Iraqi Journal of Pharmacy 23(2) (2026) 53-58

Online ISSN: 2664-2522

Print ISSN: 1680-2594

Iraqi Journal of Pharmacy

Journal homepage : https://iphr.mosuljournals.com

Research Article:

Ameliorative Effect of Ezetimibe on Inflammatory and Apoptotic Biomarkers in Methotrexate-Induced Salivary Gland Injury

Nashwah Subhi Azeez [1], Rana Khairi Attarbashee [2]

[1] Department of Prosthetic Dentistry, College of Dentistry, University of Mosul, Mosul, Iraq.

[2] Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq.

Article Information

Article history:

Received on: 17 January 2026
Revised on: 07 February 2026
Accepted on: 25 February 2026
Published on: 01 June 2026

Keywords:

Ezetimibe,
Immunohistochemistry, Salivary gland

Abstract

Background: Methotrexate (MTX) is a chemotherapeutic agent used to treat various malignancies and autoinflammatory conditions. MTX have an adverse effect on oral tissues, particularly by compromising salivary gland (SG) function. Ezetimibe is a lipid-lowering medication that has more potent anti-inflammatory, and anti-apoptotic effects. **Aims:** This research aimed to assess the protective effects of ezetimibe on SG in rats subjected to MTX - induced alterations. **Methods:** A total of 18 male albino rats were haphazardly assigned into 3 groups. Group I (vehicle control) received normal saline solution for 10 days, while the group II was induced by MTX injection (20 mg/kg, single intraperitoneal (i.p.)) on the sixth day of the experiment. Group III (Ezetimibe+ MTX) received oral Ezetimibe 10 mg/kg once daily for 10 days. SG tissues from rats were collected on day 11 and euthanized for biochemical and immunohistochemical (IHC) testing. **Results:** Ezetimibe significantly ameliorated the biochemical and IHC dysregulation caused by MTX, as evidenced by reduced levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and an elevation in B-cell lymphoma 2 (Bcl-2) level. **Conclusions:** Ezetimibe is an anti-inflammatory and antiapoptotic agent that may be a promising approach to reducing MTX toxicity in submandibular glandular tissue.

1. Introduction

Saliva has a vital role in maintaining homeostasis of the mouth cavity (1). The second-largest salivary gland (SG) is the submandibular gland, which produces an average of 65% of saliva. Glandular hypofunction may manifest in patients receiving chemotherapy and radiotherapy, resulting in a high degree of dental caries, mucositis, gingivitis, pharyngeal infections, speech problems, swallowing problems, and gustatory difficulties (2).

Methotrexate (MTX) can cause adverse effects on certain tissues despite its known antitumor, anti-inflammatory, and immunosuppressive effects (3). Animal models have shown that experimental procedures have adverse effects on secretory glands, especially the parotid and submandibular glands (4-7). Notwithstanding its clinical usefulness, considerable evidence indicates that MTX elicits cytotoxic consequences, encompassing oxidative stress, cellular apoptosis, and damage to several organs, particularly the salivary glands (8). Research conducted by (9) indicates that the detrimental effects of MTX arise from its capacity to generate free radicals, impair mitochondrial function, and activate pathways that induce apoptosis by elevating the expression of the caspase-3 gene while decreasing Bcl-2 levels. The combined effect of MTX with substances exhibiting anti-inflammatory and anti-apoptotic characteristics may mitigate its harmful effects (10, 11).

Statins, in addition to their primary function of inhibiting cholesterol biosynthesis and reducing lipid levels, may potentially warrant supplementary indications, including the treatment of conditions such as inflammation and cancer (12). Ezetimibe is a cholesterol absorption inhibitor in the intestine (13). It has multiple pharmacological effects, including antioxidation, anti-inflammation, and anti-apoptosis . Additionally, ezetimibe can manage a range of inflammatory conditions, including dyslipidemia and related atherosclerotic complications (14, 15), rheumatoid arthritis (16), cancer, and ulcerative colitis (17). Moreover, Furthermore, specific articles have highlighted the benefits of employing this medicine to maintain SG function.

*Corresponding author: *Nashwah Subhi Azeez, Department of Prosthetic Dentistry, College of Dentistry, University of Mosul, Mosul, Iraq. Email: nashwa2013@uomosul.edu.iq*

How to cite:

Azeez, N., S., Attarbashee, N., S., (2026). Ameliorative Effect of Ezetimibe on Inflammatory and Apoptotic Biomarkers in Methotrexate-Induced Salivary Gland Injury. Iraqi J. Pharm. 23(2), 53-58.

DOI: https://doi.org/10.33899/iraqij.p.2026.168717.1178

Previous research has demonstrated that ezetimibe can effectively mitigate parotid gland damage in male rats with Experimental Adjuvant Arthritis (18). This study investigated the protective effects of ezetimibe on inflammatory and antiapoptotic variables in the submandibular SG of rats received 20 mg/kg of MTX.

2. Materials and Methods

2.1. Drugs and chemicals

The methotrexate was provided by Ebewe (Austria). Ezetimibe was acquired from pharma Compass of Germany. An immunohistochemical kit was provided by Abcam (UK).

2.2.Preparation of animals

Six rats were housed in individual polypropylene cages devoid of pollutants. The cages featured a substantial wire-mesh bottom that was kept hygienic to prevent the rats from licking or contacting one another. Rats were maintained in uniform and suitable environments with a temperature range of 22 to 25 °C and a 12-hour light/dark cycle. The facility was equipped with a ventilation system that perpetually circulated the air, and it provided access to fresh water and normal ad libitum sustenance. All rats exhibited adaptation to their environment after exposure to passive preliminaries.

2.3. Experimental design

The experiment was done from May 2025 to December 2025 under document number UoM. The research proposal received approval from the Institute Animal Ethical Committee (IAEC) of the College of Dentistry, in compliance with UoM.Dent.25/1049. The study comprised 18 male albino rats, weighing 190-210 grams. These rats were randomly divided into three groups, each including six rats. Group I (vehicle control) received normal saline solution for 10 days, while the other groups, (induction, group II) was induced by MTX injection (20 mg/kg, single intraperitoneal (i.p.) on the sixth day of the experiment to induce SG damage and received oral normal saline. Group III (Ezetimibe+ MTX) received oral Ezetimibe 10 mg/kg once daily for 10 days, 5 days before induction and 5 days after induction. Before administration, the Ezetimibe was freshly prepared into suspensions in distilled water.

2.4. Preparation and sampling of animals

On day 11, rats were euthanized with a combination of ketamine and xylazine (80 mg/kg and 10 mg/kg, respectively). Subsequently, the submandibular SG tissue was dissected. Tissue biopsies were obtained and processed to create a tissue homogenate for bio-indicator analysis and immunohistochemistry.

2.5. Preparation of Tissue Homogenate

To prepare SG tissue homogenate, the excised SG tissue from several rat groups preserved it at -80 °C. The subsequent stage was homogenizing the acquired SG samples using tissue protein lysate buffer. Tissues were weighed and rinsed thoroughly in phosphate-buffered saline (PBS) (pH 7.0-7.4) at 4 °C before homogenization to remove any remaining blood. The tissues were minced and positioned on filter paper to collect the residual fluid. The tissue components were subsequently homogenized in 6-10 mL of PBS utilizing a cylindrical glass homogenizer maintained on ice. One gram of SG tissue has been maintained in a 9-milliliter phosphate-saturated buffer at pH 7.2. To homogenize the tissues, they were first mashed with a pestle and mortar before being centrifuged at 5,000 rpm on a cold setting for 15 minutes. Supernatants were transferred to an Eppendorf tube and frozen at temperatures between -80 and -20 °C.

2.6. Determination of Inflammatory biomarkers

Following vendor guidelines, TNF-α and IL-1β were measured in rat SG tissue homogenates using an enzyme-linked immunosorbent assay (MyBioSource, USA) (Cloud-Clone Corp). A 96-well plate is used to place biomarker antibodies at the start of the test. The wells are connected to specimens for extraction and baseline TNF-α or IL-1β standards via encapsulating antibodies. After rinsing with the wash buffer, the biotin antibody was applied to the pores. After removing the detached biotinylated antibody, streptavidin and horseradish peroxidase were cautiously added to the plate. By comparing optical density with standard curves, bioindicators in each sample were quantified. Spectrophotometry showed a link between optical density and bioindicators. After another wash, TMB-substrate combinations were applied to the plates, and the color indicated the marker level. The stop agent's reaction from bluish to yellow is measured at 450 nm for color severity.

2.7. Determination of Anti-apoptotic biomarker

In this study, immunohistochemistry was used to determine the presence of gene products in cells isolated in three experimental groups, namely, the control, MTX and ezetimibe+MTX treated animals. The rabbit monoclonal antibodies used for immunohistochemical staining were diluted 1:100 and were specific for Bcl -2 to be applied on paraffin-embedded gland tissue sections. Tissue slices were incubated with primary antibodies for 1 hour. They were followed by treatment with a streptavidin-biotin conjugate and DAB chromogen for visibility. Tissue slices from all animals in each group were collected for a blinded evaluation. The intensity of the immunohistochemical staining was quantified by means of "a semi-quantitative scoring system: 0 (not stained), 1 (0 -25%, weakly stained), 2 (26-50%, moderate), 3 (51-75%, intense), and 4 (76-100 %, very intense).

2.8. Statistical analysis

Data entry was conducted using SPSS 24, the latest version of the statistical software. One-way ANOVA, followed by Tukey's post hoc test, was used to compare mean values across groups. For non-parametric data, the Kruskal–Wallis test, followed by Mann–Whitney U tests for pairwise comparisons. Results are expressed as mean ± standard deviation (SD) for parametric data (normally distributed variables with homogeneous variances) and median for non-parametric data. The significance threshold was set at $p < 0.05$ for all statistical analyses.

3. Results

3.1. Impacts of ezetimibe and MTX on proinflammatory related-markers

Compared with the vehicle control group, the MTX group exhibited markedly elevated mean levels of inflammatory markers, such as TNF-α and IL-1β (p = 0.000). The MTX + ezetimibe cohort exhibited a statistically significant decrease in TNF-α and IL-1β levels relative to the MTX group (p = 0.000), as seen in **Figure 1**.

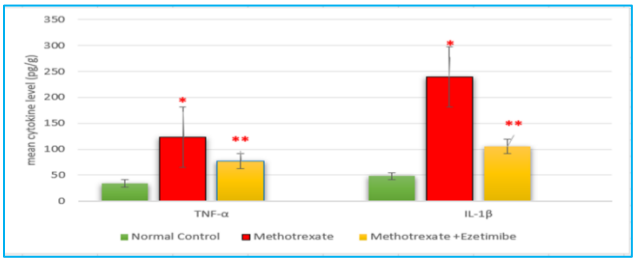

Figure 1. Depicts the impact of the Ezetimibe and MTX on pro-inflammatory markers (IL-1β and TNF-α).

The data is shown as mean ± standard deviation. *: Indicates significant differences (p = 0.000) in comparison with the control group; ** denotes substantial variances (p = 0.000) related to the MTX group. Levels of TNF-α and IL-1β are measured in (pg/g).

3.2. Impacts of Ezetimibe and MTX on anti-apoptotic -related marker

The group that received MTX showed negative to weak positive immunohistochemistry for Bcl-2, with a median of 0 (0–1), compared to the vehicle control group which showed intense to very intense positive expression with a median of 3 (3–4) (p =0.006, figure 1). on the other hand, the group that received MTX in combination with ezetimibe showed weak to moderate positive expression of immunohistochemistry for Bcl-2 with a median of 1 (1–2), (p = 0.006, **Figure 2**)

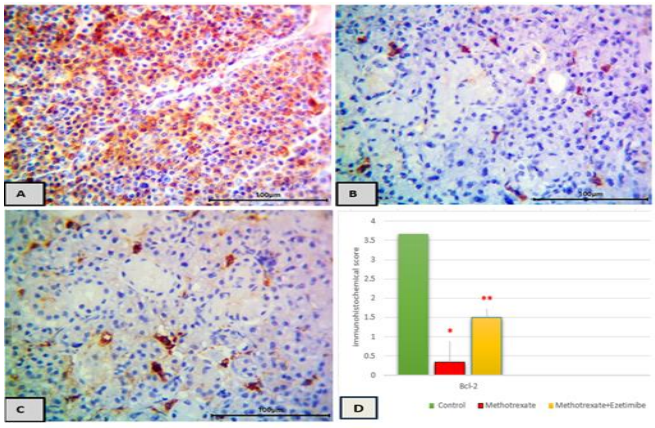

Figure 2. Effects of Ezetimibe and MTX on immunohistochemical scores of Bcl-2 among study groups A. Rats Immunohistochemical salivary tissue specimen of control group (H, 400X). **B.** Immunohistochemical salivary tissue specimen of induction (H, 400X). **C.** Immunohistochemistry salivary specimen of Ezetimibe+MTX group (H, 400X). D. Immunohistochemical scores of Bcl-2 among study groups.

4. Discussion

The present study revealed that MTX therapy significantly elevated the TNF-α, and IL-1β, relative to the control group. The results of this study align with other studies that found that MTX -induced tissue degradation is associated with increased expressions of inflammatory markers including TNF-α, and IL-1β (19-21). The results align with those of other studies who found that MTX-induced SG tissue degradation is associated with elevated expressions of IL-1β, and TNFα (4, 6). The impairment of the SG, subsequent to methotrexate administration, may also be associated with the deleterious effects of free radicals (22-24). The free radicals released from the metabolism of MTX interact with the structure of cell membrane, resulting in membrane rupture and the escape of significant antioxidant enzymes in the serum. This influences the normal concentration of this scavenger enzyme in blood and indicates a detrimental impact of MTX on the integrity of cells (23). Ezetimibe treatment significantly reduced IL-1β, and TNFα, highlighting its anti-inflammatory effects. This result aligns with previous studies indicating that Ezetimibe markedly diminished levels of proinflammatory biomarkers (25-27).

The complex mechanism underlying Ezetimibe's anti-inflammatory activity is activated by stimulating the AMPK/Nrf2 (NF-E2-related factor 2) pathway, which in animal studies decreases oxidative stress (28). To regulate the consequences of oxidant exposure, Nrf2 regulates the expression of antioxidant response genes under both stress and basal condition (29). In addition, Ezetimibe lowers blood cholesterol via inhibiting the cholesterol transporter in the small intestine, which is a different mechanism by which it works. An elevated blood oxidized low-density lipoprotein level is inversely associated with Treg cells and directly associated with Th17 cells. Thus, Ezetimibe's reduction in blood lipids should reduce Th17 cells and inflammation (25). Recent research by Weng and colleagues investigated whether Ezetimibe could improve symptoms of ankylosing spondylitis in a mouse model by acting as an anti-inflammatory agent. Ezetimibe inhibited the production of MMP3, MMP13, and ADAMTS5 induced by IL-1. Additionally, Ezetimibe regulates T-cell proliferation and the synthesis of proinflammatory cytokines by immune cells (30). Subsequent researches showed that Ezetimibe effectively controlled the rat model of ulcerative colitis by reducing inflammatory markers, such as TNF-α, IL-1β, and NF-κB (31-33).

The present study revealed that MTX therapy significantly decreased the anti-apoptotic marker (Bcl2) relative to the control group. MTX was recognized as inducing organ damage by facilitating the expression of pro-apoptotic factors and inhibiting the synthesis of anti-apoptotic factors (Bcl2) (21, 34-39). One possible explanation for this phenomenon is that the elimination of folate cofactors led to a reduction in the rate of protein synthesis, which may have induced the development of cellular lysosomes and provided distinctive indications of apoptosis. The amount of apoptotic bodies is significantly elevated in tissues treated with chemotherapeutic drug (40-42). Our results align with substantial literature demonstrating that MTX diminishes the production of anti-apoptotic bioindicators, notably Bcl2 (42-47). Several findings indicating the apoptotic and detrimental outcomes of MTX may be elucidated by adverse interactions with anti-apoptotic molecules. Notably, ezetimibe treatment significantly increased Bcl2 level, suggesting anti-apoptotic effect.

The findings indicated that ezetimibe therapy protects endothelial cells of human umbilical vein via enhanced the production of anti-apoptotic molecule, Bcl2 in vitro (48). Ezetimibe may mitigate oxidative stress and inflammation provoked via caspase-1 and IL-1β by the AMP-activated protein kinase/ Nrf2 /thioredoxin interacting protein pathway (49,50). These findings support the hypothesis that Ezetimibe may significantly mitigate MTX-induced SG changes

5. Conclusion

The current study determined that Ezetimibe provides significant protection against the detrimental effects of methotrexate on salivary gland due to its enhanced anti-inflammatory, and anti-apoptotic properties.

6. Acknowledgment

We express my gratitude to the College of Dentistry/ University of Mosul for providing the necessary facilities to do this endeavor.

7. References

1. Dinu CA, Stefan CS, Mihalache D, Carnaru MV, Rusu-Negraia M. The role of salivary glands in oral cavity homeostasis: secretion, composition and neuroendocrine control. *Romanian Journal of Oral Rehabilitation* 2025;17(1):897–909.
2. Vistoso Monreal A, Polonsky G, Shiboski C, Sankar V, Villa A. Salivary gland dysfunction secondary to cancer treatment. *Frontiers in Oral Health* 2022;3:907778.
3. Hamed KM, Dighriri IM, Baomar AF, Alharthy BT, Alenazi FE, Alali GH, et al. Overview of methotrexate toxicity: a comprehensive literature review. *Cureus* 2022;14(9):e29518.
4. Attarbashee RK, Hamodat HF, Mammdoh JK, Ridha-Salman H. The Possible effect of Bosentan on the methotrexate-induced salivary gland changes in male rats: histological and Immunohistochemical study. *Toxicology Research* 2025;14(1):tfaf007.
5. Aboulfotoh MM. Effect of cevimeline and different concentration of gum arabic on parotid salivary gland function in methotrexate-induced xerostomia: a comparative study. *BMC Oral Health* 2024;24(1):624.
6. Attarbashee RK, Al-Athari AJH, Shihab EM, Ridha-Salman H, Zigam QA, Abbas WJ, et al. Suppressive effect of rutin on methotrexate-induced salivary gland injury in rats. *Pharmakeftiki* 2025;37(2S):187.
7. Fattah HSA, Omar EM. The protective role of curcumin nanoparticles on the submandibular salivary gland toxicity induced by methotrexate in male rats. *Archives of Oral Biology* 2023;152:105717.
8. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, et al. Oral Microbiome: A Review of Its Impact on Oral and Systemic Health. *Microorganisms* 2024;12(9):1797.
9. Kızıl HE, Caglayan C, Darendelioğlu E, Ayna A, Gür C, Kandemir FM, et al. Morin ameliorates methotrexate-induced hepatotoxicity via targeting Nrf2/HO-1 and Bax/Bcl2/Caspase-3 signaling pathways. *Molecular Biology Reports* 2023;50(4):3479-88.
10. Ahmed AAM, Selim MAA, El-Sayed NM. α-Lipoic acid ameliorates oral mucositis and oxidative stress induced by methotrexate in rats. Histological and immunohistochemical study. *Life Sciences* 2017;171:51-9.
11. Taha RM, Abdel-Latif GA, Said RH. The Prospective Effect of Green Tea versus Pomegranate Peels Extracts on Submandibular Salivary Glands of Albino Rats after Methotrexate Administration (Histological and Immunohistochemical Study). *International Journal of Dentistry* 2024;2024:3290187.
12. Faggiotto A, Paoletti R. Do pleiotropic effects of statins beyond lipid alterations exist in vivo? What are they and how do they differ between statins? *Current Atherosclerosis Reports* 2000;2(1):20-5.
13. Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, Braun MP, et al. The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *Proceedings of the National Academy of sciences* 2005;102(23):8132-7.
14. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. *The New England Journal of Medicine* 2015;372(25):2387-97.
15. Tobaru T, Seki A, Asano R, Sumiyoshi T, Hagiwara N. Lipid-lowering and anti-inflammatory effect of ezetimibe in hyperlipidemic patients with coronary artery disease. *Heart Vessels* 2013;28(1):39-45.
16. Mäki-Petäjä KM, Booth AD, Hall FC, Wallace SML, Brown J, McEniery CM, et al. Ezetimibe and Simvastatin Reduce Inflammation, Disease Activity, and Aortic Stiffness and Improve Endothelial Function in Rheumatoid Arthritis. *Journal of the American College of Cardiology* 2007;50(9):852-8.
17. Mostafa RE, Abdel-Rahman RF. Ezetimibe alleviates acetic acid-induced ulcerative colitis in rats: targeting the Akt/NF-κB/STAT3/CXCL10 signaling axis. *Journal of Pharmacy and Pharmacology* 2023;75(4):533-43.
18. Seferos N, Christine T, Tsamouri M, Kotsiou A. Effects of simvastatin and ezetimibe on rats' parotid in experimental freund's adjuvant arthritis. *International Journal of Health Sciences and Research* 2017;7(06):167-72.
19. Drishya S, Dhanisha SS, Guruvayoorappan C. Antioxidant-rich fraction of Amomum subulatum fruits mitigates experimental methotrexate-induced oxidative stress by regulating TNF-α, IL-1β, and IL-6 proinflammatory cytokines. *Journal of Food Biochemistry* 2022;46(4):e13855.
20. Mammadov R, Suleyman B, Akturan S, Cimen FK, Kurt N, Suleyman Z, et al. Effect of lutein on methotrexate-induced oxidative lung damage in rats: a biochemical and histopathological assessment. *Korean Journal of Internal Medicine* 2019;34(6):1279-86.
21. Dogra A, Gupta D, Bag S, Ahmed I, Bhatt S, Nehra E, et al. Glabridin ameliorates methotrexate-induced liver injury via attenuation of oxidative stress, inflammation, and apoptosis. *Life Sciences* 2021;278:119583.
22. Yousef DM, Abd El-Fatah SS, Al-Semeh M, Amira E. Oxidative stress changes induced by methotrexate on parotid gland structure of adult male albino rat: can vitamin C ameliorate these changes? *The Medical Journal of Cairo University* 2019;87:2555-65.
23. Fathi R, Abd Allah D, Helmy I. Effect of Green tea and black seed on methotrexate induced cytotoxicity of the oral mucosa, tongue and the submandibular salivary gland of Albino rats. *Egyptian Dental Journal* 2023;69(1):413-26.

24. Ungureanu LB, Grădinaru I, Ghiciuc CM, Amălinei C, Geleţu GL, Petrovici CG, et al. Atrophy and inflammatory changes in salivary glands induced by oxidative stress after exposure to drugs and other chemical substances: a systematic review. *Medicina* 2023;59(9):1692.
25. Qin L, Yang Y-B, Yang Y-X, Zhu N, Li S-X, Liao D-F, et al. Anti-inflammatory activity of ezetimibe by regulating NF-κB/MAPK pathway in THP-1 macrophages. *Pharmacology* 2014;93(1-2):69-75.
26. Cho Y, Kim R-H, Park H, Wang HJ, Lee H, Kang ES. Effect of ezetimibe on glucose metabolism and inflammatory markers in adipose tissue. *Biomedicines* 2020;8(11):512.
27. Tamaki N, Ueno H, Morinaga Y, Shiiya T, Nakazato M. Ezetimibe ameliorates atherosclerotic and inflammatory markers, atherogenic lipid profiles, insulin sensitivity, and liver dysfunction in Japanese patients with hypercholesterolemia. *Journal of Atherosclerosis and Thrombosis* 2012;19(6):532-8.
28. Nam KT, Park JS, Kim SH, Lee M, Kim G, Min BS, et al. Ezetimibe, an NPC1L1 inhibitor, is a potent Nrf2 activator that protects mice from diet-induced nonalcoholic steatohepatitis. *Free Radical Biology and Medicine* 2016;99:520-32.
29. Taguchi K, Motohashi H, Yamamoto M. Molecular mechanisms of the Keap1–Nrf2 pathway in stress response and cancer evolution. *Genes Cells* 2011;16(2):123-40.
30. Moon J, Lee S-Y, Na HS, Lee AR, Cho K-H, Choi JW, et al. Ezetimibe ameliorates clinical symptoms in a mouse model of ankylosing spondylitis associated with suppression of Th17 differentiation. *Frontiers in Immunology* 2022;13:13:922531.
31. Jaafar F, Abu-Raghif A. Ezetimibe Ameliorates Clinical Parameters, Oxidative Stress, and Adhesion Molecules in Experimentally Induced Colitis in Male Rat Models. *Opera Medica et Physiologica* 2023;10(4):103-10.
32. Jaafar FR, Abu-Raghif A. Comparative treatment of Sulfasalazine+ Ezetimibe combination and Sulfasalazine in a rat model with induced colitis. *Journal of Medicine and Life* 2023;16(8):1165.
33. Jaafar FR, Abu-Raghif AR. The Effects of Sulfasalazine and Ezetimibe on Proinflammatory Cytokines in Male Rat with Induced Colitis: A Comparative Study. *Medical Journal of Babylon* 2024;21(3):681-5.
34. Ribeiro DA, Salvadori DM, Marques ME. Abnormal expression of bcl- 2 and bax in rat tongue mucosa during the development of squamous cell carcinoma induced by 4- nitroquinoline 1- oxide. *International journal of experimental pathology* 2005;86(6):375-82.
35. Mansouri H, Mnango LF, Magorosa EP, Sauli E, Mpolya EA. Ki-67, p53 and BCL-2 Expressions and their Association with Clinical Histopathology of Breast Cancer among Women in Tanzania. *Scientific Reports* 2019;9(1):9918.
36. de Mello Gomes Á N, Nagai MA, Lourenço SV, Coutinho-Camillo CM. Apoptosis and proliferation during human salivary gland development. *Journal of Anatomy* 2019;234(6):830-8.
37. Larsen SR, Bjørndal K, Godballe C, Krogdahl A. Prognostic significance of Ki-67 in salivary gland carcinomas. *Journal of oral pathology and medicine* 2012;41(8):598-602.
38. Hu S, Wang Y, Xu Z, Zhou Y, Cao J, Zhang H, et al. Identification of the Bcl-2 and Bax homologs from Rhipicephalus haemaphysaloides and their function in the degeneration of tick salivary glands. *Parasit Vectors* 2021;14(1):386.
39. Ali N, Rashid S, Nafees S, Hasan SK, Sultana S. Beneficial effects of Chrysin against Methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. *Molecular and Cellular Biochemistry* 2014;385:215-23.
40. Al–Moula AD, Al-Mashhadane FA, Mammdoh JaK. Effects of 6–Mercaptopurine on Salivary Glands in Rabbit. *Al-Rafidain Dental Journa*l 2012;12(2):266-73.
41. Kaplan HM, Pazarci P. Antiproliferative and Apoptotic Effects of Tempol, Methotrexate, and Their Combinations on the MCF7 Breast Cancer Cell Line. *ACS Omega* 2024;9(6):6658-62.
42. Spurlock CF, 3rd, Aune ZT, Tossberg JT, Collins PL, Aune JP, Huston JW, 3rd, et al. Increased sensitivity to apoptosis induced by methotrexate is mediated by JNK. *Arthritis Rheum* 2011;63(9):2606-16.
43. Freeman ML, Clagett BM, Moisi D, Yeh E, Morris CD, Ryu A, et al. Methotrexate Inhibits T Cell Proliferation but Not Inflammatory Cytokine Expression to Modulate Immunity in People Living With HIV. *Frontiers in Immunology* 2022;13:924718.
44. Melikova NI, Tashkenbaeva UA. Impact of combination therapy on Ki-67 and Bcl-2 expression in psoriasis. *Russian Journal of Skin and Venereal Diseases* 2023;26(4):375-82.
45. Kızıl HE, Caglayan C, Darendelioğlu E, Ayna A, Gür C, Kandemir FM, et al. Morin ameliorates methotrexate-induced hepatotoxicity via targeting Nrf2/HO-1 and Bax/Bcl2/Caspase-3 signaling pathways. *Molecular Biology Reports* 2023;50(4):3479-88.
46. Amarnani D, Machuca-Parra AI, Wong LL, Marko CK, Stefater JA, Stryjewski TP, et al. Effect of Methotrexate on an In Vitro Patient-Derived Model of Proliferative Vitreoretinopathy. *Investigative Ophthalmology and Visual Science* 2017;58(10):3940-9.
47. Wu S, Shao M, Zhang Y, Shi D. Activation of RSK2 upregulates SOX8 to promote methotrexate resistance in gestational trophoblastic neoplasia. *Laboratory Investigation* 2021;101(11):1494-504.
48. Qin J, Wang L-l, Liu Z-y, Zou Y-l, Fei Y-j, Liu Z-x. Ezetimibe protects endothelial cells against oxidative stress through Akt/GSK-3β pathway. *Current Medical Science* 2018;38(3):398-404.
49. Fathy N, Farouk S, Sayed RH, Fahim AT. Ezetimibe ameliorates cisplatin- induced nephrotoxicity: A novel therapeutic approach via modulating AMPK/Nrf2/TXNIP signaling. *The Federation of American Societies for Experimental Biology Journal* 2024;38(1):e23382.
50. Xu Y, Bai L, Yang X, Huang J, Wang J, Wu X, et al. Recent advances in anti-inflammation via AMPK activation. *Heliyon* 2024;10(13).

التأثير المُحسِّن للإيزيتيميب على المؤشرات الحيوية الالتهابية والاستماتية في إصابة الغدد اللعابية المُستحثة تجريبياً

الخلاصة

الخلفية والاهداف :الميثوتريكسيت هو دواء يُوصف لعلاج العديد من الأورام الخبيثة وحالات الالتهاب الذاتي. قد يؤثر الميثوتريكسيت سلبًا على أنسجة الفم، وخاصةً من خلال التأثير على وظيفة الغدد اللعابية. الإيزيتيميب هو دواء خافض للدهون يتميز بتأثيرات مضادة للالتهاب ومضادة للاستماتة أقوى ولقد هدفت هذه الدراسة إلى تقييم التأثيرات الوقائية للإيزيتيميب على الغدد اللعابية في الفئران التي خضعت لتغيرات في الغدد اللعابية ناتجة عن الميثوتريكسيت .**الطرق** :استخدمت هذه الدراسة ثمانية عشرجرذا بوزن 210-190 جرام. تم تصنيف الحيوانات إلى ثلاث مجموعات: الميثوتريكسيت (20 ملغم / كغم)، الميثوتريكسات بالإضافة إلى اللايزيتيميب (10 ملغم / كغم)، واالضابطة (الماء المقطر). تم إجراء كل من التحليلات البيوكيميائية والكيميائية المناعية على الغدد اللعابية. تم تشريح الغدد اللعابية للحيوانات مباشرة بعد أن تم التضحية بها في اليوم الحادي عشر .**النتائج** :على النقيض من المجموعة الضابطة، كان لدى مجموعة الميثوتريكسات مستويات أعلى بكثير من عامل نخر الورم الفا، انترلوكين-1 بيتا ((p = 0.000 ولكن مستويات اقل من بروتين لمفوما الخلايا البائية . ومع ذلك، أظهرت مجموعة الميثوتريكسات + ازيتيميب مستويات أقل بكثير من عامل نخر الورم الفا ، و انترلوكين-1 بيتا ومستويات اكثر من بروتين لمفوما الخلايا البائية على النقيض من مجموعة الميثوتريكسيت، .(p = 0.006)**الاستنتاج** :تثبت هذه النتائج أن الايزيتيميب يتمتع بحماية كبيرة ضد سمية الغدد اللعابية الناجمة عن الميثوتريكسيت في الجرذان.

الكلمات المفتاحية: ازيتيميب، الميثوتريكسيت، الكيمياء المناعية والنسيجية و الغدد اللعابية