Journal of Medicine and Health (JMH) **e-ISSN: 2442-5257**
Vol. 7 No. 2 August 2025 **https://doi.org/10.28932/jmh.v7i2.9540**

Research Article

Comparative Histopathological Effects of Several Non-Selective NSAIDs on Gastric Mucosa in Wistar Rats

Komparatif Efek Histopatologis Beberapa NSAID Non-selektif terhadap Mukosa Lambung pada Tikus Wistar

Syarinta Adenina[1]*, Patwa Amani[2], Kamalia Layal[3]

[1]Bagian Farmakologi, Fakultas Kedokteran, Universitas Sriwijaya, Palembang, Indonesia
Jl. Dokter Muhammad Ali, Sekip Jaya, Kec. Kemuning, Kota Palembang, Sumatera Selatan 30114, Indonesia
[2]Bagian Fisiologi, Fakultas Kedokteran, Universitas Trisakti, Jakarta, Indonesia
Jl. Letjen S. Parman No.1, RT.6/RW.16, Grogol, Kec. Grogol petamburan, Kota Jakarta Barat, Daerah Khusus Ibukota Jakarta 11440, Indonesia
[3]Fakultas Kedokteran dan Ilmu Kesehatan, Universitas Bangka Belitung, Bangka Belitung, Indonesia
Gang IV No.1, Balun Ijuk, Kec. Merawang, Kabupaten Bangka, Kepulauan Bangka Belitung 33172, Indonesia
**Penulis korespondensi*
Email: masayusyarintaadenina@fk.unsri.ac.id

Received: July 23, 2024
Accepted: April 23, 2025

Abstract

Non-selective NSAIDs are widely used for their anti-inflammatory effects. However, their use is limited by significant gastrointestinal side effects. Little is known about which NSAIDs cause more damage to the gastrointestinal. This study aims to compare the mucosal damage induced by various non-selective NSAIDs. Adult male Wistar rats were divided into six groups and administered different NSAIDs: mefenamic acid, diclofenac sodium, piroxicam, ibuprofen, aspirin, and a control group with distilled water. After five days of treatment, the rats were sacrificed, and their gastric tissues were examined both macroscopically and microscopically for signs of damage, including hemorrhagic injury, mucosal edema, epithelial cell loss, and inflammatory cell infiltration. All NSAID-treated groups exhibited significant gastric damage compared to the control group. Diclofenac sodium and piroxicam groups showed the most pronounced hemorrhagic injury, while piroxicam induced the most severe mucosal edema. Ibuprofen caused the highest level of inflammatory cell infiltration. Aspirin resulted in the least gastric histopathological damage score among the tested drugs. Among the tested NSAIDs, aspirin exhibited the least histopathological damage, suggesting a lower risk of gastric injury in this model

Keywords*: NSAIDs; non-selective; gastric mucosal damage;histopathological examination*

How to Cite:
Adenina S, Amani P, Layal K. Comparative histopathological effects of several non-selective NSAIDs on gastric mucosa in wistar rats. Journal of Medicine and Health. 2025; 7(2): 116-28. DOI: https://doi.org/10.28932/jmh.v7i2.9540

Research Article

Abstrak

NSAID non-selektif masih sering digunakan karena memiliki efek anti-inflamasi yang kuat. Akan tetapi penggunaannya terbatas oleh efek samping gastrointestinal yang signifikan. Informasi mengenai jenis NSAID yang menyebabkan kerusakan saluran gastrointestinal yang lebih parah masih terbatas. Penelitian ini bertujuan untuk membandingkan kerusakan mukosa yang diinduksi oleh berbagai jenis NSAID non-selektif. Tikus Wistar jantan dewasa dibagi menjadi enam kelompok dan diberi perlakuan dengan berbagai jenis NSAID, yaitu asam mefenamat, natrium diklofenak, piroksikam, ibuprofen, aspirin, serta satu kelompok kontrol yang hanya diberikan air suling. Setelah lima hari perlakuan, tikus-tikus tersebut dikorbankan, dan jaringan lambungnya diperiksa secara makroskopik maupun mikroskopik untuk mendeteksi tanda-tanda kerusakan, seperti cedera hemoragik, edema mukosa, kehilangan sel epitel, dan infiltrasi sel inflamasi. Semua kelompok yang menerima perlakuan NSAID menunjukkan kerusakan lambung yang signifikan dibandingkan dengan kelompok kontrol. Kelompok yang menerima natrium diklofenak dan piroksikam menunjukkan cedera hemoragik yang paling parah, sedangkan piroksikam menginduksi edema mukosa yang paling berat. Ibuprofen menyebabkan tingkat infiltrasi sel inflamasi tertinggi, sementara aspirin menghasilkan skor kerusakan histopatologis lambung terendah di antara obat-obatan yang diuji. Disimpulkan, di antara NSAID yang diuji, aspirin menunjukkan kerusakan histopatologis paling ringan, yang mengindikasikan risiko cedera lambung yang lebih rendah dalam model ini.

Kata kunci: OAINS; non-selektif; kerusakan mukosa gaster; pemeriksaan histopatologis

Introduction

More than 30 million people routinely consume NSAIDs as anti-inflammatories, painkillers, antiplatelet drugs, and antithrombotics. NSAIDs have a wide range of side effects such as gastrointestinal ulcers, cardiovascular, kidney toxicity, congestive heart failure, and increased blood pressure. Among these side effects, the most common effect is on the gastrointestinal tract.[1–3] Gastrointestinal tract abnormalities range from petechia to ulceration, with complications of hemorrhage and perforation.[4] Not only prolonged use, some studies have mentioned that short-term use of NSAIDs is at risk of GI complications, such as gastritis.[4,5]

Based on their chemical structure or mechanism of action, NSAIDs consist of acetylicylic acid (aspirin), propionic acid derivatives (ibuprofen), acetic acid derivatives (indomethacin and diclofenac), enolic acid derivatives (piroxicam), anthranilic acid derivatives (mefenamic acid), and COX-2 inhibitors (celecoxib).[6] Salicylates, acetic acid, enolic acid, and anthranilic acid derivatives are nonselective COX inhibitors.[7]

COXs are key enzymes that play a role in the prostaglandin synthesis from arachidonic acid. Nonselective NSAIDs can inhibit COX-1 and COX-2. COX-1 plays a role in maintaining the integrity of the gastrointestinal tract. These enzymes play a role in the production and secretion of mucus and bicarbonate, increasing mucosal blood flow and promoting the proliferation of

epithelial cells. So that if the COX-1 enzyme is inhibited, unwanted side effects will occur, especially in the gastrointestinal tract.[1,2,7,8] COX-2 selective inhibitors have been subsequently developed to avoid this risk, but some COX-2 selective inhibitors have been withdrawn due to increased cardiovascular risk.[7]

Nonselective NSAIDs remain widely used for their anti-inflammatory effects. Among the existing nonselective NSAIDs, studies have reported varying degrees of selectivity in inhibiting COX isoforms.[9] The ratios COX-1/COX-2 ibuprofen, meloxicam, and diclofenac are 0,56, >1, and >1, respectively. Ibuprofen has a ratio of <1, which indicates that this drug is more selective against COX-1, while meloxicam and diclofenac have a ratio of >1, so it is more sensitive to COX-2.[8,10]

Other studies have presented different conclusions, with some describing ibuprofen as essentially nonselective. There are also conflicting reports on diclofenac's selectivity, with some studies categorizing it as nonselective and others as COX-2 selective. Piroxicam has been found to be COX-1 selective in murine models but nonselective in human blood.[8,10] From these previous studies, we can conclude that non-selective NSAIDs have different degrees of effects on the gastrointestinal tract. Although many studies have researched about NSAID gastrointestinal toxic effects on the human gastrointestinal mucosa, however, which nonselective NSAIDs cause damage in gaster still unknown.[11] The aim of this study was to compare the mucosal damage in the gaster induced by various non-selective NSAIDs.

Methods

Animals

Adult male *Rattus norvegicus*, Wistar strain rats (200-300 g, 2-3 months old) were purchased from Abduh Tikus Palembang and used in the present investigation. To prevent bias, adult male rather than female rats were chosen because of physiological variability of female.[12,13]

Animals were acclimatized to standard laboratory conditions at a constant temperature (25 ± 0.5˚C) and light/dark (12/12 h) cycles for 14 days. Rats were kept in polycarbonate cages of 40 cm × 25 cm × 20 cm, with a density of 2 animals per cage to ensure animal welfare and minimize stress due to overcrowding. The cage is placed in a room with controlled environmental conditions including humidity (40–60%), and access to water and feed (*Ad libitum*). The standard diet composition includes water content (max 14%), protein (17-19%), fat (max 7%), crude fiber (max 7%), ash (max 8%), calcium (0.9-1.2%), total phosphorus (with enzymes, 0.6-1%), total alphatoxin (max 50 μg/kg), amino acid lysine (min 0.9%), methionine (min 0.3%), methionine + cystine (min 0.6%). The feed ingredients include corn, rice bran, soybean meal, copra, meat and

bone meal, CGM, rice fragments, DDGs, and palm oil. The protocols for animal research were systematically evaluated by the Ethics Committee Medical Faculty of Sriwijaya University (No 177-2022)

Drugs, Chemicals and Reagent Kits

All used chemicals, solvents and reagents were purchased and obtained from authorized sources and of analytical grade. Ibuprofen (Ibuprofen 400 mg tablets; Phapros Tbk, Indonesia), Piroxicam (Piroxicam 20 mg tablet; Kimia Farma, Indonesia), Mefenamic acid (Mefenamic Acid 500 mg coated tablet; Dexa Medica, Indonesia), Diclofenac sodium (Diclofenac sodium 50 mg, coated tablet; Kimia Farma, Indonesia), and Aspirine (Acetylsalicylic acid 80 mg, tablet; Medifarma Laboratories, Indonesia) were used throughout the study. The doses of each drugs were determined by using Laurence & Bacharach conversion dosage methods.[14] The therapeutic dose for rat weighing 200 g = 18/1000 × adult human therapeutic daily dose (Ibuprofen 400 mg, Piroxicam 20 mg, Mefenamic acid 500 mg, Diclofenac sodium 50 mg and aspirin 80 mg) = 7,2 mg, 0,36 mg, 9 mg, 0,9 mg and 1,476 mg.[15]

The exact LD_{50} for gastric-specific lethality remains undefined for each drugs, however the dosages used in this study were based on the standard therapeutic doses for humans, adjusted using the Laurence and Bacharach conversion method. Moreover, previous studies have demonstrated that doses exceeding those administered in our study have been associated with gastritis in rats. Studies have shown that ibuprofen's gastric toxicity in Wistar rats is dose-dependent, with mucosal damage observed even at moderate doses (e.g., 44 mg/kgBW or 8,8 mg/200 gr).[16] Lisna and Stepchenko have proved that ibuprofen 400mg can induced morphological changes such as petechial hemorrhages, foci of necrosis and the formation of erosions.[17] Others also stated that Iburpofen (50 mg/ kg or 10 mg/200 gr) can cause gastric ulceration by inhibition of serum PGE2 and TXA2 levels.[18]

The use of piroxicam (30 mg/kgBW or 6 mg/200mg) resulted in large number of lesions on the gastric mucosa.[19] Meanwhile Tilarso et all has proved that piroxicam 1.8 mg/kgBW or 0,36 mg/200 mg can caused ulcer and bleeding in gaster.[20] Mefenamic acid is a non-steroidal anti-inflammatory drug (NSAID) that is commonly used for pain relief and to reduce inflammation. The oral LD_{50} of mefenamic acid in rats is approximately 740 mg/kg or 148mg/200gr).[21] The therapeutic doses of mefenamic acid in rats typically range from 25 mg/kg to 200 mg/kg, depending on the specific experimental context and objectives.[22,23] 9 mg mefenamic acid/200 g BW also have been used by Ariasti and Muhsin to study the analgesic effect of the drugs.[24]

Research Article

Elnashar *et al.* stated that Sprague-dawley rats which received diclofenac (3,5 mg/kgBW p.o) had small ulcers in the stomach.[25] Crowe and Kinsey have stated that diclofenac can induce gastric hemorrhages at doses of ≥ 33.33 mg/kg in male mice and 100 mg/kg in female mice.[26] Aspirin oral induction dose of 400 mg/kg BW or 80 mg/200 gr have been used to induced ulcers.[27,28] Meanwhile, Marcellinus et all shows aspirin 200 mg/kg BW or 40 mg/200 mg will caused ulceration of the gastrointestinal tract (GIT) lining.[29] All substances used in this study were dissolved in distilled water.

Experimental Design

After acclimatization, animals were divided randomly into 6 groups, each group contain of 2 rats. The treatments were administered orally once daily using oral feeding tube. Before oral administration of drugs, animals were fasted for 16 h. Group I: normal control group receiving distilled water, group II: receiving mefenamic acid (63 mg/kg), group III: receiving diclofenac acid (6.3 mg/kg), group IV: receiving piroxicam (2.52 mg/kg), group V: receiving ibuprofen (5.4 mg/kg) and group VI receiving aspirin (63 mg/kg). After 5 days treatments, animals were anesthetized by ether inhalation after 1 h fasting.

Experimental studies show that NSAIDs can induce measurable gastric damage within a short timeframe (from hours to days of administration).[30–32] Previous studies have proved that 5 days of NSAID resulted in significant histopathological changes in Wistar rats, indicating that this duration is sufficient to observe NSAID-induced gastric lesions.[33] While acute damage can occur within hours or days, shorter durations may not allow sufficient time for cumulative effects to manifest fully. Prolonged exposure could also lead to complications by causing compensatory mechanisms that could affect the study outcomes.[34]

Animals were carefully dissected, and the stomachs were removed by pyloric ligation for gastric juice collection. After that, the animals were sacrificed by cervical dislocation. The gaster then prepared to morphological and microscopic examination.

Determination of gastric acidity

Gastric content was centrifuged at 4000 rpm for 10 min, and the supernatant was collected. The pH of the supernatant was examined by pH universal strips.[35,36]

Morphological and Microscopic Histopathological Examination

Rat’s gasters were cut along the smaller curvature, washed with saline (0,9% NaCl) expand, and fixed on the dissection plate with their edges held with the aid of office pins. Gaster

then photographed using an appropriate digital camera. The ulcer area will look redder in color than the normal gastric area.[37] After visualization, gaster tissues were prepared for microscopic examination. The gaster tissue was fixed in 10% neutral buffered formalin, embedded in paraffin and cut into 4 μM thick sections by slide microtome. To detect microscopic gastric injury, the sections were stained with hematoxylin and eosin using standard procedures, followed by examination under light microscopy. Histopathological changes were assessed by an experienced and qualified pathologist blinded to treatments. Gastric structure was determined on a 0-4 scale, and each tissue section was examined for hemorrhagic damage (0–4), mucosal edema (0–4), presence of inflammatory cells infiltration (0–3), and epithelial cell loss.[38]

Results

The measured gastric pH values in each group are presented in Table 1. The pH of gastric juice in the stomachs of rats in the NSAID groups was slightly decreased compared to the normal group.

The macroscopic examination of the gastric mucosa from the control group revealed healthy pink-colored gastric mucosa without any lesions, showing no visible signs of gastritis or ulcers. In contrast, redness was observed in the gaster tissue of all NSAID groups (mefenamic acid, diclofenac sodium, piroxicam, ibuprofen, and aspirin). Additionally, multiple erosions were found in the mefenamic acid group (Figure 1). In the control group, microscopic examination showed a normal histological structure lined by stratified squamous keratinized epithelium with underlying submucosa and muscularis, with no signs of hemorrhages, congestion, or mucosal epithelium disturbances (Fig. 2a).

Table 1 Gastric Content Acidity

Group	Groups	pH
I	Aquadest	6
II	Mefenamic Acid	4-5
III	Diclofenac Natrium	4-5
IV	Piroxicam	4-5
V	Ibuprofen	5
VI	Aspirin	5

Research Article

Administration of mefenamic acid, diclofenac sodium, piroxicam, ibuprofen, and aspilet all resulted in substantial variations in the gaster's histological appearance, including epithelial cell loss, hemorrhagic injury, edema, and inflammatory cell infiltration. The differences between the groups were notable, with some changes being more prominent than others. Hemorrhagic injury was more dominant in the diclofenac sodium and piroxicam groups. Edema was more pronounced in the piroxicam group. Ibuprofen administration caused more inflammatory cell infiltration than other NSAIDs. Overall, based on the total score, aspilet caused the least damage compared to other NSAIDs.

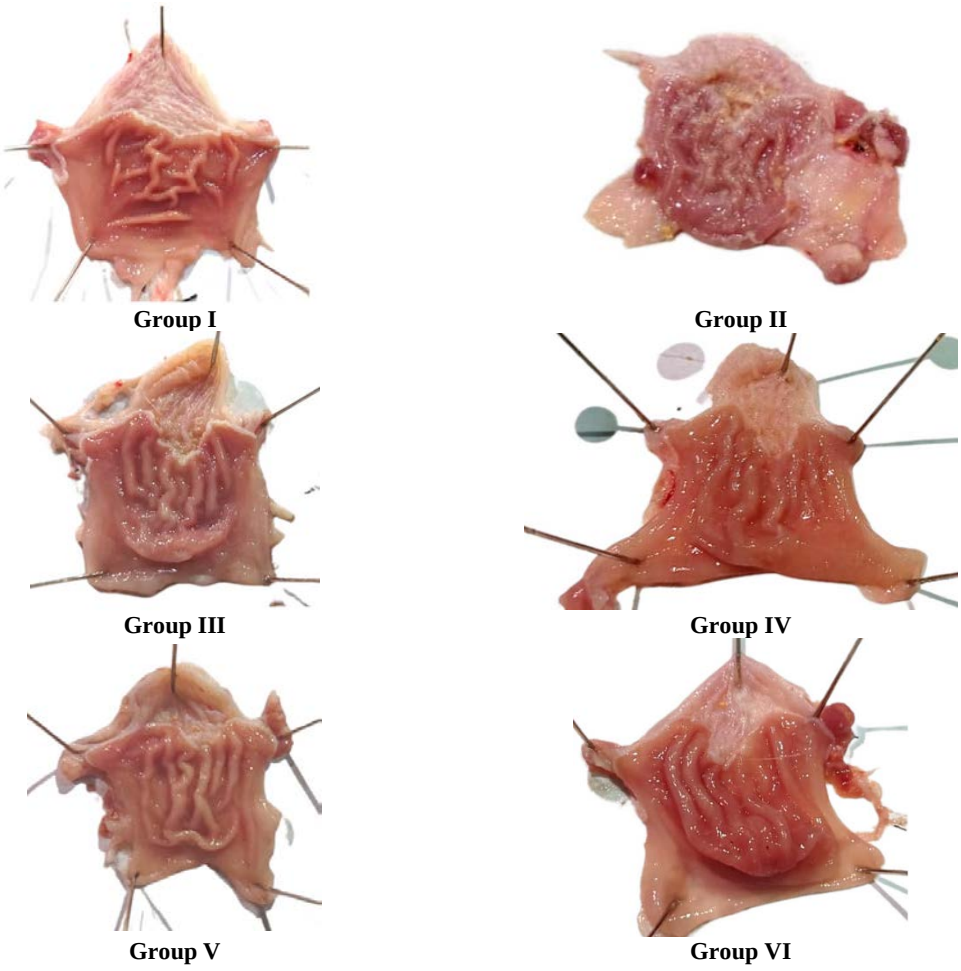
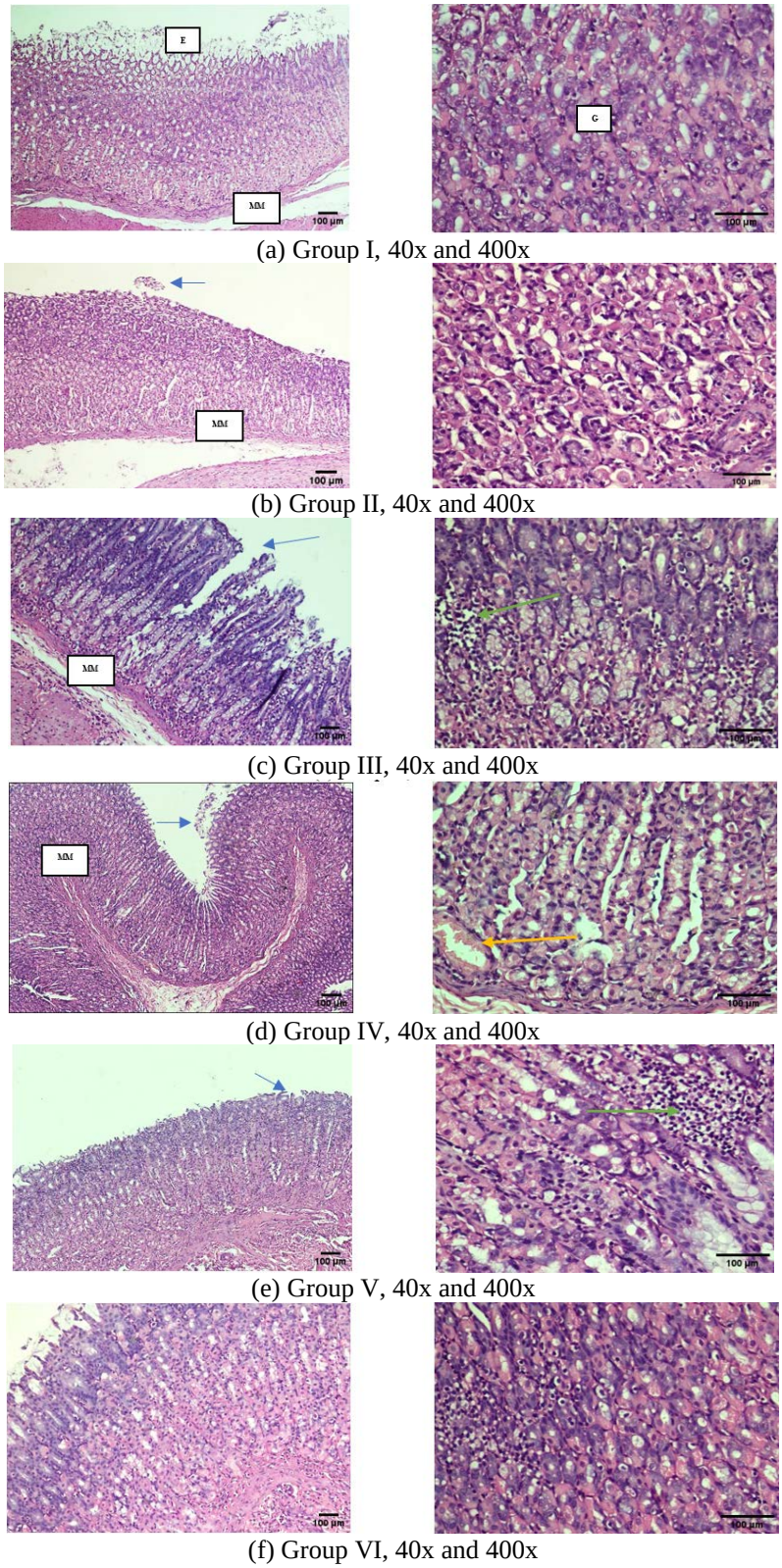


Figure 1 Gaster Macroscopic
I: Aquadest; II: Mefenamic Acid; III: Diclofenac Acid; IV: Piroxicam; V: Ibuprofen; VI: Aspilet

Research Article



(a) Group I, 40x and 400x

(b) Group II, 40x and 400x

(c) Group III, 40x and 400x

(d) Group IV, 40x and 400x

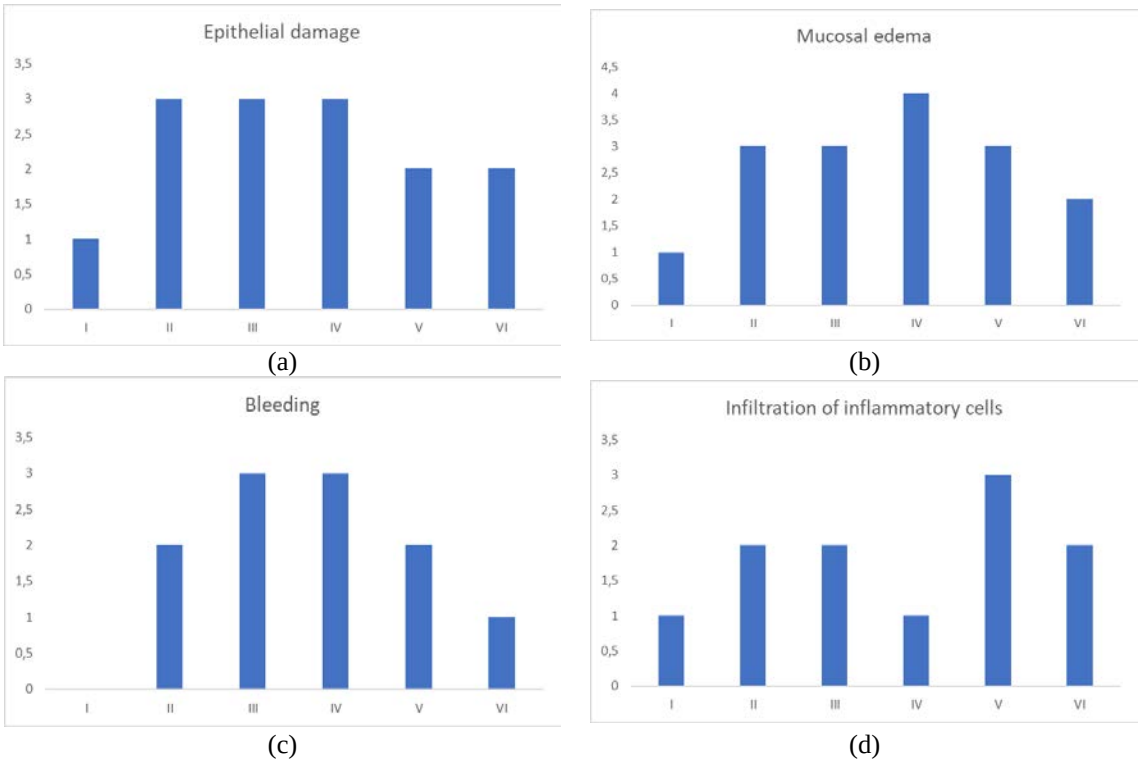
(e) Group V, 40x and 400x

(f) Group VI, 40x and 400x

Figure 2 Histological Structure of Gaster with H&E Stain, 40x and 400x

Blue arrow shows epithelial erosion, green arrow shows inflammatory cells infilitration (lymphocyte), and yellow arrow shows bleeding. I: Aquadest; II: Mefenamic Acid; III: Diclofenac Acid; IV: Piroxicam; V: Ibuprofen; VI: Aspilet.. (E): Stratified squamous keratinized epithelium; (MU): Underlying submucosa (MU); (MM): Muscularis; (G): Gastric glands.

Research Article



(a) (b)

(c) (d)

Figure 3 Histopathological Examination Tabulation
I: Aquadest; II: Mefenamic Acid; III: Diclofenac Acid; IV: Piroxicam; V: Ibuprofen; VI: Aspilet.

Discussion

NSAID are anti-inflammatory drugs that have been used widely in the world. Many studies have reported GI complications caused by these drugs. The most common diseases by NSAID usage are erosive-hemorrhagic gastritis (20-40%) and peptic ulcer (10-30%).[3] In this study, we observed that gastric juice pH are slightly lower compare with the control group. Normal gastric juice pH rats from other studies are around 6,5±0,08.[39]

Gastric pH secretion occurs due to the activity of the parietal cells H+/K+ ATPase which is controlled by acetylcholine, gastrine, histamine and intracellular signals (cAMP and calcium signalling pathways).[40] The decrease in pH occurs due to an increase in the gaster epithelial layer's permeability, resulting in the acid's reverse diffusion into the lumen. The prostaglandin inhibitory effect by NSAIDs also leads to increased gastric acid secretion.[41] However, other studies have stated that ulcers caused by NSAIDs is independent of gastric acid.[42] 21 NSAIDs caused by ulcers are characterized by hyperplasia and mucosal edema, but only a small infiltration of inflammatory cells.[43]

The gaster has a mucosal lining as the first line of defense against external stimuli.[44] Several studies have indicated that NSAIDs can induce gastrointestinal damage in the mucosa,

such as ulcers, erosion especially in epithelial cells, cell infiltration, congestion of blood vessels, and leukocyte aggregation.[45,46] Macroscopic and microscopic examinations revealed no significant differences among the NSAID groups, although some groups exhibited more pronounced damage in specific aspects, possibly due to the duration and dose of NSAID administration.[5] The condition of gastric mucosal in this study was similar to Santos et all, Tandoh et all, Eliot et all, Wang et all, and Firdaus et all. So, it can be concluded that mefenamic acid, diclofenac natrium, piroxicam, ibuprofen and aspirin could damage gaster.[37,41,47–50]

These studies proved all NSAID can induce epithelium cell loss. Gastric mucosal lession happen through local and systemic. Some NSAID can directly damage epithelial cells. Various mechanisms have been proposed for this cytotoxic action. NSAIDs with a carboxylic acid residue can enter epithelial cells in the gaster. When these cells are intracellular, the drug will be converted into an ionic form and cannot escape. This is known as ion trapping. The more drugs accumulate in the epithelial cells, the swelling of the epithelial cells will occur and cause lysis.[51] Meanwhile the systemic actions occur through its ability to suppress gastric prostaglandin synthesis.[52] Inhibition in COX-1 will cause a decrease in prostaglandins. These prostaglandins play an important role in maintaining mucosal integrity, blood flow and stimulating mucus and bicarbonate secretions.[41] A decrease in mucus and bicarbonate secretion will lead to a decrease in the effectiveness of the pH gradient of juxtamucosal that protects the epithelium.[51] NSAIDs can also trigger the production of free radicals that can interact with adenyl cyclase and alter the concentration of cAMP. The use of NSAIDs also causes microvascular damage, decreased blood flow, and increased expression of adhesion molecules and neutrophil molecules in the vascular epithelium.[37,47–49] NSAID drugs effect was dose-dependent. Drugs used in this study have lower dose compared to the previous study.[41]

In this study, hemorrhage was most frequently observed in the piroxicam and diclofenac sodium groups. Diclofenac is known to have a selective effect in inhibiting COX-2 compared to COX-1. Prostaglandins derived from COX-2 are protective against damage induced by hepatotoxins. Rats given diclofenac showed gastrointestinal damage evident in histopathological changes. Similar to the studies conducted by Mostafa et al, the diclofenac group exhibited abnormal histological structures manifested by epithelial erosion, mucosal edema, bleeding, along with inflammatory cellular infiltration in the submucosa.[53] In this study the piroxicam group was the most severe mucosal edema. Gastritis mucosal oedema can be caused by vascular changes due to inflammation.[54] The administration of aspirin in this study caused the least amount of bleeding. Low-dose aspirin in rats is known not to cause bleeding, but it can result in mucosal damage and epithelial cell exfoliation[43,51,55]

Research Article

This study has limitations that should be acknowledged, such as a small sample size will reduce the statistical power of the findings and the method used to assess gastric pH. Future studies should be conducted to investigate the effect of NSAIDs by using larger sample sizes and standardized pH meter measurements.

Conclusion

The study demonstrates that all tested NSAIDs induced histopathological damage to the gastric mucosa, although the severity and specific patterns of injury varied among them. Piroxicam induced the most pronounced mucosal edema, while ibuprofen led to the greatest inflammatory cell infiltration. Among the NSAIDs studied, aspirin exhibited the least histopathological damage; however, this finding should be interpreted cautiously, as aspirin's long-term GI safety profile remains a concern. Further research on prostaglandin levels and COX-1/COX-2 selectivity is needed to clarify the differential gastrointestinal effects of NSAIDs.

References

1. da Cunha Jácome Marques F, da Silva Pantoja P, Matos VEA, Silva RO, Damasceno SRB, Franco ÁX, et al. Galactomannan from the seeds of Caesalpinia pulcherrima prevents indomethacin-induced gastrointestinal damage via neutrophil migration. Int J Biol Macromol. 2019;141:68–75.
2. Shim KN, Kim J Il, Kim N, Kim SG, Jo YJ, Hong SJ, et al. The efficacy and safety of irsogladine maleate in nonsteroidal anti-inflammatory drug or aspirin-induced peptic ulcer and gastritis. Korean J Intern Med. 2019;34(5):1008–21.
3. Fang YF, Xu WL, Wang L, Lian QW, Qiu LF, Zhou H, et al. Effect of hydrotalcite on indometacin-induced gastric injury in rats. Biomed Res Int. 2019;2019:1–9.
4. Quinn CM, Bjarnason I, Price A. Gastritis in patients on non-steroidal anti-inflammatory drugs (NSAIDs). Histopathology. 1995;26(4):391–2.
5. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochem Pharmacol. 2020; 180:114147
6. Ioele G, Grande F, De Luca M, Occhiuzzi MA, Garofalo A, Ragno G. Photodegradation of anti-inflammatory drugs: Stability tests and lipid nanocarriers for their photoprotection. Molecules. 2021;26(19).
7. Jordan F, Tj Q, Mcguinness B, Passmore P, Jp K, C TS, et al. Aspirin and other non-steroidal anti-inflammatory drugs for the prevention of dementia. Cochrane Database Syst Rev. 2020;(4):1–66.
8. García-rayado G, Navarro M, Lanas A. NSAID induced gastrointestinal damage and designing GI-sparing NSAIDs. Expert Rev Clin Pharmacol. 2018;16(7):625–37.
9. Cryer B, Feldman M. Cyclooxygenase-1 and cyclooxygenase-2 selectivity of widely used nonsteroidal anti-inflammatory drugs. Am J Med. 1998;104(5):413–21.
10. Wong RSY. Role of nonsteroidal anti-inflammatory drugs (NSAIDs) in cancer prevention and cancer promotion. Adv Pharmacol Sci. 2019;2019:1-10.
11. Cooper C, Chapurlat R, Al-Daghri N, Herrero-Beaumont G, Bruyère O, Rannou F, et al. Safety of oral non-selective non-steroidal anti-inflammatory drugs in osteoarthritis: what does the literature say?. Drugs and Aging. 2019;36(s1):15–24.
12. Coquoz A, D. R, and Stute P. Impact of progesterone on the gastrointestinal tract: a comprehensive literature review. Climacteric. 2022;25(4):337–61.
13. Kim N. Gastroduodenal diseases BT - sex/gender-specific medicine in clinical areas. In: Kim N, editor. Singapore: Springer Nature Singapore; 2024. p. 95–136. Available from: https://doi.org/10.1007/978-981-97-0130-8_5
14. Laurence D., Bacharach A. Evaluation Of drug activities-pharmacometrics Vol-1. London: Academic Press Inc (London) LTD; 1964.p. 1–453.
15. Ajeng D, Ardiaria M, Kurniawati DM, Ayustaningwarno F. The effect of edamame milk on triglyceride levels in diabetes Wistar rats. Sport Med Curiosit J. 2022;1(2):59–64.
16. Adeyeye CM, Bricker JD, Vilivalam VD, Smith WI. Acute gastrointestinal toxic effects of suspensions of

unencapsulated and encapsulated ibuprofen in rats. Vol. 13, Pharmaceutical Research. 1996. p. 784–93.
17. Lisna AY, Stepchenko LM. Haematological changes in laboratory rats with Ibuprofen-induced gastric ulcer treated with Humilid. Theor Appl Vet Med. 2022;10(2):15–22.
18. Bastaki SMA, Padol IT, Amir N, Hunt RH. Effect of aspirin and ibuprofen either alone or in combination on gastric mucosa and bleeding time and on serum prostaglandin E2 and thromboxane A2 levels in the anaesthetized rats in vivo. Mol Cell Biochem. 2018;438(1–2):25–34.
19. Abd el wahab H, Mohamed MEM, Kamel MA. Anti-ulcer and gastroprotective effects of dexlansoprazole in experimentally induced gastric ulcer in rats. Zagazig Vet J. 2023;51(2):201–15.
20. Tilarso DP, Bachri MS, Widyaningsih W. Gastroprotective effect of Canna edulis Ker. ethanolic extract in piroxicam-induced rats. Pharmaciana. 2021;11(1):39.
21. Pfizer. Material Safety Data Sheet. 2007;1–8. Available from: http://www.fishersci.in/msds/cinnamicacid.pdf
22. Aguiar GC, Queiroz-Junior CM, Sitta GL, Amaral FA, Teixeira MM, Caliari M V., et al. Mefenamic acid decreases inflammation but not joint lesions in experimental osteoarthritis. Int J Exp Pathol. 2016;97(6):438–46.
23. Seyyedi R, Talebpour Amiri F, Farzipour S, Mihandoust E, Hosseinimehr SJ. Mefenamic acid as a promising therapeutic medicine against colon cancer in tumor-bearing mice. Med Oncol. 2022;39(2):18.
24. Ariasti M, Muhsin LB. Analgesic activity test of Inggu leaf (*Ruta angustifolia* [L.] Pers) with Tail Flick and writhing test method. 2024;10(2):139–47.
25. Adel Elnashar A, Kamal HI, Magdy MA, Gamal T, Hamid L, Ragab SFM, et al. Review on diclofenac toxicities in different organs. Ain Shams J Forensic Med Clin Toxicol. 2024;42(1):10–24.
26. Crowe MS, Kinsey SG. MAGL inhibition modulates gastric secretion and motility following NSAID exposure in mice. Eur J Pharmacol. 2017;807:198–204.
27. Simbolon RO, Dalimunthe A, Bangun H. The effect of ethanol extract of avocado leaf (Persea Americana Mill.) on reduction of gastric acid in aspirin-induced rats. J La Medihealtico. 2024;05(03):590–9.
28. Chukwudoruo SC, Okereke SC, Nwaokezie CO, Nwaekpe CG, Ochijeh FE, Okafor CP, et al. Gastro-protective effect of Ageratum conyzoides (Linn) leaf extract on aspirin-induced ulcer in albino rats. 2025;27(21):62–9.
29. Marcellinus AE, Olubunmi EO, Poh CF, Olayiwola SJ. The ameliorative effect of andrographis paniculata on aspirin-induced ulcerogenic and hepatic damage in Wistar rats. 2024;3987(2):38–48.
30. Morsi AA, Shawky LM, Shawky TM, Bahr MH, Alnasr MTA, El Bana E. Targeting NF-κB/COX-2 signaling by soyasaponin I alleviates diclofenac-induced gastric ulceration in male albino rats. Cell Biochem Funct. 2024;42(1):e3927.
31. Ansari AZ, Doshi GM. Evaluation of the anti-ulcer activity of curcumin and linseed oil in an NSAID-induced gastric ulcer model in rats. Indian J Pharm Educ Res. 2024;58(1):252–61.
32. Altuner D, Kaya T, Suleyman H. The protective effect of lercanidipine on indomethacin-induced gastric ulcers in rats. Brazilian Arch Biol Technol. 2020;63:1–8.
33. Nugroho TE, Hakim DAR, Satoto HH. Perbandingan gambaran histopatologi lambung tikus Wistar setelah pemberian deksketoprofen dan ketorolak. JAI J Anestesiol Indones. 2019;11(1):19.
34. Moro MG, Sanchez PKV, Gevert MV, Baller EM, Tostes AF, Lupepsa AC, et al. Gastric and renal effects of COX-2 selective and non-selective NSAIDs in rats receiving low-dose aspirin therapy. Braz Oral Res. 2016;30(1):1–7.
35. Fahmi A, Abdur-Rahman M, Aboul-Naser AF, Hamed MA, Abd-Alla HI, Nasr MI. Pulicaria crispa mitigates gastric ulcer induced by ethanol in rats: Role of treatment and auto-healing. Biomarkers. 2018;1–32.
36. Hatware KV, Sharma S, Patil K, Shete M, Karri S, Gupta G. Evidence for gastroprotective, anti-inflammatory and antioxidant potential of methanolic extract of Cordia dichotoma leaves on indomethacin and stress-induced gastric lesions in Wistar rats. Biomed Pharmacother. 2018;103:317–25.
37. Badriyya E, Mandasari Z, Aldi Y, Almahdy A. Gastroprotective effect of propolis against male white mice gastric ulcers induced by aspirin. Adv Heal Sci Res. 2022;40(Iccscp):98–103.
38. Nabil M, El Raey MA, Abdo W, Abdelfattah MAO, El-Shazly AM, Sobeh M, et al. Gastro-protective effects of Albizia anthelmintica leaf extract on indomethacin-induced gastric ulcer in Wistar rats: in silico and in vivo studies. Antioxidants. 2021;10(2):1–18.
39. Beiranvand M, Bahramikia S. Ameliorating and protective effects mesalazine on ethanol-induced gastric ulcers in experimental rats. Eur J Pharmacol. 2020;888:173573.
40. Tamaddonfard E, Erfanparast A, Farshid AA, Imani M, Mirzakhani N, Salighedar R, et al. Safranal, a constituent of saffron, exerts gastro-protective effects against indomethacin-induced gastric ulcer [Internet]. Vol. 224, Life Sciences. Elsevier Inc; 2019. 88–94 p. Available from: https://doi.org/10.1016/j.lfs.2019.03.054
41. Tandoh A, Danquah CA, Ossei PPS, Benneh CK, Ayibor WG, Woode E. Protective effects of andrographolide against diclofenac-induced gastric damage. Sci African. 2021;13:e00944. 42. Satoh H, Akiba Y, Urushidani T. Proton Pump inhibitors prevent gastric antral ulcers induced by NSAIDs via activation of capsaicin-sensitive afferent nerves in mice. Dig Dis Sci. 2020;65(9):2580–94.
43. Bebb J, James MW, Atherton J. Gastritis and peptic ulcer. Handb Mucosal Immunol. 1994;435–8.
44. Razzaq A, Qureshi IZ. Naproxen sodium nanoparticles are less toxic and gastroprotective agents than the conventional NSAID drug naproxen sodium in Balb/c mice. Toxicol Appl Pharmacol. 2022;452:116192.
45. AlKreathy HM, Alghamdi MK, Esmat A. Tetramethylpyrazine ameliorates indomethacin-induced gastric ulcer in rats: impact on oxidative, inflammatory, and angiogenic machineries. Saudi Pharm J. 2020;28(8):916–26.

46. Koc K, Cerig S, Ucar S, Colak S, Bakir M, Erol HS, et al. Gastroprotective effects of oleuropein and thymol on indomethacin-induced gastric ulcer in Sprague-Dawley rats. Drug Chem Toxicol. 2020;43(5):441–53.
47. Wang Z, Hasegawa J, Wang X, Matsuda A, Tokuda T, Miura N, et al. Protective effects of ginger against aspirin-induced gastric ulcers in rats. Yonago Acta Med. 2011;54(1):11–9.
48. Firdaus SB, Ghosh D, Chattyopadhyay A, Dutta M, Paul S, Jana J, et al. Protective effect of antioxidant-rich aqueous curry leaf (Murraya koenigii) extract against gastro-toxic effects of piroxicam in male Wistar rats. Toxicol Reports. 2014;1:987–1003.
49. Elliott G, Whited BA, Purmalis A, Davis JP, Field SO, Lancaster C, et al. Effect of 16,16-dimethyl PGE2 on renal papillary necrosis and gastrointestinal ulcerations (gastric, duodenal, intestinal) produced in rats by mefenamic acid. Pergamon. 1986;39(3):423–32.
50. de Cássia dos Santos R, Bonamin F, Périco LL, Rodrigues VP, Zanatta AC, Rodrigues CM, et al. Byrsonima intermedia A. Juss partitions promote gastroprotection against peptic ulcers and improve healing through antioxidant and anti-inflammatory activities. Biomed Pharmacother. 2019;111:1112–23.
51. Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? Physiol Rev. 2008;88(4):1547–65.
52. Wallace JL. How do NSAIDs cause ulcer disease? Baillieres Clin Gastroenterol. 2000;14(1):147–59.
53. Mostafa RE, El-Marasy SA, Abdel Jaleel GA, Bakeer RM. Protective effect of royal jelly against diclofenac-induced hepato-renal damage and gastrointestinal ulcerations in rats. Heliyon. 2020;6(2):e03330.
54. Patel PK, Patel SK, Dixit SK, Rathore RS. Gastritis and peptic ulcer diseases in dogs: a review. Int J Curr Microbiol Appl Sci. 2018;7(03):2475–501.
55. Choi J il, Raghavendran HRB, Sung NY, Kim JH, Chun BS, Ahn DH, et al. Effect of fucoidan on aspirin-induced stomach ulceration in rats. Chem Biol Interact. 2010;183(1):249–54.