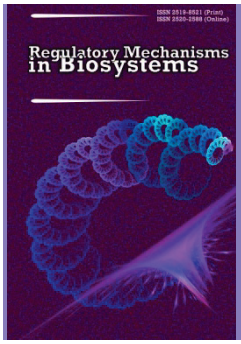

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Effect of ibuprofen on the submandibular salivary gland in rats: Histological and biochemical study

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This study aims to assess the histological and biochemical effects of ibuprofen on the submandibular salivary gland in rats. The study provides detailed data on ibuprofen's impact on oxidative stress levels and structural changes in the salivary gland. A total of 15 male rats were used, divided into three groups: the control group, left untreated; the first group, which received a moderate dose of ibuprofen (200 mg/kg/day); and the second group, which received a higher dose of ibuprofen (400 mg/kg/day). Ibuprofen was administered orally for 3 weeks. The histological results showed that the group administered ibuprofen at 400 mg/kg exhibited apoptosis and necrosis in the granular convoluted tubule cells and degeneration of the striated duct cells with sloughing. The biochemical analysis indicated a significant increase in caspase-3 concentration in the 400 mg/kg ibuprofen group compared to the control group (0.398 ± 0.001). Additionally, this high-dose group exhibited a marked decrease in total antioxidant capacity (TAC) levels (30.1 ± 1.1) compared to the control group, indicating that a high ibuprofen dose negatively affects the system's total antioxidant capacity. In conclusion, high doses of ibuprofen may cause damage to salivary gland tissue and the body's antioxidant system, as well as increase programmed cell death, raising the likelihood of cellular and tissue damage. It is important to be careful when taking amounts of ibuprofen for a long time period because it can cause health problems related to the digestive system in at risk patients. Therefore using other treatments alongside ibuprofen might reduce any effects on tissues.

Keywords: biochemical analysis; ibuprofen; salivary gland; tissues; oxidative stress; caspase-3; total antioxidant capacity.

Introduction

Many people rely on ibuprofen as a nonsteroidal inflammatory medication to relieve pain and reduce inflammation linked to different health issues, like arthritis and sports injuries or headaches. The drug works by hindering certain enzymes called cyclooxygenase (COX) (Upadhyay et al., 2021). This action decreases the production of prostaglandins that trigger pain and inflammation in the body (Jang et al., 2020). Although ibuprofen is known for its effectiveness and general safety profile, over time; several research findings indicate that prolonged or excessive consumption of ibuprofen could result in effects such as stomach ulcers and complications with the kidneys and liver functions; therefore it is crucial to further investigate the potential impact of ibuprofen on other organs that might be influenced by long term usage (Varrassi et al., 2020).

The salivary glands are parts of the digestive system, and here we make a special mention to the submandibular salivary gland for its importance in producing saliva that aids in primary digestion and protects oral tissues while also helping in swallowing food effectively (Bowers et al., 2021). Extended contact with substances or drugs can harm these glands. Disruption of the secretion of saliva affects oral and digestive processes negatively (Chibly et al., 2022).

Certain research suggests that NSAIDs could potentially impact the production of saliva and the makeup of glands; however the specific effects of ibuprofen on the submandibular gland have not been extensively studied yet (Sangalli et al., 2023).

This investigation aims to explore how ibuprofen affects the biochemical aspects of the salivary gland in rats (Daba and Bogazia, 2023). The immunohistochemical markers chosen for this research study focus on two indicators related to stress, glutathione (GSH) and malondialdehyde (MDA) (Liesche-Starnecker et al., 2020).

Glutathione functions as an antioxidant that plays a role in safeguarding cells from harm caused by free radicals and is essential for maintaining cellular equilibrium and minimizing oxidative stress levels in the body's cells (Labarrere & Kassab, 2022). On the other hand malondialdehyde is a byproduct resulting from peroxidation and is regarded as a reliable indicator reflecting oxidative damage and cellular injury (Bey et al., 2024). Oxidative stress occurs when there is an imbalance between the creation of free radicals and protective antioxidants in the body; this situation can result in harm to proteins, fats and DNA and is closely linked to the onset of numerous long term illnesses and tissue damage (Engwa et al., 2022).

This study will not involve analyzing only biochemistry but also performing examinations to evaluate any alterations in the submandibular gland caused by exposure to ibuprofen to assess potential changes in the glands structure and function resulting from prolonged drug use.

The main goal of this research is to carefully study the biochemical effects of ibuprofen on the salivary gland in rats and provide comprehensive information on how ibuprofen affects oxidative stress levels and structural modifications in the salivary gland tissue in order to enhance our scientific knowledge about the impact of NSAIDs on salivary tissue. The findings of this study may contribute to safer usage guidelines for ibuprofen and to the development of therapeutic strategies aimed at reducing the side effects associated with its prolonged use.

Materials and methods

This study involved 15 adult male rats weighing between 250–300 grams. The rats were obtained from the College of Vet- Med, University of Mosul, and housed in designated plastic cages under controlled environmental conditions, with 12-hours light and 12-hour sdark. Food and water were provided throughout the study period. Ethical approval

was obtained from the College of Dentistry, University of Mosul, in 2023. In accordance with the standards of the Ethical Committee for the Care and Use of Animals in Scientific Research, all measures were taken to minimize pain and stress in the rats during the experiments.

Pure ibuprofen (98% purity) was purchased from a certified chemical supplier from Granules India Limited – India. Ibuprofen was dissolved in sterile saline solution to achieve the desired doses.

The rats were divided into three groups, each group containing five animals:

– control group: rats were left untreated for three weeks;

– experimental group 1 (ibuprofen medium dose): rats were given a moderate dose of ibuprofen (200 mg/kg/day) orally for three weeks;

– experimental group 2 (ibuprofen high dose): rats were given a high dose of ibuprofen (400 mg/kg/day) orally for three weeks.

After the treatment period (21 days), the rats were anesthetized using ether, and then sacrificed according to ethical standards for animal care. The submandibular salivary glands were carefully removed from each rat and prepared for both histological and biochemical analyses.

Salivary gland samples were prepared to assess histological changes. Following removal, the glands were rinsed in saline solution and fixed in 10% formalin/24 hr. After fixation, the samples underwent dehydration and paraffin embedding. Tissue sections, 5 microns thick, were cut using a microtome then stain with hematoxylin and eosin (H&E) for examination of histologicalhy alteration under a microscope. The following histological variables were evaluated: (1) changes in the size and shape of the salivary gland; (2) presence of inflammatory cells or fibrosis; (3) alterations in the salivary gland ducts.

The kit for measuring caspase-3 in rat serum is the Rat Caspase-3 ELISA Kit, available from MyBioSource (USA). The kit operates on the ELISA (enzyme-linked immunosorbent assay) technique. Serum samples from rats are added to wells pre-coated with antibodies specific to caspase-3. A secondary antibody conjugated to an enzyme, such as peroxidase, is then added to bind to caspase-3. Upon adding a detection solution (substrate), a colorimetric reaction occurs, proportional to the caspase-3 concentration in the sample. The absorbance is measured with an ELISA reader, providing caspase-3 concentration levels.

The total antioxidant capacity (TAC) Assay Kit from Sigma-Aldrich (USA) operates using a method based on either electron transfer or hydrogen transfer from antioxidants in the sample to a specialized chemical substrate. This substrate reacts with the antioxidants, resulting in a color change. The color intensity can be measured by an absorbance reader (typically at a specified wavelength), and the intensity is proportional to the total antioxidant content in the sample. The results are then compared to a standard sample to determine the total antioxidant capacity in the serum.

The data is presented in the form mean ± standard error. Tukey's test was used to compare samples.

Results

The histological sections of the submandibular salivary gland of the control group showed intact architecture of the granular convoluted tubules, mucous acini and striated ducts (Fig. 1). The submandibular salivary gland of the treatment group 1 receiving ibuprofen at a dose of 200 mg/kg/day revealed mild necrosis and degeneration of the granular convoluted tubules cells and mild degeneration of the striated ducts cells (bold-arrow) and congested blood vessels (Fig. 2).

The histological section of rat submandibular salivary gland of the ibuprofen 400 mg/kg treated group showed apoptosis and severe necrosis of the granular convoluted tubules cells, and degeneration of the striated ducts cells with sloughing (Fig. 3 and 4).

Table 1 shows the serum concentration of caspase-3 after treating the rats with ibuprofen for three weeks.

Table 1

Caspase-3 concentration in serum after treatment with ibuprofen for 3 weeks (mean ± standard error, n = 5)

Groups	Caspase-3, ng/mL
Control	0.036 ± 0.001^{a}
Group one, 200 mg/kg	0.089 ± 0.002^{b}
Group two, 400 mg/kg	0.398 ± 0.001^{c}

Note: difference from the control group $P < 0.05$.

Table 2 presents the concentration of total antioxidant capacity (TAC) in the serum of rats after three weeks of ibuprofen treatment. This suggests that the higher dose of ibuprofen has a negative effect, reducing total antioxidant capacity in serum.

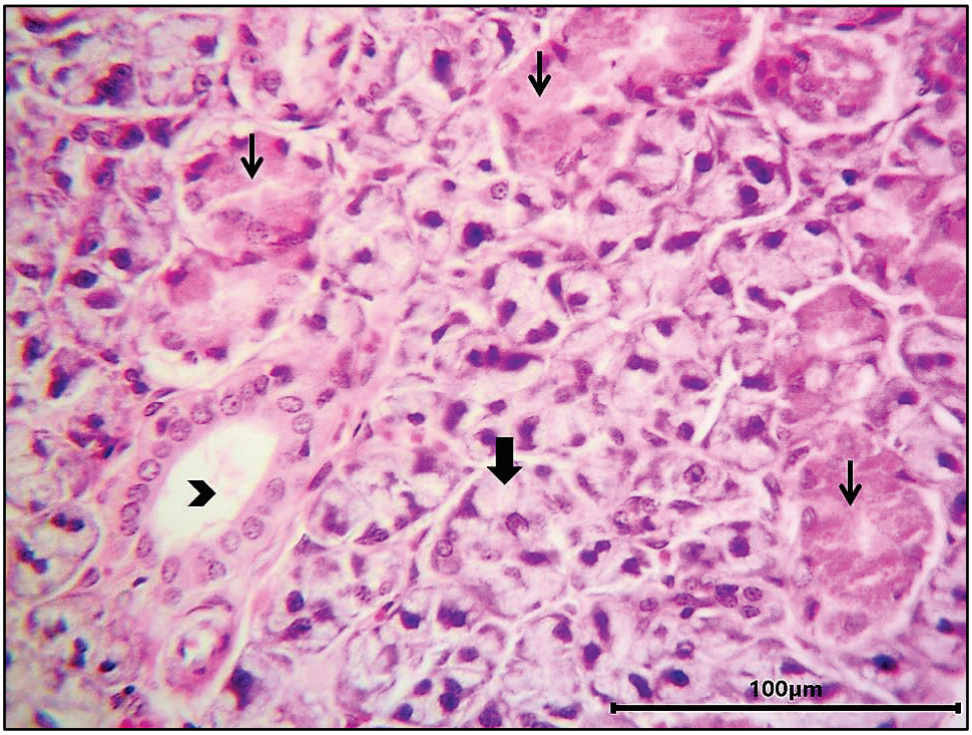

Fig. 1. Histological section of submandibular salivary gland of a rat from the control group showing normal architecture of the granular convoluted tubules (arrows), mucous acini (bold-arrow) and striated ducts (arrowhead)

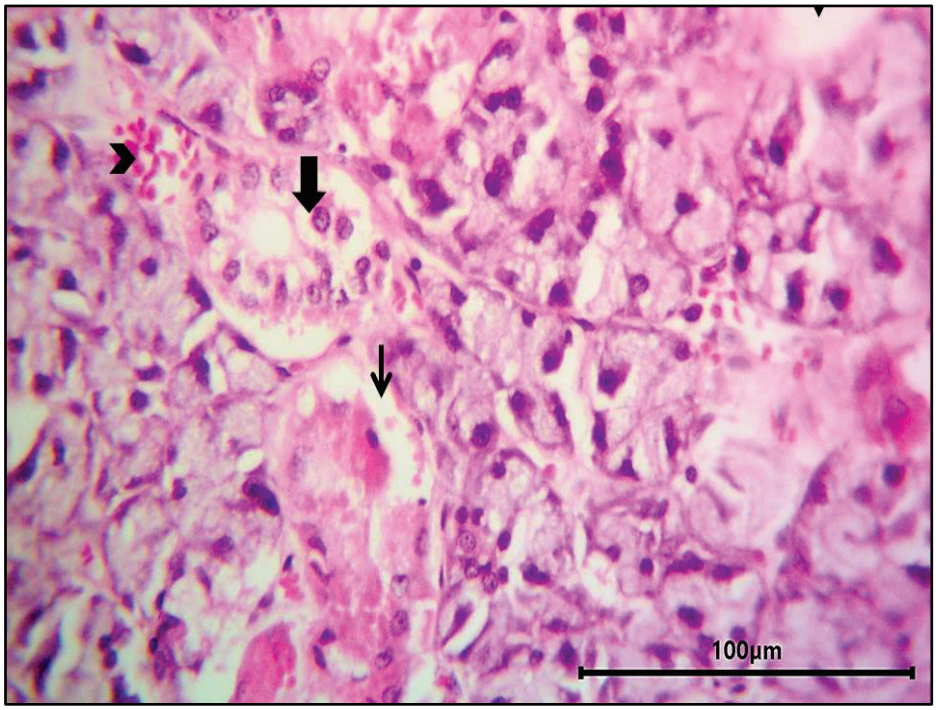

Fig. 2. Histological section of submandibular salivary gland of a rat from the ibuprofen 200 mg/kg group showing mild necrosis and degeneration of the granular convoluted tubules cells (arrows), and mild degeneration of the striated ducts cells (bold-arrow) and congested blood vessels (arrowhead)

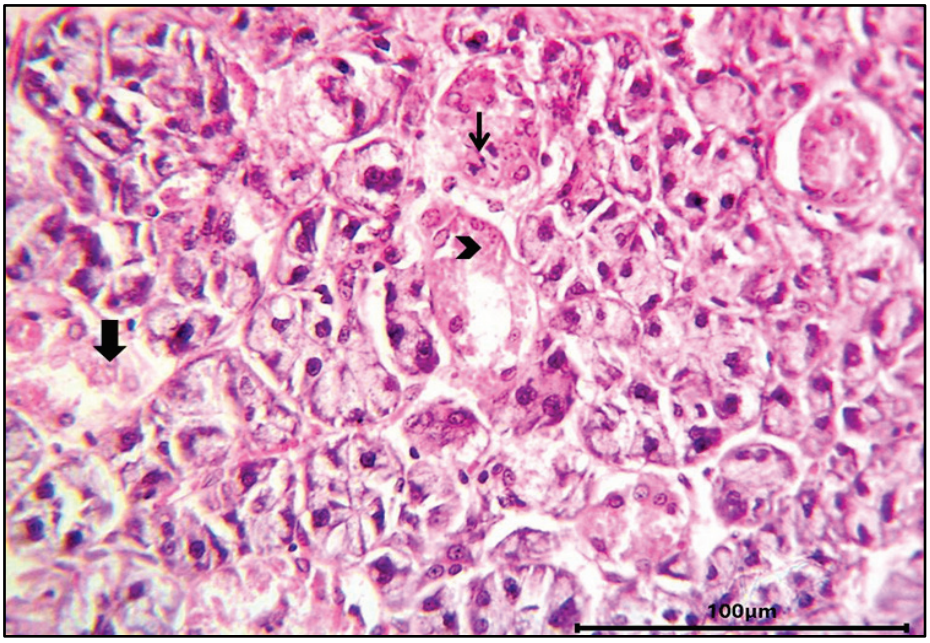

Fig. 3. Histological section of submandibular salivary gland of a rat from the ibuprofen 400 mg\kg group showing apoptosis (arrows) and necrosis (bold-arrow) of the granular convoluted tubules cells, and degeneration of the striated ducts cells with sloughing (arrowhead)

Table 2
TAC concentration in serum after treatment with ibuprofen for 3 weeks (mean ± standard error, n = 5)

Groups	TAC, mmol/L
Control	36.5 ± 1.0[a]
Group one, 200 mg/kg	35.0 ± 1.9[a]
Group two, 400 mg/kg	30.1 ± 1.1[b]

Note: see Table 1.

Discussion

In the current study, histological sections of the submandibular salivary glands in rats treated with a high dose of ibuprofen (400 mg/kg) showed notable pathological tissue changes, including necrosis and cellular damage, especially in the granular convoluted tubule cells and striated duct cells, which are essential components for the gland's function. These changes included degeneration and cellular infiltration, with evident cellular sloughing.

These findings align with studies that have demonstrated the toxic effect of high doses of ibuprofen on salivary tissues, as NSAIDs like ibuprofen can increase oxidative stress and inflammatory activity, leading to cellular imbalance in the salivary glands (Yeoh, 2025). Research has shown that prolonged usage of doses of NSAIDs leads to alterations caused by elevated oxidative levels and diminished activity of antioxidant enzymes, like glutathione peroxidase (Bindu et al., 2020). This weakens the body's defenses at a cellular level and contributes to an increased occurrence of necrosis and apoptosis, in cells (Li et al., 2023). The impact

of ibuprofen on the cells within the glands is partly due to its ability to block enzymes, which helps decrease the production of prostaglandins that trigger inflammatory reactions in the body's tissues (Ali et al., 2023).

However, this action also affects factors linked to prostaglandins by preventing them from functioning effectively and making tissues more vulnerable to oxidative harm (Bindu et al., 2020). This can result in outcomes such as decreased blood flow to tissues and impaired function of glands; thereby leading to potential side effects, like tissue damage and disruption of normal cell activities (Jasmer et al., 2020). The recent discoveries underscore the consequences of ibuprofen intake on the well being of glands – a factor that could impede saliva production crucial for digestion and oral hygiene maintenance. Biological cell death and dysfunction in the glands can result in changes to saliva makeup and potentially lead to problems like mouth infection or heightened vulnerability to oral infections (Salave et al., 2023).

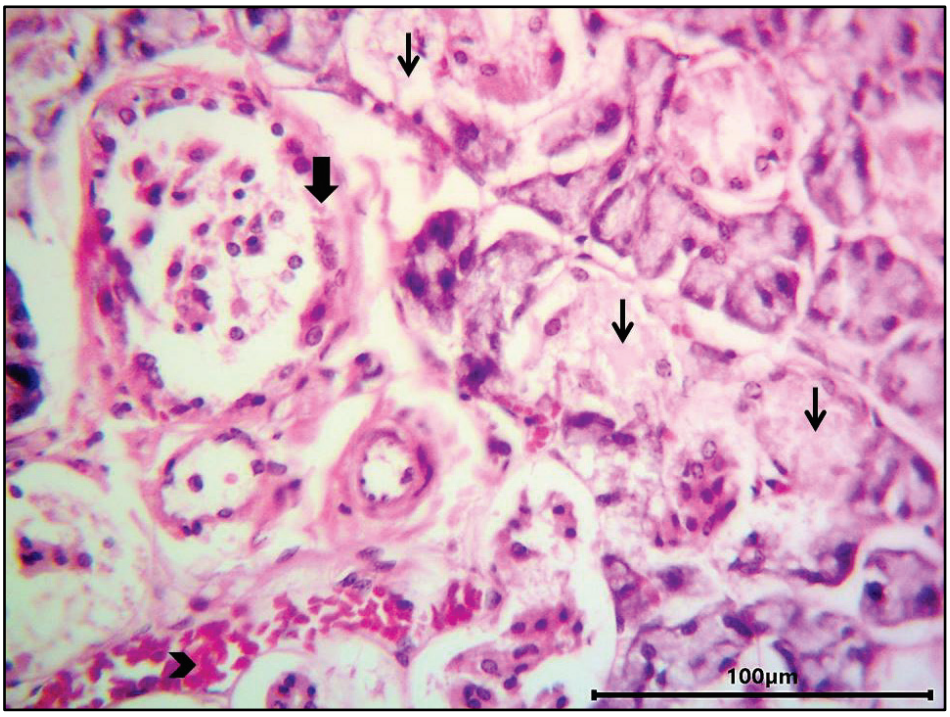

Fig. 4. Histological section of the submandibular salivary gland of a rat from the ibuprofen 400 mg/kg group showing necrosis of the granular convoluted tubules cells (arrow) and striated ducts cells with sloughing (bold-arrow)

Biochemical research results also showed a rise in caspase-3 levels following ibuprofen administration at a dosage of 400 mg/kg, suggesting a role of the drug in triggering cell death processes, with caspase-3 serving as an indicator of such activity, in the submandibular salivary glands' cellular function (Jasmer et al., 2020). The increasing impact on caspase-3 in relation to dosage suggests that elevated levels of ibuprofen could have effects on well being of tissues (Işıcı et al., 2023).

It plays a role in the cell death process. It is crucial for the signaling pathway that triggers apoptosis in reaction to different stimuli, resulting in the disintegration of vital cellular proteins and orderly cell breakdown (Rani et al., 2023).

Research indicates that taking amounts of ibuprofen can result in a level of caspase-3 activation in the tissues, which points to an increase in cell death through apoptosis in those tissues affected by it (Kolawole & Kashfi, 2022). This could be due to the impact ibuprofen has on elevating stress and lowering antioxidant levels within the body's cells (Okoroama et al., 2023). The buildup of radicals might stimulate signals that initiate apoptosis processes and trigger the activation of caspases, such as caspase-3 activation, either directly or indirectly (Wójcik et al., 2024). Some researchers suggest that ibuprofen may harm mitochondria by causing the release of caspase from the mitochondria to the cytoplasm and triggering the caspase cascade involving caspase. Once this process begins, caspase starts breaking down cellular proteins, resulting, in programmed cell death (Kolawole & Kashfi, 2022). Since caspase-3 leads to cellular breakdown and executes the apoptosis process, its increased activity may cause tissue degradation, resulting in functional damage to the targeted organs (Adamičková et al., 2024).

In the salivary glands, for example, this elevated activity may lead to the loss of secretory cells, weakening saliva production and causing dry mouth, while in organs like the kidneys or liver, it may lead to tissue and functional damage (Song et al., 2024). Further research is needed to understand the underlying mechanism of ibuprofen's effect on caspase-3 and whether this effect could result in long-term adverse outcomes for the salivary glands or other tissues (Darmadi et al., 2020). The decrease, in TAC levels among individuals exposed to 400 mg/kg of ibuprofen suggests a rise in stress mainly caused by ibuprofen's impact on cells. NSAIDs such as ibuprofen hinder COX enzymes that are crucial for producing prostaglandins for tissue defense. This hindrance could result in a buildup of radicals and oxidative harm causing a drop in TAC levels as seen in the high dose group (Gupta et al., 2021).

The results indicate that taking amounts of ibuprofen could harm the body's antioxidant defenses and raise the risk of tissue and cell damage, from oxidative stress levels. These effects might be more noticeable in organs that depend on antioxidants for protection like the liver, kidneys and salivary glands. This aligns with studies that found death and harm in salivary tissues treated with similar doses (Buss, 2023).

Conclusions

Taking ibuprofen can harm the body's antioxidant system and lead to an increase in programmed cell death, which could potentially cause damage to tissues and cells It is important to be careful when using high doses or taking ibuprofen for a long time. This is especially true for individuals with health conditions that impact the oxidative system. To minimize the potential harm on tissues it might be helpful to use antioxidant treatments along with ibuprofen.

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