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Suppressive effect of rutin on methotrexate-induced salivary gland injury in rats

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ABSTRACT

Methotrexate is an antimetabolite used in the treatment of various cancers and autoinflammatory disorders. However, it can adversely affect oral tissues, particularly impairing salivary gland function. The antioxidant and anti-inflammatory effects of rutin may counteract these toxic effects. This study aimed at examining whether rutin confers protective effects on the salivary glands of rats exposed to methotrexate. Twenty-four male rats were randomly assigned to three groups. The control group received normal saline intraperitoneally for 10 days. On day six of the experiment, the methotrexate group was administered methotrexate intraperitoneally at a dose of 20 mg/kg. The methotrexate + rutin group also received rutin intraperitoneally at 50 mg/kg once daily for 10 days. On day 11, the animals were euthanized, and their salivary gland tissues were harvested for histological and biochemical analyses. Rutin markedly ameliorated the methotrexate-induced histopathological changes and biochemical alterations, as indicated by reduced levels of the tumour necrosis factor-α and malondialdehyde, alongside elevated levels of interleukin-10 and superoxide dismutase. These findings suggest that the antioxidant and anti-inflammatory properties of rutin may offer a promising strategy for mitigating the methotrexate-associated toxicity in submandibular gland tissues.

1. Introduction

Methotrexate is a folic acid analogue widely employed in the treatment of various malignancies and autoinflammatory conditions. Despite its antitumor and anti-inflammatory properties, methotrexate may ex-

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ert harmful effects on certain tissues. Experimental studies have demonstrated its detrimental impact on secretory glands, particularly targeting the submandibular glands[1]. Clinical manifestations of salivary gland hypofunction include extensive dental caries, periodontal disease, pharyngeal infections, and disturbances in swallowing, taste, and speech. Rutin, a flavonoid glycoside derived from *Ruta graveolens* and other botanical sources (including citrus peels, buckwheat, apples, and strawberries), has been associated with multiple potential health benefits, notably antioxidant, anti-inflammatory, and antiproliferative activities[2]. The objective of this study was to investigate whether rutin attenuates histological changes, oxidative stress, and inflammatory biomarkers in the submandibular glands of rats administered 20 mg/kg of methotrexate.

2. Methodology

2.1. Drugs and chemicals

Methotrexate was obtained from Ebewe, and so was rutin; both compounds were prepared in normal saline prior to administration.

2.2. Experimental design

The experiment was conducted between April 2023 and December 2024. A total of 24 male albino rats, aged 6 to 9 weeks and weighing between 190 and 210 g, were randomly assigned to three groups (n=8 per group): (i) group I (control) that received intraperitoneal injections of normal saline once daily for 10 days, (ii) group II (methotrexate) that received a single intraperitoneal dose of methotrexate (20 mg/kg) on day six, and (iii) group III (methotrexate + rutin) that, apart from methotrexate (as in group II), also received rutin (50 mg/kg) *via* oral gavage once daily for 10 days.

2.3. Animal preparation and tissue sampling

On day 11, rats were euthanized using a combination of ketamine and xylazine (80 mg/kg). Submandibular gland tissues were subsequently dissected. Tissue samples were collected for histopathological evaluation and some were processed into homogenates for biomarker analysis.

2.4. Determination of inflammation and oxidative stress biomarkers

Levels of tumour necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), malondialdehyde (MDA), and superoxide dismutase (SOD) were quantified in the obtained rat glandular tissues using standardized ELISA kits and following the manufacturer's instructions.

2.5. Histopathological evaluation

Tissues were embedded in paraffin blocks using stainless-steel molds. Once solidified, blocks were trimmed and sectioned into 5-µm slices using a rotary microtome. Paraffin was removed by sequential treatment with heat, xylol, and ethanol. Specimens were mounted on clean glass slides and stained with haematoxylin and eosin[3].

2.6. Statistical analysis

Data were analysed using SPSS version 24. Descriptive statistics included means and standard deviations (SD). One-way analysis of variance (ANOVA) was employed in order to compare multiple group means, followed by Tukey's HSD *post hoc* test for pairwise comparisons. A *p*-value below 0.05 was considered as statistically significant.

2.7. Ethics approval

This study was approved by the Institutional Review Board of the College of Dentistry of the University of Mosul on April 20, 2024 (approval number: UoM. Dent.24/42), following review of the final protocol, topic outline, and research design.

3. Results and Discussion

The methotrexate group exhibited significantly elevated levels of oxidative stress markers – specifically, MDA (9.2 ± 1.5 nmol/mg protein) – and markedly reduced levels of antioxidative enzymes such as SOD

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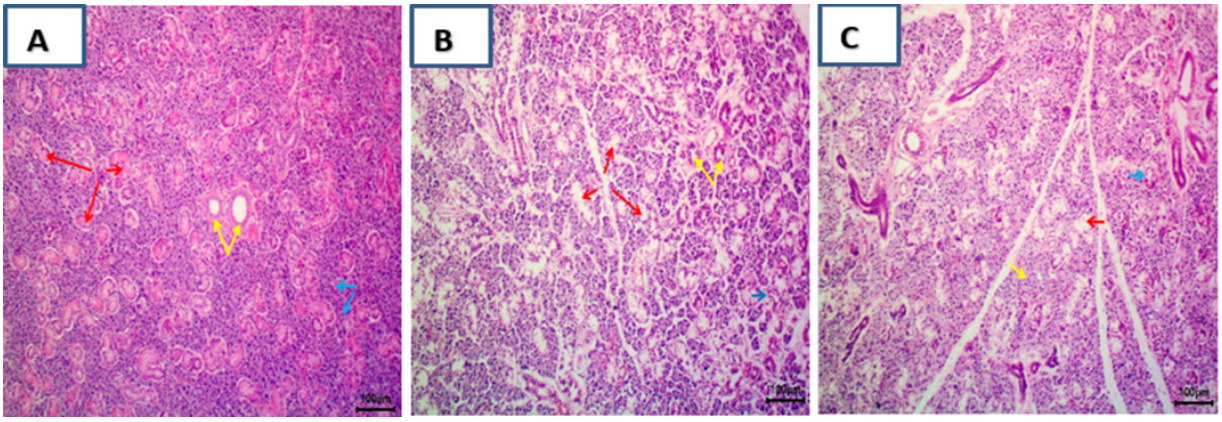

Figure 1. *Histopathological evaluation of the rat salivary glandular tissues. (A): Representative section from the control group showing normal histoarchitecture, including intact mucous acini (yellow arrows), granular convoluted tubules (red arrows), and striated ducts (blue arrows). (B): Representative section from the methotrexate-treated group exhibiting severe degenerative and necrotic changes, particularly in the granular convoluted tubules (red arrows), along with disorganized mucous acini (yellow arrows) and distorted striated ducts (blue arrow). (C): Representative section from the methotrexate + rutin group showing preserved mucous acini (yellow arrow), mildly degenerated granular convoluted tubules (red arrow), and relatively intact striated ducts (blue arrow), indicating partial restoration of the tissue's integrity. Notes: all sections stained with haematoxylin and eosin; magnification: ×100; scale bar: 100 μm.*

(18.5 ± 3.9 U/g protein), compared to the control group (MDA: 1.6 ± 0.4 nmol/mg protein; SOD: 54.6 ± 5.1 U/g protein; $p<0.05$). On the other hand, rats in the methotrexate + rutin group exhibited significantly lower levels of MDA (2.8 ± 0.8 nmol/mg protein) and significantly higher levels of SOD (42.6 ± 6.8 U/g protein), thereby demonstrating a protective effect of rutin against methotrexate-induced oxidative damage ($p<0.05$).

Similarly, the methotrexate group exhibited substantially elevated concentrations of the pro-inflammatory cytokine TNF-α (428.5 ± 21.6 pg/g) and decreased levels of the anti-inflammatory cytokine IL-10 (149.1 ± 5.4 pg/g), relatively to controls (TNF-α: 145.7 ± 9.3 pg/g; IL-10: 298.2 ± 14.6 pg/g; $p<0.05$). Rutin administration in the methotrexate + rutin group resulted in significantly reduced TNF-α levels (185.1 ± 15.2 pg/g) and increased IL-10 levels (268.8 ± 10.5 pg/g) compared to methotrexate alone ($p<0.05$).

Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and the availability of endogenous antioxidants. This imbalance provokes inflammatory cascades and sustains a destructive feedback loop that exacerbates tissue degeneration. Methotrexate is known to stimulate ROS formation, promote lipid peroxidation, and elevate MDA levels. It also inhibits key antioxidative components, including SOD and glutathione peroxidase, and increases the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6[1]. Further studies indicate that methotrexate-induced damage to salivary gland tissues is associated with elevated MDA and TNF-α levels and diminished levels of glutathione and SOD[4]. Rutin, by contrast, appears to mitigate methotrexate toxicity through its ability to enhance endogenous antioxidant defences (e.g., SOD), lower MDA, and downregulate pro-inflammatory mediators; this dual action helps reverse degenerative changes in submandibular tissues.

Targeting oxidative and inflammatory pathways may reduce lipid peroxidation and free radical generation, restoring glandular integrity. Rutin's antioxidant effect is primarily mediated *via* the suppression of oxidative stress and the inhibition of lipid peroxidation[2]. Its anti-inflammatory role is further supported by its ability to modulate nuclear factor kappa B signalling, thereby reducing tissue levels of MDA and

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cytokines such as TNF-α, IL-1β, and IL-17[5].

Histological examination revealed that the salivary glands obtained from the control group displayed normal architecture, with intact glandular ducts and densely packed serous acini (Figure 1A). The methotrexate group exhibited pronounced degenerative changes, including granular convoluted tubule necrosis, periductal fibrosis, vascular congestion, and distorted pyknotic nuclei (Figure 1B). In contrast, the methotrexate + rutin group exhibited milder histopathological features: minor ductal expansion, moderate vascular congestion, and limited acinar degeneration, with only occasional pyknotic nuclei (Figure 1C). These observations further underscore the protective effect of rutin (p<0.05).

4. Conclusion

Rutin demonstrates significant protective effects against methotrexate-induced salivary gland damage, primarily through its potent antioxidative and anti-inflammatory properties.

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Conflicts of interest

None exist.

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