RESEARCH ARTICLE

The Protective Effects Trimetazidine on Indomethacin Induced Gastric Ulcer in Rat Model

Redhaa Imad*, Huda I. Al-Qadh

Department of Pharmacology, College of Medicine, University of Baghdad, Baghdad, Iraq

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ABSTRACT

Introduction: Gastric ulcer (GU) is the most common gastrointestinal tract disorder, representing about 20% of peptic ulcer due to an imbalance between gastric defense mechanisms and aggressive factors, mainly *Helicobacter pylori* and non-steroidal anti-inflammatory drugs (NSAIDs). The study aims to evaluate trimetazidine's protective effect on histopathology, GU severity, inflammatory and oxidative stress markers (TNF- alpha, MPO and MDA) in rate model of indomethacin induced GU.

Method: Total 30 healthy male albino rats were divided into five groups each of ten (N=10). Group A: Given indomethacin vehicles (normal saline 0.9% and tween 80) orally *via* gavage tube and serve as control positive group. Group B: Induced ulcer by Indomethacin 60 mg/kg orally *via* oral gavage serve as control negative. Group C: Pretreated with trimetazidine 50 mg/kg orally by gavage tube 1-hour before administering indomethacin 60 mg/kg. At the end of study histological examination, anti-inflammatory and antioxidant markers were evaluated.

Results: TMZ 50 mg/kg pretreated groups show a significant ($p < 0.01$) reduction in GU severity score and histopathology damage score in comparison with the indomethacin ulcerated group. Moreover, pretreated groups TMZ 50 mg/kg showed a significant reduction in inflammatory markers (TNF-alpha and MPO) and oxidative stress marker (MDA) in comparison with the induction group, similar to the results of the reference drug.

Conclusion: TMZ dihydrochloride has similar efficacy as standard omeprazole drug by a decrease in oxidative stress status manifested by a reduction in lipid peroxidation indicator marker and a reduction in pro-inflammatory cytokine level and leukocyte recruitment indicator marker and finally, macroscopic and microscopic evaluation show a reduction GU severity.

Keywords: Malondi aldehyde, Myeloperoxidase, Oxidative stress, TNF-alpha, Trimethazidin.

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INTRODUCTION

Abrasion of gastric lining epithelium occurs due to an imbalance between gastric defense, protective mechanism and aggressive factors represent about 20% of peptic ulcer start as mild erosion of epithelial 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 gastrointestinal tract disease; approximately 5–10% of the populations are affected.[2] A multifactorial GU 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] GU is highly linked to *Helicobacter pylori* infection and chronic non-steroidal anti-inflammatory drugs (NSAID) use, the incidence of GU is about 80% in *H. pylori* infected than non-infected patients and 10–30% of GU is related to chronic NSAID use.[4] *H. pylori* prevalence increases with age (53.3%) and appears to be more in females (59.72 %) than in males (43.75 %).[5] GU is a multifactorial disease, with endogenous and exogenous factors involved in GU development. GU 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 GU formation by reducing gastric defense layers through inhibition of prostaglandin synthesis by blocking cyclo-oxygenase (COX) enzyme isoforms.[7] Parietal cells secret acid and considered the first line of defense mechanism against bacterial overgrowth and colonization. It enhances the absorption of some important materials, including iron, calcium and B12, overproduction 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, convert superficial erosion to

*Author for Correspondence: ridaa.emad1206b@comed.uobaghdad.edu.iq

a deeper lesion, disturb mucosal integrity and inactivates 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] Trimetazidine (TMZ) was introduced first in France in 1978 as a complementary treatment of stable angina, in 2000 European society of cardiology (ESC) recommended TMZ as a second line in the treatment of angina. TMZ seemed to decrease mean weekly angina attack by 40% and decrease weekly consumption of nitrate, recently many clinical trials have been demonstrated the beneficial protective effect of TMZ in patients undergo percutaneous intervention (PCI) or coronary artery bypass graft (CABG).[10] The study aims to evaluate TMZ's protective effect on histopathology, GU severity, inflammatory and oxidative stress markers (TNF-alpha, MPO and MDA) in rate model of indomethacin-induced GU. TMZ 10 mg/kg administration (H & E 10X)(Figure 1).

METHOD

Comparative and prospective animal study. This 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 to 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 the procedure. TMZ powder for laboratory use only with 99.7% of purity as TMZ hydrochloride (HCL). TMZ was prepared by dissolving in vehicle of normal saline and tween 80 in a dose of 12.5 mg/mL, the selection of 50 mg/kg was based on pilot study in which two doses are tested 20 and 50 mg/kg, 50 mg chosen based on macroscopic evaluation of gastric mucosal erosion severity in blinded manner by a pathologist. After 7 days of adaptation, experimental rats were randomly directed to one of five groups each group, including 10 rates, each as follows: Group A: rats orally administered 1-mL of indomethacin vehicle (only normal saline and tween 80) *via* gavage tube after 24 hours of fasting. This group served as a negative control. Group B: rats orally received 60 mg/ kg of indomethacin solution after 24 hours of fasting. This group served as positive control. Group C: After 24 hour of fasting rats pretreated orally with 50 mg/kg 0f TMZ solution 1-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.[11] Quantification of protein expression was evaluated under light microscopy at 20X. The extent of the immunohistochemical reaction of proteins, such as MDA, MPO and TNFα was measured by percentage of positively stained cells according to the following scale.[12] The software used for data analysis was SPSS (Statistical Packages for Social Sciences) version 26. The data were represented by their mean, standard deviations, and 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.

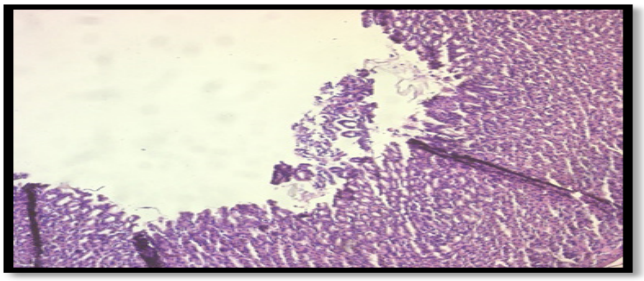

Figure 1: TMZ 10 mg/kg administration (H & E 10X).

RESULTS

The effect of TMZ on GU number and damaging percent. Oral administration of 60 mg/kg indomethacin cause a significant increase in GU number in comparison with healthy group while the pretreated group with TMZ showed a significant reduction in ulcer number in compare with ulcer control group, indomethacin shows the highest mean number score among a pretreated group and differ significantly from healthy group, TMZ 50 mg/kg showed a mean score of (1.2 ± 0.4). Additionally, indomethacin seems to cause the higher mucosal damage effect (100%), differ significantly from healthy group TMZ came next with inhibition rate (68.4%). Oral administration of 60 mg/kg indomethacin increase ulcer severity after 4 hours of administration and show a highest severity score (4 ± 0) in compare with healthy group (0 ± 0), TMZ came next in mean score of (1.1 ± 0.3) Both drugs significantly differ from healthy group as show in Table 1.

Histopathological changes of TMZ 50 mg/kg 1-hour before indomethacin 60 mg/kg administration showed in Table 2. Indomethacin administration show a high value of score damage (4 ± 0) compared with healthy group (0 ± 0); that microscopic appearance showed normal mucosa architecture. Indomethacin significantly differs from other groups and microscopic investigation revealed several histopathological changes, including erythema, mucosal layer edema, and inflammatory reaction and congestion. Microscopic appearance of TMZ damaging score (1.2 ± 0.4) (1.4 ± 0.5), respectively showed no significant difference between them but significantly differ from healthy and omeprazole pretreated group.

According to the pattern of MPO, study groups could be divided into two categories those with relatively low MOP expression group which include healthy and pretreated groups with TMZ with MPO mean score (1.2 ± 0.4) differ significantly from ulcer control group, other categories include indomethacin administered group with higher MPO expression level with mean score of (4 ± 0). For TNF-α expression indomethacin show a clearly higher score (4 ± 0) and differ significantly from the TMZ pretreated group (1.5 ± 0.5)

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

Groups	*Lesions number*	*Damage %*	*Severity score*	*Damage %*
Healthy	0 ± 0 A	0 ± 0% A	0 ± 0 A	0 ± 0% A
Indomethacin	3.8 ± 0.4 B	100 ± 11.1% B	4 ± 0 B	100 ± 0% B
Indomethacin +Trimetazidine	1.2 ± 0.4 D	31.6 ± 11.1% D	1.1 ± 0.3 D	27.5 ± 7.9% D
p-value	<0.001**	<0.001**	<0.001**	<0.001**

ANOVA test, **significant at 0.01

Table 2: Effect of TMZ on mean histopathological damages scores comparison, according to study groups.

Groups	*Histopathology damage scores*	*Damage %*
Healthy	0 ± 0 A	0 ± 0% A
Indomethacin	4 ± 0 B	100 ± 0% B
Indomethacin +Trimetazidine	1.4 ± 0.5 D	35 ± 12.9% D
p-value	<0.001**	<0.001**

ANOVA test, **significant at 0.01.

Table 3: Mean MPO scores comparison, according to study groups.

Groups	*MPO score*	*Damage %*
Healthy	1 ± 0 A	26.3 ± 0% A
Indomethacin	3.8 ± 0.4 B	100 ± 11.1% B
Indomethacin +Trimetazidine	1.2 ± 0.4 A	31.6 ± 11.1% A
p-value	<0.001**	<0.001**

ANOVA test, **significant at 0.01.

Table 4: Mean TNF scores comparison, according to study groups

Groups	*TNF score*	*Damage %*
Healthy	1 ± 0 A	25 ± 0% A
Indomethacin	4 ± 0 B	100 ± 0% B
Indomethacin +Trimetazidine	1.5 ± 0.5 C	37.5 ± 13.2% C
p-value	<0.001**	<0.001**

ANOVA test, **significant at 0.01.

Table 5: Mean MDA scores comparison, according to study groups

Groups	*MDA score*	*Damage %*
Healthy	1 ± 0 A	26.3 ± 0% A
Indomethacin	3.8 ± 0.4 B	100 ± 11.1% B
Indomethacin +Trimetazidine	1.5 ± 0.5 C	39.5 ± 13.9% C
p-value	<0.001**	<0.001**

ANOVA test, **significant at 0.01

and the healthy group. Higher MDA score had been shown in the indomethacin received group (3.8 ± 0.4) with (100%) damage that differ significantly from other study groups on the other hand pretreatment with TMZ (1.5 ± 0.5) show significantly differ from healthy group as show in Tables 3-5.

DISCUSSION

GU 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.[13] Indomethacin is one of the most ulcerogenic drugs widely used in experimental animals to induce ulcer by multi mechanisms. It induces mitochondrial structural and functional damage by impairment of stage three and four of respiration and generation of free radicals that act as signal transduction molecules to amplify inflammatory cytokines and increase neutrophil recruitment, leading to inflammatory disorders.[14] The present study showed morphological changes, including a significant increase in GU severity (numbers and length) in the ulcerated group following oral administration of indomethacin, these agree with (Song *et al.,* 2020)[15] 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.[16] In the present study, there was a significant increase in pro-inflammatory marker TNF-alpha, MPO which is an indicator of neutrophil recruitment and oxidative stress marker MDA which is compatible with previous studies due to the mentioned mechanisms.[17,18] This study may be the first study that evaluates TMZ in indomethacin-induced experimental GUs, 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.[19,20] TMZ administration 1-hour prior to indomethacin administration clearly showed a significant reduction in pro-inflammatory cytokine TNF alpha and neutrophil recruitment indicator MPO in comparison with the group received indomethacin this finding is compatible with (Jin *et al.*, 2007)[21] 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,[22] Tetik *et al.* in 1999[23] 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 GU, ROS damage gastric mucosal cells by peroxidation of phospholipids, proteins and DNA molecules.[24] Concerning the effect on MDA level in this study, the TMZ and CST pretreated group showed a significant effect compared to the 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)[25] they found that pretreatment with CST 1-hour before ethanol and pyloric legation induction of GU 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),[23] they observed a significant reduction in MDA level after intestinal ischemic- reperfusion as well as similar to the results of (Girgin *et al.*, 2000)[22] 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 leukocyte infiltration, decrease edema as well as congestion despite a decrease in mucosal damage, the detached epithelial cells were still present. Histopathological study of the gastric section of SCT and TMZ show a significant reduction in score compared with the ulcerated group. This agree with (Moawad *et al.*, 2019).[25] whose results showed a protective effect when pretreated with CST in dose-dependent fashion, as the dose increases the degree of gastric mucosal damage decrease.

CONCLUSION

TMZ dihydrochloride has a similar efficacy as standard omeprazole drug by a decrease in oxidative stress status manifested by a reduction in lipid peroxidation indicator marker. A reduction in pro-inflammatory cytokine level and leukocyte recruitment indicator marker and, finally, macroscopic and microscopic evaluation show a reduction in GU severity.

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