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Investigating the Gastroprotective Impact of Sitagliptin on Ethanol-Induced Gastric Ulcers in Rats: Insights into the NO/cGMP/KATP Signaling Pathway

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ABSTRACT

Purpose: To evaluate the gastroprotective effect of sitagliptin against ethanol-induced gastric ulcers in rats and to investigate the potential involvement of the nitric oxide (NO), cyclic guanosine monophosphate (cGMP), and ATP-sensitive potassium (KATP) channel pathway.
Methods: Seventy-seven adult male Wistar rats were randomly allocated into 11 groups (n = 7 per group). Gastric ulcers were induced by oral administration of ethanol (4 ml/kg) following 18–24 hours of fasting. Sitagliptin (25, 50, and 100 mg/kg) was administered orally 30 minutes before ethanol exposure. Pharmacological modulators, including stimulators (L-arginine, sildenafil, diazoxide) and inhibitors (Nω-nitro-L-arginine methyl ester hydrochloride, methylene blue, glibenclamide), were administered intraperitoneally 30 minutes before sitagliptin. One hour after ethanol administration, rats were sacrificed, gastric lesions were assessed, and tissue samples were analyzed for histopathological changes and inflammatory markers.
Results: Sitagliptin at 100 mg/kg significantly reduced gastric ulcer area and inflammatory markers compared to the ethanol-treated group. Co-administration with stimulatory modulators further enhanced these protective effects, whereas inhibitory agents attenuated them.
Conclusion: The findings suggest that sitagliptin exerts gastroprotective effects against ethanol-induced gastric injury, possibly involving the NO/cGMP/KATP signaling pathway. These results should be interpreted as a proof-of-concept, and further studies are required to evaluate their clinical relevance.

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1. Introduction

Gastric ulcer is a widespread illness, affecting the upper digestive tract, defined by an open sore in the stomach lining. These ulcers generally exceed 5 mm across and extend through the mucosal layer into the underlying muscularis mucosa.[1,2] Disruption in the equilibrium between processes that preserve the health of gastric epithelial cells and destructive contributors compromises the stomach's mucosal barrier, increasing susceptibility to gastric ulcer development. Protective mechanisms include mucus secretion, bicarbonate release, and adequate blood supply, and damaging factors include excessive secretion of gastric acid, heightened pepsin activity, and Helicobacter infection. Additionally, environmental and lifestyle factors, such as alcohol consumption, tobacco use, stress, and the use of medications like non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, antipsychotics, or antidepressants, further contribute to mucosal damage and increase the risk of ulcer formation.[1,3-5]

Ethanol is a well-documented inducer of gastric mucosal injury in animal models, promoting oxidative stress, inflammatory cell infiltration, and disruption of the mucosal barrier, leading to hemorrhage, edema, and ulcer formation.[4,5] These effects are mediated by increased gastric acid secretion, vascular endothelial damage, and the production of reactive oxygen species (ROS), which exacerbate inflammation and tissue injury.[3,6] Currently, therapies like proton pump inhibitors (PPIs), histamine-2 (H2) receptor blockers, antibiotics, and cytoprotective medications are commonly used, but they frequently face challenges such as adverse effects and partial ulcer resolution, highlighting the need for developing new treatment options.[5,7]

Sitagliptin is a medication used in the treatment of type 2 diabetes mellitus that inhibits DPP-4, leading to increased levels of incretin hormones, particularly glucagon-like peptide-1 (GLP-1), and subsequently increasing insulin and glucose metabolism. Beyond its metabolic effects, sitagliptin exhibits broad-spectrum tissue protective properties, including anti-inflammatory, antioxidant effects, and cytoprotective actions, which may promote mucosal healing in conditions like gastric ulcers.[8-12] Additionally, some studies have demonstrated the interplay between sitagliptin and nitric oxide (NO)-mediated pathways, offering a promising direction for further exploration of sitagliptin's effects.[10,12,13] NO is an important mediator in gastric mucosal integrity, promoting vasodilation, epithelial repair, and inhibiting inflammatory pathways.[14,15] The cyclic guanosine monophosphate (cGMP) pathway, activated by NO, enhances mucus secretion and reduces acid production.[16,17] Additionally, NO and cGMP are essential in the activation of various targets, including different types of potassium (K+) channels. Research indicates that the activation of ATP-sensitive potassium (K_{ATP}) channels may serve a protective function in the gastric mucosa.[6,18]

Therefore, this study seeks to investigate the protective role of sitagliptin against ethanol-induced gastric ulcers in rats, with a focus on elucidating the underlying mechanisms. Specifically, it investigates the role of the NO/cGMP/K_{ATP} signaling pathway, which may be essential for sitagliptin's gastroprotective effects and could lead to new treatment options.

2. Materials and methods

2.1. Animals and ethics

Seventy-seven male Wistar rats, aged 8–11 weeks and weighing 220–250 g, were obtained from the animal care facility at Mazandaran University of Medical Sciences, Iran. The rats were randomly allocated to 11 groups, each containing 7 animals. Before experiments, the rats underwent fasting for 18–24 hours. Throughout the study, they were housed in standard rat cages in a controlled environment with a temperature of 22 ± 2 °C, 40–50% humidity, and a 12-hour light/12-hour dark cycle. They had access to specialized rodent chow and municipal tap water. Animal care and experiments were conducted in compliance with the ARRIVE guidelines and the National Research Council's Guide for the Care and Use

of Laboratory Animals. The study protocol (Approval ID: IR.MAZUMS.AEC.1402.077; approval date: 2023-08-13) was reviewed and approved by the Research Ethics Committee for Laboratory Animals of Mazandaran University of Medical Sciences.

2.2. Chemicals and reagents

Sitagliptin and glibenclamide were procured from Mahban Darou Pharmaceutical Company. Ethanol was obtained from Merck. L-NAME, L-arginine, methylene blue, and diazoxide were sourced from Sigma-Aldrich. Sildenafil was acquired from Darou Pakhsh Pharmaceutical Manufacturing Company. Levels of tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) were quantified using ELISA kits supplied by Karmania Pars Gene, Iran.

2.3. Evaluation of sitagliptin's protective effect on ethanol-induced gastric injury

Rats were administered either saline or sitagliptin (25, 50, or 100 mg/kg) via oral gavage. Thirty minutes later, 4 ml/kg absolute ethanol was given orally, except to the negative control group, which received saline. The positive control group received only ethanol. One hour after ethanol administration, rats were euthanized.[6,19] After euthanasia, the stomachs were carefully excised and opened. The gastric contents were gently rinsed with cold normal saline to remove debris and blood clots. Each stomach was then spread flat on a suitable surface to avoid tissue folding. Macroscopic images of the gastric mucosa were captured using a digital camera without magnification. The total gastric surface area and the ulcerated regions were measured from the images, and the percentage of ulcerated area was calculated using the following formula:

$$\text{Percentage of ulcer} = \frac{\text{ulcer surface}}{\text{Total gastric surface}} \times 100$$

The ulcerated areas were identified as visible hemorrhagic lesions and mucosal erosions. All image analyses were performed under standardized conditions to ensure consistency and reproducibility. However, macroscopic evaluation was not performed under blinded conditions, which is acknowledged as a limitation of the study.[20]

2.4. Investigation of the NO pathway

To investigate the role of NO, rats were pretreated with nitric oxide synthase (NOS) inhibitor, Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), at doses of 10 mg/kg via intraperitoneal injection or L-arginine (200 mg/kg, intraperitoneally, as an NO precursor) 30 minutes before receiving sitagliptin (100 mg/kg, orally). Ethanol (4 mL/kg) was administered orally 30 minutes after sitagliptin, and one hour later, gastric damage was evaluated by assessing macroscopic features, while samples were collected for histopathology (fixed in 10% formalin) and biochemical analysis (stored at -70 °C).

2.5. Investigation of the cGMP pathway

To explore the cGMP pathway, rats were pretreated with methylene blue (20 mg/kg, intraperitoneally, guanylyl cyclase inhibitor) or sildenafil (5 mg/kg, intraperitoneally, cGMP activator) 30 minutes before sitagliptin (100 mg/kg, orally). After another 30 minutes, ethanol (4 ml/kg) was administered to induce gastric damage. One hour after ethanol administration, gastric damage was evaluated as previously described, and samples were processed for histopathology and biochemical assays.

2.6. Investigation of the K_{ATP} channels

To assess K_{ATP} channel involvement, rats were pretreated with glibenclamide (3 mg/kg, intraperitoneally, K_{ATP} channel inhibitor) or diazoxide (3 mg/kg, intraperitoneally, K_{ATP} channel activator) 30 minutes before sitagliptin (100 mg/kg,

orally). Ethanol (4 ml/kg) was administered 30 minutes later, and gastric damage was evaluated as previously described, one hour post-ethanol, with samples collected for histopathology and biochemical analysis.

2.7. Preparation and analysis of gastric tissue homogenates for cytokine quantification

Gastric corpus samples were carefully excised, rinsed with cold physiological saline to remove blood and impurities, and sectioned into approximately 50 mg pieces before storage at –70 °C. For experimental procedures, the frozen tissue sections were chopped into smaller fragments to facilitate homogenization. A 0.1 M phosphate buffer solution (pH 7.4) was added at a ratio of 1:10 (10 ml of buffer per gram of tissue), and the mixture was thoroughly homogenized using a tissue homogenizer to obtain a consistent suspension. The homogenate was then vortexed and centrifuged at 10,000 × g for 10 minutes at 4 °C. The resulting supernatant was carefully aliquoted into 50-µl portions in microtubes and was used to quantify inflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), by employing enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. [6,21]

2.8. Histopathological analysis

For the histopathological analysis, any excess tissue was first removed. The stomachs were then fixed in a 10% neutral-buffered formalin solution. After fixation, the tissues underwent dehydration through graded alcohols, were cleared with xylene, and embedded in paraffin. Sections of 4-6 µm thickness were prepared, deparaffinized, and stained with hematoxylin and eosin (HE) before examination under a light microscope. The evaluation was performed based on criteria outlined by Laine and Weinstein. [6,22] Each tissue section was inspected for epithelial cell loss (rated infiltration of inflammatory cells (rated from 0 to 2), with assessments conducted on a 1 cm segment of each sample. An experienced pathologist carried out the evaluations in a blind manner, without knowledge of the treatment groups.[23]

2.9. Statistical analysis

Data analysis was conducted with GraphPad Prism version 8.0.0. The results are expressed as means accompanied by standard errors (mean±SEM). Differences among groups were assessed via one-way ANOVA, with subsequent comparisons made using Tukey's post hoc analysis. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Macroscopic impact of sitagliptin on ethanol-induced gastric ulcers

According to our findings, administration of absolute ethanol (4 ml/kg) significantly increased the percentage of ulcerated gastric area compared to the saline group ($p < 0.001$). Pretreatment with sitagliptin resulted in a dose-dependent reduction in ulcer size, with significant decreases observed at doses of 50 mg/kg and 100 mg/kg compared to the ethanol group (Figures 1 and 2; $p < 0.05$). Given this dose-dependent relationship, we selected a sitagliptin dosage of 100 mg/kg for the subsequent experiments.

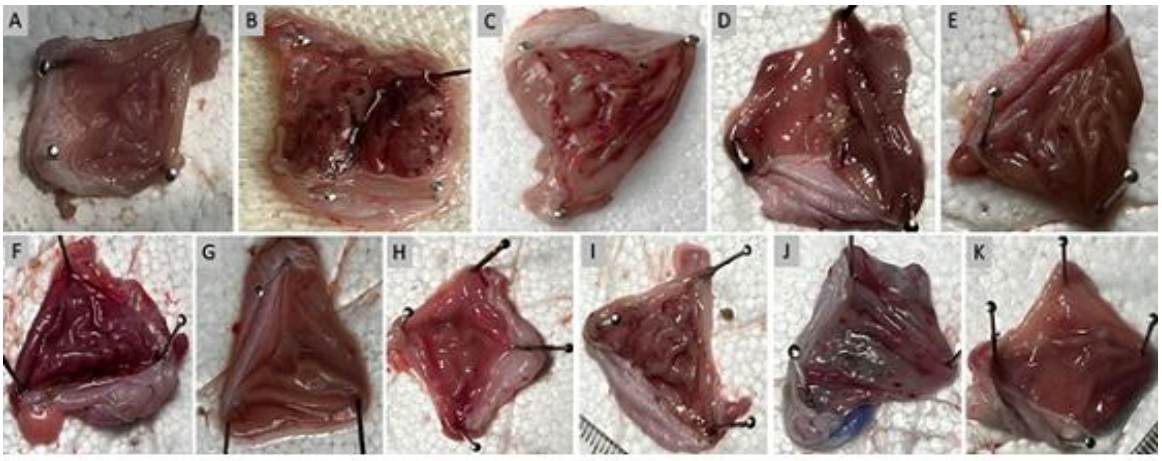

Figure 1. Measurement of the lesion's scope. A: Saline, B: Ethanol, C: Sitagliptin 25, D: Sitagliptin 50, E: Sitagliptin 100, F: L-Name + Sitagliptin 100, G: L-Arginine + Sitagliptin 100, H: Diazoxide + Sitagliptin 100, I: Glibenclamide + Sitagliptin 100, J: Methylene blue + Sitagliptin 100, K: Sildenafil + Sitagliptin 100

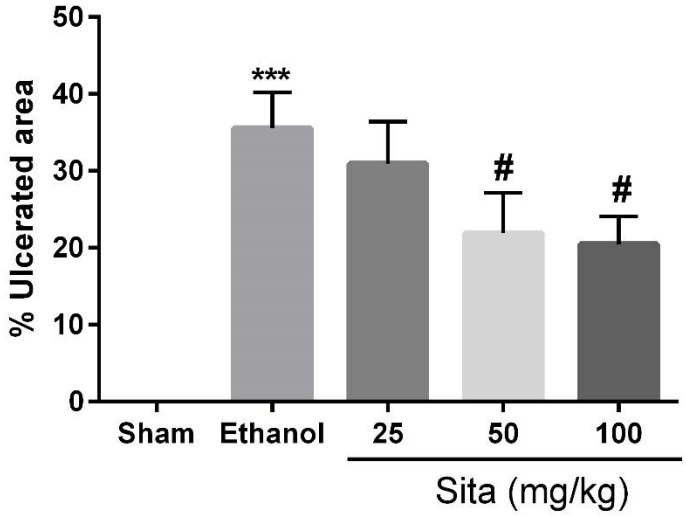

Figure 2. Comparison of ulcerated area percentage among control groups and different dosages of sitagliptin. Sham: saline (negative control), Ethanol: positive control, Sita: sitagliptin. * Significant difference with the negative control group (*** $p<0.001$). # Significant difference with the positive control group (# $p<0.05$).

3.2. Role of the NO pathway in sitagliptin's gastroprotective effect: Macroscopic features

Co-administration of L-arginine (200 mg/kg, an NO precursor) with sitagliptin (100 mg/kg) significantly reduced the gastric ulcerated area compared to the ethanol-treated group ($p < 0.05$). However, this combination did not yield a statistically significant difference compared to sitagliptin alone. In contrast, co-treatment with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, 10 mg/kg, an NOS inhibitor) and sitagliptin (100 mg/kg) significantly increased the ulcerated area compared to sitagliptin alone (Figures 1 and 3; $p < 0.05$).

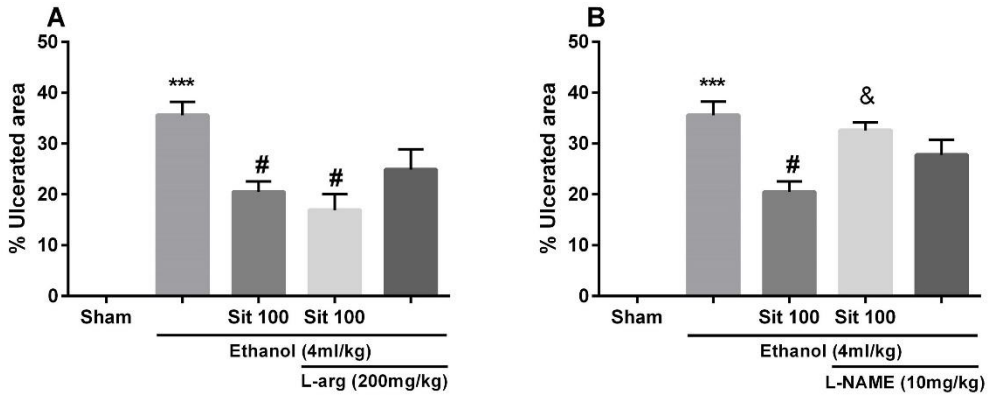

Figure 3. The effect of sitagliptin (100 mg/kg) in Combination with L-arg (200 mg/kg) and L-NAME (10 mg/kg) on ethanol-induced gastric ulceration. Sham: saline (negative control), ethanol: positive control, Sita: sitagliptin, L-arg: L-arginine, L-NAME: Nω-nitro-L-arginine methyl ester hydrochloride. * Significant difference with the negative control group (*** p<0.001). # Significant difference with the positive control group (# p<0.05). & Significant difference with the positive control group (& p<0.05).

3.3. Role of the cGMP pathway in sitagliptin's gastroprotective effect: Macroscopic features

The combination of sildenafil (5 mg/kg, a cGMP activator) with sitagliptin (100 mg/kg) significantly reduced the gastric ulcer area compared to the ethanol-treated group ($p < 0.05$), with no significant difference observed between this combination, sildenafil alone, or sitagliptin alone. Conversely, co-administration of methylene blue (20 mg/kg, a guanylyl cyclase inhibitor) with sitagliptin (100 mg/kg) significantly increased the ulcerated area compared to sitagliptin alone (Figures 1, 3, and 4; $p < 0.05$).

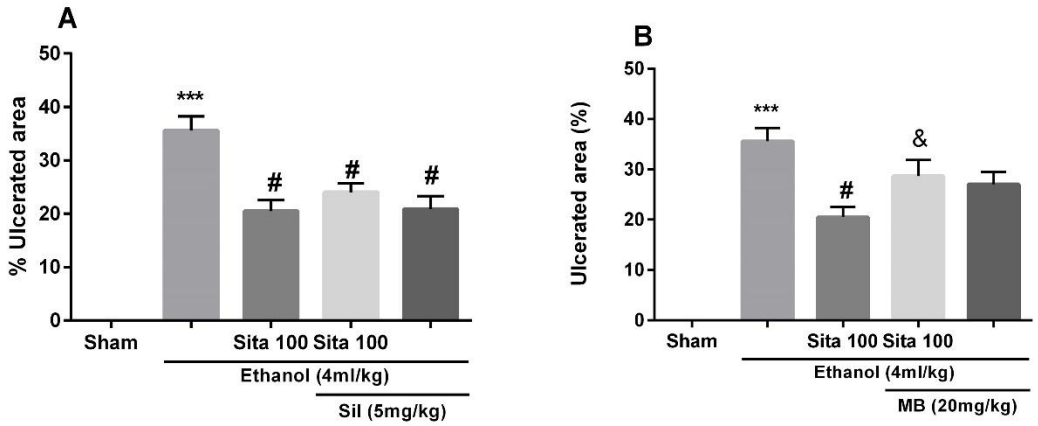

Figure 4. The effect of sitagliptin (100 mg/kg) in combination with Sil (5 mg/kg) and MB (20 mg/kg) on ethanol-induced gastric ulceration. Sham: saline (negative control), Ethanol: positive control, Sita: sitagliptin, Sil: sildenafil, MB: methylene blue. * Significant difference with the negative control group (*** p<0.001). # Significant difference with the positive control group (# p<0.05). & Significant difference with the positive control group (& p<0.05).

3.4. Role of the K_{ATP} channel in sitagliptin's gastroprotective effect: Macroscopic features

Pretreatment with diazoxide (3 mg/kg, a K_{ATP} channel activator) combined with sitagliptin (100 mg/kg) significantly decreased the gastric ulcer area compared to the ethanol-treated group ($p < 0.05$), showing no significant difference from diazoxide alone or sitagliptin alone. However, the combination of glibenclamide (3 mg/kg, a K_{ATP} channel inhibitor) with sitagliptin (100 mg/kg) significantly increased the ulcer area compared to sitagliptin alone (Figures 1, 4, and 5; $p < 0.05$).

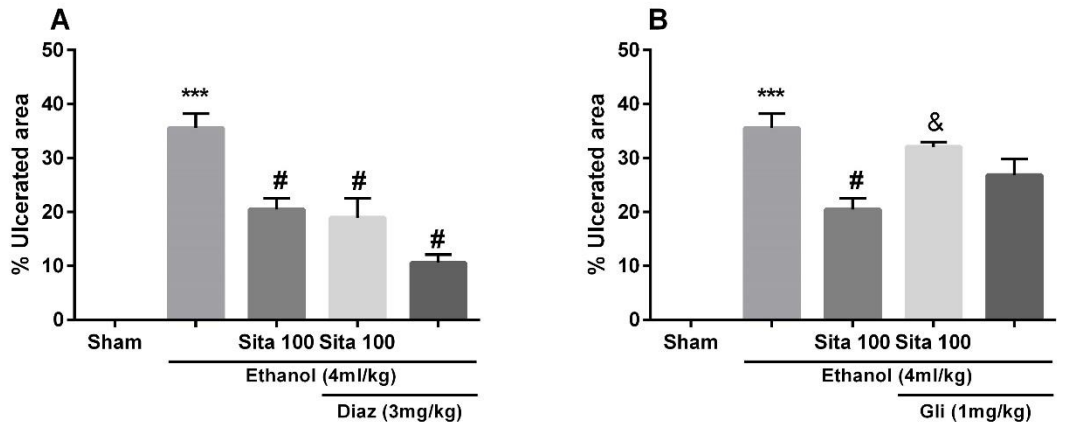

Figure 5. The effect of sitagliptin (100 mg/kg) in combination with diazoxide (3 mg/kg) and glibenclamide (1 mg/kg) on ethanol-induced gastric ulcer area. Sham: saline (negative control), Ethanol: positive control, Sita: sitagliptin, Diaz: diazoxide, Gli: glibenclamide. * Significant difference with the negative control group (*** $p<0.001$). # Significant difference with the positive control group (# $p<0.05$). & Significant difference with the positive control group (& $p<0.05$).

3.5. Histopathological findings

Micrographs of gastric tissue from all groups revealed distinct structural differences (Figure 6). The negative control group displayed a completely normal stomach structure, with intact mucosal, submucosal, muscular, and serosal layers. In contrast, ethanol-treated rats exhibited severe gastric damage, including hemorrhagic streaks and spots, submucosal infiltration of inflammatory cells, edema, and widespread gastric epithelial necrosis (Figure 6). Histopathological analysis confirmed that ethanol administration caused extensive epithelial cell loss, hemorrhage, inflammatory cell infiltration, and erosions of the lamina propria mucosae. The control group showed no damage across these parameters. Sitagliptin treatment at 25 mg/kg and 100 mg/kg mitigated ethanol-induced damage in a dose-dependent manner, with the higher dose offering greater protection, particularly against hemorrhage. Combination treatments of sitagliptin 100 mg/kg with stimulatory agents (L-arginine, sildenafil, diazoxide) were highly effective, significantly reducing damage across all parameters, with notable improvements in epithelial cell preservation. However, combinations with inhibitory agents (L-NAME, methylene blue, glibenclamide) were less effective, indicating that these agents may diminish sitagliptin's protective effects (Table 1 and Figure 6).

Table 1. The presented data represent median values, with the corresponding minimum and maximum scores provided in parentheses. Sita: sitagliptin, L-arg: L-arginine, L-NAME: Nω-nitro-L-arginine methyl ester hydrochloride, Diaz: diazoxide, Gli: glibenclamide, Sil: sildenafil, MB: methylene blue. *P<0.05 vs Saline group; #P<0.05 vs Ethanol group; &p< 0.05 vs. Ethanol + Sita 100 group.

	epithelial cell loss (0-3)	Hemorrhage (0-4)	inflammatory cell infiltration (0-2)	Erosions of the lamina propria mucosae (0-4)	Total scores (0-13)
Normal Saline	0	0	0	0	0 (0-0)
Ethanol	3 (3-3)*	4 (3-4)*	2 (1-2)*	3 (3-4)*	12 (10-13)*
Sita 25	2.5 (2-3)	2 (2-3)	1 (1-2)	2.5 (2-3)	8 (7-11)
Sita 100	2 (2-3)	2 (1-2)#	1 (0-1)	2 (1-2)	7 (4-8)#
L-arg+Sita 100	1 (1-2)#	2 (1-2)	1 (0-1)	1.5 (1-2)#	5.5 (3-7)
L-Name+Sita 100	2 (2-3)	3 (2-3)	2 (1-2)	3 (3-3)	10 (8-11)&
Diaz+Sita 100	1 (1-2)#	2 (1-2)#	1 (0-1)	1.5 (1-2)#	5.5 (3-7)#
Gli+Sita 100	2 (2-3)	2 (1-3)	1.5 (1-2)	2 (2-3)	7.5 (6-11)
Sil +Sita 100	1 (1-2)#	2 (1-2)#	1 (1-2)	2 (1-2)	6 (4-8)#
MB+Sita 100	2 (2-3)	2 (2-3)	2 (1-2)	3 (2-3)	9 (7-11)&

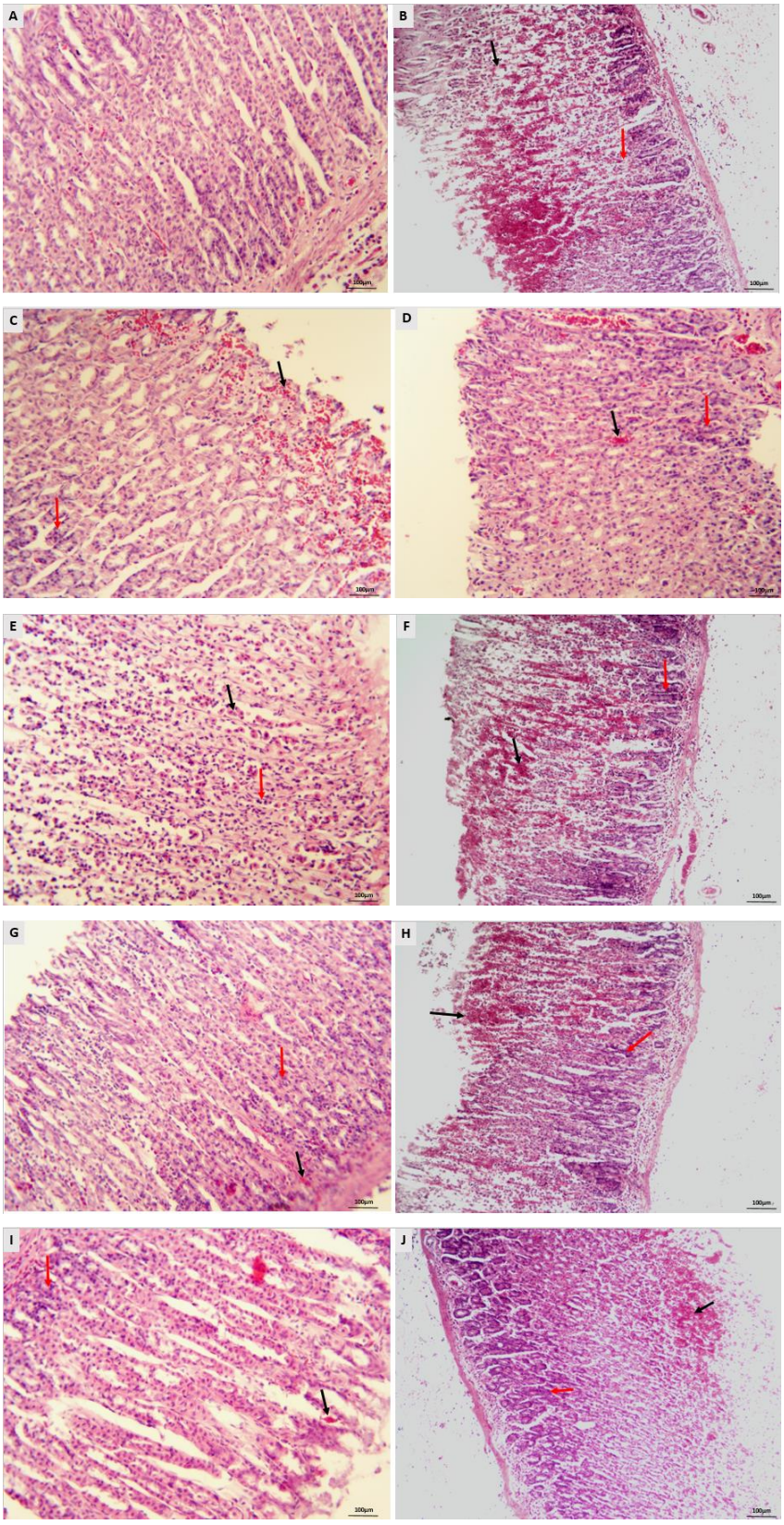

Figure 6. Micrographs of the gastric tissue structure in all groups. A. Negative control group with normal structure. B. Positive control group, gastric mucosa in ethanol-received rats showing areas of hemorrhage in the mucosal layer (black arrow) and inflammatory cell infiltration (red arrow). C. sitagliptin25+Ethanol, D. sitagliptin100+Ethanol, E. L-arginine +Sitagliptin 100+Ethanol, F. L-Name +Sitagliptin 100+ Ethanol, G. Diazoxide +Sitagliptin 100+ Ethanol, H. glibenclamide +Sitagliptin 100+ Ethanol, I. sildenafil +Sitagliptin 100+ Ethanol, J. methylene blue +Sitagliptin 100+ Ethanol. The gastric tissue in the L-arginine, Diazoxide, and sildenafil

groups that received a high dose of sitagliptin showed fewer gastric lesions. Staining: Hematoxylin and Eosin. Magnification: X 100. Scale bar = 100 μm.

3.6. Measurement of TNF-α and IL-1β in different treatment groups

The TNF-α and IL-1β measurements in gastric mucosa (Figure 7 and Figure 8) show that ethanol markedly induced inflammation, with TNF-α and IL-1β rising versus saline controls ($p < 0.001$ for both). Sitagliptin at 100 mg/kg significantly lowered TNF-α compared with the ethanol group ($p < 0.01$), indicating an anti-inflammatory effect, but co-administration with inhibitory agents altered this benefit: glibenclamide (3 mg/kg), methylene blue (20 mg/kg), or L-NAME (10 mg/kg) plus sitagliptin all elevated TNF-α levels relative to sitagliptin alone ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively), suggesting these agents may counteract sitagliptin's anti-inflammatory action in the gastric mucosa. Conversely, while combination treatments of glibenclamide plus sitagliptin (3 mg/kg + 100 mg/kg) and methylene blue plus sitagliptin (20 mg/kg + 100 mg/kg) reduced IL-1β levels compared with the ethanol group ($p < 0.05$), they did not differ significantly from sitagliptin alone (Figure 8).

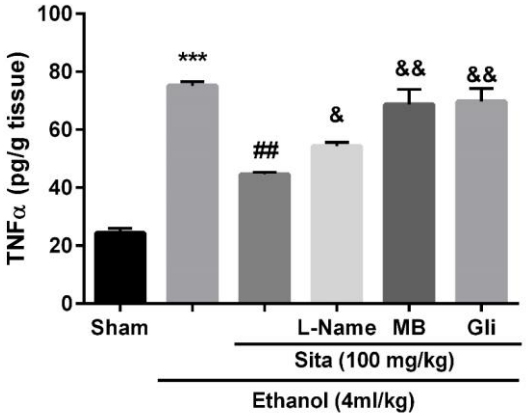

Figure 7. Comparison of TNF-α Levels in experimental animal groups. Sham: saline (negative control), Ethanol: positive control, Sita: sitagliptin, L-NAME: Nω-nitro-L-arginine methyl ester hydrochloride, MB: methylene blue, Gli: glibenclamide. * Significant difference with the negative control group (*** $p<0.001$). # Significant difference with the positive control group (## $p<0.01$). & Significant difference with the positive control group (& $p<0.05$, && $p<0.01$).

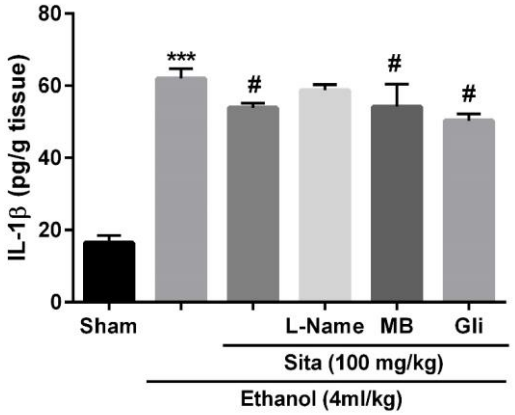

Figure 8. Comparison of IL-1β Levels in experimental animal groups. Sham: saline (negative control), Ethanol: positive control, Sita: sitagliptin, L-NAME: Nω-nitro-L-arginine methyl ester hydrochloride, MB: methylene blue, Gli: glibenclamide. * Significant difference with the negative control group (*** $p<0.001$). # Significant difference with the positive control group (# $p<0.05$).

4. Discussion

Our findings indicate that sitagliptin exhibits gastroprotective effects in a dose-dependent manner, with the most significant effect observed at a dose of 100 mg/kg. Based on this finding, we selected this dose for the remaining experiments, where it demonstrated consistent effects across all assessed parameters, including macroscopic and histopathological evaluations, as well as reductions in inflammatory cytokines. Furthermore, our investigations into the underlying mechanisms suggest that the gastroprotective effects of sitagliptin may be mediated through the NO/cGMP/K_{ATP} signaling pathway.

Sitagliptin has demonstrated promising therapeutic effects across various systems, including the cardiovascular, digestive, urinary, and nervous systems. These effects are attributed to its potential antioxidant, anti-inflammatory, and anti-apoptotic properties.[19,24-26] Previous studies on its effects on gastric ulcers in rat models have yielded mixed results, which may depend on factors such as the ulcer induction method, the presence of diabetes, and the dietary conditions of the animals.

Consequently, we further investigated the gastroprotective effects of sitagliptin along with its potential mechanisms using an ethanol-induced gastric ulcer model. Ethanol-induced gastric injury is a well-established model for evaluating the protective effects of pharmacological agents on gastric mucosa.[1,3,5,6]

NO is a vital biological mediator involved in various physiological processes, including vasodilation, neurotransmission, and immune response.[27] In the gastrointestinal tract, NO plays an essential role in maintaining mucosal integrity by regulating blood flow, enhancing tissue perfusion, stimulating mucus and bicarbonate secretion, and modulating inflammatory responses. It has been reported that NO exerts some of its effects via stimulation of guanylate cyclase, leading to increased production of cGMP. Additionally, both NO and cGMP have been shown to activate the K_{ATP} channels, which are involved in gastric mucosal defense mechanisms.[6,28,29]

Previous studies have suggested that sitagliptin exerts protective effects against tissue injury by modulating the NO pathway.[13,30] To further elucidate this mechanism, we employed L-arginine and L-NAME as pharmacological modulators. Our findings showed that L-arginine enhanced, whereas L-NAME attenuated, the gastroprotective effects of sitagliptin. While these findings support the involvement of the NO/cGMP/K_{ATP} pathway, the present conclusions are based on pharmacological modulation. Direct biochemical measurements (e.g., NO or cGMP levels) were not performed; therefore, the proposed mechanism should be considered suggestive rather than definitive.

cGMP is essential to the gastric mucosal defense system, and previous studies have highlighted the significance of this messenger molecule in NO-mediated gastric protection. Anna Berg et al.[16] demonstrated that the inhibition of gastric acid secretion via NO occurs through a cGMP-dependent pathway involving the activation of guanylate cyclase. Additionally, Archer et al.[31] showed that the NO/cGMP pathway promotes vasodilation and enhances blood flow. To further elucidate the role of cGMP in the gastroprotective effects of sitagliptin, we investigated the impacts of two pharmacological agents: methylene blue, a cGMP inhibitor, and sildenafil, a phosphodiesterase-5 inhibitor that increases cGMP levels. Co-administration of sitagliptin (100 mg/kg) with methylene blue significantly increased gastric ulcer percentage and TNF-α levels compared to the sitagliptin-only group, thereby negating sitagliptin's gastroprotective effect. Our findings align with earlier research, indicating that some pharmacological agents exert their gastroprotective effects against ethanol-induced gastric ulcers by influencing the NO/cGMP signaling pathway.[6,28,32]

K_{ATP} channels are integral membrane proteins involved in maintaining cellular excitability and homeostasis.[33] Research indicates that KATP channels play a role in defense mechanisms.[34] Moreover, pharmacological agents targeting these

channels have demonstrated anti-ulcer effects, promoting mucosal protection and reducing inflammation.[6,28,32] In this study, we investigated the effect of sitagliptin on K_{ATP} channels by co-administering diazoxide (a potassium channel activator) and glibenclamide (a potassium channel inhibitor) along with sitagliptin. Our findings revealed that co-administration of sitagliptin (100 mg/kg) with diazoxide significantly reduced the percentage of ulcerated gastric tissue compared to the positive control group. In contrast, administration of glibenclamide counteracted sitagliptin's protective effects against ethanol-induced mucosal damage.

The observed reductions in inflammatory cytokines and improvements in histopathological parameters are consistent with activation of the NO/cGMP/K_{ATP} pathway, supporting a mechanistic link between pathway modulation and gastroprotection. In addition, alternative mechanisms, including antioxidant effects and GLP-1–mediated pathways, may also contribute to the observed protective effects of sitagliptin. The absence of control groups receiving the pharmacological modulators alone represents a limitation of this study, as the intrinsic effects of these agents on the gastric mucosa cannot be completely excluded. The doses used in this study were selected to achieve robust pharmacological effects and may not directly correspond to clinically relevant dosing regimens. Therefore, these findings should be interpreted as a proof-of-concept. In addition, it should be noted that all assessments were conducted at a single early time point (1 hour after ethanol administration), which primarily reflects acute gastroprotective effects rather than mucosal healing or long-term recovery.

4.1. Study Limitations

This study has several limitations that should be acknowledged. First, independent modulator-only control groups (e.g., L-NAME, L-arginine, sildenafil, methylene blue, diazoxide, and glibenclamide) were not included; therefore, the intrinsic effects of these agents on gastric mucosa cannot be completely excluded. Second, direct biochemical measurements of gastric NO and/or cGMP levels were not performed to confirm pathway activation. Third, the acute experimental design, involving a single high-dose pretreatment and a short observation period, limits the conclusions to immediate cytoprotective effects rather than long-term mucosal healing.

5. Conclusions

The present study suggests that sitagliptin exerts gastroprotective effects against ethanol-induced gastric ulcers in rats. These findings highlight the potential of sitagliptin as a candidate for reducing gastric mucosal injury, possibly through involvement of the NO/cGMP/K_{ATP} signaling pathway. However, these results should be interpreted as a proof-of-concept, as the study was conducted in an acute experimental model and at doses that may not directly correspond to clinically relevant regimens. Therefore, further studies are required to evaluate the translational relevance of these findings, including investigations using clinically relevant dosing strategies, chronic models of gastric injury, and ultimately, clinical studies. In addition, future research is needed to further elucidate the precise molecular mechanisms underlying the observed effects.

Author contributions

B.G and G.H designed the study, contributed to the study concepts, data analysis, and reviewed the paper. P.M, F.T, M.S, T.T, and S.B contributed to the definition of intellectual content, experimental studies, and project administration. L.K contributed to the literature research, writing the text, and reviewing the paper. All authors read and approved the final manuscript.

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Ethical Approval

The study was reviewed and approved by the Research Ethics Committee of Mazandaran University of Medical Science, Institutional Review Board (IR.MAZUMS.AEC.1402.077), and was performed under the Declaration of Helsinki.

Competing Interests

There is nothing to declare.

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Data Availability Statement

The data underlying this article are available in the article and in its online supplementary material.

References

1. Alamoudi JA, El-Masry TA, El-Nagar MMF, El Zahaby EI, Elmorshedy KE, Gaballa MMS, et al. Chitosan/hesperidin nanoparticles formulation: a promising approach against ethanol-induced gastric ulcers via Sirt1/FOXO1/PGC-1α/HO-1 pathway. *Front Pharmacol* 2024;15:1433793. doi: 10.3389/fphar.2024.1433793
2. Islam H, Siddiqui A, Islam R, Islam T, Ahmed S, Fahim M, et al. NSAID-induced Gastric Ulcer Disease: A Deleterious Connection. *Discov Med* 2024;36(188):1789-99. doi: 10.24976/Discov.Med.202436188.165
3. Albalawi M, Khateeb S. Enhanced Therapeutic Efficacy of Omeprazole Nanosuspension in Ethanol-Induced Gastric Ulcer: A Focus on Oxidative Stress and Inflammatory Pathways. *Biomolecules* 2025;15(6). doi: 10.3390/biom15060902
4. Kwon SC, Kim JH. Gastroprotective effects of irsogladine maleate on ethanol/hydrochloric acid induced gastric ulcers in mice. *Korean J Intern Med* 2021;36(1):67-75. doi: 10.3904/kjim.2018.290
5. Taher RF, Abd El Ghany EM, El-Gendy ZA, Elghonemy MM, Hassan HA, Abdel Jaleel GA, et al. In vivo anti-ulceration effect of Pancratium maritimum extract against ethanol-induced rats via NLRP3 inflammasome and HMGB1/TLR4/MYD88/NF-κβ signaling pathways and its extract metabolite profile. *PLoS One* 2025;20(4):e0321018. doi: 10.1371/journal.pone.0321018
6. Medeiros JV, Gadelha GG, Lima SJ, Garcia JA, Soares PM, Santos AA, et al. Role of the NO/cGMP/K(ATP) pathway in the protective effects of sildenafil against ethanol-induced gastric damage in rats. *Br J Pharmacol* 2008;153(4):721-7. doi: 10.1038/sj.bjp.0707605
7. Amir N, Abu Damir H, Ghazal-Aswad K, Hassan M, Adem M, Ali MA, et al. Withania coagulans fruit extract antiulcerogenic effect: comparative study with lansoprazole and ranitidine in rats. *Front Med (Lausanne)* 2025;12:1544422. doi: 10.3389/fmed.2025.1544422
8. Chen YC, Ho CC, Yi CH, Liu XZ, Cheng TT, Lam CF. Exendin-4, a glucagon-like peptide-1 analogue accelerates healing of chronic gastric ulcer in diabetic rats. *PLoS One* 2017;12(11):e0187434. doi: 10.1371/journal.pone.0187434
9. Famurewa AC, Asogwa NT, Ezea SC. Antidiabetic drug sitagliptin blocks cyclophosphamide cerebral neurotoxicity by activating Nrf2 and suppressing redox cycle imbalance, inflammatory iNOS/NO/NF-κB

response and caspase-3/Bax activation in rats. *Int Immunopharmacol* 2023;116:109816. doi: 10.1016/j.intimp.2023.109816

10. Moraes RM, Lima GM, Oliveira FE, Brito AC, Pereira RC, Oliveira LD, et al. Exenatide and Sitagliptin Decrease Interleukin 1β, Matrix Metalloproteinase 9, and Nitric Oxide Synthase 2 Gene Expression But Does Not Reduce Alveolar Bone Loss in Rats With Periodontitis. *J Periodontol* 2015;86(11):1287-95. doi: 10.1902/jop.2015.150278
11. Ning MM, Yang WJ, Guan WB, Gu YP, Feng Y, Leng Y. Dipeptidyl peptidase 4 inhibitor sitagliptin protected against dextran sulfate sodium-induced experimental colitis by potentiating the action of GLP-2. *Acta Pharmacol Sin* 2020;41(11):1446-56. doi: 10.1038/s41401-020-0413-7
12. Wójcicka G, Pradiuch A, Fornal E, Stachniuk A, Korolczuk A, Marzec-Kotarska B, et al. The effect of exenatide (a GLP-1 analogue) and sitagliptin (a DPP-4 inhibitor) on asymmetric dimethylarginine (ADMA) metabolism and selected biomarkers of cardiac fibrosis in rats with fructose-induced metabolic syndrome. *Biochem Pharmacol* 2023;214:115637. doi: 10.1016/j.bcp.2023.115637
13. Ibrahim MA, Geddawy A, Abdel-Wahab S. Sitagliptin prevents isoproterenol-induced myocardial infarction in rats by modulating nitric oxide synthase enzymes. *Eur J Pharmacol* 2018;829:63-9. doi: 10.1016/j.ejphar.2018.04.005
14. Calatayud S, Sanz MJ, Canet A, Bello R, de Rojas FD, Esplugues JV. Mechanisms of gastroprotection by transdermal nitroglycerin in the rat. *Br J Pharmacol* 1999;127(5):1111-8. doi: 10.1038/sj.bjp.0702649
15. Polte T, Oberle S, Schröder H. Nitric oxide protects endothelial cells from tumor necrosis factor-alpha-mediated cytotoxicity: possible involvement of cyclic GMP. *FEBS Lett* 1997;409(1):46-8. doi: 10.1016/s0014-5793(97)00480-8
16. Berg A, Redéen S, Grenegård M, Ericson AC, Sjöstrand SE. Nitric oxide inhibits gastric acid secretion by increasing intraparietal cell levels of cGMP in isolated human gastric glands. *Am J Physiol Gastrointest Liver Physiol* 2005;289(6):G1061-6. doi: 10.1152/ajpgi.00230.2005
17. Brown JF, Keates AC, Hanson PJ, Whittle BJ. Nitric oxide generators and cGMP stimulate mucus secretion by rat gastric mucosal cells. *Am J Physiol* 1993;265(3 Pt 1):G418-22. doi: 10.1152/ajpgi.1993.265.3.G418
18. Gomes AS, Lima LM, Santos CL, Cunha FQ, Ribeiro RA, Souza MH. LPS from Escherichia coli protects against indomethacin-induced gastropathy in rats--role of ATP-sensitive potassium channels. *Eur J Pharmacol* 2006;547(1-3):136-42. doi: 10.1016/j.ejphar.2006.07.028
19. Chang MW, Chen CH, Chen YC, Wu YC, Zhen YY, Leu S, et al. Sitagliptin protects rat kidneys from acute ischemia-reperfusion injury via upregulation of GLP-1 and GLP-1 receptors. *Acta Pharmacol Sin* 2015;36(1):119-30. doi: 10.1038/aps.2014.98
20. Cross SE, Naylor IL, Coleman RA, Teo TC. An experimental model to investigate the dynamics of wound contraction. *Br J Plast Surg* 1995;48(4):189-97. doi: 10.1016/0007-1226(95)90001-2
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54. doi: 10.1016/0003-2697(76)90527-3
22. Laine L, Weinstein WM. Histology of alcoholic hemorrhagic "gastritis": a prospective evaluation. *Gastroenterology* 1988;94(6):1254-62. doi: 10.1016/0016-5085(88)90661-0
23. Yang Y, Yin B, Lv L, Wang Z, He J, Chen Z, et al. Gastroprotective effect of aucubin against ethanol-induced gastric mucosal injury in mice. *Life Sci* 2017;189:44-51. doi: 10.1016/j.lfs.2017.09.016

24. Li Y, Vaughan KL, Wang Y, Yu SJ, Bae EK, Tamargo IA, et al. Sitagliptin elevates plasma and CSF incretin levels following oral administration to nonhuman primates: relevance for neurodegenerative disorders. *Geroscience* 2024;46(5):4397-414. doi: 10.1007/s11357-024-01120-4

25. Mosaoa RM, Al-Rabia MW, Asfour HZ, Alhakamy NA, Mansouri RA, El-Agamy DS, et al. Targeting SIRT1/AMPK/Nrf2/NF-κB by sitagliptin protects against oxidative stress-mediated ER stress and inflammation during ANIT-induced cholestatic liver injury. *Toxicology* 2024;507:153889. doi: 10.1016/j.tox.2024.153889

26. Zhou Y, Guo Z, Yan W, Wang W. Cardiovascular effects of sitagliptin - An anti-diabetes medicine. *Clin Exp Pharmacol Physiol* 2018;45(7):628-35. doi: 10.1111/1440-1681.12953

27. Palmer RM. The L-arginine: nitric oxide pathway. *Curr Opin Nephrol Hypertens* 1993;2(1):122-8. doi: 10.1097/00041552-199301000-00018

28. Behmanesh MA, Rasekhian A, Kiani F, Dehghandoost M, Dezfuli DA, Ghorbanzadeh B. The nitric oxide-cyclic GMP-K(ATP) channels pathway contributes to the effects of montelukast against gastric damage induced by ethanol. *Alcohol* 2023;113:33-40. doi: 10.1016/j.alcohol.2023.05.008

29. Kubes P, Wallace JL. Nitric oxide as a mediator of gastrointestinal mucosal injury?-Say it ain't so. *Mediators Inflamm* 1995;4(6):397-405. doi: 10.1155/s0962935195000640

30. Al-Awar A, Almási N, Szabó R, Takacs I, Murlasits Z, Szűcs G, et al. Novel Potentials of the DPP-4 Inhibitor Sitagliptin against Ischemia-Reperfusion (I/R) Injury in Rat Ex-Vivo Heart Model. *Int J Mol Sci* 2018;19(10). doi: 10.3390/ijms19103226

31. Archer SL, Huang JM, Hampl V, Nelson DP, Shultz PJ, Weir EK. Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. *Proc Natl Acad Sci U S A* 1994;91(16):7583-7. doi: 10.1073/pnas.91.16.7583

32. da Silva Monteiro CE, Franco Á X, Sousa JAO, Matos VEA, de Souza EP, Fraga CAM, et al. Gastroprotective effects of N-acylarylhydrazone derivatives on ethanol-induced gastric lesions in mice are dependent on the NO/cGMP/K(ATP) pathway. *Biochem Pharmacol* 2019;169:113629. doi: 10.1016/j.bcp.2019.113629

33. Olson TM, Terzic A. Human K(ATP) channelopathies: diseases of metabolic homeostasis. *Pflugers Arch* 2010;460(2):295-306. doi: 10.1007/s00424-009-0771-y

34. Peskar BM, Ehrlich K, Peskar BA. Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in the rat. *J Pharmacol Exp Ther* 2002;301(3):969-74. doi: 10.1124/jpet.301.3.969