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Does Ibuprofen affect the taste buds? Histological and biochemical study

O. W. Saleh, B. N. Al Hussary, O. M Ameen

University of Mosul, Mosul, Iraq

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Department of Basic Science, College of Dentistry, University of Mosul, Mosul, Iraq. E-mail: omer.saleh@uomosul.edu.iq, bananalhussary@uomosul.edu.iq, omar.mazin@uomosul.edu.iq

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Ibuprofen is one of the nonsteroidal inflammatory medications which can reduce the body's natural defense against harmful substances and increase stress on the body's cells and tissues. This research was conducted to explore the impact of using ibuprofen and its potential effects on taste perception in rats by examining changes in the structure of taste buds or biochemical functions through histological and biochemical analysis. In the experiment 15 rats were divided into three groups: the control group, the low-dose group receiving 150 mg/kg of ibuprofen, and the high-dose group receiving 300 mg/kg for two weeks. Upon examination, under a microscope we found that rats taking ibuprofen develooed parakeratosis (thickening of tissue layer) a decrease in taste buds and thickening of mucosa along with the presence of cells beneath the tissue layer . The results from the analysis showed oxidative stress in the high dose group as indicated by reduced levels of glutathione and increased malondialdehyde (MDA) compared to both the control and low dose groups. These findings suggest that extended or high doses of ibuprofen could potentially harm the structure and function of tongue tissue. A dosage of 300 mg/kg of ibuprofen seemed to cause reactions related to stress that disrupted the balance of antioxidants in the body; this was evidenced by a decrease in glutathione levels and an increase in MDA levels. On the other hand ,the dosage of 150 mg/kg did not bring about changes in these markers of oxidative stress, implying that the body may have the ability to adjust to moderate levels of ibuprofen induced oxidative stress without notable biochemical or structural alterations. In general, these findings suggest that prolonged use of ibuprofen at high doses could harm the structure and function of tongue tissue, potentially resulting in issues with taste perception.

Keywords: Ibuprofen; histological changes; oxidative stress; tongue.

Introduction

One of the nonsteroidal anti-inflammatory drugs (NSAIDs) is ibuprofen which is often recommended for its pain relief and anti-inflammatory benefits, for conditions like headaches and muscle pain. The mechanism of action of ibuprofen is primarily attributed to its ability to inhibit cyclooxygenase (COX) enzymes, specifically COX-1 and COX-3 – which are involved in the biosynthesis of prostaglandins, key mediators of inflammation and pain (Machado et al., 2021; Ribeiro et al., 2022). Even though ibuprofen is commonly used for therapy and in practice due to its overall effectiveness, taking it in doses or over a long period has sparked worries because of the possible harm it may cause to various parts of the body.

The mouth area is crucial for functions such as chewing food and speaking clearly; it also significantly influences our ability to taste flavors by using specific sensory structures called taste buds, located mostly in certain parts of the tongue like the fungiform and circumvallate papillae. The taste buds consist of a group of gustatory receptor cells that identify five primary taste sensations: sweets, sour, salt, bitter and umami (Salta & Du, 2024). The correct operation of taste buds is crucial not only for pleasure but also for dietary intake, eating habits, and general well-being. Receptor cells responsible for tasting can naturally renew themselves about every 10 to 14 days; in certain situations, however, they can be negatively impacted by external factors like certain medications or treatments such as chemotherapy or radiation therapy, as well as long-term use of drugs like NSAIDs (Crawford et al., 2021; Alabdaly, 2023). Substances that interfere with the oxidative balance can disrupt the turnover of taste bud cells, harm their environment, and cause changes in how tastes are perceived.

Ibuprofen can potentially harm tissues by causing oxidative stress, which is a significant factor in its toxic effects. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense systems of the body. While low levels of ROS play essential roles in cell signaling, elevated ROS levels can lead to cellular injury, DNA damage, protein oxidation, and lipid peroxidation (Upadhyay et al., 2021). Ibuprofen, particularly at high doses or when used long-term, has been shown to increase the generation of ROS, thereby overwhelming antioxidant systems and disrupting cellular homeostasis (Martemucci et al., 2022). This includes the depletion of crucial antioxidants such as glutathione (GSH), a tripeptide that plays a central role in neutralizing free radicals and maintaining redox balance. Simultaneously, increased levels of malondialdehyde (MDA), a byproduct of lipid peroxidation, serve as a biomarker indicating the extent of oxidative tissue damage (Demirci-Cekic et al., 2022).

Although numerous studies have evaluated the gastrointestinal, renal, and hepatic side effects of NSAIDs, limited attention has been given to their potential impact on the structure and function of oral tissues, particularly the sensory systems involved in taste. Given that the tongue is directly exposed to systemic circulation and is highly vascularized, it is plausible that medications like ibuprofen could affect the histological architecture and biochemical status of lingual tissues (Kotowska-Rodziewicz et al., 2023). Alterations in the taste buds or surrounding epithelium may compromise taste function, leading to symptoms such as dysgeusia (distortion of taste), hypogeusia (reduced taste sensitivity), or ageusia (complete loss of taste). Despite the critical role of taste in daily life, research concerning the effects of common drugs like ibuprofen on the gustatory system remains scarce. It is important to know whether these drugs can affect taste perception not to evaluate their safety but to give proper advice on using them – especially for patients, with existing oral or systemic issues.

The main goal of this research is to explore how ibuprofen impacts the taste buds of rats by conducting examinations using both histological and biochemical analysis.In particular the study centers on examining any alterations in the structure of the epithelium and taste bud shape and analyzing stress markers like glutathione and malondialdehyde.This investigation aims to shed light on the dangers linked to high volume use of ibuprofen relating to taste perception and oral tissue health.

Materials and methods

All procedures performed on animals followed the guidelines set by both the institution and international standards for animal research

in labs. The Ethical Committee for Animal Care and Use in Scientific Research approved the plan. Steps were taken to limit the animal count and alleviate any distress during the study period to ensure their well-being, and ethical treatment was maintained.

In this research study fifteen healthy adult male Wistar rats were used, with each rat weighing between 180–220 grams. The rats were obtained from an animal breeding facility. They were given a week to adjust before starting the experimental processes.

The rats were kept in plastic cages filled with bedding that was replaced on a regular basis.The lab environment was meticulously managed to keep a temperature, around 22 ± 2 °C and a humidity level of 50–60%. The animals were kept under a 12-hour light/dark cycle to mimic natural circadian rhythms. During the entire experimental period, all rats had free access to standard laboratory chow and clean drinking water.

Pure ibuprofen powder was obtained from Pioneer Pharmaceuticals (Sulaymaniyah, Iraq). The required dosages were prepared freshly each day by dissolving the ibuprofen in distilled water to achieve accurate dosing concentrations. The solutions were prepared under sterile conditions and administered to the rats via oral gavage to ensure precise delivery of the drug.

The fifteen rats were randomly assigned into three experimental groups, each consisting of five animals (n = 5):

Group I – control group: rats in this group received no treatment and served as the negative control. They were administered an equivalent volume of distilled water via oral gavage for 14 consecutive days.

Group II – low-dose Ibuprofen group: rats in this group received 150 mg/kg body weight of ibuprofen orally once daily for a period of 14 days.

Group III – high-dose Ibuprofen group: rats in this group were administered 300 mg/kg body weight of ibuprofen via oral gavage daily for 14 days.

The dosage levels were selected based on previously published toxicological studies that investigated subacute exposure to NSAIDs in rodent models.

At the end of the treatment period, all animals were humanely euthanized under deep anesthesia. Tongue tissues were carefully dissected and immediately fixed in 10% neutral-buffered formalin for 48 hours. After fixation, tissues were processed through a standard paraffin-embedding protocol. Sections of 5 µm thickness were cut using a microtome and mounted on glass slides.

The sections were stained using hematoxylin and eosin staining to observe general histological architecture. The stained slides were examined under a light microscope for structural alterations in the lingual epithelium, morphology of taste buds, signs of epithelial thickening, parakeratosis, and cellular changes beneath the mucosal surface. Photomicrographs were taken for documentation and analysis.

In order to assess the biochemical impact of ibuprofen on tongue tissue, two key markers of oxidative stress were evaluated.

Glutathione (GSH) levels were measured using Ellman's method, a well-established spectrophotometric technique. In this assay, the sulfhydryl group of reduced glutathione reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) – also known as Ellman's reagent – resulting in the formation of a yellow-colored compound (5-thio-2-nitrobenzoic acid) that absorbs light at 412 nm. The absorbance was recorded using a UV-Visible spectrophotometer, and the concentration of GSH was calculated using a standard calibration curve (Alisik et al., 2019).

Malondialdehyde (MDA), an end-product of lipid peroxidation, was quantified using the thiobarbituric acid reactive substances (TBARS) assay. This method involves the reaction of MDA with thiobarbituric acid (TBA) under high temperature and acidic conditions, producing a pink-colored MDA-TBA adduct. The absorbance of the resulting complex was measured at 532 nm using a spectrophotometer. MDA levels were expressed in nmol/mg protein, providing an index of lipid peroxidation and tissue oxidative damage.

All data collected from the histological and biochemical assays were analyzed using IBM SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Results were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) test was performed to compare the means of different groups. When significant differences were found, the post hoc Tukey's test was applied to determine specific inter-group differences. A P-value less than 0.05 was considered statistically significant for all analyses.

Results

The histological section of rat tongue of the control group showed normal architecture of the keratin layer, mucosa with numerous taste buds' papillae, healthy submucosa and skeletal muscles (Fig. 1). Histological section of rat tongue of the ibuprofen 150 mg/kg treated group revealed mild thickening of the keratin layer (parakeratosis), mild decrease in the number of taste buds, mild thickening of the mucosa (Fig. 2). In the section of a rats tongue from the group treated with ibuprofen at 300 mg/kg, there were observed a thickening of the keratin layer (parakeratosis) loss of taste buds accompanied by mucosa thickening and the presence of cells infiltrating the submucosa (Fig. 3).

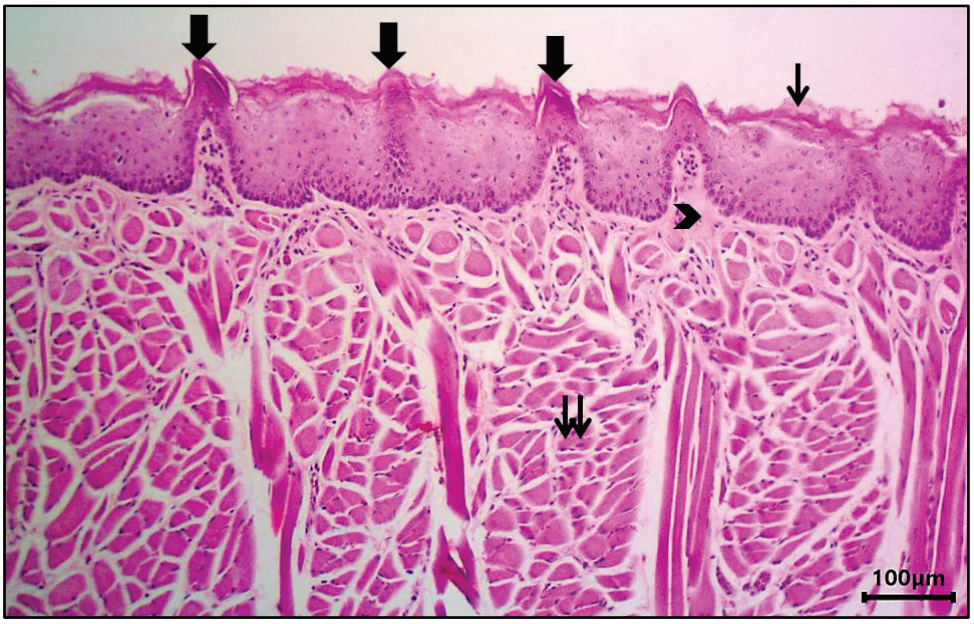

Fig. 1. Histological section of rat tongue of the control group showing normal architecture of the keratin layer (arrow), mucosa with numerous taste buds' papillae (bold-arrow), healthy submucosa (arrowhead) and skeletal muscles (double-arrow); hematoxylin and eosin staining

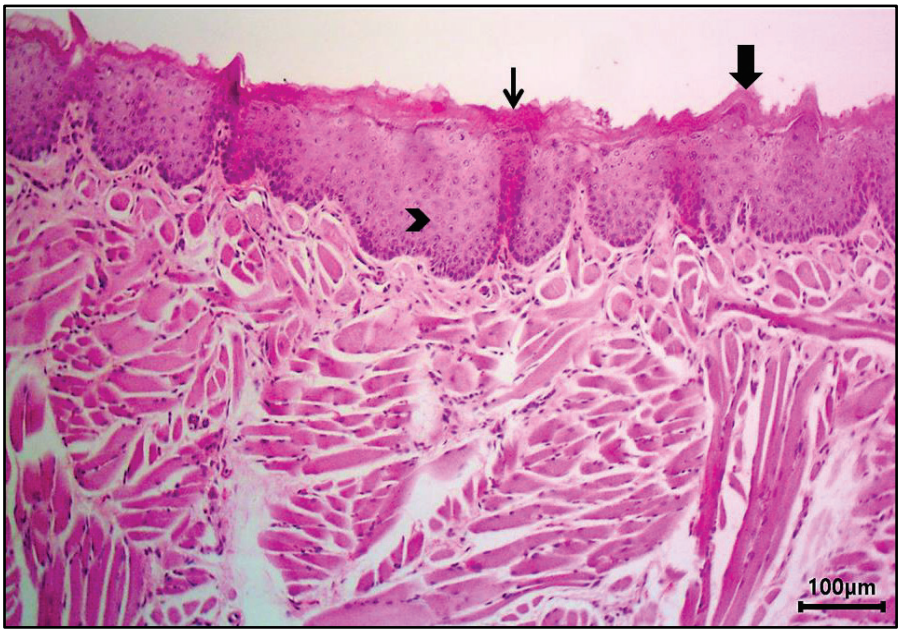

Fig. 2. Histological section of rat tongue of the ibuprofen 150 mg/kg treated group showing mild thickening of the keratin layer (parakeratosis) (arrow), mild decrease in number of taste buds (bold-arrow), mild thickening of the mucosa (arrowhead); hematoxylin and eosin staining

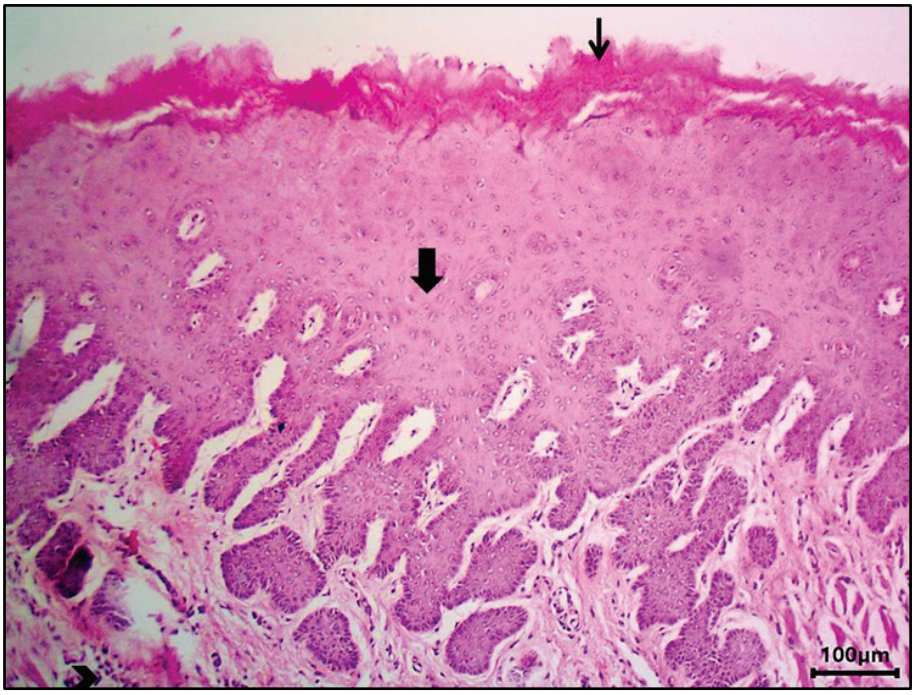

Fig. 3. Histological section of rat tongue of the ibuprofen 300 mg/kg treated group showing thickening of the keratin layer (parakeratosis) (arrow), disappearing of taste buds with thickening of mucosa (bold-arrow), and infiltration of inflammatory cells in the submucosa; hematoxylin and eosin staining

Table 1 shows the levels of glutathione, in the blood after two weeks of ibuprofen therapy. This shows variances among the three study cohorts; the control group and the groups administered 150 or 300 mg/kg of ibuprofen respectively.The results are displayed as values with error, for each group. The control group displayed the amount of glutathione at 18.3 ± 0.7 µM/mL. The group that was given a dosage of 150 mg/kg experienced a dip in the glutathione level to 17. The group that was given 300 mg/kg exhibited a decrease in glutathione concentration to 15.1 ± 0.4 µM/mL, lower than the control group and the lower dose group which had statistically significant differences, from them. Based on the data in Table 2, the levels of malondialdehyde (MDA), in the serum were measured following the two week course of ibuprofen treatment. The animals who took 150 mg/kg of ibuprofen experienced a rise in MDA levels to 2 ± 0 µM/mL, which was higher than the control group's levels but did not show any difference. The animals who were given 300 mg/kg of ibuprofen exhibited a rise in MDA concentration reaching 4.0 ± 0.3 µM/mL in comparison, to both the control group and the group that received a dosage.

Discussion

The present study explored the histopathological and biochemical impacts of ibuprofen on the structural integrity and oxidative stress status of tongue tissue in rats. The findings indicate that high-dose and prolonged administration of ibuprofen can lead to significant histological changes and oxidative imbalance, both of which may contribute to altered taste perception and tissue functionality. When ibuprofen, a commonly used prescription anti-inflammatory drug, is taken ,it works mainly by blocking certain enzymes known as cyclooxygenas-

es (COX-1 and COX-2). This action helps lower the production of prostaglandins and lessen swelling and discomfort (Tsikas, 2017; Richards & Edwards, 2020).

Table 1
Glutathione concentration in serum after treatment with ibuprofen for 2 weeks (x ± SE, n = 5)

Groups	Glutathione, µmol/g tissue
Control	18.6 ± 0.8^{a}
Group one, 150 mg/kg	17.2 ± 0.1^{a}
Group two, 300 mg/kg	15.1 ± 0.4^{b}

Table 2
Malondialdehyde concentration in serum after treatment with Ibuprofen for two weeks (x ± SE, n = 5)

Groups	MDA, µM/mL
Control	1.5 ± 0.2^{a}
Group one, 150 mg/kg	2.6 ± 0.6^{a}
Group two, 300 mg/kg	5.6 ± 0.5^{b}

Yet this blocking effect can lead to issues when taken in large amounts or over a prolonged period of time. The examination of tissue samples in this research revealed changes in the structure of rats' tongues after receiving a high dose of ibuprofen (300 mg/kg). Observations included disrupted cell arrangement under the surface epithelium, thickening of the mucosa, and the presence of parakeratosis. These suggest that the tongue tissues underwent remodeling due to stress induced by exposure to the compound. The presence of parakeratosis, where nuclei are retained in the keratinized layer, is often seen as a response to long-term irritation or injury (Toivola et al., 2024). When ibuprofen is involved in this situation, it might act as a chemical stressor that disrupts the process of cell turnover and encourages keratinization as a defense mechanism against further damage. This thickened protective layer of cells could unintentionally hinder the ability of taste buds to sense flavors by changing their surroundings and blocking the entry of taste substances (Mahajan & Gupta, 2021).

Furthermore, the observed mucosal thickening may indicate an inflammatory or regenerative response. Prolonged NSAID use has been shown to elicit mucosal proliferation and epithelial hyperplasia in various tissues, possibly as a result of sustained irritation and tissue repair processes (Risso et al., 2020). The presence of cellular abnormalities beneath the mucosa further supports the notion of ongoing inflammatory and reparative mechanisms in the tongue tissue following high-dose ibuprofen exposure. Taste buds are highly specialized epithelial structures involved in the perception of gustatory stimuli (Vishvakarma et al., 2023). Any morphological disruption to these structures may compromise their function. The reduction in taste buds observed in the ibuprofen-treated group aligns with the hypothesis that NSAIDs, particularly at high concentrations, can impair the integrity of these sensory cells (Saeed et al., 2023).

The reduction in taste bud number or function can potentially lead to dysgeusia (distorted taste) or hypogeusia (reduced taste sensitivity), both of which may have significant implications for food intake, nutrition, and quality of life. Although taste bud cells exhibit regenerative capabilities, prolonged exposure to cytotoxic agents such as ibuprofen could interfere with their normal turnover or induce apoptotic pathways, leading to reduced cell viability and function (Sheikh & Perry, 2021; Holzer-Geissler et al., 2022). This phenomenon is clinically relevant, as patients undergoing long-term NSAID therapy sometimes report alterations in taste perception, although this association remains underreported and underinvestigated in both clinical and experimental settings. Biochemical analysis of tongue tissue revealed that high-dose ibuprofen treatment led to significant oxidative stress, as evidenced by decreased levels of reduced glutathione (GSH) and elevated levels of malondialdehyde (MDA).

Glutathione is a key intracellular antioxidant responsible for detoxifying reactive oxygen species (ROS) and maintaining redox homeostasis (Engwa et al., 2022). The decrease in GSH levels among the rats who took 300 mg/kg of ibuprofen implies that their antioxidant defense system may have been overpowered. This could be a result of ibuprofen metabolism leading to an increase in ROS production (Wang & Kang, 2020). These findings align with research indicating that NSAIDs can cause oxidative stress in different organs such as the liver, kidneys, and gastrointestinal tract (Mahmoud et al., 2022).

On the other hand, rats given 150 mg/kg of ibuprofen did not display alterations in glutathione levels in comparison to the control group, suggesting that at lower dosages the body's natural antioxidant defense system can manage the oxidative stress (Fincham et al., 2023). This varying effect based on dosage highlights the significance of considering the dosage range when using ibuprofen. Malondialdehyde, a well-known byproduct of lipid peroxidation, serves as a reliable indicator of oxidative damage to cell membranes (Lısıcı et al., 2023). The significant increase in MDA levels in the high-dose group reflects substantial lipid peroxidation and membrane disruption in tongue tissue. This finding reinforces the conclusion that excessive ibuprofen intake disrupts the cellular redox balance and initiates peroxidative damage (Świacka et al., 2021). Interestingly, while the 150 mg/kg group exhibited a slight, non-significant increase in MDA, this suggests a mild oxidative impact that was effectively neutralized by the available antioxidant defense, further supporting the dose-dependent toxicity hypothesis (Ping et al., 2020).

The likely mechanism underlying these changes involves ibuprofen-induced inhibition of COX enzymes, which while beneficial for reducing inflammation, may inadvertently lead to upregulation of alternate pathways that generate ROS, such as cytochrome P_{450} metabolism and mitochondrial dysfunction (Banerjee & Maric, 2023). This ROS overproduction, if not adequately neutralized by antioxidants like glutathione, can cause extensive damage to DNA, proteins, and lipids, triggering inflammation and tissue degeneration. Moreover, ibuprofen-induced oxidative stress may activate signaling pathways such as NF-κB and MAPK, which are known to contribute to inflammation, apoptosis, and impaired cellular regeneration in various tissues (Bindu et al., 2020). The tongue, being a highly regenerative and metabolically active organ, is especially vulnerable to such perturbations.

These findings collectively suggest that while ibuprofen is generally safe at therapeutic doses, prolonged or high-dose exposure carries the risk of localized tissue toxicity, particularly in oral tissues that come into direct contact with the drug. This warrants caution in over-the-counter use and highlights the need for further research on NSAID-associated oral side effects.

Recommendations for future research investigating longer-term effects beyond 14 days of administration. Evaluating reversibility of histological and biochemical changes after drug withdrawal. Exploring the co-administration of antioxidants (e.g., vitamin C, vitamin E, N-acetylcysteine) to counteract ibuprofen-induced oxidative stress. Conducting clinical studies to assess taste alteration symptoms in human subjects on prolonged NSAID therapy.

Conclusion

This study highlights the fact that prolonged or high-dose administration of ibuprofen (300 mg/kg) can negatively affect tongue tissue structure and function in rats. Histological alterations, such as thickened keratin layers and reduction of the number of taste buds, suggest potential impairment in taste perception. Biochemically, high-dose ibuprofen induced oxidative stress, marked by decreased glutathione and increased malondialdehyde levels, indicating cellular damage. In contrast, the lower dose (150 mg/kg) caused minimal changes, implying that the body may adapt to moderate oxidative stress without significant harm. These findings suggest caution in long-term or high-dose ibuprofen use due to its potential to disrupt oral tissue health and antioxidant balance.

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