Journal of Science and Technology
ISSN: 2456-5660 Volume 8, Issue 11 (Nov -2023)
www.jst.org.in *DOI:https://doi.org/10.46243/jst.2023.v8.i11.pp83- 93*

Comparative Study of the Impact of Ranitidine and Sitagliptin on Acetic Acid-Induced Gastric Ulcer Healing in Rats

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To Cite this Article
D.Dhachinamoorthi, M.Rajan, K Balaram Kumar, L. Ramachandra Reddy**, "Comparative Study of the Impact of Ranitidine and Sitagliptin on Acetic Acid-Induced Gastric Ulcer Healing in Rats"** *Journal of Science and Technology, Vol. 08, Issue 11- Nov 2023, pp83-93*

Article Info
Received: 29-09-2023 Revised: 07-11-2023 Accepted: 18-11-2023 Published: 28-11-2023

Abstract
Cyclooxygenase-2 (COX-2) and Inducible Nitric Oxide Synthase (iNOS) are two of the many promoting factors that control the complicated process of gastric ulcer healing. In most cases, delayed stomach ulcer healing is linked to diabetes mellitus. In order to compare the effects of ranitidine and sitagliptin (dipeptidyl peptidase-4 inhibitor) on the healing of stomach ulcers, the current study was created. Forty male albino rats, split into four equal groups, participated in the current study: Groups 1 and 4 are the normal control group, the gastric ulcer model group, the sitagliptin-treated group, and the ranitidine-treated group, respectively. The stomach of the rats was taken for histological analysis and immunohistochemical evaluation of COX-2 and iNOS ten days after ulcer induction, and the rats were then killed. This study found that the sitagliptin-treated group had much worse gastric ulcer healing than the ranitidine-treated group. This was demonstrated by the stomach's histological investigation, which showed a significantly bigger ulcerated region and poor ulcer base maturation. In comparison to the ulcer model group and the ranitidine-treated group, the sitagliptin-treated group exhibited a substantial decrease in mean vascular density (MVD), COX-2, and iNOS expression. A strong positive association between iNOS and COX-2 was discovered, suggesting that they work in concert. COX-2 and iNOS were found to have a substantial positive connection with MVD, indicating that they had a proangiogenic effect. Given these findings, it is unclear if sitagliptin is recommended for diabetic patients who already have a stomach ulcer. Future investigations involving humans are required to validate our initial experimental findings.
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Introduction

These days, gastric ulcers are regarded as one of the most preventable gastrointestinal conditions. [1] The main cause of its development is an imbalance between aggressive and mucosal-protecting elements, such as NSAIDs and Helicobacter pylori activity in the stomach mucosa [2].Multiple variables influence the dynamic process of gastric ulcer healing, which includes angiogenesis, epithelium regeneration, and base maturation (reduction of the ulcer base size) [3, 4]. Two of the most crucial elements that promote gastric ulcer healing are COX-2 (cyclooxygenase-2) and iNOS (inducible nitric oxide synthase) [5-7]. Prostaglandins (PGs), which have stimulatory effects on ulcer healing, are produced when COX-2 is present. By maintaining increased blood flow at the ulcer margin, stimulating angiogenesis in the ulcer base, and inhibiting inflammatory neutrophil accumulation through the downregulation of adhesion molecule surface expression, iNOS-derived Nitric Oxide (NO) aids in the healing of gastric ulcers [8, 9–10]. It was recently

shown that the more well-known COX-2 pathway and the iNOS-based inflammatory process cross-link. The two inflammatory systems' synergistic molecular interaction may provide more insight into how they promote healing in gastric ulcers [11]. Patients with diabetes are more susceptible to stomach ulcers. because diabetes impairs the stomach mucosa's antioxidant defense mechanism [12, 13]. Furthermore, diabetic neuropathy may cause diabetic patients with gastric ulcers to perceive normal gastrointestinal sensations less clearly, and they are more likely to have bleeding [14]. Additionally, diabetes may be linked to delayed gastric ulcer healing because of a substantial reduction in stomach microcirculation, which may be caused by a decrease in mucosal prostaglandins [15]. Furthermore, it has been noted that elevated proinflammatory cytokine production and hyperglycemia cause a prolonged inflammatory response, which may be the cause of the ulcer area's delayed healing [16]. These earlier results make it necessary to investigate how antidiabetic medications affect the healing of stomach ulcers. Recently developed medications called dipeptidyl peptidase-4 (DPP-4) inhibitors are used to treat type 2 diabetes. According to recent research, DPP-4 inhibitors or similar substances may have strong inflammatory-modifying effects via modulating the production of cytokines. [17] To the best of our knowledge, no research has been done to compare the effects of DPP-4 inhibitors and ranitidine, a histamine 2 antagonist that is one of the most significant substances shown to treat gastric ulcers and aid in their healing primarily through its effect on COX2 and iNOS [18,19].

Thus, the goal of this research is to compare the effects of ranitidine and sitagliptin (a DPP-4 inhibitor) on rats that are mending stomach ulcers.

2. Materials and methods

2.1. Experimental Animals

All experiments were performed in accordance with national animal care guidelines and were preapproved by the Ethics Committee at Faculty of Medicine, Alexandria University.

The present study was conducted on 40 male Wistar albino rats weighing from 150 to 200 g. The rats were obtained from the Animal House at the Faculty of Medicine, Alexandria University. They were housed under optimal laboratory conditions (relative humidity 85±2%, temperature 22±1°C and 12 h light and 12 h dark cycle). All through the study, rats were fed on standard commercial pellet diet and had free access to drinking water.

2.2. Animal Grouping

Four groups of ten rats each were created from the rats: Rats in Group 1 (the typical control group) had unrestricted access to drinking water devoid of additives. Group 2: (gastric ulcer model), where rats were given unrestricted access to drinking water without any additives and stomach ulcers were produced in them. Group 3: (ranitidine treated group): starting on day 3 and continuing for 7 days after gastric ulcer formation, rats in this group were given ranitidine added to their drinking water at a rate of 50 mg/kg orally daily [20]. Rats in Group 4 (the sitagliptin-treated group) were given sitagliptin mixed into their drinking water at a daily oral dosage of 30 mg/kg starting on day three and continuing for seven days after the creation of a gastric ulcer. Because sitagliptin has a half-life of two hours in rats [21] compared to 13 hours in humans [22], the dosage of 30 mg/kg/d is much greater than the human dose. Because of its short half-life, it had to be continuously administered by drinking water rather from being taken once daily like people do. [23] To guarantee that every rat got the precise dosage in the drinking water, the Boston University-USA Institutional Animal Care and Use Committee (IACUC) procedure for introducing a new substance to the drinking water was adhered to [24].

2.3. Induction of Gastric Ulcer

Rats were given halothane anesthesia after an 18-hour fast, and, in accordance with Okabe and Amagase's 2005 [25] instructions, 0.2 mL of 100% acetic acid was applied to the serosal surface for 60 seconds to cause stomach ulcers. The pathogenic characteristics and healing process of this stomach ulcer model are quite similar to those of real ulcers, which is why it was selected. Rats were killed by administering an intraperitoneal injection of sodium pentobarbital ten days after gastric ulcer induction. Following their removal, the stomachs were opened along their larger curvature, cleaned with saline, and then preserved in 10% buffered formalin.

2.4. Pathological Assessment of Ulcer Healing

The stomachs were grossly examined for pathological changes. The ulcerated area (mm) was quantified using the following equation: $S = \pi (d1/2) X (d2/2)$ where, S represented the ulcerated area (mm), d1 and d2 the longest longitudinal and transverse diameters of the ulcer [26].

Representative sections were routinely processed. 5 µm-thick sections were cut and stained with the conventional Haematoxylin and

Eosin (H&E) stain and examined by the light microscope for histopathological assessment. Masson trichrome stain was used to highlight fibrosis. The degree of inflammation, degeneration and thickness (maturation) of ulcer base were semi-quantitatively assessed at the ulcer bed. Length of regenerated mucosa (mm) was also measured.

2.5. Immunohistochemistry for iNOS and COX-2

Graded alcohols were used to rehydrate the tissue slices that had been deparaffinized. The avidin-biotinylated immunoperoxidase technique was used for immunohistochemical staining. For ten minutes, 3% hydrogen peroxide was used to stop the endogenous peroxidase activity. Sections were microwaved in 10 mM citrate buffer (pH 6.0) to retrieve antigen. COX-2 (clone SP21, rabbit monoclonal antibody), iNOS (rabbit polyclonal antibody), and prediluted primary antibodies were used. The Ultra Vision Detection System Anti-Polyvalent, HRP/DAB (Ready-To-Use), was used to find the bound antibodies. Every run included both positive and negative controls. The detection technology and primary antibodies were acquired from Thermo Fisher Scientific Inc.'s Lab Vision Corporation in the United States.

2.6. Computerized Image Analysis (CIA)

Histological sections immunostained for iNOS and COX-2 were subjected to quantitative estimate of the total area of positive response using image analyzer software (Digimizer ® Version 4.1, MedCalc Software Belgium).

The mean total area of positive response was computed and binary pictures were created for measurement.

2.7. Assessment of Microvessel Density (MVD)

As previously mentioned, sections were immunostained using the vascular marker CD31 (rabbit polyclonal antibody). (Figure 1d) After that, MVD was computed as previously mentioned [27].

2.8. Statistical Analysis

The Statistical Package for Social Science (SPSS® Statistics 20) was used to examine the data. The Kolmogorov-Smirnov test was used to determine if the distributions of quantitative variables were normal. The mean and standard deviation were used to characterize quantitative values that were normally distributed. Their means were compared using the independent t-test. The median, minimum, and maximum were used to characterize both quantitatively and qualitatively improperly distributed ordinal values. Spearman's correlation coefficient was used to test correlations. A comparison of their distributions was made using the Mann-Whitney (U) test. A 5% threshold was used to determine statistical significance ($p < 0.05$).

3. Results

3.1. Induction of gastric ulcer resulted in Significant Histopathological Changes and Increased MVD.

When rats were given acetic acid gastrically, all identified pathological conditions increased statistically significantly.

The ulcer model group's mean ulcerated area (mm) ($p<0.001$), deteriorated mucosa ($p<0.001$), inflammatory exudates ($p<0.001$), ulcer base thickness ($p<0.001$), and length of regenerated mucosa (mm) ($p<0.001$) were all different from the normal control group (Table 1 and Figure 1). Furthermore, the model group's MVD was substantially higher than that of the normal control group ($p <0.001$). (Table 1).

3.2. Induction of Gastric Ulcer Significantly Induced COX-2 and iNOS Expression

The ulcer model group's stomachs showed elevated expression of COX-2 and iNOS, which was statistically significantly greater than that of the normal control group, which did not express either of these molecules ($p<0.001$). (Figure 2, 5, 6; Table 1). The inflammatory cells near the ulcer base had the highest expression levels of COX-2 and iNOS (Figure 2).

3.4. Sitagliptin administration significantly impaired gastric ulcer healing

When sitagliptin was taken orally for seven days, the healing of stomach ulcers was significantly impaired, as shown by pathology, in contrast to the ulcer model group (Figure 1). Table 1 and Figure 1, 3 show that the

ulcerated area in the sitagliptin-treated group was approximately nine times broader than in the model group ($p < 0.001$).
The sitagliptin-treated group showed less mucosal regeneration and more severe inflammatory alterations (intensity of inflammatory exudate and mucosal degradation) than the ulcer model group, although the differences were not statistically significant. However, there was a strong negative correlation ($\rho = -0.477$) between the severity of inflammatory exudate and COX-2 expression.
In comparison to the model group, the sitagliptin-treated group exhibited substantially lower levels of MVD, COX-2, and iNOS expression ($p<0.001$). (Figure 2, 4-6; Table 1). In the sitagliptin-treated group, COX-2 and iNOS expression was higher near the ulcer borders and lower in the inflammatory cells in the ulcer base. (Fig. 2)
There was a significant negative correlation between the mean ulcerated area (mm2), MVD ($\rho = -0.635$, $p = 0.004$), iNOS expression ($\rho = -0.702$, $p = 0.001$), and COX-2 expression ($\rho = -0.652$, $p = 0.002$). The sitagliptin-treated group's ulcer base maturation was substantially worse than that of the model group ($U = 20$, $p = 0.023$). It also had a significant negative correlation with the expression of iNOS ($\rho = -0.548$, $p = 0.015$) and COX-2 ($\rho = -0.508$, $p = 0.026$).

3.5. Ranitidine administration significantly improved Gastric Ulcer Healing

When compared to the ulcer model group and the sitagliptin-treated group, oral ranitidine administration significantly reduced the following pathological changes: mean ulcerated area (mm) ($p<0.001$), degenerated mucosa ($p<0.001$), inflammatory exudates ($p<0.001$), thickness of ulcer base ($p<0.001$), and length of regenerated mucosa (mm) ($p<0.001$). (Figure 1, Table 1)
The group treated with ranitidine showed a substantial increase in MVD, COX-2, and iNOS expression.

compared to the group treated with sitagliptin ($p<0.001$). (Figure 4-6, Table 1)

4.4. Positive Correlation Between iNOS, COX-2 and MVD

COX-2 and iNOS expression were shown to be positively correlated in the present research in a statistically significant way ($p = <0.001$). Additionally, there was a statistically significant positive connection between iNOS expression and MVD ($\rho = 0.540$, $p = 0.017$) and COX-2 expression and MVD ($\rho = 0.510$, $p = 0.026$).

4. Discussion

The current investigation found that oral sitagliptin treatment impaired the healing of gastric ulcers in rats by causing significant alterations in gastric histopathology and a reduction in CO2 and iNOS expression in the gastric mucosa.
A gastric ulcer is defined by morphological investigations as a local excavation caused by active inflammation that compromises the stomach's mucosal integrity [28]. Comparing the model group to the normal control group, the current research found substantial abnormalities in the gastrointestinal histopathology, including a large ulcerated area, deteriorated mucosa, and the presence of inflammatory exudates. Other investigations revealed the same histological alterations [2, 29].
In addition to being crucial for the preservation of the integrity of the stomach mucosa, iNOS and COX-2 also play a significant role in the angiogenesis, ulcer base maturation, and regulation of inflammatory responses that occur during ulcer healing [30–33]. Furthermore, it has been suggested that NO contributes significantly to ulcer healing by creating a gelatinous layer that covers the ulcer bed. This layer is made up of mucus and necrotic cells and is based on fibrin. It serves as a barrier to avoid direct contact with the stomach luminal contents [34]. Moreover, it has been hypothesized that other mediators, including NO, may perform the protective roles of PGs in the stomach [35]. According to the current research, the gastric model group had significantly higher levels of both iNOS and COX2 expression than the normal control group, whose stomach tissue did not exhibit either of these markers. This result is consistent with recent research that found that COX-2 and iNOS are often not detectable in the majority of healthy tissues, with their expression only being triggered in inflammatory locations [36, 37]. Significant COX-2 and iNOS expression was found in the ulcer bed in the model group of the present

investigation. According to Tatemichi et al. [6] and Shigeta et al. [5], iNOS and COX-2 expression surged during the fast healing period and was restricted to the ulcer bed, which is consistent with that conclusion. A statistically significant positive connection between COX-2 and iNOS expression was found in the present investigation. This discovery adds credence to the recent discovery of a synergistic molecular connection between the iNOS and COX-2 pathways, demonstrating the interdependence of these two systems and their potential role as a key mechanism in inflammatory responses [11, 38, 39].
Since the neovasculature facilitates the delivery of nutrients to the repairing tissue, angiogenesis is another crucial element that plays a key role in the healing of stomach ulcers [40]. When compared to the normal control group, the ulcer model group's MVD [one of the most widely used methods to measure angiogenesis] [26] was significantly higher.

was substantially favorably connected with the mucosal regeneration length. Furthermore, there was a positive association found between MVD and the expression of iNOS and COX-2. These results imply that by controlling angiogenesis, iNOS and COX-2 may aid in the healing process of ulcers. Konturek et al. provided more evidence for this [41]. He said that NO promotes angiogenesis at the ulcer base, which aids in the repair of stomach ulcers. Leahy et al. [42] as well. claimed that the angiogenic stimulating effects of PGs generated from COX-2 are comparable.
The current research investigated the impact of sitaglitpin (DPP-4 inhibitor), a newly approved oral antidiabetic medication, on the healing process of gastric ulcers since diabetes mellitus is linked to delayed gastric ulcer healing. The serine protease DPP-4 is extensively distributed throughout the body and is expressed as an ectoenzyme on the surface of T-lymphocytes, endothelial cells, and in a circulating form. Despite having a wide range of possible substrates, this enzyme seems to be particularly important for the inactivation of the incretin hormones gastric inhibitory peptide (GIP) and glucagon-like peptide-1 (GLP-1) [43].
Compared to the ulcer model group and the ranitidine-treated group, which shown improvement in the stomach healing process, the sitagliptin-treated group in the present research had considerably worse gastric ulcer healing. It is well known that ranitidine improves gastric healing by inhibiting histamine 2 receptors in the stomach mucosa. The sitagliptin-treated group's ulcerated area was noticeably greater and the ulcer base's maturation was noticeably worse than that of the ulcer model group and the ranitidine-treated group. Additionally, compared to the ulcer model group and the ranitidine-treated group, the sitagliptin-treated group had more severe inflammatory alterations and less mucosal regeneration; however, these differences did not achieve statistical significance.
In the current investigation, the sitagliptin-treated group exhibited substantially lower levels of COX-2, iNOS, and MVD expression in comparison to the ulcer model group and the ranitidine-treated group. This was confirmed much further by our discovery of a

The mean ulcerated area on the one hand and COX-2, iNOS, and MVD expression on the other were significantly correlated negatively in the sitagliptin-treated group. Additionally, there was a strong negative correlation between COX-2 and iNOS expression and the severity of inflammatory alterations and the thickness (maturation) of ulcer base in the sitagliptin-treated group. These findings imply that sitagliptin inhibits both COX-2 and iNOS, which impairs the mechanisms involved in ulcer healing, particularly angiogenesis. This is consistent with the findings of other researchers who found that the treatment of COX-2 and iNOS inhibitors significantly inhibited the regeneration of mucosal tissue, the maturation of the ulcer base, and the regression of angiogenesis in the rat stomachs under investigation [5, 44]. COX-2 and iNOS were mostly expressed near the ulcer edges in the sitagliptin-treated group of the current research, with less strong expression at the ulcer base. This likely has a negative impact on ulcer healing. According to Tarnawski et al. [3], iNOS was shown to have a negative effect on ulcer healing if it was expressed near the ulcer margin, which is a crucial region for ulcer healing since it supplies new epithelial cells (the regeneration zone).

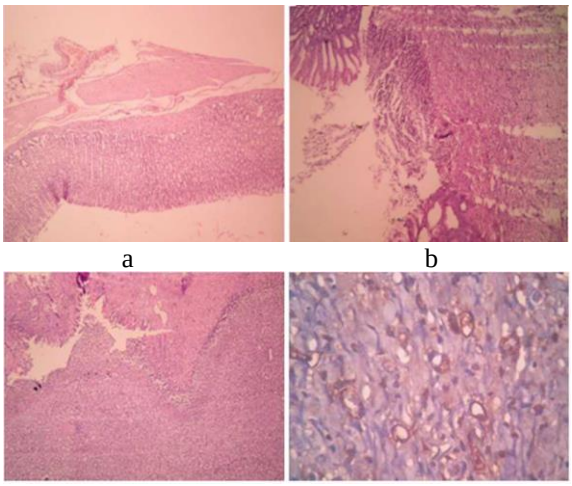
The impact of sitagliptin administration on iNOS expressions in different tissues has not been thoroughly studied. According to Nader et al. [45], sitagliptin therapy significantly reduced both the NO content and the mRNA expression of iNOS in a mouse model of allergic airway illness. But in rats that had artificially caused myocardial infarction, Ye et al. [46] shown that sitagliptin had no impact on COX-2 activity. The impact of incretins and incretin mimetics on iNOS expression was investigated by several researchers. According to Salehi et al. [49], GLP-1 activated the cAMP/PKA pathway in diabetic rat islets, suppressing excessive NO production and iNOS activity. Additionally, Belin et al. [48] showed that GLP-1 decreased NO generation by raising cAMP levels in islets stimulated by high glucose and IL-1β, respectively. Furthermore, exenatide, a GLP-1 agonist, was shown by Kang et al. [49] to reduce cytokine-induced iNOS protein production.

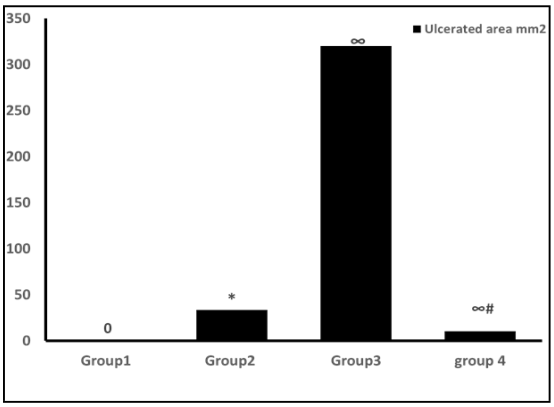
5. Tables and figures

Table 1. Comparison between different assessed parameters among the test groups assessed 7 days after the induction of gastric ulcer.

	Group 1: normal control	**Group 2: ulcer model group**	**Group 3: sitagliptin treated group**	**Group 4: ranitidine treated group**
A-Ulcerated area mm^2 Md (Max-Min)	0.00	33.77(0.00-435.90)*	320.05(62.83-589.05)$^{\infty}$	10.60(0.00-94.25) $^{\infty\#}$
B-Degenerated mucosa Mdn (Min-Max)	0.00	1(0-3) *	2(1-3)	3.50(0.00-10.00) $^{\infty\#}$
C-Length of regenerated mucosa (mm) Mdn (Min-Max)	0.00	4.00(0.00-20.00) *	0(0.00-5.00)	20.00(.00-30.00) $^{\infty\#}$
D-iNOS mean area Mdn (Min-Max)	0.00	211.21(5.70-332.49) *	16.22(2.30-102.61) $^{\infty}$	106.86(8.31-147.30)$^{\infty\#}$
E-COX2 mean area M±SD	***0±00***	175.29±76.15*	62.79±50.08$^{\infty}$	197.30±64.99$^{\infty\#}$
F-MVD M±SD	2.40±0..97	7.60±0.97*	4.90±1.20$^{\infty}$	11.10±0.88$^{\infty\#}$

Please take notice of parameters A, B, C, and D. The model group, normal control group, and treatment groups were compared for statistical significance using the Mann-Whitney test, and the data was shown as a median. With reference to parameters E and F The data were shown as M± SD, and the statistical significance of the model group, normal control group, and treatment groups was evaluated using Tukey's test. P is less than 0.001 against group 1, #P is less than 0.001 against group 2, and ∞P is less than 0.001 against group 2.





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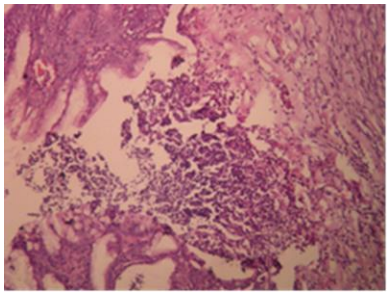

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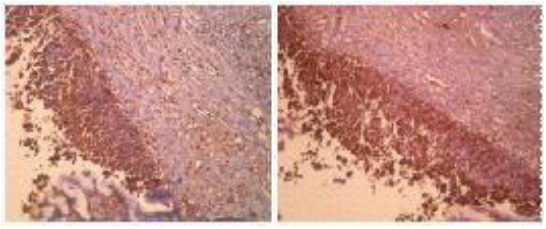
Fig 1: Histopathological alterations in the groups under study: (a) The mucosal surface of the normal control group is undamaged and free of fibrosis and inflammation (H&E, 40x). (b) A model of a gastric ulcer that displays a moderately sized ulcerated region with necroinflammatory debris covering the ulcer base (H&E, 40x). The group receiving sitagliptin exhibited (c) a big ulcer with a swollen ulcer base and severe inflammation (H&E, 40x); (d) CD31 immunostain-highlighted microvessels. (200x) (f) Group treated with ranitidine demonstrates a decrease in ulcer size (H&f, x100).



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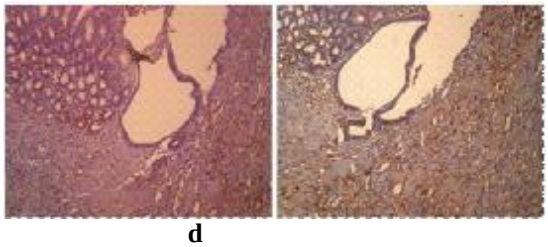

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Fig 2 Under 100x original magnification, the immunohistochemical expression of COX-2 and iNOS in the groups under study: High levels of iNOS (a) and COX-2 (b) expression in the ulcer base are shown in the upper panel of an acid-induced gastric ulcer model. Lower panel: The group treated with sitagliptin has moderate expression at the ulcer borders and decreased expression of COX-2 (d) and iNOS (c) at the ulcer base.

Fig 3 Comparing the histological evaluation of the ulcerated region in groups G1 (normal control group), G2 (ulcer model group), G3 (sitagliptin treatment), and G4 (ranitidine). Notes: The Mann-Whitney test was used to establish the statistical significance between the various groups: * $P < 0.001$ vs group 1, ∞$P < 0.001$ versus group 2, #$P < 0.001$ versus group 3.

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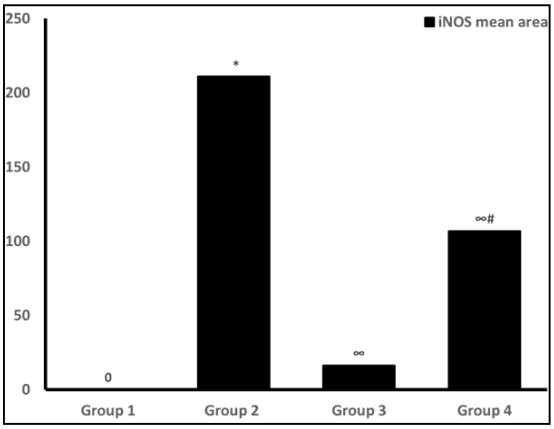

Fig 4 Comparison of the iNOS area across groups G1 (normal control group), G2 (ulcer model group), G3 (sitagliptin treatment), and G4 (ranitidine). Notes: The Mann-Whitney test was used to establish the statistical significance between the various groups: * P < 0.001 vs group 1, ∞P < 0.001 versus group 2, and #P < 0.001 versus group 3.

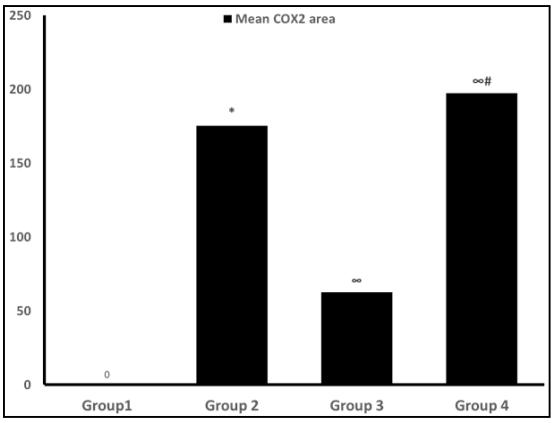

Fig 5 Comparing the COX2 area of groups G1 (normal control group), G2 (ulcer model group), G3 (sitagliptin treated), and G4 (ranitidine). Notes: Tukey's test was used to establish the statistical significance between the various groups, * P

< 0.001 versus group 1, $^{\infty}P < 0.001$ versus group 2, $^{\#}P < 0.001$ versus group 3

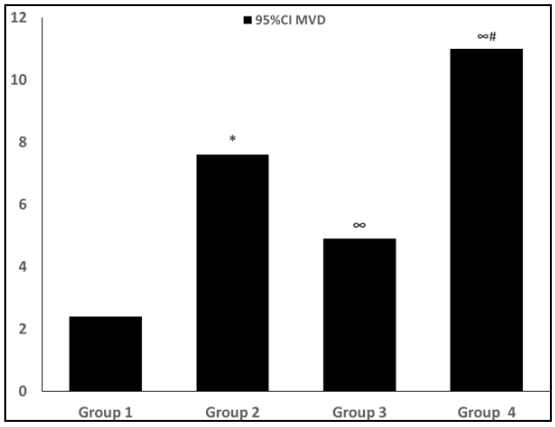

Fig 6 Comparison of the 95% MVD across groups G1 (normal control group), G2 (ulcer model group), G3 (sitagliptin treatment), and G4 (ranitidine). Notes: Tukey's test was used to establish the statistical significance between the various groups: * $P < 0.001$ vs group 1, ∞$P < 0.001$ versus group 2, #$P < 0.001$ versus group 3.

6. Conclusion

When compared to ranitidine, sitagliptin was shown to considerably hinder the healing of stomach ulcers in rats, probably via inhibiting the production of COX-2 and iNOS. To support its prescription for diabetic individuals who already have a stomach ulcer, further research is required.

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