

## AMELIORATIVE EFFECT OF L-CARNITINE AGAINST HISTOPATHOLOGICAL CHANGES INDUCED BY METHOTREXATE ON THE LINGUAL MUCOSA OF ALBINO RATS

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### ABSTRACT

**Background and objectives:** Methotrexate (MTX) is a chemotherapeutic drug that is used in the treatment of many cancers and chronic inflammatory illnesses. Oral mucositis is a common side effect of MTX, which may lead to the discontinuation of chemotherapy. The study aimed to evaluate the possible protective effect of L-carnitine (LC) on the lingual mucosa of MTX-treated Albino rats.

**Material and Methods:** Forty male albino rats were used in this experiment. The rats were divided into four experimental groups: Group 1 (negative control group), Group 2 (LC group), Group 3 (MTX group), and Group 4 (LC+MTX treated group). Rats were euthanized separately on day 14<sup>th</sup>. Tongue specimens were processed for hematoxylin & eosin staining and immunohistochemically prepared for TNF- $\alpha$ , COX-2, and Bax expression. The data was analyzed and expressed statistically by the one-way ANOVA test.

**Results:** The negative control and LC groups showed normal histological structures of the lingual mucosa. The MTX group showed destruction in normal histological structure, while the group that took LC as a protective agent before MTX induction, showed a marked protection to the lingual mucosa from the destructive effect of MTX. Immunohistochemical results revealed mild to moderate immune reactivity to TNF- $\alpha$ , COX-2, and Bax in the negative control, LC, and LC+MTX groups, while a strong immune reactivity was observed in the MTX group ( $p < 0.05$ ).

**Conclusion:** The current study proved that LC could act as a prophylactic agent before MTX treatment to prevent oral mucositis.

**KEYWORDS:** Methotrexate, Oral mucositis, L-carnitine, TNF- $\alpha$ , COX-2, Bax.

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## INTRODUCTION

Methotrexate (MTX), a chemotherapeutic drug, is commonly used to treat variety of cancers, including osteosarcomas, lung, breast, head and neck cancers.<sup>1</sup> MTX is also commonly used to treat chronic inflammatory illnesses such as rheumatoid arthritis<sup>2</sup> and systemic lupus erythematosus.<sup>3</sup> Its mechanism of action was discovered to be primarily dependent on the inhibition of DNA synthesis, which inhibits the proliferation of these aberrant cells.<sup>4</sup> MTX is an anti-metabolite (anti-vitamin) of folic acid (FA, vitamin B9) that indirectly inhibits cell division through the blockage of folate-related enzymes, mainly dihydrofolate reductase (DHFR). It catalyzes the conversion of dihydrofolate to tetrahydrofolate (THF). THF is an important coenzyme in various transmethylation events in the pyrimidine and purine nucleotide synthesis pathways, which are required for DNA strand synthesis, repair, and replication.<sup>5</sup> The inhibition of intracellular THF formation by MTX results in cell growth interruption and metabolic imbalance, which leads to apoptosis.<sup>6</sup>

It is not surprising that the most susceptible cells to the cytotoxic effect of MTX are most visible in actively dividing cells, primarily in the S phase of the cell cycle, and in highly proliferating cancer cells. This indicates that folate antagonism is related to MTX's anti-tumor activity.<sup>7</sup> Unfortunately, MTX has cytotoxic effects on normal tissues undergoing rapid cellular turnover, such as the oral mucosa, gastrointestinal tract, and bone marrow cells that explains why oral mucositis an initial manifestation of MTX toxicity.<sup>8</sup>

Oral mucositis (OM) is the most serious complication of anticancer therapy.<sup>9</sup> It begins in the epithelium and progresses to invade the connective tissue. Mucosal thinning that induced by apoptosis and depletion of the epithelial basal layer with eventual denudation are characteristic features for OM.<sup>10</sup> Loss of epithelial integrity may increase the risk of bacteremia, fungemia, and sepsis whereas

the oral cavity is considered the home for wide range of bacteria.<sup>11</sup> It can lead to major secondary consequences such as difficulties in eating and swallowing, throat or mouth pain, xerostomia, infection, and malnutrition, all of which can lead to patient death.<sup>12</sup> Additionally, the MTX has harmful effect on the salivary glands through the acini's histological destruction, the ducts dilatation, and changing the composition of the salivary secretion.<sup>13</sup>

Effective antibacterial, anti-inflammatory, anti-ulcer, and antioxidant drugs may be helpful in treating oral mucositis associated with MTX. L-carnitine (LC), a water-soluble quaternary amine, has a wide range of biological effects, such as anti-inflammatory, antioxidant, neuroprotective, cardioprotective, gastroprotective, cytoprotective, and antiapoptotic characteristics.<sup>14</sup> Nearly 75% of the LC that the body stores come from food, while the remaining 25% is produced internally from lysine and methionine.<sup>15</sup>

Reactive oxygen species (ROS) and several pro-inflammatory cytokines have been implicated in the pathophysiology of OM.<sup>16</sup> MTX has been shown to raise the levels of tumor necrosis factor alpha (TNF- $\alpha$ ), an indicator of inflammation, interleukin-1 beta (IL-1), and malondialdehyde (MDA), an oxidant parameter in the oral mucosa.<sup>17</sup>

L-carnitine can be used as a scavenger for free oxygen radicals. Additionally, it acts as a powerful non-enzymatic antioxidant that protects the cell, mitochondrial membrane, and DNA integrity against free oxygen radicals. Also, it is considered as an enhancer of antioxidant enzymes like glutathione peroxidase.<sup>18</sup>

Oxidative stress leads to production of ROS that effect on initiation of the nuclear factor-kB signaling pathway, which is important for the regulation of many genes implemented in inflammatory responses, such as TNF- $\alpha$ , cyclooxygenase-2 (COX-2), inducible nitric oxide synthase, and caspase family of proteases leading to apoptosis. Hence LC neutralizes ROS generated by oxidative stress and protect against apoptosis.<sup>19</sup>

The current study was undertaken to investigate the possible protective effect of LC on the lingual mucosa of MTX-induced OM in the albino rats.

### Material and Methods

**Ethical statement:** This study was approved by the research ethical committee (REC), Faculty of dentistry, Suez Canal University, approval No. (673/2023).

**Chemicals:** MTX 50 mg per 2 ml of solution was purchased from Haupt Pharma GmbH – Germany (imported by: RAMCO). LC was purchased from SEDICO Pharmaceuticals, for: Arab Co. for Pharmaceutical & Medicinal Plants (MEPACO – MEDIFOOD) Egypt.

**Animals and housing:** Forty healthy male albino rats weighing 200-250 grams were supplied and kept in the animal house, Faculty of Dentistry, Suez Canal University (Ismailia, Egypt). They were kept under normal laboratory conditions and at normal temperature ( $24\pm 5^{\circ}\text{C}$ ). They were fed a standard diet and tap water *ad libitum* under good ventilation throughout the experimental period.

**Inclusion and exclusion criteria:** Male albino rats weighing from 200-250 grams and with normal mucosa without wounds, inflammation, or any lesions were included. Any rat with systemic illness, injuries, diarrhea, or infections was excluded.

**Experimental design:** According to Charan and Biswas (2013)<sup>20</sup>, the sample size was calculated and divided equally in each group. The forty rats were randomly divided into the four groups (n=10). **Group 1** (negative control) didn't receive any type of treatment till the end of experiment. **Group 2** (LC group) was injected intraperitoneally (i.p.) with LC 500 mg/kg per day for 14 days.<sup>21</sup> **Group 3** (MTX group) received a single i.p. injection of 80 mg/kg of MTX.<sup>22</sup> **Group 4** (LC+MTX group) was injected i.p. with LC 500 mg/kg per day for 14 days, and at day 5; a single i.p. injection of 80 mg/kg MTX was administered. All rats were weighed at the beginning and end of the experiment.

**Euthanasia:** On the 14<sup>th</sup> day, all rats were euthanized by Carbon dioxide (CO<sub>2</sub>) inhalation, and the tongue tissues were dissected and preserved for 24 hours in a 10% neutral buffered formalin solution. The dead experimental animals were disposed of by burning in the Animal Aching Unit of the Faculty of Medicine, Suez Canal University.

**Histopathological analysis:** The samples were subsequently processed, paraffin-embedded, sectioned into 5  $\mu\text{m}$  pieces, mounted, and stained with hematoxylin and eosin for light microscopic analysis to assess the overall histopathological alterations.

**Immunohistochemical (IHC) analysis:** From each paraffin block, 5  $\mu\text{m}$  sections were cut and mounted on positively charged slides. The immunostaining was performed using: The rabbit polyclonal antibody to TNF- $\alpha$  (Gene Tex International Corporation; Cat. No. GTX110520), the rabbit polyclonal antibody to Cox-2 (Thermo Fisher Scientific, Anatomical Pathology, UK, Cat. No. RB-9072-R7) and rabbit polyclonal mouse antibody to Bax (Santa Cruz Biotechnology, Cat. No. sc526). The steps of IHC staining were followed according to manufacturer's instructions. All microscopic analysis were done by using a light microscope (Olympus, Tokyo, Japan) by two independent oral pathologists. TNF- $\alpha$ , Cox-2, and Bax immunoreactivity were assessed in the nucleus and cytoplasm of epithelial cells.

**Morphometric analysis:** Quantitative analysis was performed through using image analyzer computer system (image J / Fiji 1.46). It was used to measure the filiform papillae's length & width, fungiform papillae's length, thicknesses of the epithelium in the ventral surface, width of blood vessel and cystic degeneration of Von Ebner salivary gland. The digitizing of the slides under 400X objective magnification enabled us to count the number of immune-positive cells as well as the number of the remaining unstained ones. So, the fraction of the positive cells for TNF- $\alpha$ , Cox-2 and Bax in five photograph fields was calculated.

### Statistical analysis

Through using the SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels  $< 0.05$  (P- Value), all statistical analysis were calculated. All data were in the form of Mean  $\pm$  Standard deviation (SD). One-way ANOVA was used to compare between the groups. Duncan's tests were used to evaluate the statistical significance.

## RESULTS

### Clinical findings:

There was a significant loss of body weight in MTX-treated group ( $208 \pm 8.1$ ) in comparison to control group and LC group ( $293 \pm 13.2$  &  $288 \pm 10.3$ ) respectively. There was marked improvement in the general health of albino rats in LC+MTX group ( $256 \pm 6.3$ ) in comparison to positive control group ( $208 \pm 8.1$ ) (Table 1).

### Histopathological assessment

**The negative and self-control (LC) groups** showed no difference histologically between both groups. There were normal histological features of surface keratinized stratified squamous epithelium. The dorsal surface revealed sharp conical projections of filiform papillae in the same directions with pointed tips. Their length ranged from  $1117 \pm 15.7$  &  $1109 \pm 29.4$  and their width was  $235 \pm 7.6$  &  $241 \pm 5.9$  in both groups respectively (fig.1A-E). Fungiform papillae were few. They had one taste bud on its upper surface with smooth keratinized epithelial covering. Their length was  $368 \pm 1.3$  and  $355 \pm 9.4$  respectively (fig.1B). The ventral surface showed normal thickness ( $745 \pm 14.1$  &  $736 \pm 25.3$ ) of keratinized stratified squamous epithelium with rete ridges. The underlying connective tissue appeared normal with minimal infiltration of inflammatory cells. The blood vessels appeared normal with no dilatation ( $262 \pm 10.4$  &  $280 \pm 11.5$ ). The skeletal muscle fibers

were running in different directions (fig.1C-F). Normal microscopic picture of Von Ebner salivary gland was observed without any cystic degeneration ( $125 \pm 18.1$  &  $137 \pm 12.4$ ) (fig.1D).

**In MTX group**, there were massive changes for both the dorsal and ventral surfaces of the lingual mucosa. The dorsal surface showed atrophied filiform papillae in different directions with loss of their conical shape. The length and width of filiform papillae showed a highly significant difference ( $389 \pm 9.5$  &  $337 \pm 7.6$ ) in comparison to the negative control group. Also, separation of the keratin layer from the underlying epithelium was prominent (fig.2A). There was a destroyed taste bud within disfigured fungiform papillae. Its width changed into  $126 \pm 4.6$  (fig.2B). Atrophied ventral surface thickness with focal thinning of the keratin layer was recorded ( $235 \pm 3.8$ ). The underlying connective tissue showed severe infiltration of inflammatory cells and disorganized lamina propria. Widening of blood vessels engorged with RBCs ( $2154 \pm 30.5$ ) was markedly observed (fig.2C). There was progressive cystic degeneration ( $1243 \pm 23.6$ ) of the Von Ebner salivary gland (fig.2D).

**In the LC+MTX group**, there was marked improvement of the lingual mucosa in comparison with the MTX group. On the dorsal surface, the normal conical shape of filiform papillae with pointed tips (fig.3A), normal shape of fungiform papillae with well-formed taste buds were restored (fig.3B). These papillae restored their normal dimensions ( $979 \pm 16.4$  &  $238 \pm 6.9$  &  $352 \pm 4.2$ ). The ventral surface of the tongue revealed moderate restoration of its thickness ( $521 \pm 7.9$ ). The underlying connective tissue showed moderate infiltration of inflammatory cells, congested blood vessels with minimal dilatation ( $875 \pm 12.4$ ), and normal architecture of skeletal muscles (fig.3C). There was mild cystic degeneration ( $565 \pm 11.5$ ) of the Von Ebner salivary gland (fig.3D).

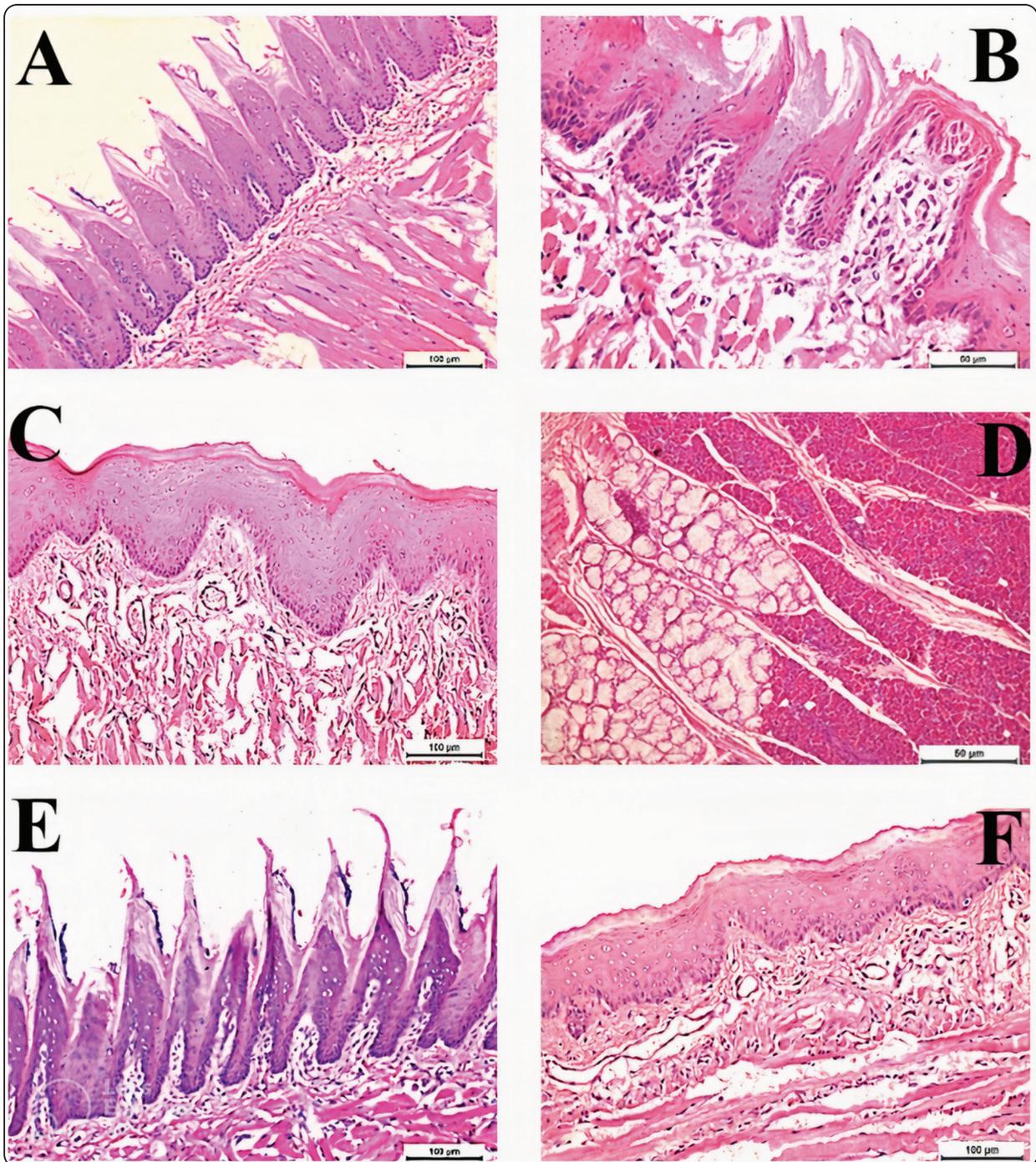


Fig. (1) (A-D) photomicrographs of lingual mucosa in the negative control group showing (A) sharp conical projections of filiform papillae with pointed tips, and normal underlying connective tissue with minimal inflammation. (B) normal appearing fungiform papillae with smooth keratinized epithelial covering. (C) normal keratinized stratified squamous epithelium in ventral surface of the tongue, skeletal muscle fibers running in different directions. (D) normal microscopic picture of Von Ebner salivary gland. (E&F) photomicrographs of lingual mucosa in LC group showing normal histological features on dorsal and ventral surface of the tongue respectively. [Negative control group (A-D); H&E-stained sections. LC group (E&F); H&E-stained sections. Magnifications: A, C, E & F:  $\times 10$  / B & D  $\times 40$ ]

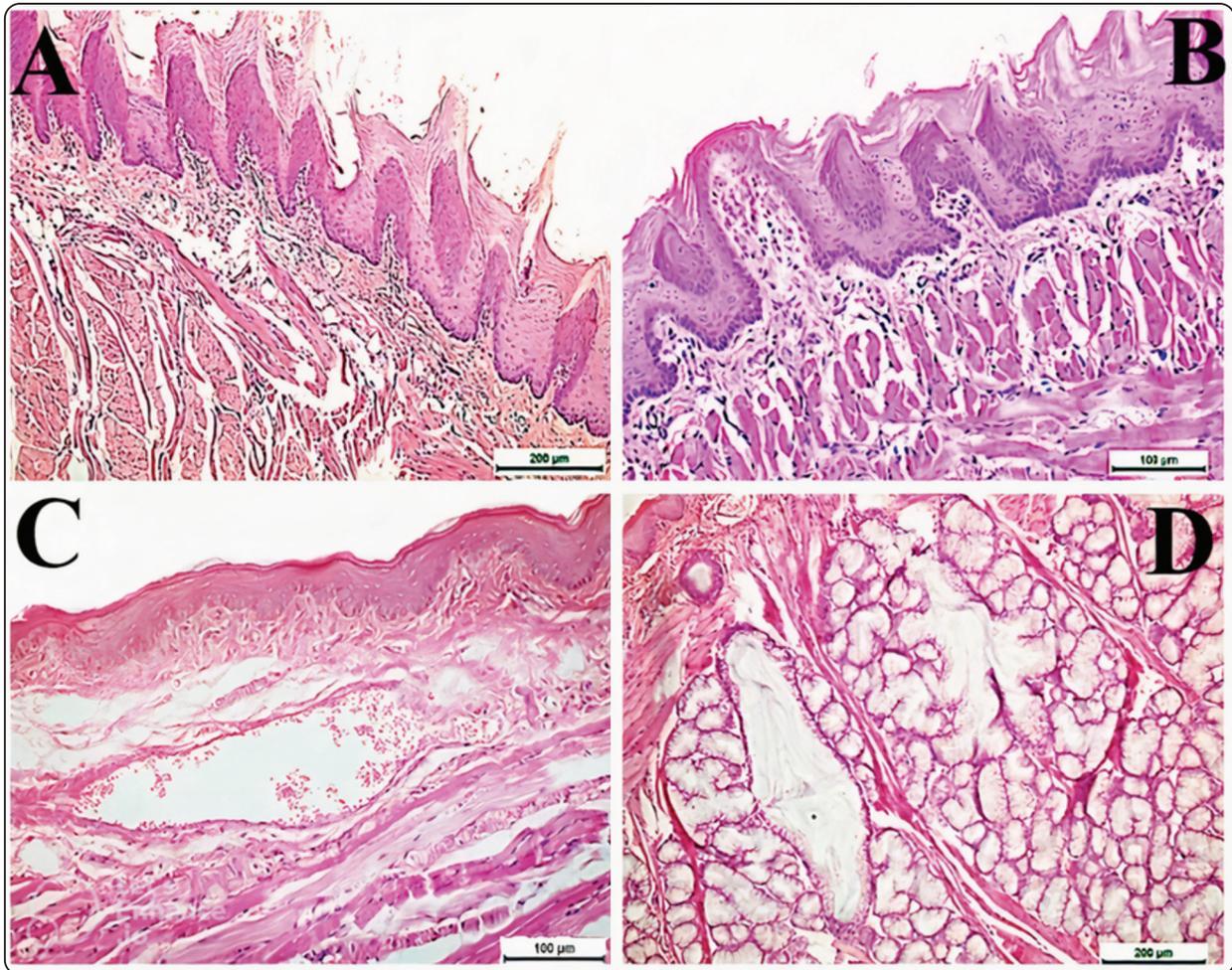


Fig. (2) Photomicrographs of lingual mucosa in MTX-treated group showing (A) atrophied filiform papillae in different directions with loss of their conical shape. (B) disfigured fungiform papillae with destroyed taste buds, disorganized lamina propria with marked inflamed connective tissue. (C) atrophied ventral surface and widening of blood vessels engorged with RBCs. (D) progressive cystic transformation of Von Ebner salivary gland. [MTX-treated group; H&E-stained sections. Magnifications: A & D:  $\times 4$  / B & C  $\times 10$ ]

#### Immunohistochemical findings (table 1, figure 4):

Photomicrographs of the lingual mucosa showed the cytoplasmic expression of TNF- $\alpha$  protein all over epithelial thickness except the keratin layer in different groups. Mild positive immune reactivity, represented by brown color, was showed in the negative control ( $72.1 \pm 2.5$ ) and LC groups ( $70.5 \pm 4.8$ ) (fig 4 A&D) while, MTX group showed severe positive immune reactivity ( $114.8 \pm 3.9$ ) (fig 4 G). Significant improvement was found in

LC+MTX group in comparison with MTX group as mild immune reactivity ( $76.3 \pm 7.4$ ) (fig 4 J).

Photomicrographs of the lingual mucosa showed the cytoplasmic and nuclear expression of Cox-2 protein in different groups. In the negative control and LC groups were showed mild immune reactivity, represented by brown color, limited to basal and parabasal layer ( $25.6 \pm 3.4$  &  $30.5 \pm 3.8$ ) respectively (fig 4 B&E). In MTX group, severe immune reactivity, represented by brown color,

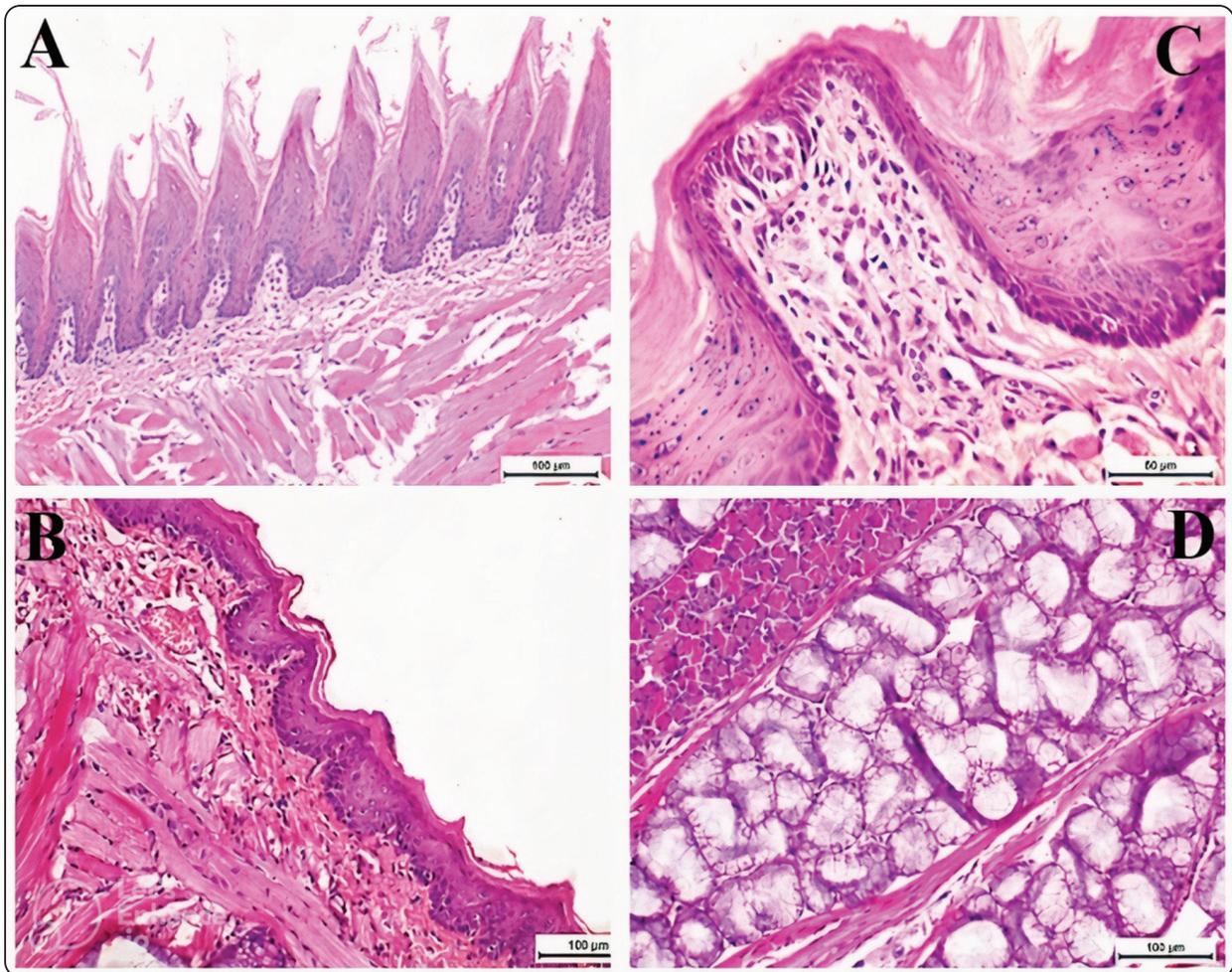


Fig. (3) Photomicrographs of lingual mucosa in carnitine- prophylactic group showing (A) restoration of normal conical shape of filiform papillae with pointed tips (B) restoration the normal shape of fungiform papillae. (C) moderate restoration of ventral surface thickness with moderate inflammation of the underlying connective tissue. (D) mild cystic transformation of Von Ebner salivary gland. [LC+MTX group; H&E-stained sections. Magnifications: A, C & D:  $\times 10$  / B:  $\times 40$ ]

extended all over epithelial thickness was shown ( $91.5 \pm 4.3$ ) (fig 4 H). In LC+MTX group, moderate immune reactivity ( $56.8 \pm 3.6$ ) extended half the epithelial thickness (fig 4 K).

Photomicrographs of the lingual mucosa showed the cytoplasmic expression of Bax protein, represented by brown color, all over epithelial

thickness except the keratin layer in different groups. In the negative control and LC groups mild positive immune reactivity ( $41.2 \pm 2.1$  &  $39.8 \pm 3.2$ ) was demonstrated (fig 4 C&F). In MTX group, severe positive immune reactivity ( $105.1 \pm 1.4$ ) was shown (fig 4 I). In LC+MTX group, moderate positive immune reactivity ( $53.6 \pm 2.8$ ) was presented (fig 4 L).

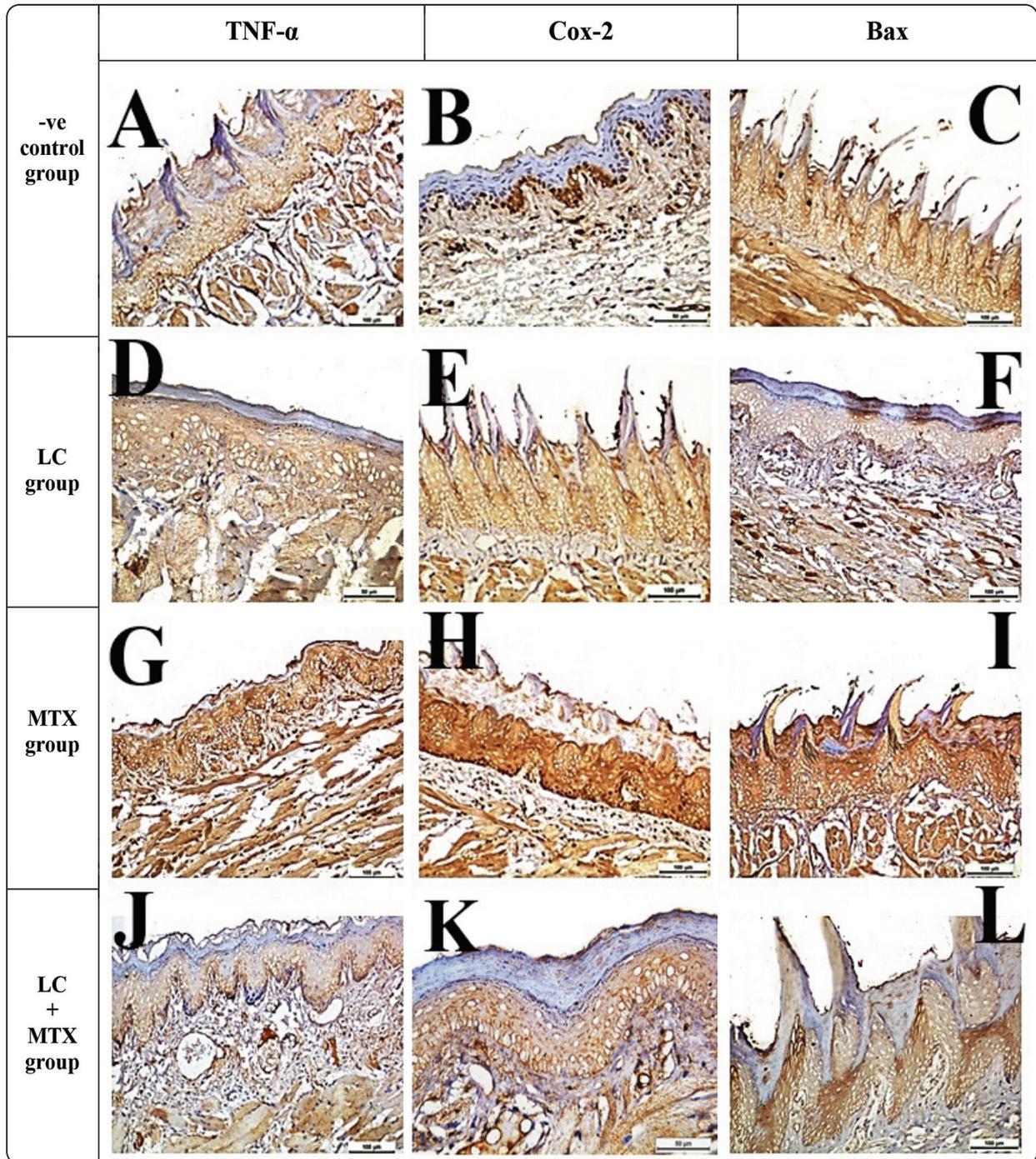


Fig. (4) Photomicrographs of the lingual mucosa showing variable immunohistochemical expression of different proteins. The negative control and LC groups showing mild immune reactivity for TNF- $\alpha$ , Cox-2 and Bax. The MTX group shows severe immune reactivity for TNF- $\alpha$ , Cox-2 and Bax. The LC\_MTX group shows mild immune reactivity for TNF- $\alpha$  and moderate immune reactivity with Bax and Cox-2. [Cox-2 images show nuclear and cytoplasmic expressions while TNF- $\alpha$  and Bax images only show cytoplasmic expressions]. [Magnifications: A, C, E, F, G, H, I, J & L: x10 / B, D & K: x40]

TABLE (1) Statistical immune reactivity and histomorphometric results:

	-ve control group	LC group	MTX group	LC+MTX group
<b>Animal weight</b>	293±13. <sup>2</sup> a	288±10. <sup>3</sup> a	208±8. <sup>1</sup> c	256±6.3 <sup>b</sup>
<b>Filiform length</b>	1117±15. <sup>7</sup> a	1109±29. <sup>4</sup> a	389±9.5 <sup>c</sup>	979±16.4 <sup>b</sup>
<b>Filiform width</b>	235±7.6 <sup>b</sup>	241±5.9 <sup>b</sup>	337±7.6 <sup>a</sup>	238±6.9 <sup>b</sup>
<b>Fungiform length</b>	368±1. <sup>3</sup> b	355±9.4 <sup>b</sup>	126±4.6 <sup>a</sup>	352±4.2 <sup>b</sup>
<b>Ventral thickness</b>	745±14. <sup>1</sup> a	736±25. <sup>3</sup> a	235±3.8 <sup>c</sup>	521±7.9 <sup>b</sup>
<b>Vessel width</b>	262±10.4 <sup>c</sup>	280±11.5 <sup>c</sup>	2154±30.5 <sup>a</sup>	875±12.4 <sup>b</sup>
<b>Cystic width</b>	125±18.1 <sup>d</sup>	137±12. <sup>4</sup> c	1243±23.6 <sup>a</sup>	565±11.5 <sup>b</sup>
<b>TNF-<math>\alpha</math></b>	72.1±2.5 <sup>c</sup>	70.5±4.8 <sup>c</sup>	114.8±3.9 <sup>a</sup>	76.3±7.4 <sup>b</sup>
<b>Cox 2</b>	25.6±3.4 <sup>c</sup>	30.5±3.8 <sup>c</sup>	91.5±4. <sup>3</sup> a	56.8±3.6 <sup>b</sup>
<b>Bax</b>	41.2±2.1 <sup>c</sup>	39.8±3. <sup>2</sup> c	105.1±1. <sup>4</sup> a	53.6±2. <sup>8</sup> b

**\*\*a,b,c,d; mean significant difference between groups using independent T-test at P value  $\leq 0.05$**

## DISCUSSION

Although the chemotherapeutic drug, MTX is used to treat a variety of cancers, including head and neck cancers, but it may negatively affect normal cells in the oral cavity, causing oral mucositis (OM).<sup>9</sup> It can significantly affect nutrition intake, oral health, and the quality of life of the patients. It is characterized by presence of pain, ulcers, inflammation, bleeding, decreasing saliva, and infections.<sup>23</sup> In the present study, when LC was given as a protective agent before MTX injection, it significantly prevented OM.

After a single dose of intraperitoneal injection of 80 mg/kg MTX, massive histological changes were observed on the lingual mucosa of rats in the current study. There was noticeable atrophy on the lingual mucosa's dorsal and ventral sides, atrophied papillae in different directions. In addition, dilatation of blood vessels and the infiltration of inflammatory cells together with a disorganized lamina propria. This alteration of the histological structure is consistent with previous studies by Ahmed et al.,<sup>24</sup> and Fathi et al.,<sup>25</sup> who proposed

that the submucosal layer's components may sustain damage prior to the overlying epithelium becoming injured as the cause of OM. Rahnama et al.,<sup>26</sup> reported that chemotherapy destroyed the epithelial cells resulting in atrophy in the form of diffuse or localized ulcerative lesions. This was explained by the cytotoxic effect on the epithelial cell layer, which causes the DNA of progenitor cells from the basal cell layer to be damaged.

In our study, the administering LC prior to MTX reduced the severity and extent of MTX-induced OM. It maintained an almost normal thickness of keratinous and epithelial layers of the lingual mucosa with subsidence of inflammatory signs. This is consistent with previous findings of El-Rouby et al.,<sup>27</sup> and İpek et al.<sup>28</sup> Carnitine derivatives are essential for glucose and lipid metabolism in cells and indicate cellular resistance to toxic effects.<sup>29</sup> LC is an effective antioxidant mediator. The protection of cell membrane and DNA against damage was induced by free oxygen radicals can through different pathways, including the activation of antioxidant enzymes like glutathione, and maintaining the mitochondrial transport chain.<sup>30</sup>

In the present study, there was progressive cystic degeneration of Von Ebner salivary gland, and these results were in accordance with previous findings.<sup>31,32</sup> It might be because MTX damaged and inhibited the proliferation of both normal and aberrant salivary gland cells. In our study, the epithelial lining and the ducts showed a noticeable improvement in the LC+MTX group. This improvement may be due to the antioxidant and anti-inflammatory properties of LC. The tongue tissues of the LC and healthy groups did not show any pathologic findings. These results were in consistent with Amin et al study.<sup>30</sup>

Congested blood vessels might be related to the release of multiple pro-inflammatory cytokines such as TNF, IL-1B, and prostaglandins which increase vascular permeability and enhance the uptake of MTX to the oral mucosa.<sup>31</sup>

The expression of TNF- $\alpha$  protein in this study was significantly increased in the rats given MTX, compared with healthy, LC, and LC+MTX groups. Bayramoglu et al.,<sup>33</sup> and İpek et al.,<sup>28</sup> were in accordance with the current results. It has been suggested that the signaling molecules; mitochondrial ROS plays a key role in the regulation of inflammation through the production of pro-inflammatory cytokines.<sup>34</sup> Furthermore, Wu et al.,<sup>35</sup> assessed the relationship between oxidant and pro-inflammatory cytokine found that cells with increased levels of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 had lower levels of GSH and enzymatic antioxidants. Calò et al.,<sup>36</sup> reported that by controlling the inflammatory response, LC treatment significantly decreased the expression of TNF- $\alpha$ . It has been demonstrated that free radical-induced oxidative stress greatly reduces DNA damage and raises the level of TNF- $\alpha$ .

Our immunohistochemical finding reported that there was an increase in COX2 expression in MTX group in lingual mucosal tissue in comparison with control, LC, and LC+MTX groups. Fathi et al.,<sup>25</sup> supported our findings through increasing the expression of COX2 in MTX-treated group indicating the presence of severe inflammation.

Logan et al.,<sup>37</sup> illustrated the role of COX-2 in the pathogenesis of OM after cytotoxic chemotherapy. Prostaglandins (PGs) and matrix metalloproteinase enzymes (MMP-9) are released by COX-2, worsening the inflammatory state, and causing more tissue damage. Results of the current study confirmed the anti-inflammatory effect LC that showed a downregulation of COX-2 expression in LC+MTX group. These results were in consistent with other experimental studies that aimed to protect the mucosa from damage caused by various irritants.<sup>28</sup>

Our results showed a significant increase in Bax in the MTX-treated group in comparison with MTX+LC group. Because MTX is involved in nucleotide metabolism, it can cause cytotoxicity by increasing the production of ROS, which damages DNA and causes cell permeability. This causes the mitochondria to release cytochrome-c (cyto-c) into the cytosol, which in turn causes MMP-9 to be lost and starts the apoptotic cascade that includes bcl-2, Bax, and cyto-c release.<sup>38</sup> On the other hand, LC reduces the buildup of long chain fatty acids surrounding the mitochondria, which may prevent the permeability and depolarization of the mitochondrial membrane and ultimately prevent cell apoptosis.<sup>39</sup> Additionally, LC can reduce cell apoptosis by improving antioxidant defense systems, reducing oxidative stress, and blocking caspase activity.<sup>40</sup>

## CONCLUSION

In conclusion, MTX had deleterious effects on lingual mucosa of albino rats. LC provided a protective action against MTX-induced lingual mucositis through its anti-inflammatory, antiapoptotic, antioxidant and free radical scavenging ability.

## RECOMMENDATIONS

Additional detailed biochemical investigations are required to know the precise mechanisms mediating the effect of LC against MTX. Follow-ups are needed to exclude the long-term side effects.

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