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 rates, each with 10 (N=10): Group A: Oral Indomethacin vehicles (normal saline0.9% and tween80) 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 (p0.01) 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 secret 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

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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 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 heathy 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 of12.5 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 rates, 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 cilostazole 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 TNFα 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 0.4) compared to other groups, while omeprazole20mg/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 ± 0 A	0% ± 0% A
Indomethacin	3.8 ± 0.4 B	100% ± 11.1% B
Indomethacin + Omeprazole	0.4 ± 0.5 C	10.5% ± 13.6% C
Indomethacin + Cilostazol	1.4 ± 0.5 D	36.8% ± 13.6% D
P-value	<0.001**	<0.001**
*ANOVA test, **significant at 0.01.*		

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 ± 0 A	0% ± 0% A
Indomethacin	4 ± 0 B	100% ± 0% B
Indomethacin + Omeprazole	0.4 ± 0.5 C	10% ± 12.9% C
Indomethacin + Cilostazol	1.4 ± 0.5 D	35% ± 12.9% D
P-value	<0.001**	<0.001**
*ANOVA, **significant at 0.01.*		

Oral administration of 60mg /kg of indomethacin increases ulcer severity after 4 hours and shows a highest severity score (4± 0) in compare with a healthy group (0± 0), omeprazole reveals the lowest mean

score of ulcer severity (0.4 ±0.5) among the pretreated group with significant difference from healthy and ulcer control group, cilostazol in a mean score of (1.4± 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

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% ± 0% A
Indomethacin	4 ± 0 B	100% ± 0% B
Indomethacin + Omeprazole	0.4 ± 0.5 C	10% ± 12.9% C
Indomethacin + Cilostazol	1.2 ± 0.4 D	30% ± 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 MOP 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% ± 0% A
Indomethacin	3.8 ± 0.4 B	100% ± 11.1% B
Indomethacin + Omeprazole	1.2 ± 0.4 A	31.6% ± 11.1% A
Indomethacin + Cilostazol	1.2 ± 0.4 A	31.6% ± 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% ± 0% A
Indomethacin	4 ± 0 B	100% ± 0% B
Indomethacin + Omeprazole	1.2 ± 0.4 AC	30% ± 10.6% AC
Indomethacin + Cilostazol	1.2 ± 0.4 AC	30% ± 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% ± 0% A
Indomethacin	3.8 ± 0.4 B	100% ± 11.1% B
Indomethacin + Omeprazole	1.2 ± 0.4 AC	31.6% ± 11.1% AC
Indomethacin + Cilostazol	1.2 ± 0.4 AC	31.6% ± 11.1% AC
P-value	<0.001**	<0.001**
*ANOVA test, **significant at 0.01*		

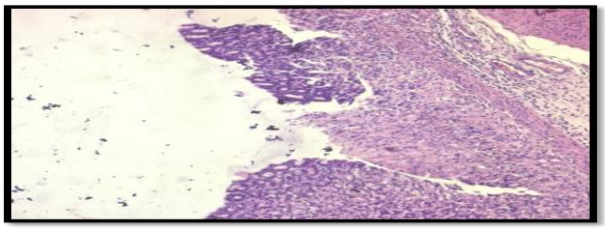

Fig 1: Histopathology effect of indomethacin.

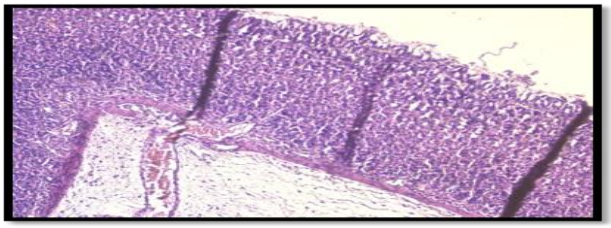

Fig 2: Histopathology effect of Cilostazol.

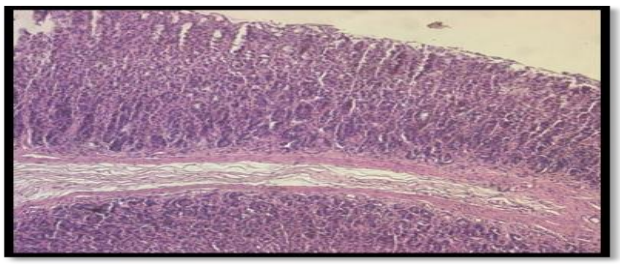

Fig 3: Histopathology effect of omprazol.

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-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 [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 alpha 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 legation 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.

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