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EFFECT OF SITAGLIPTIN ON INDOMETHACIN-INDUCED GASTRIC ULCERS IN MICE

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ABSTRACT

An imbalance between defensive and offensive factors leads to damage to gastric mucosal and thus ulcers. Helicobacter pylori and nonsteroidal anti-inflammatory drugs (such, indomethacin) are the most important causes of gastric ulcers. Sitagliptin is one of the antidiabetic drugs that demonstrated an anti-inflammatory and antioxidant role in many diseases. In this study, we investigated the protective effect of sitagliptin on indomethacin-induced gastric ulcers in mice and compared it with famotidine effect. Adult females Balb/c mice were divided into four groups (n=7 in each); group 1 (normal control), group 2 (induced-ulcer, non-pretreated), group 3 (sitagliptin 21 mg/kg), group 4 (famotidine 8.6 mg/kg). We administered drugs orally for 15 days, then induced gastric ulcers by a single oral dose of indomethacin (300 mg/kg). Histological findings showed that indomethacin resulted in severe damage to the gastric mucosa in mice, as histological examination showed the presence of deep ulcerations that extended through the entire mucosal membrane, damaged the muscularis mucosa and accompanied by inflammatory infiltrates. sitagliptin did not prevent or reduce the lesions caused by indomethacin, and there was a statistically significant difference between sitagliptin and famotidine. In conclusion, sitagliptin did not show a protective effect on indomethacin-induced gastric ulcers in mice.

KEYWORDS: Gastric ulcers, Sitagliptin, Famotidine, Indomethacin, Ulcer index.

INTRODUCTION

Gastric ulcers are common gastrointestinal diseases.[1,2] An imbalance between defensive and offensive factors leads to damage to gastric mucosal and thus ulcers.[3] Defensive factors include mucus, prostaglandin (PG), gastric mucosal blood flow, and bicarbonate, while offensive factors include Helicobacter pylori (H. pylori) infection and the chronic use of nonsteroidal anti-inflammatory drugs (NSAIDs), alcohol and smoking.[4,5,6]

Helicobacter pylori, a gram-negative bacterium, is a major cause of gastric ulcers because it infects the mucous layer and the epithelium of the stomach[7], leading to inflammation and ulceration.[8] Chronic use of NSAIDs is the second most common cause of gastric ulcers.[9] These drugs are usually prescribed for their anti-inflammatory effects[10] by inhibiting COX enzymes, which are involved in the synthesis of many inflammatory mediators.[11] Despite their therapeutic benefits, they cause gastric ulcers by preventing the synthesis of prostaglandins by cox1 enzyme.[11] This results in a decrease in the production of gastric mucus, bicarbonate, and a decrease in mucosal blood flow.[9] In addition, NSAIDs cause the generation of reactive oxygen species (ROS) and the upregulation of proinflammatory cytokines as Tumor Necrosis Factor-alpha (TNF-α) beside to the other mechanisms independent of prostaglandins and contribute to the development of NSAIDs ulcers.[11]

Sitagliptin, the first Dipeptidyl peptidase-4 (DPP-4) inhibitors approved in 2006 for the treatment of type 2 diabetes (T2D)[12], which prevents glucagon-like peptide-1 (GLP-1) degradation by inhibiting of DPP-4 enzyme, thus inhibits glucagon secretion and stimulates insulin secretion.[13] Recently, researchs have focused on the anti-inflammatory and antioxidant properties of sitagliptin.[14,15]

Given the high occurrence rate of gastric ulcers in diabetes patients, research on the impact of antidiabetic medicines like sitagliptin on gastric ulcers is quite interesting. To the best of our knowledge, few studies have investigated the effect of sitagliptin on gastric ulcers, but their results showed contradictory between its protective effect on gastric ulcers and its negative effect on ulcer healing.[16,17,18]

However, there have been no studies in the literature compared the effect of sitagliptin on gastric ulcer prevention with that of the Histamin 2 antagonist (famotidine), which has been proven effective in gastric ulcer treatment and prevention.

MATERIALS AND METHODS

Drugs

Indomethacin, sitagliptin and famotidine were obtained from Syrian pharmaceutical factories. Indomethacin was suspended in 1% aqueous solution of carboxymethyl cellulose (CMC), Famotidine was suspended in 0.5% aqueous solution of CMC, and sitagliptin was dissolved in 0.5% aqueous solution of CMC. All solutions were prepared freshly.

Animals

For this experiment, we used 28 female Balb/c mice whose weight ranged between 18-34 g. The experiment was conducted in the experimental animal laboratory at the Faculty of Pharmacy, Tishreen University, Syria. Housing condition was carefully controlled with temperatures of 25±2°C, humidity of 50±15%, and a 12-h light/dark cycle. Mice had free access to a standard rodent diet and water throughout the study. All experimental procedures followed regulatory guidelines for the care and use of laboratory animals.

Experimental design

Mice were divided into 4 group (n=7) in each group:
Group 1 (Normal group): received oral vehicle (CMC 0.5% solution) for 15 days
Group 2 (Indomethacin group): received oral vehicle (CMC 0.5% solution) for 15 days
Group 3 (Sitagliptin group): received sitagliptin (21 mg/kg) for 15 days
Group 4 (Famatiodine group): received famotidine (8.6 mg/kg) for 15 days
The drug doses were calculated depending on Human Equivalent Dose (HED)[19]

Ulcer induction and gastric tissue collection

After 15 days, the mice were fasted from food with free access to water for 24 hrs. All mice were given indomethacin in a single oral dose (300 mg/kg), except for the first group which was given an equivalent amount of CMC 1%. We tested the indomethacin dose before starting work.

All experiments were performed during the same time of the day to avoid diurnal variations of the putative regulators of gastric functions. The mice were sacrificed 6 hour following indomethacin administration by an overdose of ether. We removed stomach and opened along the greater curvature. The stomachs were fixed in formalin 10% solution for histopathological examination.

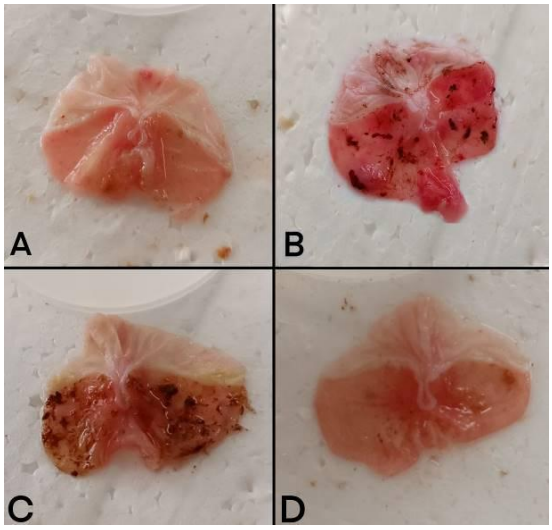

Figure 1: Macroscopic Changes in the gastric mucosa. (a) normal group. (b) indomethcin group. (c) sitagliptin group. (d) famotidine

Histopathological examination of gastric tissue

Alcohol was used in increasing concentrations to dehydrate the formalin-fixed samples, then they were finally embedded in paraffin. The specimens were cut into 5-µm-thick sections, stained with hematoxylin and eosin (H&E), and examined by a pathologist unaware of the applied treatment protocol using an light microscope. The damage of the stomach wall layers was scored for its severity and its depth by using a scale ranges from 0 to 5 (table 1).

Table 1: Scoring System.

Score	Gastric mucosa
0	normal gastric mucosa
1	superficial erosions of mucosa (One third of mucosa)
2	medium depth lesions of mucosa (Two-thirds of mucosa)
3	deep lesions of mucosa (all of mucosa)
4	deep lesions of the mucosa with damage to the muscularis mucosa but not through (ulcer onset)
5	ulcers which are extended through muscularis mucosa

Ulcer Index & Protective Ratio

Gastric mucosal lesions were expressed in terms of the ulcer index according to the method of Khallouf et al.[20]

The ulcer index (UI) was calculated from the equation:
Ulcer Index (UI) = mean of intensity in a group + [number of ulcer positive animals/total number of animals] × 2

The percentage protective ratio was calculated from the equation:[21]
Protective ratio = 100 - (UI pretreated group/ UI control group) × 100

Satatistical analysis

Ulcer score data were analyzed by the Kruskal-Wallis test followed by the Mann–Whitney test for multiple comparisons. p-value<5% was considerd as statistically significant for all comparisons.

RESULT

Histopathological study

In the normal group, the gastric epithelium was normal without any erosions (Figure 2A).

Indomethacin administration caused deep ulcers that extended through the entire mucosal layer and damaged the muscularis mucosa. Severe inflammatory infiltrates were also observed (Figure 2B).

Pretreatment with sitagliptin did not show any changes in the lesions seen in the indomethacin group, wherein the lesions were also deep and affected the muscularis mucosa. Moreover, they also accompanied by severe inflammatory infiltrates (Figure 2C).

In comparison, the mucosa in famotidine group was protected and showed only superficial to moderately deep erosions and lacked the pathological changes seen in the indomethacin and sitagliptin groups. However, sparse inflammatory cells were noted in Famotidine group (Figure 2D).

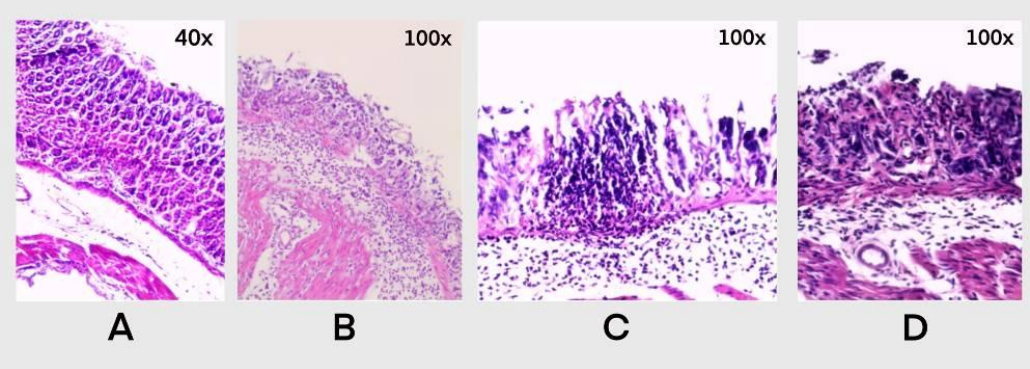

Figure 2: Histological assessment of gastric tissues using H&E stain. (A) normal group showed normal mucosa without any ulcer. (B) Indomethacin group showed deep ulcers that penetrated the entire mucosal layer with damage to the muscularis mucosa. (C) Sitagliptin groups respectively showed deep ulcers. (D) Famotidine group showed moderately deep erosions in the epithelium.

Effect of sitagliptin on ulcer score, ulcer index and protective ratio in indomethacin induced gastric ulcers

Pretreatment with sitagliptin did not achieve a significant reduction in ulcer grade compared with the indomethacin group; the differences were without statistically significant ($P > 0.05$).

There was statistically significant difference in ulcer score in the sitagliptin group compared with the famotidine group ($P < 0.05$).

Table 2: Effect of sitagliptin on indomethacin induced ulcer.

Parameter Group	Ulcer score Mean ± SD	Ulcer index	Protective ratio
Normal	0.000 ± 0.000	-	-
Indomethacin	3.33 ± 0.81	3.33	-
Sitagliptin	3.28 ± 0.487	3.28	1.50%
Famotidine	1.142 ± 0.899	1.14	65.76%

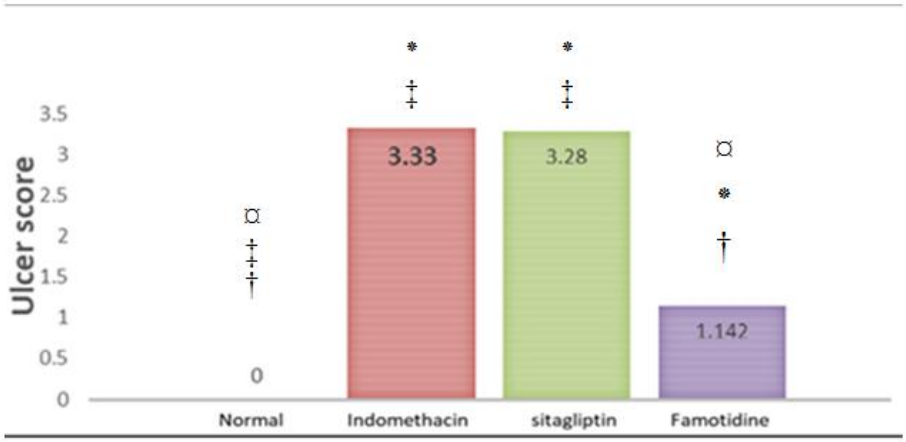

Fig. 3: p<0.05*compared to normal group, p<0.05¤compared to indomethacin group, p<0.05† compared to sitagliptin group, p<0.05‡ compared to famotidine group.

DISCUSSION

Administration of a toxic dose of indomethacin (300 mg/kg) resulted in severe damage to the gastric mucosa in mice, as histological examination showed the presence of deep ulcerations that extended through the entire mucosal membrane, damaged the muscularis mucosa and accompanied by inflammatory infiltrates.

Pretreatment with sitagliptin (21 mg/kg) did not cause any statistically significant changes in ulcer score reported in the indomethacin group, with presence of inflammatory infiltrates. In contrast, the famotidine group showed a significant reduction in ulcer score, with a statistically significant difference between this group and both the sitagliptin and indomethacin groups.

Our study was based on the histological examination of stomachs taken from experimental mice. Measurement of inflammatory mediators and oxidative stress markers was not included in our study. Strict criteria were applied to evaluate the severity of mucosal lesions, where we distinguished between erosions, in which the damage is limited to the mucosal epithelium and lamina propria, and ulcers, where the whole mucosa with muscularis mucosa was involved. Rofaeil et al.[16] and Fouad et al.[17] suggested that sitagliptin has gastro-protective properties by reduction both TNF-α and Malondialdehyde (MDA), but without mentioning the criteria used in the assessment of the mucosal damage. Differences in animal models or substances used might explain this discrepancy.

CONCLUSION

The current study showed that sitagliptin does not have any effect on gastric ulcers, and therefore its use will not protect against ulcers. More detailed studies, serological and histological, are needed to determine the exact mechanism and effect of sitagliptin on gastric ulcers, either protective or aggravating.

REFERENCES

1. Mariam A. Kadhem, Ahlam A. Abdul-niby, Huda K. Khassaf. Study the effect of Ethanolic extract of Anethum graveolens L. on Aspirin induced Gastric Ulcer in Male Guinea Pigs. Research J. Pharm. and Tech, 2018; 11(9): 3793-3798.
2. Sweety Saini, Chandana Majee. Antiulcer activity of Lagerstroemia indica leaves in Indomethacin Induced Gastric Ulcer in Rats. Research Journal of Pharmacy and Technology, 2021; 14(8): 4408-2.
3. Mahardian Rahmadi, M. Shofwan Haris, Anggraini Kusuma, Annisa Septiana Ahmad, Arina Dery Puspitasari, Dinda Monika Nusantara Ratri, Chrismawan Ardianto. Role of 5-HT1A Receptor on Fluvoxamine induced Gastrointestinal Mucosa Protection and Healing in Animal with Stress-Induced Gastric Ulcer. Research Journal of Pharmacy and Technology, 2023; 16(2): 709-4.
4. Kiran P. Narkhede, Trilochan Satapathy, Bibhas Pandit. Protective effect of Cod Liver Oil in Experimentally Induced Gastric Ulceration in Rats. Research J. Pharm. and Tech, 2019; 12(1): 05-10.
5. Sadeel A. Shanshal, Ali Saleh Noori, Jaafar Atheer Ghazi, Abdullah Tahseen Dahham, Abdulrahman Samer Mohamed Saleh, Harith Kh. Al-Qazaz. Impact of peptic ulcer disease on the quality of life:

A Cross Sectional Study. Research Journal of Pharmacy and Technology, 2022; 15(7): 3267-2.
6. Rana Alsamaan, Farah Alhakim. Study of the protective effect of Pomegranate peel Ethanolic Extract on Gastric ulcer caused by stress on rats. Research Journal of Pharmacy and Technology, 2023; 16(1): 86-0.
7. Manisha Bhatti, Divya Dhawal Bhandari, Jitender Singh. Review on Peptic ulcer and its effective Management and Treatment with Herbals. Research Journal of Pharmacy and Technology, 2022; 15(8): 3580-8.
8. P. Manimekalai, P. Maheshwari, R. Velmurugan, M. Gurumoorthy, S. Hansraj Kumar, G. Vijayakumar. Gastro Protective effect of Standardized Ethanolic leaf extract of Indigofera tinctoriao on experimental Gastric Ulcers in Rats. Research J. Pharm. and Tech, 2018; 11(2): 527-531.
9. K. Jaswanth, C. Kiran Kumar, P. Venkatesh. A review on peptic ulcer. UPI Journal of Pharmaceutical, Medical and Health Sciences, 2022; 5(1): 19-26.
10. Akram Nezam, Dima Al Diab, Nouma Hasan. In-vitro Anti-inflammatory activity of Total Phenolic content of some fruit juices in Syria. Research Journal of Pharmacy and Technology, 2021; 14(7): 3685-8.
11. Abdel-Tawab, M. S., Tork, O. M., Mostafa-Hedeab, G., Hassan, M. E., & Elberry, D. A. Protective effects of quercetin and melatonin on indomethacin induced gastric ulcers in rats. Reports of biochemistry & molecular biology, 2020; 9(3): 278.
12. A. Kavyasree, P. Geetha, P. Shanmugasundaram. A Review: Comparison of Efficacy of Liraglutide Versus Sitagliptin add-on-to Metformin in Type 2 Diabetes Mellitus patients. Research Journal of Pharmacy and Technology, 2021; 14(4): 2291-5.
13. Ankita, Keerti Bhardwaj, Navneet Khurana, Ashish Sutte, Gopal Khatik. Identification of Dipeptidyl peptidase-4 (DPP-4) inhibitors as Potential Antidiabetic agents using Molecular docking study. Research J. Pharm. and Tech, 2020; 13(11): 5257-5262.
14. Ayman E El-Sahar, Marwa M Safar, Hala F Zaki, Amina S Attia, Afaf A Ain-Shoka. Sitagliptin attenuates transient cerebral ischemia/reperfusion injury in diabetic rats: implication of the oxidative-inflammatory-apoptotic pathway. Life Sci, 2015; 126: 81-6.
15. Yujiang Ji, Sanatkumar B. Nyamagoud, Sreeharsha Nagaraja, Anurag Mishra, Shiva K. Gubbiyappa, Yogendra Singh. Sitagliptin protects liver against aflatoxin B1-induced hepatotoxicity through upregulating Nrf2/ARE/HO-1 pathway. Bio Factors, 2020; 46(1): 76-82.
16. Remon R. Rofaeil, Asmaa S. Mohamed. Gastroprotective effect of sitagliptin in experimentally-induced peptic ulcer in rats. Minia Journal of Medical Research, 2020; 31(2): 253-256.
17. Amr A. Fouad, Moataz Mohamedalhasan Ali, Heba M. Hafez. Ameliorative Effects of Sitagliptin against Indomethacin-Induced Gastric Ulcer in Rats. Journal for Re Attach Therapy and Developmental Diversities, 2023; 6(9s): 1485-1492.
18. Amina Unis, Eman Abdelzaher. Sitagliptin impairs healing of experimentally induced gastric ulcers via inhibition of inos and cox-2 expression. American Journal of Pharmacology and Toxicology, 2013; 8.3: 107-119.
19. Anroop B Nair, Shery Jacob. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm., 2016; 7(2): 27-31.
20. Sara Khallouf, Rana Makhous, Rana Issa. Efficacy of olmesartan, irbesartan and telmisartan against acute indomethacin-induced gastric ulcers in mice. Bulletin of Pharmaceutical Sciences. Assiut, 2022; 45(2): 1005-1012.
21. Ousman Ahmed a, Teshome Nedi b, Ebrahim M. Yimer c. Evaluation of anti-gastric ulcer activity of aqueous and 80% methanol leaf extracts of Urtica simensis in rats. Metabolism Open, 2022; 14.