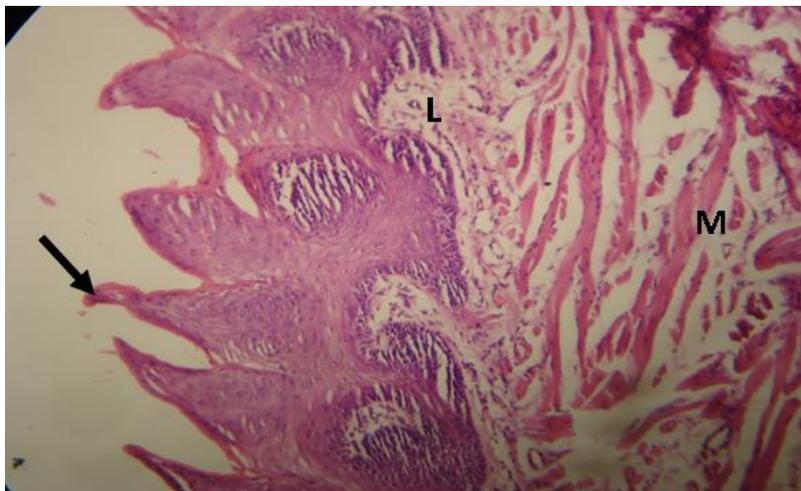


Figure (3): light micrograph of the dorsal surface of tongue of rabbit in group I, showing a thick stratified squamous epithelium forming the lingual papillae (black arrow). The lamina propria have long papillae(L).The mucosa is bound to connective tissue surrounding muscles (M)of the tongue. H&E.[X-75].

The histological appearance of ventral surface of tongue shows that a thin non keratinized stratified squamous epithelium

bound to underlying muscle by a narrow lamina Figure (4).

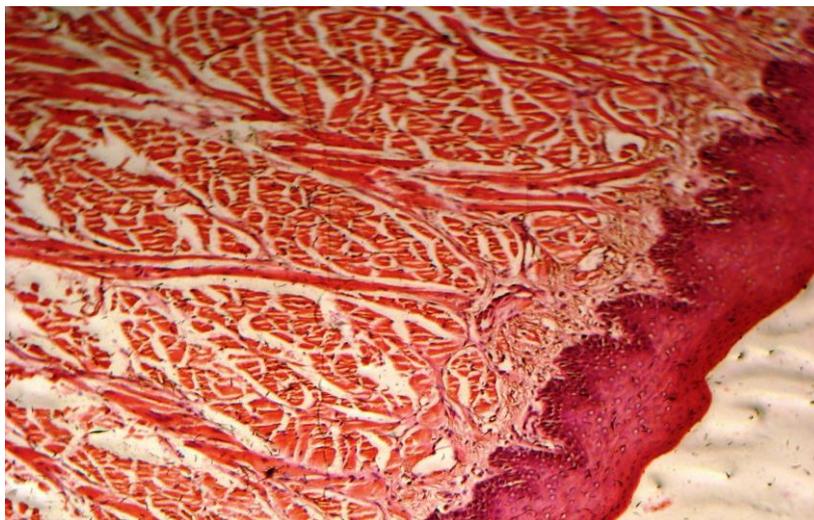


Figure (4): light micrograph of ventral surface of tongue of rabbit in group I , showing non keratinized stratified squamous epithelium(black arrow) bound to underlying muscle(M) by a narrow lamina(L). H&E.[X- 90].

*Treated group*

Table (1): All animals in Group II &III showed a gradual and regular reduction in body weight over the eight weeks after

administration of methotrexate. While the control group shows increase in the body weight.

Table (1): Body weight (gm) of rabbits before and after treatments

Animal No	Before treatments	After Treatments	Note
1	1320	1340	Control Group I
2	1455	1480	
3	1650	1670	
4	1350	1280	Single dose weekly Group II
5	1500	1345	
6	2025	1480	
7	1535	1423(died)	Twice dose weekly Group III
8	1265	1123(died)	
9	1700	1476(died)	

*Group II:*

The histological appearance of the buccal mucosa shows that the covering epithelium is non keratinized stratified squamous thinner than control, some cells show vacuolation in the cytoplasm Figure

(5). The lamina propria have fewer and shorter slender papillae and congestion of the blood vessels, fatty infiltration and distortion of attachments of mucosa to underlying muscle Figure (6).

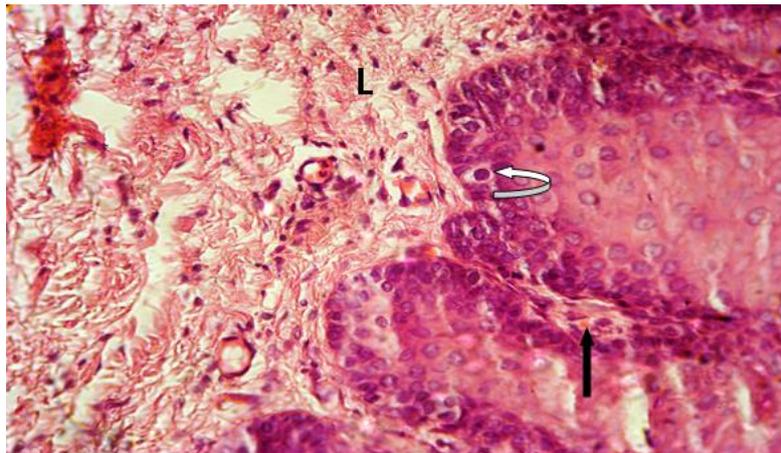


Figure (5): light micrograph of the treated buccal mucosa of rabbit in group II showing the covering epithelium (black arrow) thinner than control, some cells show vacuolation in the cytoplasm(white arrow). The lamina propria (L) have fewer and shorter slender papillae. H&E.[X- 370].

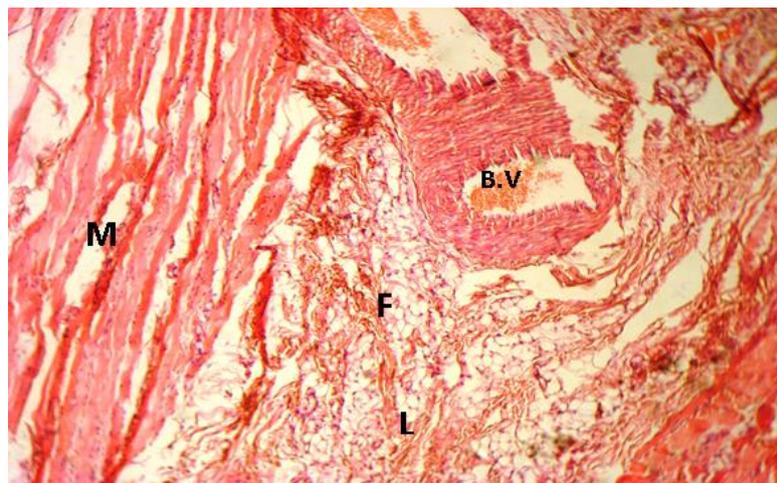


Figure (6): light micrograph of the treated buccal mucosa of rabbit group II showing congestion of the blood vessels (B.V ), fatty infiltration (F) in the lamina propria (L) and distortion of attachments of mucosa to underlying muscle(M). H&E.[X- 90].

The minor salivary glands of the buccal mucosa shows loss of architecture, inflammatory cellular infiltration, disarrangements of acini, vacuolation in the acinar cells. The duct cells showed atrophy and shrinkage with a large space around the duct Figure (7).

The histological appearance of the

floor of mouth shows distortion and loose attachments of the submucosa to the underlying muscle Figure (8).

The minor salivary glands shows deposition of homogenous material, and inflammatory cellular infiltration between the acini Figure (9).

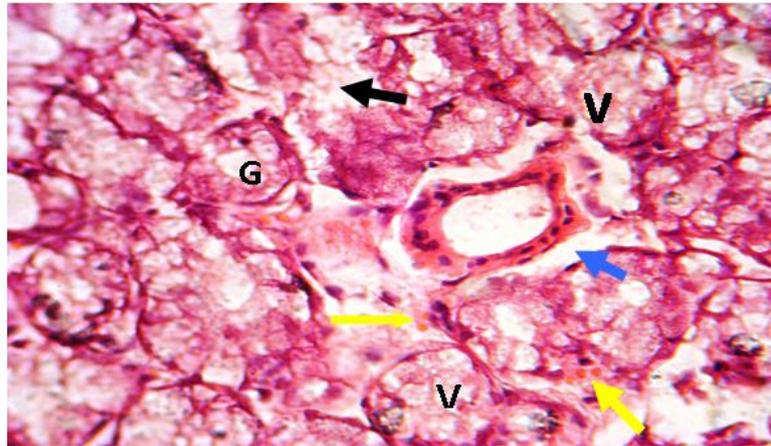
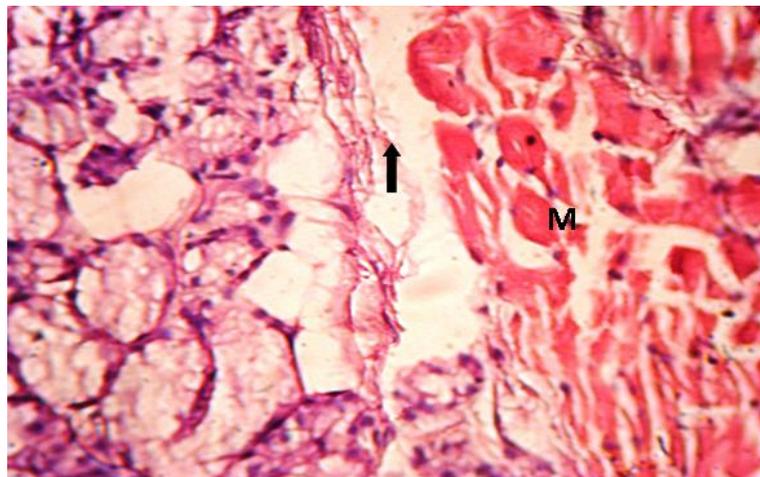


Figure (7): light micrograph of the treated buccal mucosa of rabbit in group II showing loss of architecture of minor salivary glands(G),inflammatory infiltration (yellow arrow),disarrangements of acini (black arrow) and vacuolation in the acinar cells(V). The duct cells showed atrophy and shrinkage with large space around the duct(blue arrow) . H&E.[X- 450]



Figure(8): light micrograph of treated floor of mouth of rabbit in group II showing distortion and loose attachments of the submucosa (black arrow) to the underlying muscle(M). H&E.[X- 450].

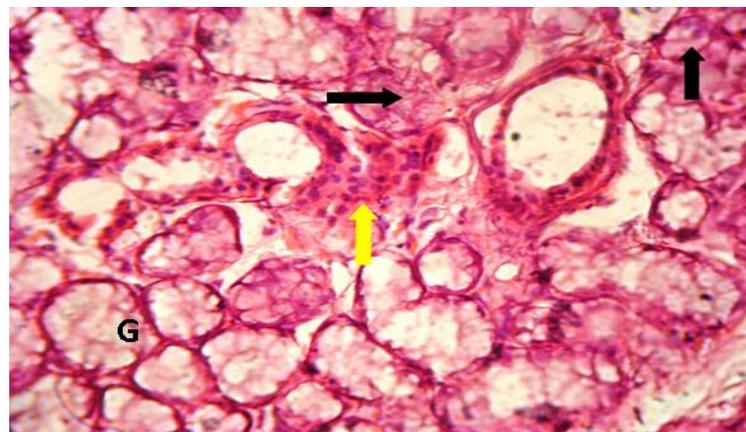


Figure (9): light micrograph of the treated floor of mouth of rabbit in group II showing deposition of homogenous material(black arrow), and inflammatory infiltration (yellow arrow) between the acini of the minor salivary glands(G) . H&E. [X- 370]

The histological appearance of tongue shows an epithelial atrophy, with lingual papillae loss in the dorsal surface . The lamina propria have short papillae Figure (10). Considerable distortion of arrangements in the musculature of tongue inflammatory cellular infiltration and congestion of blood vessels Figure (11). The

ventral surface of the tongue shows loss in the orientation of the muscle Figure (12).

*Group III:*

Death of all animals during the second week after receiving methotrexate twice weekly (no sectioning obtained).

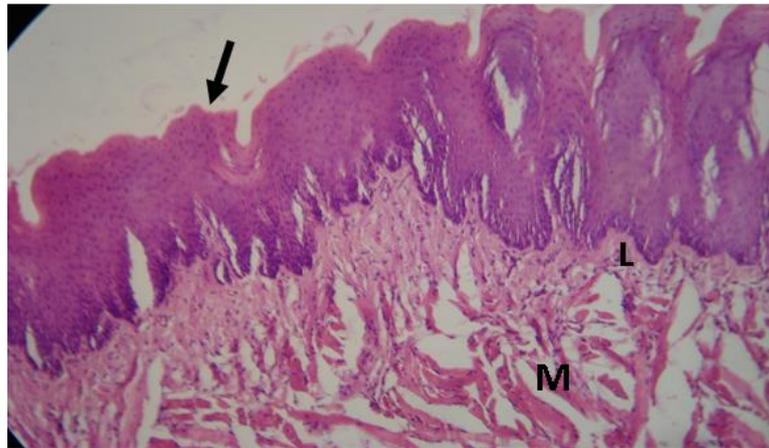


Figure (10): light micrograph of treated dorsal surface of tongue of rabbit in group II showing epithelial atrophy, with lingual papillae loss (black arrow) . The lamina propria have short papillae(L), distortion of arrangements in the muscles of tongue(M). H&E. [X- 75].

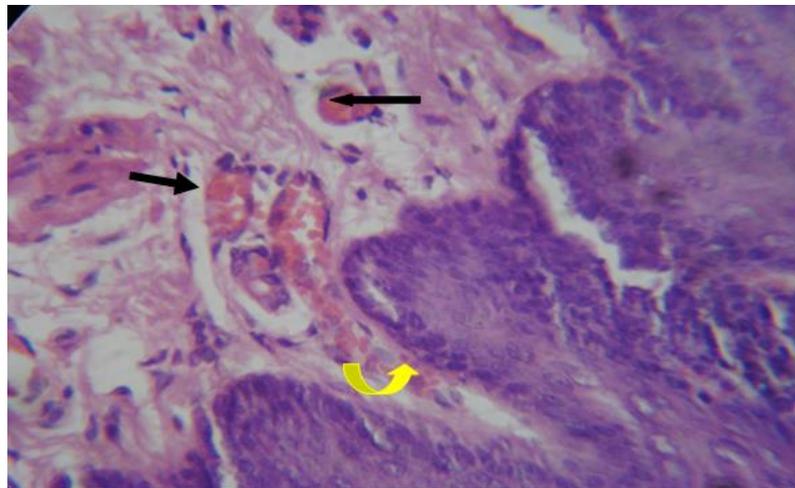


Figure (11): light micrograph of treated dorsal surface of tongue of rabbit in group II showing inflammatory infiltration (yellow arrow) and congestion of blood vessels in the lamina propria(black arrow) . H&E.[X- 350].

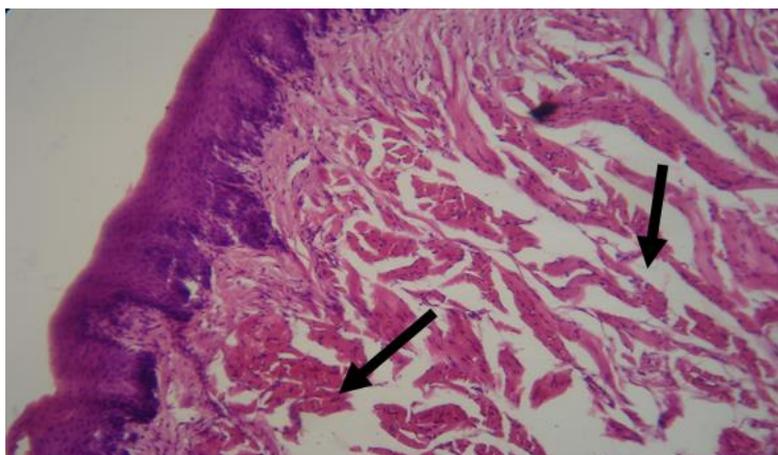


Figure (12): light micrograph of treated ventral surface of tongue of rabbit in group II showing loss of orientation of the muscle (black arrows) .  
H&E.[X- 75]

### DISCUSSION

The pathogenesis of the toxic effects of methotrexate on oral mucosa is not well studied. This study confirms the original finding on the adverse side effects of methotrexate as a chemotherapeutic agent on oral mucosa of adult male rabbits. As currently used, low dose methotrexate is now first – line therapy for the treatment of rheumatoid arthritis not responsive to nonsteroidal anti-inflammatory drugs alone.<sup>(6)</sup> The side effects of methotrexate like oral ulceration, vomiting, white patches on the mouth and lips was not observed in this study. While other literatures reported the oral effects range from non healing ulcers to lymphoma-like lesions.<sup>(7)</sup> This may be due to the low-dose regime of methotrexate used in our study. Oral ulceration can occur as a side effect of methotrexate therapy. This may be due to lack of folic acid supplementation or over-dosage due to confusion regarding its once-weekly regime.

The decrease in the thickness of the covering epithelium of the buccal mucosa which is noticed in our study with shortening of the dermal papillae. Inflammatory cell infiltration in the lamina propria is in agreement with the other studies which demonstrate that treatment with methotrexate was associated with a damage to tissues in the form of epithelial atrophy, inflammatory cell infiltration in the lamina propria. This is due to increase in the cell death and decrease in the cell

number.<sup>(8,9)</sup> The loss of architecture, inflammatory infiltration, disarrangements of acini, vacuolation in the acinar cells of the minor salivary glands in the buccal mucosa, floor of the mouth is in agreement of the other studies which show that methotrexate induces loss of architecture, with disarrangements and marked swelling of the acini.<sup>(4)</sup> These marked changes in the minor salivary glands of oral mucosa suggests suppression and or disruption of protein synthesis of abnormal protein this can lead to formation of cytolysosome, some may be an evidence of the distinctive process called shrinkage necrosis, this process has been described as apoptosis in a number of tissues and the apoptotic bodies which are found in small numbers in normal tissues and greatly increased in tissues which have been subjected to chemotherapy.<sup>(10-12)</sup> The congestion of the blood vessels in the lamina propria of oral mucosa may be due to vasculitis after methotrexate therapy. Some authors have suggested that methotrexate induced cutaneous small-vessel vasculitis in patients with collagen vascular disease who were treated with low-dose methotrexate.<sup>(13)</sup> Other studies demonstrate that administration of a single dose of methotrexate intraperitoneally does not exert an acute cytotoxic effect on rapidly replicating oral tissue like the mucosa or on tissue with a slow turnover like the parotid.<sup>(14)</sup> The distortion of arrangements and loss of orientation in the musculature of tongue,

these side effects of methotrexate are probably due to inhibition of synthesis of dihydrofolate reductase, which is essential to maintain the cellular tetrahydrofolate pool during purine and thymidine synthesis. Being a high affinity inhibitor of dihydrofolate reductase, methotrexate is a pro-oxidant compound that causes depletion of the dihydrofolate pool and directly affects the synthesis of thymidilate, suppressing DNA synthesis. Therefore methotrexate affects not only tumor cells but also rapidly dividing cells such as gastrointestinal mucosa, where it inhibits epithelial proliferation and induce apoptosis.<sup>(8,15,16)</sup> Recently, it has demonstrated that methotrexate causes significant reduction in the antioxidant enzymes levels, sensitizing the cells to reactive oxygen species (ROS).<sup>(17)</sup>

The decrease in the weight of the animals in group II and Group III after treatment is mostly due to the loss of appetite due to the anti cancer therapy (Table 1).

In the third group there was a lethal effect on the animal during the second week after receiving methotrexate twice weekly. The cause of death of some animal may be dose related, as the oral dose should be taken once weekly and not more.<sup>(18)</sup>

### CONCLUSION

The constant cellular division associated with epithelial renewal renders the oral cavity so susceptible to damage by the anticancer therapies. low-dose methotrexate lead to oral toxicity, dental practitioners should be aware of the possible oral effects that have so far been largely unrecognized of low-dose methotrexate which is used for a variety of conditions, particularly rheumatoid arthritis.

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