Iraqi Journal of Pharmacy 22(1) (2025) 8-13

Online ISSN: 2664-2522

Print ISSN: 1680-2594

Iraqi Journal of Pharmacy

Journal homepage : https://iphr.uomosul.edu.iq/

Research Article:

Impact of Bosentan in Ameliorating Methotrexate-Induced Tongue Toxicity in Rats

Rana Khairi Attarbashee [1]

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

Article Information

Article history:

Received on: 13 November 2024
Revised on: 08 January 2025
Accepted on: 10 January 2025
Published on: 01 March 2025

Keywords:

Bosentan,
Methotrexate,
Oxidative stress,
Tongue toxicity

Abstract

Background and objective: Although methotrexate has been utilized over many years, adverse effects are frequent throughout therapy, especially among cancer patients. The study examined the efficacy of bosentan as a protective therapy against tongue toxicity induced by methotrexate. **Methods:** This study employed twenty-four 300–400 g Wister-albino rats. The animals were categorized into three groups: methotrexate (80 mg/kg), methotrexate (80 mg/kg) plus bosentan (60 mg/kg), and control healthy (distilled water). The tongue mucosa was subjected to biochemical and immunohistochemical analysis. The animals' tongue mucosa was dissected immediately following they were euthanized on day 16. **Results:** In contrast to the control group, the Methotrexate group had significantly higher levels of TNF-α, IL-1β and MDA ($p < 0.05$). However, the Methotrexate+Bosentan group displayed considerably lower levels of TNF-α, IL-1β and MDA in contrast to the Methotrexate group, ($p < 0.05$). **Conclusion:** These findings demonstrate that bosentan protect considerably against methotrexate-induced tongue toxicity in rats.

2025 Iraqi Journal of Pharmacy. Published by University of Mosul, Iraq,. This is an open access article licensed under CC BY: (https://creativecommons.org/licenses/by/4.0)

1. Introduction

Methotrexate acts as a folic acid blocker that works as an antiproliferative drug (1). Methotrexate is utilized for the treatment of choriocarcinoma, and juvenile acute lymphoblastic leukemia. Methotrexate is a highly successful treatment for rheumatoid arthritis (RA), psoriasis, and other rheumatic problems, in addition to multiple sclerosis (MS) and other autoimmune diseases (2). Low dosages of methotrexate could be used for treatment of inflammatory diseases, but large doses for treating cancer should be used (1,3). Moderate to severe inflammatory illnesses may result in modest to severe side effects, which can lead to therapy termination (4). These adverse effects can occur throughout the treatment of inflammatory disorders. Among the most serious effects of methotrexate is mucositis. One of the most important negative impacts of cancer treatment is oral toxicities, like stomatitis and mucositis (6). A condition known as mucositis involves inflammation and ulceration in the mucosal tissue extending through the mouth towards the anus (7). Oral mucositis can limit the amount of medicine taken, heighten the risk of infection, make feeding challenging, impede therapy, and lengthen hospital stays (8). Pro-inflammatory cytokines and reactive oxygen species (ROS) have been linked to the development of mucositis (9). Interleukin 1 beta (IL-1β), an oxidant in the oral mucosa, tumor necrosis factor (TNF-α), a marker of inflammation, and malondialdehyde (MDA) have all been associated with increased levels of methotrexate (10).

While a number of drugs are available to prevent or lessen the intensity of mucositis, there isn't a 100% effective treatment. Based on this research, effective anti-inflammatory and antioxidant drug could assist to avoid methotrexate-associated mucositis.

*Corresponding author: *Rana Khairi Attarbashee, Department of Dental Basic Sciences, College of Dentistry, University of Mosul, Mosul, Iraq.*
Email: drranakhaire@uomosul.edu.iq

How to cite:
Attarbashee, R., Kh., (2025). Impact of Bosentan in Ameliorating Methotrexate-Induced Tongue Toxicity in Rats. Iraqi J. Pharm. 22(1), 8-13.
DOI: https://doi.org/10.33899/iraqij.p.2025.155197.1121

Bosentan was the first orally available mixed endothelin receptor antagonist given approval by the FDA for use in pulmonary arterial hypertension (11, 12). Preclinical studies suggest that bosentan may treat a number of inflammatory disorders, like: arthritic conditions (13), cancer (14), uveitis (15), and depression (16). Bosentan is a useful tool for examining the impact of endotheline-1 on disease and its possibilities for treatment. Bosentan reduces the deleterious effects of endothelin while also having vasodilating, anti-inflammatory, antioxidant, and anti-apoptotic characteristics. These functions may explain bosentan's effectiveness in treating pulmonary diseases by slowing the course of the condition and increasing survival rates (17). The impact of bosentan on rats' oxidative and inflammatory tongue injury resulting from methotrexate was evaluated in this study.

2. Methods

2.1. Ethical approve

The study procedure was authorized by "the institutional animal ethics committee at Al-Mosul University's College of Dentistry" (UoM.Dent/A.L.14/22).

2.2. Drugs and Chemicals

The methotrexate was supplied by Ebewe (Austeria). Bosentan was purchased from a Cipla (India). I mmunohistochemical kit was supplied by Abcam (UK).

2.3. Preparation of drugs

During the day of the trial, bosentan and methotrexate were prepared fresh before being administered. Distilled water was employed to suspend estimated medications. Previous studies were utilized to determine the bosentan dose of 60 mg/kg (18). To induce oral mucositis, methotrexate (80 mg/kg) was administered (19).

2.4. Animal Model and Treatment

Twenty-four mature male albino rats weighing 300-400 g were kept in polyvinyl cages (4 rats /cage) at a"standard temperature (22°C ± 3°C) and a 12-hour light/dark cycle". Animals were randomly split into 3 groups :

i. Control group was given a standard saline solution for 15 days.
ii. Mucositis group received one intraperitoneal injection (80 mg/kg) of methotrexate on day 8 (19),
iii. The methotrexate+bosentan group was supplemented using 60 mg/kg bosentan before the illness induction (7 days) and 7 days after the onset of illness induction.

2.5. Drug-Induced Tongue toxicity Model

The rats were placed in the animal house for seven days with no therapy to assist them adjust to their new surroundings. From the first to the 8th day, the Methotrexate+ bosentan subgroup received 60 mg/kg orally. On the 8th day, all groups except the control were given one intraperitoneal (IP) methotrexate injection at 80 mg/kg. Following the 8th day of therapy, the Methotrexate+ bosentan rats received 60 mg/kg bosentan until the fifteenth day. On the 16th day, the animals received anesthesia with Ketamine plus Xylazine (80 mg/kg) via the same syringe. The rat's tongues have been selected for biochemical analysis and immunohistochemistry **Figure 1**.

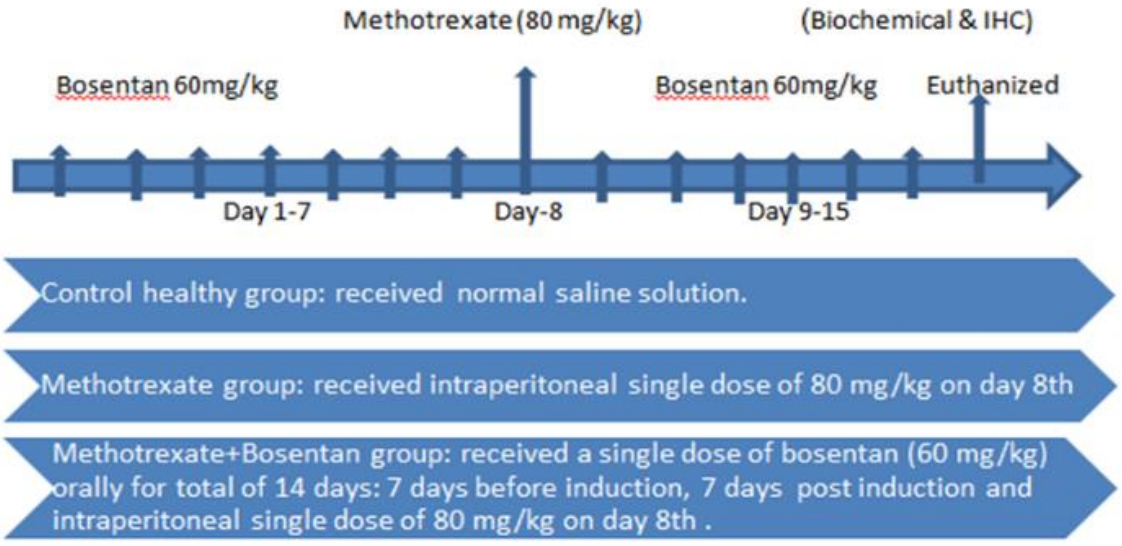

Figure 1. Experimental design of study groups

2.6. Biochemical examination:
2.6.1. Measurement of Inflammatory Biomarkers

TNF-α and IL-1β (MyBioSource, USA) were recognized in rat tongue tissue homogenates employing a widely used sandwich technique "(enzyme- linked immunosorbent assay) according to vendor guidelines (Cloud-Clone Corp). The antibodies biomarker is initially put on a 96-well plate as part of the test. The extract specimens and baseline standards' TNF-α or IL-1β are bound to the wells using encapsulating antibodies. After that, the pore gaps were rinsed with wash buffer and coated with an antibody to biotin. The plate was carefully supplied with streptavidin and horseradish peroxidase after the elimination of the detached biotin-connected antibody" (20–22).

By comparing optical density with standard curves, the number of different bio-indicators in every sample was determined. "Spectrophotometry results for optical density and bioindicator quantity were correlated. After another wash, TMB-substrate combinations were added to the plates, and the color produced was used to indicate the matched marker level. When there is a change from a bluish color to yellow using the stop agent", the color severity is measured at 450 nm (23-24).

2.6.2. Measurement of Oxidative Biomarker

The basic concept of the immunohistochemistry (IHC) study implemented in this work is the use of specific antibodies to find the gene protein within the cells of the untreated group, methotrexate, and methotrexate-bosentan groups. "The primary antibody (rabbit polyclonal to MDA (Anti –MDA antibody ab6463) was then applied to slices of paraffin-fixed tongue tissues. The secondary antibody plus a 3,30-diaminobenzidine (chromogen) was then applied". (25, 26). The tissue slices were stained with hematoxylin. To allow for blind examination, segments from every animal in every group were utilized. "Semi-quantitative scoring of positive staining is employed for quantifying the evaluation of immunohistochemistry: Score 0 = no stain; score 1 = 25%; score 2 = 26-50%; score 3 = 51-75%; score 4 = 67-100%." (27, 28).

2.7. Statistical analysis

A statistical program (SPSS 24) made exclusively for social scientists, was utilized to enter the data. The standard deviation (SD) and mean constitute aspects of the descriptive statistical analysis. The data was examined using appropriate statistical techniques and depicted graphically. "The ANOVA test was used to compare groups, followed by the Tukey test as a post-hoc test. The statistical significance level was chosen at $p < 0.05$" (29).

3. Results

3.1. Impact of tested drugs on inflammatory indicators

The Methotrexate group had considerably more levels of both of the inflammatory markers; TNF-α and IL-1β; than the ones in the control group ($p < 0.05$). Even so, **Figure 2**. illustrates levels of TNF-α and IL-1β in the Methotrexate+Bosentan group were significantly lower than those seen in the Methotrexate group ($p < 0.05$).

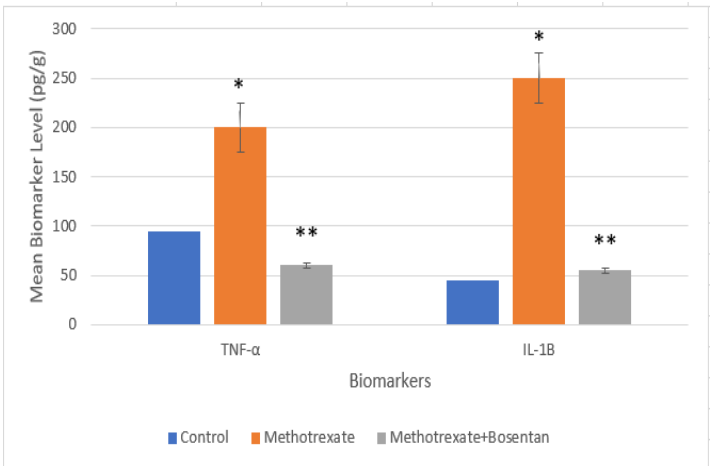

Figure 2. illustrates how the tested drugs affect pro-inflammatory indicators (IL-1β and TNF-α). *

Indicates significant changes ($p<0.05$) from that of control group; ** implies substantial differences ($p<0.05$) from that of Methotrexate group. The Data is represented as mean ± standard deviation. The expression of TNF-α and IL-1β levels are expressed as pg/g .

3.2. Effects of tested drugs on oxidative biomarker

In comparison to the control group, the methotrexate group's IHC levels for the oxidative biomarker MDA were considerably greater ($p < 0.05$). However, as seen in **Table 1**; and **Figure 3**, the Methotrexate+Bosentan group revealed a significant reduction in IHC levels of the oxidative marker as compared to the Methotrexate group ($p < 0.05$).

Table 1. Effects of tested drugs on oxidative marker (MDA).

Marker	Study groups (mean ± standard deviation)		
	Control (n=8)	Methotrexate (n=8)	Methotrexate+Bosentan (n=8)
MDA	*0.88± 0.354*	*3.88± 0.354**	*1.75±0.463***

The mean ± standard deviation is utilized to display the data; * implies significant variations ($p<0.05$) from the control group, and ** designates significant variations ($p<0.05$) from the Methotrexate group.

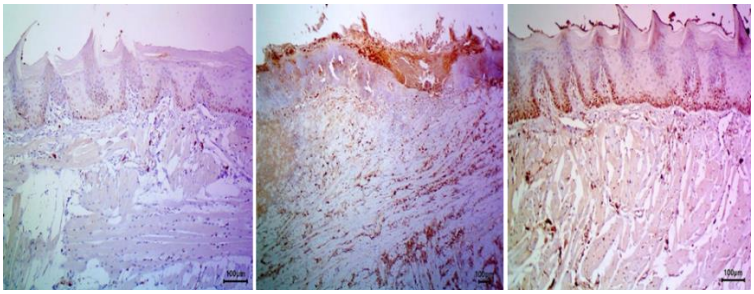

Figure 3. depicts the impact of the tested drugs on tongue immunohistochemistry analysis (MDA).

A. Immunohistochemistry of MDA in the control group of rat tongue exhibited mild positive expression. Hematoxylin at a magnification of 100x. B. MDA immunohistochemistry in the rat tongue Methotrexate group exhibits high positive expression. Hematoxylin at a magnification of 100x. C. MDA immunohistochemistry in the Methotrexate+Bosentan group of rat tongue exhibited moderately positive expression. Hematoxylin at a magnification of 100x .

4. Discussion

Methotrexate is extensively used as a cytotoxic chemotherapy drug, although its effectiveness is limited owing to the adverse effects (19). Methotrexate has a tendency to cause serious adverse effects, such as mucositis (5). Mucositis results in mucosal hemorrhage, ulceration as well as inflammation of the mucosal tissue (30). The current study showed fluid retention, hyperemia, ulcerations, and bleeding in the tongue tissues of rats administered methotrexate, which is comparable with prior findings (31).

Pro-inflammatory cytokines and reactive oxygen species appeared to play a role in the progression of mucositis. To study methotrexate-induced mucositis, this study examined TNF-α, IL-1β and MDA levels within tongue tissues. The study demonstrated that rats receiving methotrexate had significantly elevated levels of TNF-α, IL-1β and MDA among their tongue tissue when compared with the control and bosentan groups. Methotrexate treatment raises reactive oxygen species, which encourages a marker for lipid peroxidation, MDA (32, 33). The investigation suggested that methotrexate promoted tissue destruction through an inflammatory process, as seen in elevated levels of inflammation-promoting cytokines like: TNF-α, IL-1β, and Il-6 (34). A recent study discovered that methotrexate-induced tissue damage is related to increased levels of inflammatory and oxidative mediators like IL-1β, TNFα, MDA, and myeloperoxidase (MPO) (35). Methotrexate-induced organ damage has been associated with higher levels of TNF-α, and MDA (36). Nevertheless, bosentan's anti-inflammatory and antioxidant properties are now increasingly being identified (37, 38, 30). Research shows that bosentan diminishes TNF-α and MDA levels in damaged tissues while improving GSH activity (39). Bosentan reduces superoxide anion-provoked IL-1β and TNF-α production, and decreases neurotrophic MPO production (40). Additionally, other studies noticed that bosentan significantly improved antioxidant indicator activity, especially GSH, while inhibiting MDA formation in weaker tissues (41, 42). More researches indicate that bosentan enhances chemotactic cytokines level, particularly TNF-α, and IL-1β in damaged tissue (43).

5. Conclusion

Biochemical and immunohistochemical studies were used to assess the development of oxidative and inflammatory damage in methotrexate-treated rat tongue tissues.

Bosentan inhibited methotrexate-induced MDA, TNF-α, and IL-1β levels in tongue tissues. These data show that bosentan could be effective in protecting against methotrexate-induced tongue toxicity.

6. Acknowledgements

I am really appreciative of the facilities provided by the College of Dentistry/ University of Mosul, which enhanced this work's quality.

7. References

1. Maksimovic V, Pavlovic-Popovic Z, Vukmirovic S, Cvejic J, Mooranian A, Al-Salami H, et al. Molecular mechanism of action and pharmacokinetic properties of methotrexate. *Molecular Biology Reports* 2020;47(6):4699-708.
2. Řiháček M, Pilatova K, Štěrba J, Pilný R, Valík D. Nové poznatky ve farmakologii methotrexátu: diagnostické možnosti a klinický význam [New findings in methotrexate pharmacology: diagnostic possibilities and impact on clinical care]. *Klinika dětské onkologie* 2015;28(3):163-70.
3. Howard SC, McCormick J, Pui CH, Buddington RK, Harvey RD. Preventing and managing toxicities of high-dose methotrexate. *Oncologist* 2016;21(12):1471-82.
4. Wang W, Zhou H, Liu L. Side effects of methotrexate therapy for rheumatoid arthritis: a systematic review. *European Journal of Medicinal Chemistry* 2018; 158:502-16.
5. Maiguma T, Hayashi Y, Ueshima S, Kaji H, Egawa T, Chayama K, et al. Relationship between oral mucositis and high-dose methotrexate therapy in pediatric acute lymphoblastic leukemia. *International Journal of Clinical Pharmacology and Therapeutics* 2008;46(11):584-90.
6. Epstein JB, Thariat J, Bensadoun RJ, Barasch A, Murphy BA, Kolnick L, et al. Oral complications of cancer and cancer therapy: from cancer treatment to survivorship. *Cancer Journal for Clinicians* 2012;62(6):400-22.
7. Sema Y, Kenan D, İbrahim B, Can A, Gulsum U, Cigdem E, et al. The effectiveness of grape extract in rats with methotrexate induced intestinal mucositis. *Journal of Medical Science* 2012;32(6):1660-7.
8. Curra M, Gabriel AF, Ferreira MB, Martins MA, Brunetto AT, Gregianin LJ, et al. Incidence and risk factors for oral mucositis in pediatric patients receiving chemotherapy. *Support Care Cancer* 2021;29(11):6243-51.
9. Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, HauerJensen M, et al. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer* 2004;100(9 Suppl):1995-2025.
10. Kuduban O, Mazlumoglu MR, Kuduban SD, Erhan E, Cetin N, Kukula O, et al. The effect of hippophae rhamnoides extract on oral mucositis induced in rats with methotrexate. *Journal of Applied Oral Science* 2016;24(5):423-30.
11. Blanco-Colio LM, Tuñón J, Martín-Ventura JL, Egido J, Antiinflammatory and immunomodulatory effects of statins. *Kidney International* 2003;63(1):12-23.
12. Duarte T, Da Cruz IB, Barbisan F, Capelleto D, Moresco RN, Duarte MM. The effects of rosuvastatin on lipid-lowering, inflammatory, antioxidant and fibrinolytics blood biomarkers are influenced by Val16Ala superoxide dismutase manganese-dependent gene polymorphism. *Pharmacogenomics Journal* 2016;16(6):501-506.
13. Maheshwari RA, Balaraman R, Sailor, GU, Sen DB. Protective effect of simvastatin and rosuvastatin on trinitrobenzene sulfonic acidinduced colitis in rats. *Indian Journal of Pharmacology* 2015; 47:17-21.
14. Abdel-Daim MM, Abdeen A. Protective effects of rosuvastatin and vitamin E against fipronil-mediated oxidative damage and apoptosis in rat liver and kidney. *Food and Chemical Toxicology* 2018;114: 69-77.
15. Abdeena A, Aboubakr M, Elgazzar D, Abdo M, Abdelkader A, Ibrahim S, Elkomy A. Rosuvastatin attenuates piroxicam-mediated gastric ulceration and hepato-renal toxicity in rats. *Biomedicine & Pharmacotherapy* 2019; 110:895-905.
16. Yahyazadeh A. Effect of curcumin on rat sublingual gland exposed to cyclophosphamide. *Cukurova Medical Journal* 2021;46(3):897-903.
17. Chang CT, Hsiang CY, Ho TY, Wu CZ, Hong HH, Huang YF. Comprehensive assessment of host responses to 5-fluorouracil-induced oral mucositis through transcriptomic analysis. *PloS One* 2015;10(8): e0135102.
18. Hogaboam CM, Muller MJ, Collins SM, Hunt RH. An orally active non-selective endothelin receptor antagonist, bosentan, markedly reduces injury in a rat model of colitis. *European Journal of Pharmacology* 1996;15;309(3):261-9.
19. Sultan AA, Ali AH, Kamal KA. Immunohistochemical study of the effect of green tea extract on methotrexate- induced oral mucositis in albino rats. *Journal of Cytology and Histology* 2014;5(3):1-7.
20. Hasan, A.M. and F.K. Gatea, Novel effect of topical Roquinimex and its combination with Clobetasol on an imiquimod-induced model of psoriasis in mice. *Naunyn-Schmiedeberg's Archives of Pharmacology* 2024. 397(7): 5219-5232.
21. Almudaris, S.A. and F.K. Gatea, Effects of topical Ivermectin on imiquimod-induced Psoriasis in mouse model – Novel findings. *Pharmacia* 2024. 71: 1-14.
22. Bash, A.A. and A.K.H. Abd, Effect of Salix alba barks on experimentally induced obesity in rats. *Annals of Tropical Medicine and Public Health* 2020, 23(5): 421-434.
23. Bash, A.A. and A.K.H. Abd, Effect of cilostazol on experimentally induced hyperglycemia in rats.

Biochemical and Cellular Archives 2020. 20(1): 1329-1336.
24. Alzubaidy, F.M., R.S. Almuttairi, and A.A. Bash, The Effects of Yasmin on the Histology and Histochemistry of Liver and Kidney in Rabbits. *International Journal of PharmTech Research* 2016. 9(11): 246-251.
25. Hassan, Z., T. Hassan, and A. Abu-Raghif, Evaluation the Effectiveness of Phenolic Compound of Salvia frigida on Induced Atopic Dermatitis in Experimental Mice. *Iraqi Journal of Pharmaceutical Sciences* 2022. 31(1): 154-166.
26. Yahiya, Y.I., et al., Role of Iberin as an anti-apoptotic agent on renal ischemia-reperfusion injury in rats. *Journal of Medicine and Life* 2023. 16(6): 915-919.
27. Atarbashe, R.K. and A. Abu-Raghif, Comparative treatment of induced ulcerative colitis in male rat model by using cinnarizine and sulfasalazine. *Iraqi Journal of Veterinary Sciences* 2020. 34(2): 465-472.
28. Hernandez-Rodriguez, J., et al., Tissue production of pro-inflammatory cytokines (IL-1β, TNFα and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. *Rheumatology* 2004. 43(3): 294-301.
29. Nundy, S., et al., Understanding Medical Biostatistics. How to Practice Academic Medicine and Publish from Developing Countries? *A Practical Guide* 2022: 95-116.
30. Attarbashee, R. K.; Mammdoh, J. K.; Al-Kazzaz, S. G., The protective effect of bosentan on methotrexate-induced oral mucositis in rats. *Iraqi Journal of Veterinary Sciences* 2023, 37 (3), 629-635.
31. Mammdoh, J. K.; Attarbashii, R. K. A.; Al Mola, A. D. H., Protective effect of rosuvastatin on cyclophosphamide-induced oral toxicity in rats: Histological and immunohistochemical Study. *Research Journal of Pharmacy and Technology* 2023, 16 (2), 759-762.
32. Ali, N., et al., Beneficial effects of Chrysin against Methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. *Molecular and cellular biochemistry* 2014. 385: 215-223.
33. Mahmoud, A.M., et al., Methotrexate hepatotoxicity is associated with oxidative stress, and down-regulation of PPARγ and Nrf2: Protective effect of 18β-Glycyrrhetinic acid. *Chemico-Biological Interactions* 2017. 270: 59-72.
34. Drishya, S., S.S. Dhanisha, and C. Guruvayoorappan, 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):13855.
35. Mammadov, R., et al., Effect of lutein on methotrexate-induced oxidative lung damage in rats: a biochemical and histopathological assessment. *The Korean Journal of Internal Medicine* 2019. 34(6): 1279-1286.
36. Dogra, A., et al., Glabridin ameliorates methotrexate-induced liver injury via attenuation of oxidative stress, inflammation, and apoptosis. *Life Sciences* 2021. 278: 119583.
37. Rafikova, O., et al., Bosentan inhibits oxidative and nitrosative stress and rescues occlusive pulmonaryhypertension. *Free Radical Biology and Medicine* 2013. 56: 28-43.
38. Fontoura, D., et al., Myocardial and anti-inflammatory effects of chronic bosentan therapy in monocrotaline-induced pulmonary hypertension. *Revista Portuguesa de Cardiologia* 2014. 33(4): 213-222.
39. Yayla, M., et al., Protective effect of Et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. *European Journal of Pharmacology* 2014. 726: 87-95.
40. Serafim, K.G., et al., Bosentan, a mixed endothelin receptor antagonist, inhibits superoxide anion-induced pain and inflammation in mice. *Naunyn-Schmiedeberg's Archives of Pharmacology* 2015. 388(11): 1211-21.
41. Visnagri, A., et al., Endothelin receptor blocker bosentan inhibits hypertensive cardiac fibrosis in pressure overload-induced cardiac hypertrophy in rats. *Cardiovascular Endocrinology & Metabolism* 2013. 2(4): 85-97.
42. Alkan, E., et al. The Effect of Bosentan, an Endothelin-1 Receptor Antagonist on Testicular Tissue Oxidant and Antioxidant Levels in Diabetic Rats. *Journal of Urology* 2015; 193: e986-e987.
43. Donate, P.B., et al., Bosentan, an endothelin receptor antagonist, ameliorates collagen-induced arthritis: the role of TNF-α in the induction of endothelin system genes. *Inflammation Research* 2012. 61: 337-348.

تاثير البوسنتان في تخفيف سمية اللسان الناجمة عن الميثوتريكسيت في الجرذان

الخلاصة

المقدمة: على الرغم من استخدام الميثوتريكسيت على مدى سنوات عديدة، إلا أن الآثار الضارة متكررة طوال فترة العلاج، خاصة بين مرضى السرطان. تناولت الدراسة فعالية البوسنتان كعلاج وقائي ضد سمية اللسان الناجمة عن الميثوتريكسيت. **الطرق**: استخدمت هذه الدراسة أربعة وعشرين جرذا بوزن 300-400 جرام. تم تصنيف الحيوانات إلى ثلاث مجموعات: الميثوتريكسيت (80 ملغم / كغم)، الميثوتريكسات بالإضافة إلى البوسنتان (60 ملغم / كغم)، والضابطة (الماء المقطر). تم إجراء كل من التحليلات البيوكيميائية والكيميائية المناعية على الغشاء المخاطي للسان. تم تشريح الغشاء المخاطي للسان الحيوانات مباشرة بعد أن تم التضحية بها في اليوم الخامس عشر. **النتائج**: على النقيض من المجموعة الضابطة، كان لدى مجموعة الميثوتريكسات مستويات أعلى بكثير من TNF-α، IL-1β و> MDA ($p<0.05$). ومع ذلك، أظهرت مجموعة الميثوتريكسات + بوسنتان مستويات أقل بكثير من TNF-α، وIL-1β، وMDA على النقيض من مجموعة الميثوتريكسات، ($P <0.05$). **الاستنتاج**: تثبت هذه النتائج أن البوسنتان يتمتع بحماية كبيرة ضد سمية اللسان الناجمة عن الميثوتريكسيت في الجرذان.

الكلمات المفتاحية: بوسنتان، الميثوتريكسيت، الإجهاد التأكسدي، سمية اللسان