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Comparative study of effect of ranitidine and sitagliptin on healing of acetic acid induced gastric ulcer in rats

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Abstract
Gastric ulcer healing is a complex process that is regulated by several promoting factors including Cyclooxygenase-2 (COX-2) and Inducible Nitric Oxide Synthase (iNOS). Diabetes mellitus is usually associated with delayed gastric ulcer healing. Hence, the current study was designed to compare the effect of sitagliptin (dipeptidyl peptidase-4 inhibitor) with ranitidine on gastric ulcer healing. The present study was conducted on 40 male albino rats that were divided into four equal groups: Group 1: normal control group, group 2: gastric ulcer model, Group 3: sitagliptin treated group, Group 4: ranitidine treated group. Rats were sacrificed ten days after ulcer induction and stomach was removed for histopathological examination and Immuno histochemical evaluation of COX-2 and iNOS. This study revealed that gastric ulcer healing was significantly impaired in the sitagliptin-treated group when compared with ranitidine treated group, evidenced by histopathological examination of stomach showing significantly larger ulcerated area and ulcer base maturation impairment.COX-2 and iNOS expression as well as mean vascular density (MVD) were significantly diminished in the sitagliptin-treated group as compared to ulcer model group as well as ranitidine treated group. A significant positive correlation was found between COX-2 and iNOS implying their synergistic action. A significant positive correlation was found between MVD on one hand and COX-2 and iNOS on the other hand pointing to their proangiogenic effect. This results raise the question of whether sitagliptin is advisable in diabetic patients with pre-existing gastric ulcer. Our preliminary experimental findings need to be substantiated by future human studies.

Keywords: ranitidine, sitaglitpin, gastric ulcer, rats

Introduction

Gastric ulcer is considered as one of the highly prevent gastrointestinal disorders nowadays. [1] Its pathogenesis rely mostly on an imbalance between mucosal protecting factors and aggressive factors e.g. NSAIDs and helicobacter pylori activation in gastric mucosa [2].

Gastric ulcer healing is a dynamic process encompassing epithelial regeneration, angiogenesis and maturation of the base (reduction of the ulcer base size) and is regulated by multiple factors [3, 4]. COX-2 (cyclooxygenase-2) and iNOS (inducible nitric oxide synthase) are among the most important healing-promoting factors for gastric ulcer [5-7]. COX-2 induces the synthesis of Prostaglandins (PGs) that have stimulatory effects on ulcer healing. [8] iNOS-derived Nitric Oxide (NO) contributes to gastric ulcer healing through maintenance of an increased blood flow at the ulcer margin and stimulation of angiogenesis in the ulcer base as well as inhibition of inflammatory neutrophil accumulation via downregulation of surface expression of adhesion molecules [9, 10]. Recently, it was shown that the iNOS-based inflammatory pathway cross-link with the more well- known COX-2 pathway. This synergistic molecular interaction between the two inflammatory systems may cast more light on their healing promoting effects on gastric ulcer [11].

Diabetic patients are more vulnerable to develop gastric ulcers as diabetes leads to impairment of the antioxidant defense system of the gastric mucosa [12, 13]. In addition, diabetic patients with gastric ulcers may suffer from reduced perception of the typical gastrointestinal symptoms due to diabetic neuropathy and they are at increased risk of bleeding [14]. Furthermore, diabetes may be associated with delayed healing of gastric ulcer due to significant decrease in the gastric microcirculation possibly resulting from reduction in mucosal prostaglandins [15]. Moreover, it was reported that hyperglycemia together with the increased production of proinflammatory cytokines result in sustained inflammatory reaction and thus may be responsible for the delay of healing at the ulcer area [16]. Such previously stated reports necessitate studying the effect of antidiabetic drugs on gastric ulcer healing.

Dipeptidyl Peptidase-4(DPP-4) inhibitors are recently introduced drugs used for treatment of type 2 diabetes. Recent studies demonstrated that DPP-4 inhibitors or related compounds may possess marked inflammatory modifying effects through modulation of cytokine production. [17] To the best of our knowledge, there have been no studies in the literature comparing the effect of DPP-4 inhibitors on gastric ulcer healing with a histamine 2 antagonist (ranitidine)which is one of the most important compounds proved to treat gastric ulcer and to promotes its healing mostly through its effect on

iNOS and COX2 [18, 19].
Accordingly, the purpose of this study is to evaluate the effect of sitagliptin (DPP-4 inhibitor) on gastric ulcer healing rats and comparing its effect with that of ranitidine.

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

Rats were divided into 4 groups of 10 rats each:
Group 1: (normal control group) in which rats had free access to drinking water without any additive.
Group 2: (gastric ulcer model) in which gastric ulcer was induced in rats and they had free access to drinking water without any additive.
Group 3: (ranitidine treated group): in which rats received ranitidine added to the drinking water, at a dose of 50 mg/kg orally every day [20], beginning on day 3 and continuing for 7 days following gastric ulcer induction.
Group 4: (sitagliptin-treated group) in which rats received sitagliptin added to the drinking water, at a dose of 30 mg/kg orally every day, beginning on day 3 and continuing for 7 days following gastric ulcer induction. The dose of 30 mg/kg/d is considerably higher than the human dose because sitagliptin has a half-life of two hours in rats [21] versus 13 h in humans [22]. This short half-life necessitated continuous administration through drinking water instead of the once-a-day dosing used in humans. [23] The Institutional Animal Care and Use Committee (IACUC) protocol of Boston University-USA for adding a novel compound to the drinking water was followed in order to ensure that each rat received the exact dose in the drinking water [24].

2.3. Induction of Gastric Ulcer

After fasting for 18 h, rats were anesthetized, using halothane and gastric ulcers were induced by application of 0.2 mL of acetic acid (100%) to the serosal surface for 60 sec as described by Okabe and Amagase, 2005 [25]. This model of gastric ulcer was chosen as it highly resembles human ulcers in terms of both pathological features and healing process.
Ten days following gastric ulcer induction, rats were sacrificed by an overdose of intraperitoneally injected sodium pentobarbital. The stomachs were removed, opened along the greater curvature and rinsed with saline then they were fixed 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

The deparaffinized tissue sections were rehydrated in graded alcohols. Immuno histochemical staining was performed using an avidin-biotinylated immunoperoxidase methodology. The endogenous peroxidase activity was quenched by using hydrogen peroxide 3% for 10 min. For antigen ret retrieval, sections were microwaved in 10mM citrate buffer (pH 6.0). Prediluted primary antibodies, COX-2 (clone SP21, rabbit monoclonal antibody) and iNOS (rabbit polyclonal antibody) were used. The bound antibodies were detected by the Ultra Vision Detection System Anti- Polyvalent, HRP/DAB (Ready-To-Use). Positive and negative controls were included in all runs. Primary antibodies and detection system were purchased from Lab Vision Corporation, Thermo Fisher Scientific Inc., USA.

2.6. Computerized Image Analysis (CIA)

Quantitative estimation of the total area of positive reaction was done on histological sections immunostained for iNOS and COX-2 using image analyzer software (Digimizer ® Version 4.1, MedCalc Software, Belgium).
Binary images for measurement were generated and the mean total area of positive reaction was calculated.

2.7. Assessment of Microvessel Density (MVD)

Sections were immunostained by the vascular marker, CD31 (rabbit polyclonal antibody) as described above. (Fig. 1d) MVD was then calculated as previously described [27].

2.8. Statistical Analysis

Data were analyzed using Statistical Package for Social Science (SPSS® Statistics 20). The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test. Quantitative normally distributed variables were described using mean and standard deviation. Independent t-test was used to compare their means. Both quantitative abnormally distributed and Qualitative ordinal variables were described using median, minimum and maximum. Correlations were tested using Spearman's correlation coefficient. Mann-Whitney (U) test was used to compare their distributions. Statistical Significance was judged at the 5% level ($p \leq 0.05$).

3. Results

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

Gastric serosal application of acetic acid in rats resulted in statistically significant increase in all observed pathological

changes in ulcer model group compared to normal control group: 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) ($p<0.001$) (Table 1 and Figure 1).
In addition, MVD was significantly increased in the model group compared to normal control group ($p <0.001$). (Table 1).

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

COX-2 and iNOS expression were induced in the stomachs of ulcer model group with a statistically significant higher expression compared to the normal control group that lacked their expression ($p<0.001$). (Table 1, Figure2, 5, 6). COX- 2 and iNOS were most intensely expressed in inflammatory cells at the ulcer base (Figure 2).

3.4. Sitagliptin administration significantly impaired gastric ulcer healing

Oral administration of sitagliptin for seven days resulted in pathologically proven significant impairment of gastric ulcer healing as compared to the ulcer model group (Figure 1). The ulcerated area in the sitagliptin-treated group was significantly larger (nearly 9 times wider) than the model group ($p < 0.001$) (Table 1 and Figure 1, 3).
Although inflammatory changes (intensity of inflammatory exudate and mucosal degeneration) were severer and mucosal regeneration was less pronounced in the sitagliptin-treated group compared to the ulcer model group, the results did not reach statistical significance. However, the intensity of inflammatory exudate was significantly negatively correlated with COX-2 expression ($\rho = -0.477$).
COX-2 and iNOS expression as well as MVD were significantly diminished in the sitagliptin-treated group compared to the model group ($p<0.001$) respectively. (Table1, Figure 2, 4-6). The expression of COX-2 and iNOS in the sitagliptin-treated group was more
pronounced at the ulcer margins with less intense expression in inflammatory cells at the ulcer base. (Figure 2)
The mean ulcerated area (mm2) was significantly negatively correlated with COX-2 expression ($\rho = - 0.652$, $p = 0.002$); iNOS expression ($\rho = -0.702$, $p = 0.001$); and MVD ($\rho = -0.635$, $p = 0.004$). Maturation of ulcer base was significantly impaired ($U = 20$, $p = 0.023$) in the sitagliptin-treated group compared to the model group. In addition, it was significantly negatively correlated with COX-2 expression ($\rho = -0.508$, $p = 0.026$); and iNOS expression ($\rho = -0.548$, $p = 0.015$).

3.5. Ranitidine administration significantly improved Gastric Ulcer Healing

Oral administration of ranitidine resulted in significant amelioration in pathological changes compared to the ulcer model group and the sitagliptin treated group: 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) ($p<0.001$). (Table 1, Figure 1, 2)
COX-2 and iNOS expression as well as MVD were significantly increased in the ranitidine-treated group compared to sitagliptin treated group ($p<0.001$). (Table 1, Figure 4-6)

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

The current study showed a statistically significant positive correlation between COX-2 and iNOS expression ($p = <0.001$). A statistically significant positive correlation was also found between COX-2 expression and MVD ($\rho = 0.510$, $p = 0.026$) on one hand and iNOS expression and MVD ($\rho = 0.540$, $p = 0.017$) on the other hand.

4. Discussion

In the present study, oral administration of sitagliptin resulted in impairment of gastric ulcer healing in the form of severe gastric histopathological changes and decreased expression of CO2 and iNOS in gastric mucosa of rats.
According to morphological studies gastric ulcer refers to a disruption of the mucosal integrity of the stomach with local excavation due to active inflammation [28]. The present study revealed significant gastric histopathological changes (large ulcerated together with degenerated mucosa and presence of inflammatory exudates) in model group when compared to the normal control group. Same histopathological changes were reported in other studies [2, 29].
iNOS and COX-2 represent important lines of defense necessary for maintenance of gastric mucosal integrity and are important factors in ulcer healing processes including angiogenesis, ulcer base maturation and modulation of inflammatory reactions [30-33]. Moreover it has been proposed that NO plays an important role in ulcer healing by forming a gelatinous coat covering the ulcer bed, consisting of a fibrin-based gel with mucus and necrotic cells, which acts as a protective barrier preventing direct contact with the gastric luminal contents [34]. Furthermore, it has been postulated that protective functions of PGs in the stomach could be carried out by other mediators, in particular NO [35]. The present study revealed significant increased expression of both iNOS and COX2 in the gastric model group when compared to the normal control group where gastric tissue lacked expression of both markers. Such finding is accordance with other studies where it was concluded that COX-2 and iNOS are normally undetectable in most normal tissues; their expression being induced only at inflammatory sites [36, 37]. In the current study, significant expression of COX-2 and iNOS was detected in ulcer bed in the model group. In agreement with that finding, Tatemichi *et al.* [6] and Shigeta *et al.* [5] stated that iNOS and COX- 2 expression peaked during the rapid healing phase and were limited to ulcer bed.
In the current study, a statistically significant positive correlation between COX-2 and iNOS expression was detected. Such finding further supports the recent identification of a synergistic molecular interaction between COX-2 and iNOS pathways proving that these two systems are related and may represent a major mechanism in inflammatory responses [11, 38, 39].
Angiogenesis is an another important factor that play a pivotal role in gastric ulcer healing since the neovasculature promotes nutrient supply to the healing tissue [40]. In the present study, MVD [one of most commonly used techniques to quantify angiogenesis) [26] was significantly increased in the ulcer model group when compared to the normal control group and

was significantly positively correlated with the length of regenerated mucosa. In addition, a positive correlation was detected between iNOS and COX-2 expression on one hand and MVD on the other hand. Such findings suggest that iNOS and COX-2 may contribute to ulcer healing process through regulation of angiogenesis. This was further supported by Konturek *et al* [41]. Who reported that NO stimulates angiogenesis in the ulcer base, contributing to gastric ulcer healing. Also, Leahy *et al* [42]. Stated that COX-2-derived PGs have similar angiogenic stimulating effects.

As diabetes mellitus is associated with delayed gastric ulcer healing, the present study examined the effect of one of the recently introduced oral antidiabetic drugs sitaglitpin (DPP-4 inhibitor) on healing process of gastric ulcer. DPP-4 is a serine protease that is widely distributed throughout the body, expressed as an ectoenzyme on endothelial cells, on the surface of T-lymphocytes and in a circulating form. Although there are many potential substrates for this enzyme, it seems to be especially critical for the inactivation of incretin hormones: GLP-1 (glucagon like peptide -1) and Gastric Inhibitory Peptide (GIP) [43].

In the current study, gastric ulcer healing was significantly impaired in the sitagliptin-treated group compared to the ulcer model group and the ranitidine treated group which showed amelioration of gastric healing process. The mechanism behind the improvement of gastric healing by ranitidine is known to be through blocking histamine 2 receptors in gastric mucosa. Compared to the ulcer model group and to the ranitidine treated group, the ulcerated area in the sitagliptin-treated group was significantly larger and maturation of ulcer base was significantly impaired. In addition, inflammatory changes were severer and mucosal regeneration was less pronounced in the sitagliptin-treated group compared to the ulcer model group and the ranitidine treated group, however, these results did not reach statistical significance.

Expression of COX-2, iNOS and MVD in the present study were significantly diminished in the sitagliptin-treated group compared to the ulcer model group and the ranitidine treated group. This was further substantiated by our finding of a significant negative correlation in the sitagliptin treated group between the mean ulcerated area on one hand and COX-2 expression, iNOS expression and MVD on the other hand. In addition, the intensity of inflammatory changes and thickness (maturation) of ulcer base in the sitagliptin treated group were significantly negatively correlated with COX-2 and iNOS expression. Such results suggest that sitagliptin acts as inhibitor of both COX-2 and iNOS leading to impairment of ulcer healing processes specially angiogenesis. This is in accordance with other researchers who reported that administration of COX-2 and iNOS inhibitors resulted in significant prevention of mucosal regeneration and maturation of the ulcer base as well as regression of angiogenesis in the examined rat stomachs [5, 44]. In the sitagliptin-treated group in the present study, COX-2 and iNOS were mostly expressed at the ulcer margins with less intense expression at the ulcer base which probably has a deleterious effect on ulcer healing. This is in accordance with Tarnawski *et al*. [3] who reported that iNOS were to act detrimentally on ulcer healing if it is expressed at the ulcer margin which is an important area for ulcer healing, supplying new epithelial cells (regenerating zone).

Few studies have investigated the effect of sitagliptin administration on iNOS expressions in various tissues. Nader *et al*. [45] have shown that NO content as well as the mRNA expression of iNOS was remarkably decreased by sitagliptin treatment in murine model of allergic airway disease. On the other hand, Ye *et al*. [46] have shown that sitagliptin had no effect on COX-2 activity in experimentally induced myocardial infarction in rats.

Other researchers explored the role of incretins and incretin mimetics on iNOS expression. Salehi *et al.* [49] reported that GLP-1 suppressed excessive NO generation and iNOS activity in diabetic rat islets via the activation of cAMP/PKA system. Also, Belin *et al* [48] demonstrated that GLP-1 reduced NO production through increasing the level of cAMP in high glucose- and IL-1β-stimulated islets respectively. In addition, Kang *et al* [49] showed that exenatide (GLP-1 agonist) decreased cytokine induced iNOS protein expression.

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$^{\infty\#}$

Note: for parameters A, B, C and D Data was presented as median and the statistical significance between the treated groups, normal control group, and model group, was determined using Mann-Whitney test. For parameters E and F Data was presented as M± SD and The statistical significance between the treated groups, normal control group, and model group, was determined using Tukey's test. *, $P < 0.001$ versus group 1, $^{\infty}P < 0.001$ versus group 2, $^{\#}P < 0.001$ versus group 3.

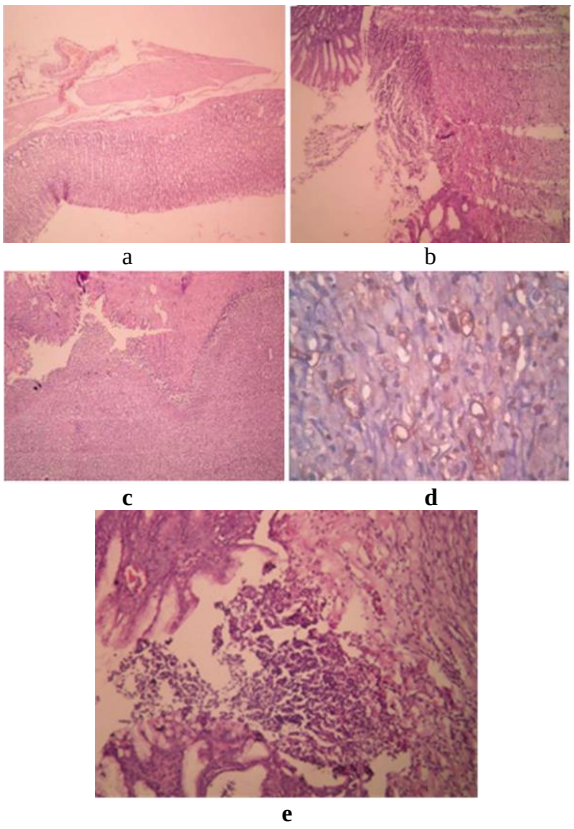

Fig 1: Histopathological changes in the studied groups: (a): Normal control group showing intact mucosal surface with absent inflammation and fibrosis (H&E, 40x). (b): Gastric ulcer model; showing ulcerated area of moderate size and the ulcer base is covered by necroinflammatory debris (H&E, 40x). Sitagliptin-treated group showing (c) large-sized ulcer with thickened ulcer base and intense inflammation (H&E, 40x); (d) (d) Microvessels highlighted by CD31 immunostain. (200x) (e) Ranitidine treated group showing reduction in ulcer size (H&E, x100).

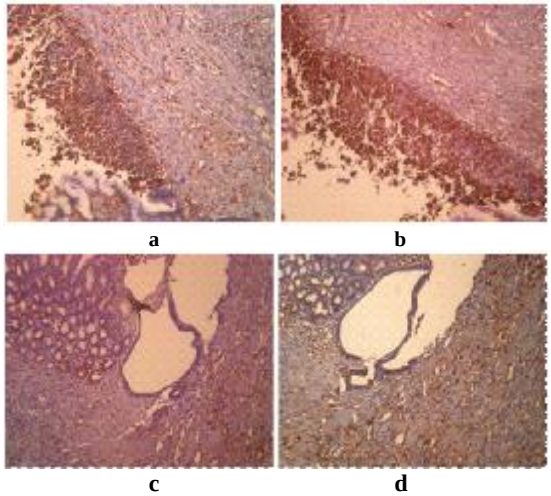

Fig 2: Immunohistochemical expression of iNOS and COX-2 in the studied groups under 100x original magnification: Upper panel: Acid-induced gastric ulcer model showing intense iNOS (a) and COX-2 (b) expression in the ulcer base. Lower panel: Sitagliptin-treated group showing diminished iNOS (c) and COX-2 (d) expression in ulcer base with moderate expression at the ulcer margins.

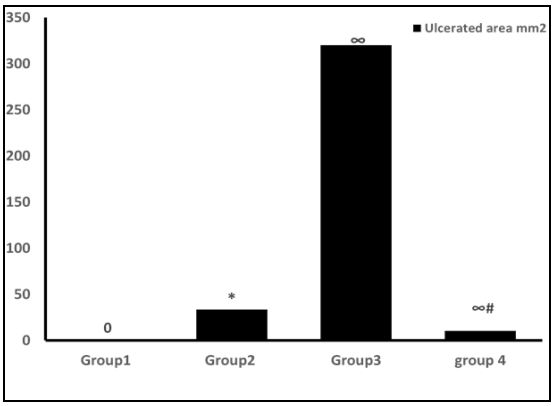

Fig 3: Comparison between group G1 (normal control group), group G2 (ulcer model group), group G3 (sitagliptin treated) and group G4 (ranitidine) regarding histopathological assessment of the ulcerated area. Notes: The statistical significance between the different groups was determined using Mann-Whitney test, * $P < 0.001$ versus group 1, $^{\infty}P < 0.001$ versus group 2, $^{\#}P < 0.001$ versus group 3.

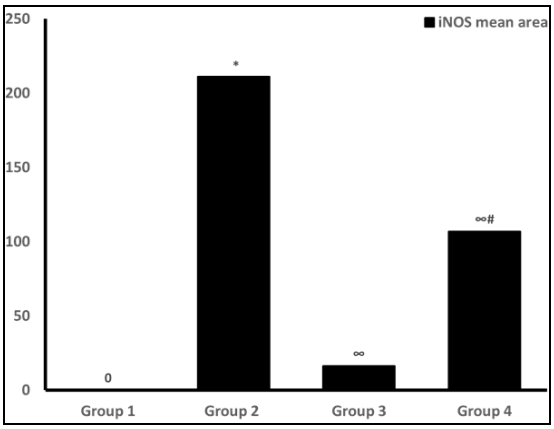

Fig 4: Comparison between group G1 (normal control group), group G2 (ulcer model group), group G3 (sitagliptin treated) and group G4 (ranitidine) regarding iNOS area. Notes: The statistical significance between the different groups was determined using Mann-Whitney test, * $P < 0.001$ versus group 1, $^{\infty}P < 0.001$ versus group 2, $^{\#}P < 0.001$ versus group 3.

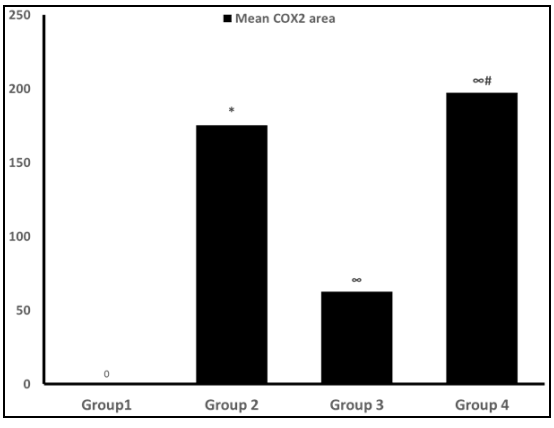

Fig 5: Comparison between group G1 (normal control group), group G2 (ulcer model group), group G3 (sitagliptin treated) and group G4 (ranitidine) regarding COX2 area. Notes: The statistical significance between the different groups was determined using Tukey's test, * $P < 0.001$ versus group 1, $^{\infty}P < 0.001$ versus group 2, $^{\#}P < 0.001$ versus group 3

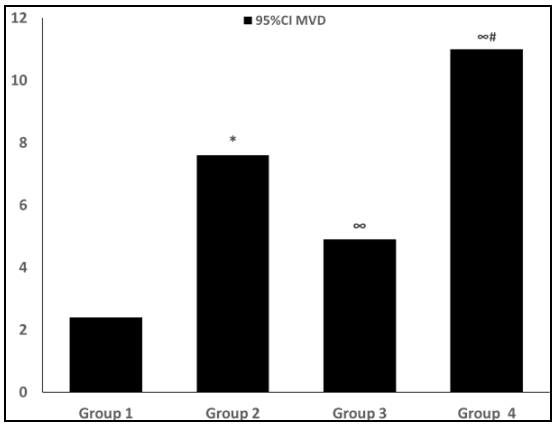

Fig 6: Comparison between group G1 (normal control group), group G2 (ulcer model group), group G3 (sitagliptin treated) and group G4 (ranitidine) regarding 95%CI MVD. Notes: The statistical significance between the different groups was determined using Tukey's test, * $P < 0.001$ versus group 1, $^{\infty}P < 0.001$ versus group 2, $^{\#}P < 0.001$ versus group 3.

6. Conclusion

Sitagliptin was found to significantly impair gastric ulcer healing in rats when compared to ranitidine, possibly through inhibition of iNOS and COX-2 expression. Further studies are needed to justify its prescription to diabetic patients with preexisting gastric ulcer.

7. References

1. Ahmed OAA, UA Fahmy R, Bakhaidar MA, El-Moselhy MA, Alfaleh ASF, Ahmed ASA Hammad, *et al*. Pumpkin Oil–Based Nanostructured Lipid Carrier System for Antiulcer Effect in NSAID-Induced Gastric Ulcer Model in Rats. Int J Nanomedicine. 2020; 15:2529-2539. https://doi.org/10.2147/IJN.S247252
2. Wang CY Yuan, Pan H, Hsu AC, Chen J, Liu J, Li P, *et al*. Protective Effect of Ocotillol, the Derivate of Ocotillol-Type Saponins in *Panax* Genus, against Acetic Acid-Induced Gastric Ulcer in Rats Based on Untargeted Metabolomics. Int J Mol Sci. 2020; 21(7):2577. doi: 10.3390/ijms 21072577. PMID: 32276345; PMCID: PMC7177626.
3. Tarnawski A, Tanoue K, Santos AM, Sarfeh IJ. Cellular and molecular mechanism of gastric ulcer healing. Is the quality of mucosal scar affected by treatment? Scand J. Gastroenterol. 1995; 210:9-14. DOI: 10.3109/00365529509090261.
4. Sato T, Amano H, Ito Y, Eshima K, Minamino T, *et al*. NSAID, aspirin delays gastric ulcer healing with reduced accumulation of CXCR4+VEGFR1+ cells to the ulcer granulation tissues. Biomed. Pharmacother, 2013; 67(7):607-613.DOI 10.1016/j.biopha.2013.01.009.
5. Shigeta J, Takahashi S, Okabe S. Role of cyclooxygenase-2 in the healing of gastric ulcers in rats. J. Pharmacol. Exp. Ther. 1998; 286:1383-1390. PMID: 9732401
6. Tatemichi M, Ogura T, Sakurazawa N, Nagata H, Sugita M, *et al*. Roles of inducible nitric oxide synthase in the development and healing of experimentally induced gastric ulcers. Int J. Exp. Pathol. 2003; 84:213-220. DOI: 10.1111/j.1365-2613.2003. 00357.x
7. Chatterjee A, Khatua S, Chatterjee S, Mukherjee S, Mukherjee A, *et al*. Polysaccharide-rich fraction of Termitomyces eurhizus accelerate healing of indomethacin induced gastric ulcer in mice. Glycoconj J. 2013; 30(8):759-68. doi: 10.1007/s10719-013-9479-5. PMID: 23715800.
8. Takahashi M, Maeda S, O0gura K, Terano A, Omata M. The possible role of Vascular Endothelial Growth Factor (VEGF) in gastric ulcer healing: Effect of sofalcone on VEGF release *in vitro*. J. Clin. Gastroenterol, 1998; 27:S178-S182. DOI: 10.1097/00004836-199800001-00029.
9. Salzman A, Eaves-Pyles T, Linn S, Denenberg A, Szabo C. Bacterial induction of inducible nitric oxide synthase in cultured human intestinal epithelial cells. Gastroenterology, 1998; 114:93-102.PMID: 9428223.
10. Konturek SJ, Brzozowski T, Majka J, Pytko- Polonczyk xJ, Stachura J. Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. Eur. J. Pharmacol. 1993; 239:215-217. DOI: 10.1016/0014-2999(93)90997-V.
11. Kim SF, Huri DA, Snyder SH. Inducible nitric oxide synthase binds, s nitrosylates and activates cyclooxygenase-2. Science. 2005; 310:1966-1966. DOI: 10.1126/science.1119407
12. Owu DU, Obembe AO, Nwokocha R, Edoho IE, Osim EE. Gastric ulceration in diabetes mellitus: Protective role of vitamin C. ISRN Gastroenterol, 2012, 362805-362805. PMID:22778975
13. Konturek PC, Brzozowski T, Burnat G, Szlachcic A, Koziel J, *et al*. Gastric ulcer healing and stress-lesion preventive properties of pioglitazone are attenuated in diabetic rats. J. Physiol. Pharmacol. 2010; 61:429-436. PMID: 20814070.
14. Boehme MW, Autschbach F, Ell C, Raeth U. Prevalence of silent gastric ulcer, erosions or severe acute gastritis in patients with type 2 diabetes mellitus-- a cross-sectional study. Hepatogastroenterology, 2007; 54:643-648. PMID: 17523341.
15. Brzozowska I, Targosz A, Sliwowski Z, Kwiecien S, Drozdowicz D, *et al*. healing of chronic gastric ulcers in diabetic rats treated with native aspirin, Nitric Oxide (NO)-derivative of aspirin and Cyclooxygenase (COX)-2 inhibitor. J. Physiol. Pharmacol. 2004; 55:773-790. PMID: 15613743.
16. Cosentino F, Eto M, De Paolis P, Van Der Loo B, Bachschmid M, *et al*. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: Role of protein kinase C and reactive oxygen species. Circulation, 2003; 107:1017-1023. DOI: 10.1161/01.CIR.0000051367.92927.07.
17. Alonso N, Julian MT, Puig-Domingo M, Vives- Pi M. Incretin hormones as immunomodulators of atherosclerosis. Front Endocrin. 2012; 3:112-112. DOI: 10.3389/fendo.2012.00112.
18. De Araújo ER, Guerra GC, Araújo DF, De Araújo AA, Fernandes JM, *et al*. Gastroprotective and Antioxidant Activity of *Kalanchoe brasiliensis* and *Kalanchoe pinnata* Leaf Juices against Indomethacin and Ethanol-Induced Gastric Lesions in Rats Int J Mol Sci. 2018;

19(5):1265.
19. Fang YF, WL Xu, Wang L, Lian QW, Qiu LF, *et al*. Effect of Hydrotalcite on Indometacin-Induced Gastric Injury in Rats. Hindawi BioMed Research International. 2019; 2019:4605748.
20. Afonso-Pereira F, Murdan S, Sousa JJ, Veiga F, Basit AW. Sex Differences in Excipient Effects: Enhancement in Ranitidine Bioavailability in the Presence of Polyethylene Glycol in Male, but Not Female, Rats, Int J Pharm. 2016; 506(1-2):237-41.
21. Beconi MG, Reed JR, Teffera Y, Xia YQ, Kochansky CJ, *et al*. Disposition of the dipeptidyl peptidase 4 inhibitor sitagliptin in rats and dogs. Drug Metab. Dispos. 2007; 35:525-532. DOI: 10.1124/dmd.106.013110.
22. Dhillon S. Sitagliptin: A review of its use in the management of type 2 diabetes mellitus. Drugs. 2010; 70:489-512. DOI: 10.2165/11203790-000000000-00000.
23. Chen B, Moore A, Escobedo L, Koletsky M, Hou D. Sitagliptin lowers glucagon and improves glucose tolerance in prediabetic obese rats. Exp. Biol. Med., 2011; 236:309-314. DOI: 10.1258/ebm.2010.010161
24. IACUC. Additives for the drinking water for rats and mice. Boston University Research Committees, 2013.
25. Okabe S, Amagase K. An overview of acetic acid ulcer models--the history and state of the art of peptic ulcer research. Biol. Pharm. Bull. 2005; 28:1321-1341. DOI: 10.1248/bpb.28.1321.
26. Kang JM, Kim N, Kim B, Kim JH, Lee BY, *et al*. Enhancement of gastric ulcer healing and angiogenesis by cochinchina momordica seed extract in rats. J. Korean Med. Sci. 2010; 25:875-881.DOI: 10.3346/jkms.2010.25.6.875.
27. Dai XP, Li JB, Liu ZQ, Ding X, Huang CH, *et al*. Effect of Jianweiyu yang granule on gastric ulcer recurrence and expression of VEGF mRNA in the healing process of gastric ulcer in rats. World J. Gastroenterol. 2005; 11:5480-5484. PMID: 16222740.
28. Valle DL. Peptic Ulcer Diseases and Related Disorders. In: Harrison's Principles of Internal Medicine, Harrison, T.R. and D.J. DeAngelo (Eds.), McGraw-Hill, New York, ISBN-10: 0071203591, 2002, 1649-65.
29. Shahrokhi N, Keshavarzi Z, Khaksari M. Ulcer healing activity of Mumijo aqueous extract against acetic acid induced gastric ulcer in rats. J Pharm Bioallied Sci. 2015; 7(1):56-9. doi: 10.4103/0975-7406.148739. PMID: 25709338; PMCID: PMC4333629.
30. Takahashi S, Fujita T, Yamamoto A. Role of nuclear factor-kappaB in gastric ulcer healing in rats. Am. J. Physiol. Gastrointest Liver Physiol. 2001; 280:G1296-G1304. PMID: 11352824.
31. Stachura J. Healing of chronic gastric ulcerations by 1-arginine. Role of nitric oxide, prostaglandins, gastrin and polyamines. Digestion. 1995; 56:463-471. DOI: 10.1159/000201277.
32. Akiba Y, Nakamura M, Mori M, Hidekazu Masaya O Soichiro HK, *et al*. Inhibition of inducible nitric oxide synthase delays gastric ulcer healing in the rat. J. Clin. Gastroenterol. 1998; 27:S64-S73. DOI: 10.1097/00004836-199800001-00011.
33. Dharmani P, Kumar V, Srivastava S, Palit G. Ulcer healing effect of anti-ulcer agents: A comparative study. Internet J. Acad. Physician Assist. 2002; 3:2.
34. Allen A, Leonard AJ, Sellers LA. The mucus barrier:Its role in gastroduodenal mucosal protection. J. Clin. Gastroenterol. 1988; 10:S93-S98.DOI: 10.1097/00004836-198812001-00015.
35. Wallace JL. Prostaglandins, NSAIDs and gastric mucosal protection: Why doesn't the stomach digest itself? Physiol. Rev. 2008; 88:1547-1565. DOI: 10. 1152 /physrev.00004.200
36. Mitchell JA, Larkin S, Williams TJ. Cyclooxygenase-2: Regulation and relevance in inflammation. Biochem. Pharmacol. 1995; 50:1535-1542.DOI: 10.1016/0006-2952(95)00212.
37. Okazaki M, Shimizu I, Ishikawa M, Fujiwara S, Yamamoto H, *et al*. Gastric mucosal levels of prostaglandins and leukotrienes in patients with gastric ulcer after treatment with rabeprazole in comparison to treatment with ranitidine. J. Med. Invest. 2007; 54:83-90. DOI: 10.2152/jmi.5.
38. Fang M, Jaffrey SR, Sawa A, Ye K, Luo X, *et al*. Dexras1: A G protein specifically coupled to neuronal nitric oxide synthase via CAPON. Neuron, 2000; 28:183-193. DOI: 10.1016/S0896-6273(00)00095-7.
39. Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science. 1995; 269:1737-1740. DOI: 10.1126/science.7569905
40. Takahashi S, Shigeta J, Inoue H, Tanabe T, Okabe S. Localization of cyclooxygenase-2 and regulation of its mRNA expression in gastric ulcers in rats. Am. J. Physiol. 1998; 275:G1137-G1145.PMID: 9815044
41. Konturek SJ, Brzozowski T, Majka J, Pytko- Polonczyk x J, Stachura J. Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. Eur. J. Pharmacol. 1993; 239:215-217. DOI: 10.1016/0014-2999(93)90997-V.
42. Leahy KM, Koki AT, Masferrer JL. Role of cyclooxygenases in angiogenesis. Curr. Med. Chem. 2000; 7:1163-1170. DOI: 10.2174/0929867003374336.
43. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology. 2007; 132:2131-2157. DOI: 10.1053/j.gastro.2007.03.054.
44. Tatemichi M, Ogura T, Sakurazawa N, Nagata H, Sugita M, *et al*. Roles of inducible nitric oxide synthase in the development and healing of experimentally induced gastric ulcers. Int J. Exp. Pathol. 2003; 84:213-220. DOI: 10.1111/j.1365- 2613.2003.00357.x
45. Nader MA, El-Awady MS, Shalaby AA, El-Agamy DS. Sitagliptin exerts anti-inflammatory and anti-allergic effects in ovalbumininduced murine model of allergic airway disease. Naunyn Schmiedebergs Arch. Pharmacol. 2012; 385:909-919. DOI: 10.1007/s00210-012-0772-9
46. Ye Y, Keyes KT, Zhang C, Perez-Polo JR, Lin Y, *et al*. The myocardial infarct size-limiting effect of sitagliptin is PKA-dependent, whereas the protective effect of pioglitazone is partially dependent on PKA. Am. J. Physiol. Heart Circ. Physiol. 2010; 298:H1454-H1465. DOI: 10.1152/ajpheart.00867.2009
47. Salehi A, Abaraviciene SM, Jimmenez-Feltstrom JO, Efendic CGS, Lundquist I. Excessive islet NO eneration

in type 2 diabetic GK rats coincides with abnormal hormone secretion and is counteracted by GLP-1. PLoS ONE. 2008; 3:e2165-e2165. DOI: 10.1371/journal.pone.0002165

48. Belin VD, Mabley JG, James RFL, Swift SM, Clayton HA, *et al*. Glucagon decreases cytokine induction of nitric oxide synthase and action on insulin secretion in RINm5F cells and rat and human islets of Langerhans. Cytokine, 1999; 11: 585-592. DOI: 10.1006/cyto.1998.0486
49. Kang JH, Chang SY, Jang HJ, Kim DB, Ryu GR, *et al*. Exendin-4 inhibits interleukin-1binduced iNOS expression at the protein level, but not at the transcriptional and posttranscriptional levels, in RINm5F b-cells. J. Endocrinol. 2009; 202:65-75. DOI: 10.1677/ JOE-08-0507.