

The protective effects of Cilostazol on indomethacin induced gastric ulcer in rat model

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Abstract:

Introduction: Gastric ulcer is the most common gastrointestinal tract disorder, representing about 20% of peptic ulcers. To examine the preventive impact of Cilostazol on histology, gastric ulcer severity, inflammatory and oxidative stress markers (TNF-alpha, myeloperoxidase and Malondialdehyde) in an indomethacin-induced gastric ulcer model. **Method:** 50 albino male rats, each with 10 (N=10): Group A: Oral Indomethacin vehicles (normal saline 0.9% and tween 80) as positive control group. Group B: Indomethacin 60mg/kg orally through oral gavage as negative control. Group C: Omeprazole 20mg/kg gavage 1 hour before 60mg/kg oral Indomethacin. Group D: Cilostazol 10mg/kg via gavage tube 60mg/kg Indomethacin orally 1 hour before. **Results:** Cilostazol 10 mg/kg pretreatment groups exhibit a substantial (p<0.01) decrease in stomach ulcer severity and histopathological damage score. Pretreated groups with cilostazol 10mg/kg reduced inflammatory indicators (TNF-alpha and Myeloperoxidase) and oxidative stress marker (Malondialdehyde), comparable to the reference medication. **Conclusion:** Cilostazol with cilostazol 1 hour before indomethacin administration shows a reduction and improvement in the damaging effect of indomethacin, both drugs were equally effective and show similar efficacy as standard omeprazole drug by a decrease in oxidative stress status manifested by a reduction in lipid peroxidation indicator marker as well as reduction in pro-inflammatory cytokine level and leukocyte recruitment indicator marker.

Keywords: cilostazol, trimethazidin, oxidative stress, TNF-alpha, malondi aldehyde and myeloperoxidase.

1. Introduction:

Abrasion of gastric lining epithelium occurs due to imbalance between gastric defense, protective mechanism and aggressive factors represent about 20% of peptic ulcer start as mild erosion of epithelia lining of the stomach lumen and extend deeper to muscularis mucosa or submucosa in 5 mm in diameter or greater [1]. Gastric ulcer (GU) is the most widespread disease of the gastrointestinal tract approximately 5-10% of populations are affected [2]. A multifactorial gastric ulcer disease leads to prevalence differences between countries and appears to be more prevalent in developing countries and densely populated regions than in developed countries due to low socioeconomics and hygiene habits [3]. Gastric ulcer is highly linked to *Helicobacter Pylori* (*H. pylori*) infection and chronic non-steroidal anti-inflammatory drugs (NSAID) use, the incidence of gastric ulcer is about 80 percent in *H. Pylori* infected than non- infected patients and 10-30. percent of gastric ulcer is related to chronic NSAID use [4]. *H. pylori* prevalence increases with age (53.3%) and appear to be more in female 59.72 % than male 43.75 % [5]. Gastric ulcer is a multifactorial disease, endogenous and exogenous factors involved in GU development. Gastric ulcer is highly related to *H. Pylori* infection a common human pathogen responsible for ulcer formation usually found beneath the mucus layer, multi virulent factors involved in *H. pylori* toxic effect [6]. NSAIDs play an important role in gastric ulcer formation by reducing gastric defense layers through inhibition of prostaglandin synthesis by blocking cyclo-oxygenase

(COX) enzyme isoforms [7]. Parietal cells secrete acid and considered the first line of defense mechanism against bacterial overgrowth and colonization also enhance absorption of some important materials include iron, calcium and B12, over production of gastric acid cause mucosal damage by entering the gastric lumen through channels in mucus layer created by high glandular hydrostatic pressure during secretion thus lead to convert superficial erosion to a deeper lesion, disturb mucosal integrity and inactivate acid liable factors [8]. Analgesic, anti-inflammatory and antipyretic weak acid drugs with good gastrointestinal absorption, highly protein binding and bioavailability, it's most widely used worldwide especially by the elderly to relieve rheumatoid arthritis pain [9]. Numerous Quinolinon derivatives have been synthesised and studied to develop an anti-platelet and vasodilator drug; cilostamide has effective anti-platelet and vasodilator activity; however, the amide moiety side chain causes tachycardia; therefore, efforts have been made to remove the side chain and reduce the side effect [10]. FDA authorised CSZ as an antiplatelet and vasodilator for IC to increase blood flow and oxygen supply to legs, reduce discomfort, and increase walk distance [11]. The aim of study is to examine the preventive impact of Cilostazol on histology, gastric ulcer severity, inflammatory and oxidative stress markers (TNF-alpha, myeloperoxidase and Malondialdehyde) in an indomethacin-induced gastric ulcer model.

2. Method

Comparative and prospective animal study. This
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study was conducted at Mustansyria University's Research Center for Cancer and Medical Genetics from January 2022 and finished in August 2022. This study was performed on 30 healthy albino male rats, between (11-12) weeks in age and (200-300) gm in weight. All rats were starved for at least 24 hours before indomethacin administration since prior feeding has been proven to decrease the ulcerogenic action of some drugs; on the day of the experiment water was held two hours before the beginning of procedure. Cilostazol powder for laboratory use only with 99.7% of purity as Cilostazol hydrochloride (HCL). Cilostazol prepared by dissolving in vehicle of normal saline and tween 80 in a dose of 12.5 mg /ml, the selection of 50mg/kg based on pilot study in which two doses are tested 20mg/kg and 50mg/kg, 50mg chosen based on macroscopic evaluation of gastric mucosal erosion severity in blinded manner by pathologist. After 7 days of adaptation, experimental rats were randomly directed to one of five groups each group include 10 rats, each as follows: **Group A:** rats orally administered 1ml of indomethacin vehicle (only normal saline and tween 80) via gavage tube after 24 h of fasting. This group served as a negative control. **Group B:** rats orally received 60mg/kg of indomethacin solution after 24 h of fasting. This group served as a positive control. **Group C:** after 24 h of fasting, rats were pretreated orally with 20 mg/kg of omeprazole solution one hour before indomethacin solution induction. **Group D:** after 24 h of fasting, rats pretreated orally with 10 mg/kg of cilostazol solution one hour before indomethacin solution induction. Tissue of rats of all groups was

harvested at the end of experiment and histopathological changes of stomach of each rat were evaluated and scored as follows [12]. Quantification of protein expression was evaluated under light microscopy at 20X. The extent of the immunohistochemically reaction of proteins, such as MDA, MPO and TNF α was measured by percentage of positively stained cells according to the following scale [13]. The software used for data analysis was SPSS (Statistical Packages for Social Sciences) version 26. The data were represented by their mean, standard deviations as well as 95% confidence level in graphs. The analysis of variances (ANOVA) test was used to compare the variable mean according to study groups, then least the square differences test was used as a post-hoc test to find the comparable differences among groups.

3. Results

Oral administration of 60mg/kg of indomethacin causes a significant increase in gastric ulcer number compared to a healthy group, while pretreated groups with omeprazole, cilostazol show a significant reduction in ulcer number compared to the ulcer control group. Indomethacin has the highest mean number score (3.8 $\pm$ 0.4) compared to other groups, while omeprazole 20mg/kg has the lowest mean number score (0.4). Indomethacin appears to induce the most mucosal injury (100%), omeprazole demonstrated a greater inhibition rate (89.5%), and both medicines vary substantially from omeprazole and the healthy group. As in table 1.

Table 1: Effect of cilostazol on gastric lesions number score and damage percentage of indomethacin ulcerated rats, according to study groups.

Groups	Lesions number	Damage %
Healthy	0 $\pm$ 0 A	0% $\pm$ 0% A
Indomethacin	3.8 $\pm$ 0.4 B	100% $\pm$ 11.1% B
Indomethacin + Omeprazole	0.4 $\pm$ 0.5 C	10.5% $\pm$ 13.6% C
Indomethacin + Cilostazol	1.4 $\pm$ 0.5 D	36.8% $\pm$ 13.6% D
P-value	<0.001**	<0.001**

ANOVA test, **significant at 0.01.

Oral administration of 60mg/kg of indomethacin increases ulcer severity after 4 hours and shows a highest severity score (4 $\pm$ 0) in compare with a healthy group (0 $\pm$ 0), omeprazole reveals the lowest mean score of ulcer severity (0.4 $\pm$ 0.5) among the pretreated group with significant difference from healthy and ulcer control group, cilostazol in a mean score of (1.4 $\pm$ 0.5). Table contrasts omeprazole and

healthy group (2). Indomethacin showed a high damaging effect (100%) compared to the healthy group (0%), omeprazole showed the highest ulcer severity score inhibition rate (90%) compared to the pretreated group, and cilostazol 10mg/kg showed no significant difference in decreasing ulcer severity but differed significantly from the healthy and omeprazole pretreated groups. As show in table 2.

Table 2: Effect of cilostazol on gastric lesions severity score and damage percentage of indomethacin ulcerated rats, according to study groups.

Groups	Severity score	Damage %
Healthy	0 $\pm$ 0 A	0% $\pm$ 0% A
Indomethacin	4 $\pm$ 0 B	100% $\pm$ 0% B
Indomethacin + Omeprazole	0.4 $\pm$ 0.5 C	10% $\pm$ 12.9% C
Indomethacin + Cilostazol	1.4 $\pm$ 0.5 D	35% $\pm$ 12.9% D
P-value	<0.001**	<0.001**

ANOVA, **significant at 0.01.

Histopathological changes of cilostazol 10mg/kg 1

hour before indomethacin 60 mg/kg administration,

Indomethacin administration shows a high value of damage score (4 ± 0) in compare with a healthy group (0 ± 0) that microscopic appearance showed normal architecture of mucosa, indomethacin significantly differ from other groups and

microscopic investigation revealed severe histopathological changes include erythema, edema of the mucosal layer as well as inflammatory reaction and congestion.

Table 3: Effect of cilostazol on mean histopathological damages scores comparison, according to study groups.

Groups	Histopathology damage scores	Damage %
Healthy	0 ± 0 A	$0\% \pm 0\%$ A
Indomethacin	4 ± 0 B	$100\% \pm 0\%$ B
Indomethacin + Omeprazole	0.4 ± 0.5 C	$10\% \pm 12.9\%$ C
Indomethacin + Cilostazol	1.2 ± 0.4 D	$30\% \pm 10.6\%$ D
P-value	$<0.001^{**}$	$<0.001^{**}$

ANOVA test, ** significant at 0.01

According to the pattern of MPO, study groups could be divided into two categories those with relatively low MPO expression groups which include healthy and pretreated groups with omeprazole, cilostazol with MPO mean score (1 ± 0) and differ

significantly from the ulcer control group, other category include indomethacin administered group with higher MPO expression level with mean score of (4 ± 0) table (4).

Table 4: Mean MPO scores comparison, according to study groups.

Groups	MPO score	Damage %
Healthy	1 ± 0 A	$26.3\% \pm 0\%$ A
Indomethacin	3.8 ± 0.4 B	$100\% \pm 11.1\%$ B
Indomethacin + Omeprazole	1.2 ± 0.4 A	$31.6\% \pm 11.1\%$ A
Indomethacin + Cilostazol	1.2 ± 0.4 A	$31.6\% \pm 11.1\%$ A
P-value	$<0.001^{**}$	$<0.001^{**}$

ANOVA test, ** significant at 0.01.

For TNF- α expression, indomethacin showed a clearly higher score (4 ± 0) and differ significantly from other groups on the other hand pretreated groups with omeprazole and cilostazol appeared to

have the lowest score (1.2 ± 0.4) with no significant difference from a healthy group (1 ± 0), and cilostazol pretreated group but significantly differ from healthy group table (5).

Table 5: Mean TNF scores comparison, according to study groups.

Groups	TNF score	Damage %
Healthy	1 ± 0 A	$25\% \pm 0\%$ A
Indomethacin	4 ± 0 B	$100\% \pm 0\%$ B
Indomethacin + Omeprazole	1.2 ± 0.4 AC	$30\% \pm 10.6\%$ AC
Indomethacin + Cilostazol	1.2 ± 0.4 AC	$30\% \pm 10.6\%$ AC
P-value	$<0.001^{**}$	$<0.001^{**}$

ANOVA test, ** significant at 0.01.

Higher MDA score had been shown in indomethacin received group (3.8 ± 0.4) with (100%) damage that differ significantly from other study groups, while pretreatment with omeprazole and cilostazol showed

a similar protective effect (68.4%) in a score (1.2 ± 0.4) that non significantly differ from a healthy group (1 ± 0) on the other hand pretreatment significantly differ from a healthy group table (6).

Table 6: Mean MDA scores comparison, according to study groups.

Groups	MDA score	Damage %
Healthy	1 ± 0 A	$26.3\% \pm 0\%$ A
Indomethacin	3.8 ± 0.4 B	$100\% \pm 11.1\%$ B
Indomethacin + Omeprazole	1.2 ± 0.4 AC	$31.6\% \pm 11.1\%$ AC
Indomethacin + Cilostazol	1.2 ± 0.4 AC	$31.6\% \pm 11.1\%$ AC
P-value	$<0.001^{**}$	$<0.001^{**}$

ANOVA test, **significant at 0.01

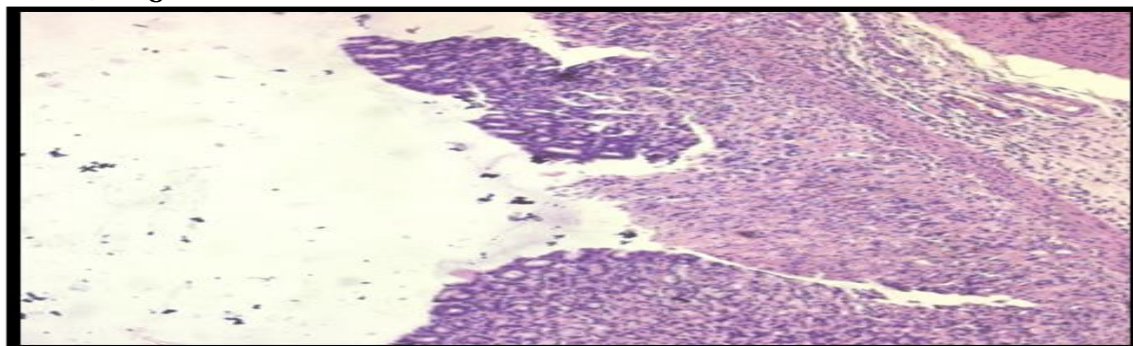


Fig 1: Histopathology effect of indomethacin.

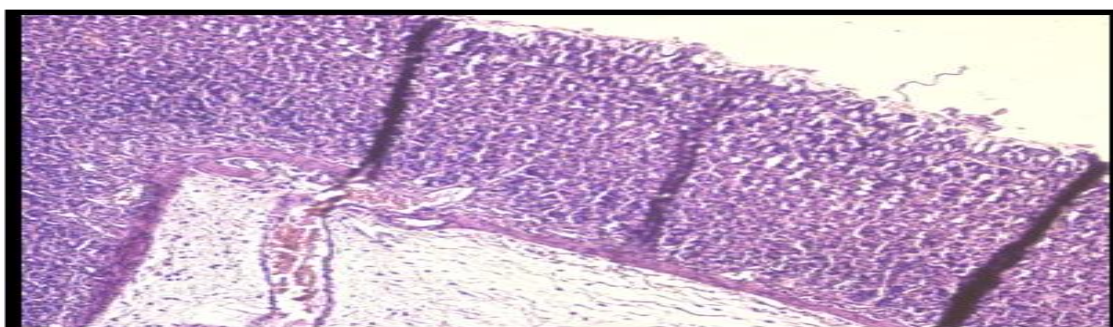


Fig 2: Histopathology effect of Cilostazol.

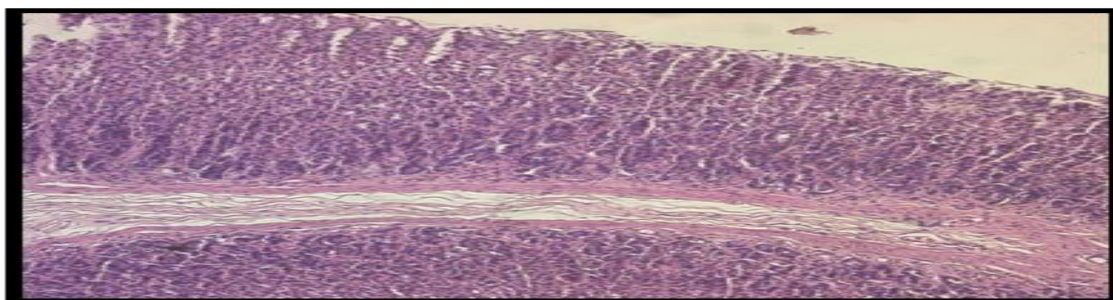


Fig 3: Histopathology effect of omeprazole.

4. Discussion

Gastric ulcer is one of the most common gastrointestinal diseases due to environmental conditions, lifestyle and an increase in NSAIDs consumption. NSAIDs are a class of medications approved by the FDA for the treatment of acute pain, fever and inflammation, because of worldwide use of this class especially by elderly the risk of gastrointestinal side effects increase include gastritis, peptic ulcer, bleeding and perforation by multiple mechanisms^[14]. Indomethacin is one of the most ulcerogenic drugs that is widely used in experimental animal to induce ulcer by multi mechanism and induce mitochondrial structural and functional damage by impairment of stage three and four of

respiration and generation of free radicals that act as signal transduction molecule to amplify inflammatory cytokines and increase neutrophil recruitment that's lead to inflammatory disorders^[15]. The present study showed morphological changes include a significant increase in gastric ulcer severity (numbers and length) in the ulcerated group following oral administration of indomethacin, these agree with (Song et al., 2020)^[16] whose study showed an increase in ulcer severity due to the mentioned mechanisms. Mitochondrial oxidative stress (MOS) consider as important PG independent pathway of IND induced gastric mucosal injury that leads to an increase in pro-inflammatory reaction in parallel with increase in neutrophil infiltration which is involved in the pathogenesis of gastric mucosal lesion by overproduction of reactive oxidant, thus the

inflammation plays a crucial role in the gastric injury pathogenesis^[17]. In the present study, there was a significant increase in pro-inflammatory marker TNF- α , MPO which is an indicator of neutrophil recruitment and oxidative stress marker MDA which is compatible with previous studies due to the mentioned mechanisms^[18, 19]. This study may be the first study that evaluates cilostazol in indomethacin induced experimental gastric ulcers, the significant decrease in ulcer severity may be correlated with its anti-inflammatory and antioxidant properties, previous studies showed that pretreatment with TMZ appeared a reduction in infarct area of the heart that developed in a rabbit model of myocardial ischemia and macroscopic improvement in intestinal injury after ischemic reperfusion in rat's model^[20, 21]. Cilostazol administration 1 hour prior to indomethacin administration clearly showed a significant reduction in pro-inflammatory cytokine TNF α and neutrophil recruitment indicator MPO in comparison with the group received indomethacin this finding compatible with (Jin et al., 2007)^[22] who showed the ability of intraperitoneal administration of cilostazol to attenuate inflammation induced by aspirin. A few years ago TMZ showed anti-inflammatory, antioxidant protective effects in organs include kidney, liver, heart, retina and pancreas from drugs' toxic effects and ischemia by reducing oxidative damage, maintaining normal cellular and mitochondrial function and block pro-inflammatory cytokines by interfering with NF KB pathway^[23], Tetik et al. in 1999^[24] showed the ability of TMZ to decrease pro-inflammatory cytokines in rate intestinal reperfusion and decrease neutrophil infiltrations. Disruption of normal cellular hemostasis by redox signals contribute to disease in every organ include the development of a gastric ulcer, ROS damage gastric mucosal cells by peroxidation of phospholipids, proteins and DNA molecules^[25]. Concerning the effect on MDA level in this study, both TMZ and CST pretreated group showed a significant effect compared to ulcerated group, however, there was no significant difference in CST and TMZ pretreated group compared to omeprazole pretreated group. These results were compatible with those reported by (Moawad et al., 2019)^[26] they found that pretreatment with CST 1 hour before ethanol and pyloric ligation induction of gastric ulcer produce a significant reduction in oxidative damage effect due to inhibition of lipid peroxidation and increase antioxidant level. TMZ effects in the current study were compatible with those of (Tetik et al., 1999)^[24], they observed a significant reduction in MDA level after intestinal ischemic- reperfusion as well as similar to results of (Girgin et al., 2000)^[23] that indicate a significant reduction in peroxidation level in ulcerative colitis laboratory induced rats, in parallel with the increase in antioxidant level. Histological examination of the pretreated group with TMZ and CST confirm the anti-inflammatory and antioxidant properties by reduction of leukocytes infiltration, decrease edema as well as congestion despite a

decrease in mucosal damage the detached epithelia cells were still present. Histopathological study of gastric section of SCT and TMZ show a significant reduction in score compared with ulcerated group this agree with (Moawad et al., 2019)^[26] whose results showed a protective effect when pretreated with CST in dose dependent fashion, as the dose increase the degree of gastric mucosal damage decrease.

5. Conclusion

The current study spotted the light on the cytoprotective activity of cilostazol (phosphodiesterase III inhibitor) on the gastric mucosal injury. Cilostazol (10mg/kg) 1 hour before indomethacin administration show a reduction and improvement in the damaging effect of indomethacin, both drugs were equally effective and show similar efficacy as standard omeprazole drug by a decrease in oxidative stress status manifested by a reduction in lipid peroxidation indicator marker as well as reduction in pro-inflammatory cytokine level and leukocyte recruitment indicator marker and finally, macroscopic and microscopic evaluation show a reduction gastric ulcer severity.

References

1. Kuna, L., Jakab, J., Smolic, R., Raguz-Lucic, N., Vcev, A. and Smolic, M. Peptic ulcer disease: a brief review of conventional therapy and herbal treatment options. *Journal of clinical medicine*, 2019, 8(2), p.179.
2. Lanasa, A. and Chan, F.K. Peptic ulcer disease. *The Lancet*, 2017, 390(10094), pp.613-624.
3. Malaty, H.M. Epidemiology of Helicobacter pylori infection. *Best Practice & Research Clinical Gastroenterology*, 2007, 21(2), pp.205-214.
- Szeto, C.C., Sugano, K., Wang, J.G., Fujimoto, K., Whittle, S., Modi, G.K., Chen, C.H., Park, J.B., Tam, L.S., Vareesangthip, K. and Tsoi, K.K. Non-steroidal anti-inflammatory drug (NSAID) therapy in patients with hypertension, cardiovascular, renal or gastrointestinal comorbidities: joint APAGE/APLAR/APSDE/APSH/APSNI/PoA recommendations. *Gut*, 2020, 69(4), pp.617-629.
- Majeed, P.D. and Khoshnaw, K.J.S. Seroprevalence of Helicobacter pylori infection among patients with gastroduodenal disorders in Erbil city. *Diyala Journal of Medicine*, 2020, 18(1), pp.91-101.
- Celli, J.P., Turner, B.S., Afdhal, N.H., Keates, S., Ghiran, I., Kelly, C.P., Ewoldt, R.H., McKinley, G.H., So, P., Erramilli, S. and Bansil, R. Helicobacter pylorus moves through mucus by reducing mucin viscoelasticity. *Proceedings of the National Academy of Sciences*, 2009, 106(34), pp.14321-14326.
4. Amandeep, K., Robin, S., Ramica, S. and Sunil, K. Peptic ulcer: A review on etiology and pathogenesis. *Int Res J Pharm*, 2012, 3(6), pp.34-38.
5. McColl, K.E., Gillen, D. and El-Omar, E. The role of gastrin in ulcer pathogenesis. *Best Practice &*

Research Clinical Gastroenterology, 2000, 14(1), pp.13-26.

6. Shin, D., Lee, S.J., Ha, Y.M., Choi, Y.S., Kim, J.W., Park, S.R. and Park, M.K. Pharmacokinetic and pharmacodynamics evaluation according to absorption differences in three formulations of ibuprofen. *Drug Design, Development and Therapy*, 2017, 11, p.135.

7. Nishi, T., Kimura, Y. and Nakagawa, K., (2000). Research and development of cilostazol: an antiplatelet agent. *Yakugaku Zasshi: Journal of the Pharmaceutical Society of Japan*, 120(12), pp.1247-1260.

8. Hobbs, S.D., Marshall, T., Fegan, C., Adam, D.J. and Bradbury, A.W. (2007). The effect of supervised exercise and cilostazol on coagulation and fibrinolysis in intermittent claudication: a randomized controlled trial. *Journal of vascular surgery*, 45(1), pp.65-70.

9. Bangun, H., Arianto, A. and Rehngenanana, E. Anti-ulcer Effect of Gastroretentive Drug Delivery System of Alginate Beads Containing Turmeric Extract Solid Dispersion. *Open Access Macedonian Journal of Medical Sciences*, 2021, 9(A), pp. 19–27.

10. Hernandez-Rodriguez, J., Segarra, M., Vilardell, C., Sanchez, M., Garcia-Martinez, A., Esteban, M. J., Queralto, C., Grau, J. M., Urbano-Marquez, A. and Palacin, A. Tissue production of pro-inflammatory cytokines (IL-1 β , TNF α and IL-6) correlates with the intensity of the systemic inflammatory response and with corticosteroid requirements in giant-cell arteritis. *Rheumatology. Oxford University Press*, 2004, 43(3), pp. 294–301.

11. Drini, M. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. *Australian prescriber*, 2017, 40(3), p.91.

12. Maity, P., Bindu, S., Dey, S., Goyal, M., Alam, A., Pal, C., Mitra, K. and Bandyopadhyay, U. Indomethacin, a non-steroidal anti-inflammatory drug, develops gastropathy by inducing reactive oxygen species-mediated mitochondrial pathology and associated apoptosis in gastric mucosa: a novel role of mitochondrial aconitase oxidation. *Journal of Biological Chemistry*, 2009, 284(5), pp.3058-3068.

13. Song, H., Hou, X., Zeng, M., Chen, X., Chen, X., Yang, T., Xu, F., Peng, J., Peng, Q., Cai, X. and Yu, R. Traditional Chinese Medicine Li-Zhong-Tang accelerates the healing of indomethacin-induced gastric ulcers in rats by affecting TLR-2/MyD88 signaling pathway. *Journal of ethnopharmacology*, 2020, 259, p.112979.

14. Bindu, S., Mazumder, S., Dey, S., Pal, C., Goyal, M., Alam, A., Iqbal, M.S., Sarkar, S., Siddiqui, A.A., Banerjee, C. and Bandyopadhyay, U.

Nonsteroidal anti-inflammatory drug induces proinflammatory damage in gastric mucosa through NF- κ B activation and neutrophil infiltration: Anti-inflammatory role of heme oxygenase-1 against nonsteroidal anti-inflammatory drug. *Free Radical Biology and Medicine*, 2013, 65, pp.456-467.

15. Küçükler S, Kandemir FM, Yıldırım S. Protective effect of chrysin on indomethacin induced gastric ulcer in rats: role of multi-pathway regulation [published online ahead of print, 2022 Jan 14]. *Biotech Histochem*. 2022;1-14.

16. Antonisamy, P., Arasu, M.V., Dhanasekaran, M., Choi, K.C., Aravinthan, A., Kim, N.S., Kang, C.W. and Kim, J.H. Protective effects of trigonelline against indomethacin-induced gastric ulcer in rats and potential underlying mechanisms. *Food & function*, 2016, 7(1), pp.398-408.

17. Ruixing, Y., Wenwu, L. and Al-Ghazali, R. Trimetazidine inhibits cardiomyocyte apoptosis in a rabbit model of ischemia-reperfusion. *Translational research*, 2007, 149(3), pp.152-160.

18. Wu, S., Chang, G., Gao, L., Jiang, D., Wang, L., Li, G., Luo, X., Qin, S., Guo, X. and Zhang, D. Trimetazidine protects against myocardial ischemia/reperfusion injury by inhibiting excessive autophagy. *Journal of Molecular Medicine*, 2018, 96(8), pp.791-806.

19. Jin, M., Wada, I., Horikawa, Y., Matsushashi, T., Hatakeyama, N., Oyake, J. and Watanabe, S. Attenuation of gastric mucosal inflammation induced by aspirin through inhibition of selective type III phosphodiesterase in rats. *Digestive diseases and sciences*, 2007, 52(5), pp.1355-1359.

20. Girgin, F., Karaoglu, O., Erkus, M., Tuzun, S., Ozutemiz, O., Dincer, C., Batur, Y. and Tanyalcin, T. Effects of trimetazidine on oxidant/antioxidant status in trinitrobenzenesulfonic acid-induced chronic colitis. *Journal of Toxicology and Environmental Health Part A*, 2000, 59(8), pp.641-652.

Tetik, C., Özden, A., Çalli, N., Bilgihan, A., Bostanci, B., Yis, Ö. and Bayramoğlu, H. Cytoprotective effect of trimetazidine on 60 minutes of intestinal ischemia-reperfusion injury in rats. *Transplant international*, 1999, 12(2), pp.108-112.

21. Suzuki H, Nishizawa T, Tsugawa H, Mogami S, Hibi T. Roles of oxidative stress in stomach disorders. *J Clin Biochem Nutr*. 2012;50(1):35-39.

22. Moawad, H., El Awdan, S.A., Sallam, N.A. et al. Gastroprotective effect of cilostazol against ethanol- and pylorus ligation-induced gastric lesions in rats. *Naunyn-Schmiedeberg's Arch Pharmacol*, 2019, 392, pp.1605–1616.