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Baicalein mitigates Indomethacin-Induced Gastric Ulcer in Rats: Involvement of the PI3K/Akt/NF-*k*B pathway

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- PI3K

Abstract

Background: Peptic ulcer is a major health concern. Compared to other NSAIDs, indomethacin (IND) typically has a higher ulcerogenic potential. As a flavonoid, baicalein (Baic) has anti-inflammatory and antioxidant qualities. Objective: to evaluate the gastroprotective impact of Baic on stomach ulcers caused by IND and to identify any possible underlying processes. Material and Methods: Thirty male rats were split into three: control, Ulcer, Ulcer+Baic (10/group). Gastric ulcer index values, gastric PH, gastric PGE2, gastric MDA, gastric SOD, gastric TNF-α, gastric IL-1β, and gastric genes expression of NF-kB, PI3K, and Akt were evaluated. In addition to histolopathological and COX2 immunohistochemical evaluation of gastric tissue were performed. Results: The Ulcer group showed substantially higher gastric ulcer index values, gastric MDA, gastric TNF-α, gastric IL-1β, and gastric gene expression of NF-kB and gastric COX2 immunoreaction with dramatically decline in gastric PH values, gastric SOD, gastric PGE2 and gastric genes expression of PI3K and Akt compared to the control. Baic dramatically improved IND-induced changes in gastric tissue. Conclusion: Baic has gastroprotective benefits in rats with IND-induced ulcers via modifying the Akt/Pi3k/NF-kB signaling pathway and by having anti-inflammatory and antioxidant qualities.

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Introduction

About 5–10% of people worldwide suffer from peptic ulcers [1].It is a complicated, multifactorial condition that can result from an imbalance between hostile factors like Helicobacter pylori (H. pylori) infection, gastric acid, pepsin, and the mucosal-bicarbonate barrier and gastroprotective factors like prostaglandin E2 (PGE-2) secretion [1]. Eventually, the mucosa may release more proinflammatory cytokines. The increase in reactive oxygen species production by neutrophil cells that have infiltrated the inflammatory area exacerbates the ulcerative process [2].A high-spice diet and NSAIDs have all contributed to the rising incidence of stomach ulcers [1].

The most frequent cause of gastrointestinal illnesses is non-selective NSAID analgesics, which inhibit the formation of mucosal barrier layers. NSAIDs cause roughly 25% of gastritis worldwide [3].Indomethacin (IND), an NSAID derived from indole, is commonly employed to initiate this model and induces prostaglandin synthesis inhibition, increased stomach acid secretion, oxidative stress, regional blood flow is reduced, and tissue regeneration is suppressed [4].

Oxidative stress and damage to the stomach mucosa are closely related. Reactive oxygen species (ROS) that are created in excess can cause oxidative damage to stomach tissue. By upregulating protein inflammatory cytokines, ROS can also exacerbate acute inflammation [5].Therefore, one viable therapeutic option for gastric ulcers will be to improve the antioxidant action of gastric tissues and decrease the in vivo damage of ROS to the stomach mucosa.

A transcription factor called NF-κB has a vital regulatory role in regulating a number of inflammation-associated genes.NF-κB is activated in gastric ulcer lesions by oxidative stress [6].

One of the essential intracellular signaling pathways, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway regulates a wide range of target proteins, such as NF-κB and GSK-3β, to control cell metabolism, proliferation, apoptosis and differentiation [7,8].

Once PI3K is activated, Akt is phosphorylated and activated as a result. Activated Akt moves from the membrane, and inactivates GSK3β. By phosphorylating transcription factors that control apoptosis, GSK3β contributes to some apoptotic signals cascades by activation of pro-apoptotic proteins and repression of survival-promoting factors are induced by GSK3β, thus its phosphorylation/inactivation improves cell survival [9].

Proton pump inhibitors, histamine H2 receptor antagonists and antacids are the most regularly prescribed drugs for stomach ulcers. However, prolonged use of these medications might result in a wide range of negative side effects; as a result, their usage is restricted [10].

In order to limit tissue damage, treatment for stomach ulcers should focus on repairing this protective barrier as well as lowering gastric acid, inflammation, and oxidative stress. When used to treat stomach ulcers, natural compounds from plants and diets have demonstrated encouraging results and are generally safe gastro-protective drugs [11].

The traditional Chinese herb baicalein (Baic) is derived from the root of ScutellariabaicalensisGeorgi. It has several biological characteristics, such as anti-inflammatory, antioxidant, and anti-apoptotic

effects [12], in addition to antiulcer, antipyretic, analgesic, anticancer, antioxidant, and wound healing properties [13].

This motivates us to investigate the potential underlying processes including referral to the Akt/Pi3k/NF-kB signaling pathway as well as the gastroprotective impact of Baic on IND-induced gastric ulcer.

Materials and Methods

Animals

We followed the rules established by the Animal Experimentation Ethics Committee of Menoufia University's Faculty of Medicine when conducting this work with IRB No: 1/2025Bio 19-2.Thirty adult male Wister rats, weighing 155–175g, were used. The rats were housed in housing with a temperature range of 20 to 24 °C. Regular rat food and tap water were also freely available to them.

Experimental design

Rats were randomly divided into three groups of ten each.

Control group: animals were orally given 1 ml0.5% sodium carboxymethylcellulose (CMC-Na) (cat. no. 21904; MilliporeSigma) once daily for 4 weeks.

IND-induced gastric ulcer (Ulcer group): Rats received a single IND dosage (50 mg/kg) via gastric gavage dissolved in 1 ml CMC on day 14 of the trial, as previously described [14], and were administered 1 ml CMC-Na orally once daily for 4 weeks.

IND-induced gastric ulcer Baic-treated (Ulcer+Baic) group: Beginning on day one and continuing for four weeks until the study's conclusion, animals were given 100 mg/kg/day of Baic (Must, Chengdu, China) dissolved in 1 ml CMC-Na orally [15, 16].Animals were given IND (single dosage; 50 mg/kg) on day 14. Rats were given Baic one hour prior to the administration of IND.

On the final day, rats were fasted overnight. Subsequently, serum separation for biochemical analysis was carried out in the morning after retroorbital blood sample collection. Finally, the rats were decapitated while under general anesthesia with 1% pentobarbital (50 mg/kg intraperitoneal injection).The gastric pylorus was tied off prior to stomach dissection along the larger curvature in order to assess gastric ulcers and acidity.

Three portions were taken out of each stomach. The 1st half was maintained at -80 (until biochemical analysis), the 2nd half was used for RNA extraction in the form of crude tissue, and the 3rd half was used for immunohistochemical and histological testing.

Gastric acidity Determination: Using a micropipette (1000 ml), gastric juice was extracted after each stomach was cleaned with phosphate-buffered saline (1 ml).The pH of the collected gastric juice was measured using a PH meter. [17].

IND-induced gastric lesion macroscopic assessment:

After cleaning the mucosa with regular saline, it was visually inspected for the presence of ulcers and bleeding. As previously mentioned, stomach mucosal damage was evaluated using the ulcer index [18].The total number of ulcer index lesions in each rat's severity factor. Additionally, a 0–3 scale was used to assess the severity factor based on the lesion's length. Lesions smaller than 1 mm have a severity value of 1, lesions 1-4 mm have a severity factor of 2, and lesions larger than 4 have

a severity factor of 3. If there are no lesions, the severity factor is equal to 0.

Gastric homogenate preparation:

Weighing and homogenizing gastric specimens by adding phosphate-buffered saline (PBS) (50 mM), PH 7.4, subsequently homogenized utilizing a tissue homogenizer (MPW120; MPW Medical Instruments,China). Afterward, the resultant supernatant was collected as well as kept at -80 for assessment of gastric TNF-a, PGE2, IL-1β, MDA and SOD levels. In an ice-cold centrifuge, crude tissue homogenate was spun for 15 min at 10 000 rpm

Measurement of IL-1β, TNF-a, and PGE2 levels in stomach homogenate was performed using rat enzyme-linked immunosorbent assay (ELISA) kit (TNF-a: R and D Systems Inc-Minneapolis-USA), (IL-1β: ab100768-Abcam-Cambridge-UK), (PGE2:MBS262150-MyBioSource-San Diego-CA-USA) as per manufacturer's guidelines. Colorimetric kits were utilized for measuring MDA, and SOD in stomach homogenate (Biodiagnostic.-Giza-Egypt).

Quantitativeassayofgeneexpressionusing reverse transcriptase polymerase chainreactiontechnique(RT-PCR)

RT-PCR was used to determine the relative mRNA levels of the PI3K, Akt, and NF-kB genes in the stomach tissue. As instructed by the manufacturer, total RNA was extracted from tissues using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA).After being separated, RNA was kept at -80°C until it was required. During the first PCR step, complementary DNA (cDNA) (reverse transcription) was synthesized using ThermoScriptTM RT reagent kits (Invitrogen).After that, cDNAs were amplified in PCR tests using SYBR Green Mix kits (Stratagene, USA). A cycle threshold (Ct) value was obtained for every amplification curve.Glyceraldehyde-3-phosphate dehydrogenase was chosen as the reference gene.7500 ABI PRISM (Applied Biosystems, USA) v.2.0.1 was used to analyze the data. The expression of the PI3K, Akt, and NF-kB genes were measured using the comparative ΔΔCt method. The forward primer for Akt was (*TCACCTCTGAGACCGACACC*), and the reverse primer was(*ACTGGCTGAGTAGGAGAACTGG*). The forward primer for PI3K was (*AGCTGGTCTTCGTTTCCTGA*), and the reverse primer was (*GAAACTTTTTCCCACCACGA*).The NF-kB forward primer was (TCGACCTCCACCGGATCTTTC). The reverse primer was (GAGCAGTCATGTCCTTGGGT). B-actin gene was endogenous control.

Histopathological analysis

Prior to being placed in paraffin, the stomach tissue was fixed at 10% neutral buffered formalin (pH=7.0), dried in ethyl alcohol, and cleaned in xylol. Sections were stained using both a special stain of Periodic Acid Schiff's (PAS) stain and regular Hematoxylin& Eosin (H&E) stain:

Gastric IHC staining for Cyclooxygenase-2 (COX-2)

Cyclooxygenase-2 (COX-2) was used to stain the stomach tissue using immunohistochemistry (IHC) using a rabbit polyclonal anti-COX-2 antibody (Catalog No. A1253; Abclonal, Woburn, United States).

Interpretation of COX-2 IHC results: In the investigated cases and control specimens, any number of gastric mucosal cells exhibiting brown granular cytoplasmic staining was deemed

positive. The three groups under study had their stomach tissues evaluated for:

1. Expression percentage: In stomach tissues, positive cells were counted and reported as a percentage of 200 cells in the whole region at 100× magnification.

2. Staining intensity: classified as strong (+++), moderate (++), or mild (+).

3. Histo-score (H score): Using the following formula, the H score was determined for each positive specimen:H score is equal to 1 × % of cells that are lightly stained, 2 × % of cells that are moderately stained, and 3 × % of cells that are highly stained [19].

Statistical analysis

The mean ± standard deviation (SD) was used to represent all data. Version 16 of the SPSS software (SPSS Inc., Chicago, IL, USA) was used to analyze the data. To find significance between the groups, a post hoc Tukey test was used after a one-way analysis of variance (ANOVA).Statistical significance was defined as a P value of less than 0.05.

Results:

Compared to control group, the ulcer group showed dramatically lower gastric PH values, gastric SOD, gastric PGE2, and gastric gene expression of PI3K and Akt, while also exhibiting significantly higher gastric ulcer index values, gastric MDA, gastric TNF-α, gastric IL-1β, and gastric gene expression of NF-kB. In contrast to the Ulcer group, the Ulcer+Baic group had significantly higher gastric PH values, gastric SOD, gastric PGE2, and gastric gene expression of PI3K and Akt, while the Ulcer+Baic group had significantly lower levels of gastric ulcer index values, gastric MDA, gastric TNF-α, gastric IL-1β, and gastric gene expression of NF-kB. Table 1

Table (1): Gastric ulcer index, PH, MDA, SOD, PGE2, TNF-α, IL-1β, Gastric PI3K, Akt, and NF-kB genes expression evaluation in the research groups (total 30 rats, 10 for each group)

	Control group	Ulcer group	Ulcer+Baic group
Macroscopic ulcer Index	0.3±0.2	45.2±3.2*	19.7±2.01*#
Gastric PH	5.2±0.37	3.18±0.29*	4.2±0.28*#
Gastric MDA (nmol/ gm. Tissue)	4.2±0.91	19.7±3.01*	12.8± 2.08*#
Gastric SOD (U/gm. Tissue)	6.2±0.233	1.6±0.21*	3.9±0.38*#
Gastric PGE2(pg/mg.tissue)	35.12±1.9	19.7±2.03*	26.1±0.9*#
Gastric TNF-α (Pg/ml)	23.58±2.37	39.32±2.31*	30.8±1.9*#
Gastric IL-1β (Pg/ml)	50.3±1.9	94.3±3.48*	72.8±3.49*#
Gastric PI3K gene expression	1	0.38±0.07*	0.59±0.05*#
Gastric Akt gene expression	1	0.39±0.04*	0.68±0.03*#
Gastric NF-kB gene expression	1	2.7±0.19*	1.82±0.07*#

* Significant compared with control, # Significant compared with Ulcer.

Histopathological results

The control group showed normal histology of the gastric mucosa (Fig. 1A). The ulcer groups showed sever disruption of the mucosal surface with apparent gastric ulcer (Fig. 1B). The Ulcer+Baic group showed improvement in the mucosal surface (Fig. 1C)

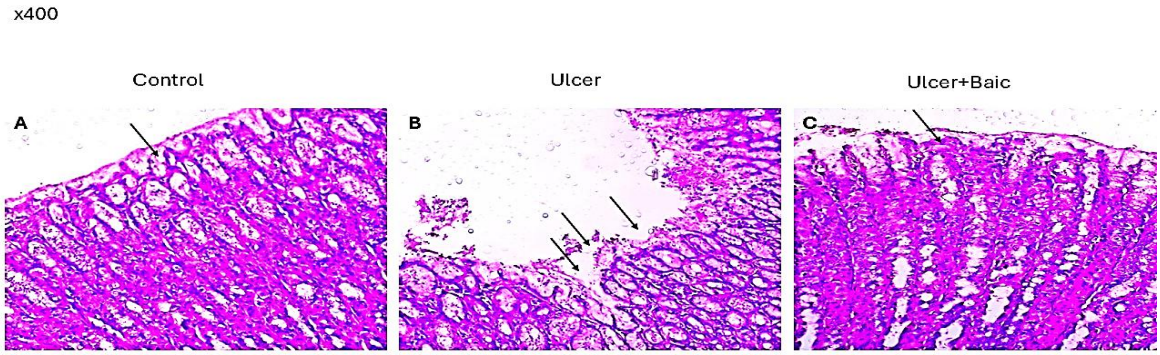

Fig.(1): H& E-stained gastric sections of the studied groups: (A) is a photomicrograph of gastric tissue of the control group showing normal gastric mucosa (black arrow). (B) is a photomicrograph of ulcer group showing sever disruption of the mucosal surface with apparent gastric ulcer (arrows). (C) is a photomicrograph of the ulcer+Baictreated group showing improvement in the mucosal surface (arrow). (x 400).

According to the PAS stain, the ulcer group's mean AS intensity value was much lower than the control groupp's (15±3.2 vs. 59±o.52, respectively, p<0.05). While the ulcer+Baic group's PAS intensity was dramatically greater than the ulcer group's (42±1.5 vs. 15±3. 2, respectively, p<0.05). (Fig. 2A-D).

Compared to the control group, the ulcer group's mean COX2 intensity levels were substantially higher (70±1.4 vs. 20±o.33, respectively, p<0.05).The Ulcer+Baic group's COX2 intensity was substantially greater than the control group's (p<0.05), although it was significantly lower than the ulcer group's (39±0.24 vs. 70±1.4, respectively, p<0.05).(Fig. 2E-H).

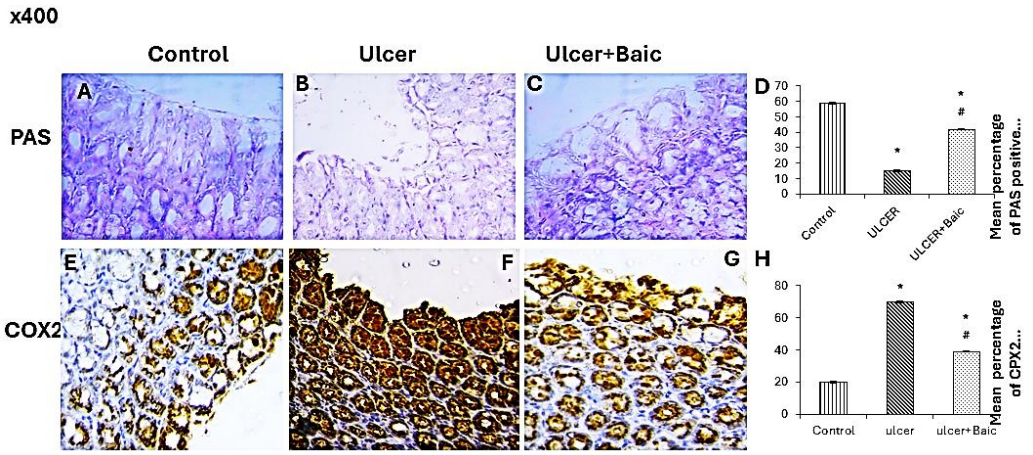

Fig. (2):Representative micrographs of the different experimental groups showing a significant decline in PAS stain reaction in the ulcer group when compared to control group and a significant positive PAS stain reaction in the ulcer+Baic group compared to ulcer (A-D). Also, there is a significant increase of the COX2 (E-H) immunoreaction in the ulcer group compared to control and a significant decrease in the ulcer + Baic group compared to ulcer. (x400)

Discussion

Investigating if Baic supplementation might shield rats against IND-induced stomach ulcers was the primary goal of this investigation. Therefore, measurements of gastric ulcer index, oxidative stress indicators (gastric MDA and SOD), inflammatory markers (gastric TNF-α andIL-1β), and PGE2 in the stomach mucosa were made in order to investigate the protective impact of Baic and the underlying likely processes. Along with

the expression of the gastric PI3K, Akt, and NF-kB genes, COX-2 immunostaining was also carried out in the stomach.

This study's noteworthy finding was that Baic mitigated IND-induced peptic ulcer in rats by diminishing the oxidative stress and inflammatory responses, restoring the levels of PGE-2 and reducing the level of COX-2 in the stomach and by modulating PI3K/Akt/NF-*k*B pathway.

The current study confirmed that IND caused stomach ulcers, which is consistent with previous results [20]. Histopathological and macroscopical results highlighted this.

In addition, rats given IND had a markedly lower stomach pH level and a higher ulcer index compared to control. By lowering the pH of gastric juice, the high concentration of hydrogen ions is a harmful factor that promotes stomach injury. IND significantly alters the hydrophobicity of the membrane. This can change the "gate keeping functions" of cells, which can result in inevitable damage to the stomach mucosa, including erosions and ulcers, due to the formation of membrane pores. Increased gastric content, overall acidity, and a resulting drop in gastric pH might result from severe disruption of the stomach mucosa [21].

This observation may be explained by a possible breakdown in the stomach mucosa, which acts as a catalyst for prooxidant activity and initiates the generation of reactive oxygen species (ROS) by lipoperoxidation. As a result, this mechanism impairs the mucosa's antioxidant capacity and triggers an inflammatory reaction [22].

However, as compared to ulcer group, our results showed a substantial rise in the stomach PH and a drop in ulcer index values in Ulcer+Baic, indicating that Baic possesses gastroprotective qualities. Additionally, this is consistent with earlier research [16].In a similar way, Baic increased the pH value while decreasing the amount of stomach secretion and total acid output. Its anti-secretory actions is exerted by inhibiting histaminergic pathways. Furthermore, in vitro H+, K+-ATPase activity was partly reduced by Baic [23].

Additionally, the here-in observed decline in the gastric PGE-2 and the upsurge in COX-2 immunoreactivity in gastric tissue of Ulcer group and this goes in line with previous study [2].

The cyclooxygenase inducible isoform COX-2 is a typical pro-inflammatory mediator in gastrointestinal injuries [24].It has also been observed that gastric ulceration induces COX-2 mRNA and protein expression [25].Suppression of prostaglandin synthesis causes vascular injury, reduces cell turnover, and diminishes mucosal blood flow, bicarbonate, and mucus output [26].

Indomethacin has been shown to reduce the flow of blood through the stomach mucosa and block blood microvessels. The decrease in stomach mucosal blood flow promotes the recruitment of neutrophils and leukocytes in gastric tissue [27].Therefore, IND's reduction of prostaglandin production may be the cause of the stomach ulcers shown in this research.

According to the current study, the ulcer group's COX-2 expression was higher than that of the control group. Other investigations have consistently shown elevated levels of COX-2

mRNA in gastric ulcers caused by indomethacin [28].

Thankfully, the administration of Baic in the current study led to a downregulation of COX-2 expression, most likely as a result of its anti-inflammatory mode of action. Important biochemical processes in the pathophysiology of gastric ulcers have been identified, including the inhibition of PG synthesis by IND which results in the infiltration of neutrophils and leucocytes in gastric tissue and the production of free radicals [29].

The results of this investigation show that, in comparison to controls, the stomach tissues of the IND group had significantly lower PGE2 levels. This discovery is consistent with the results that Sabiu et al. [30] obtained. When IND is administered, cyclooxygenase is suppressed, which prevents the synthesis of PGs and causes gastrointestinal damage [31].

PGE2 is clearly an essential modulator of the gastrointestinal mucosal defense. By encouraging the release of mucus and bicarbonate and preventing the production of stomach acid, PGE2 is a significant cytoprotective endothelial vasodilator that aids in ulcer healing [32].According to a prior study, Baic significantly raised the PGE2 level [16].

The pathophysiology of stomach ulcers is likely linked to oxidative stress, which is triggered by the production of ROS [33].Therefore, the comparable gastric mucosal damage in the rats treated with indomethacin may be partially explained by the increased generation of ROS and antioxidant depletion seen in our investigation. This is consistent with earlier research [2].

Indomethacin's pro-oxidant actions are well documented; neutrophil infiltration in the stomach mucosa can produce free radicals such ROS [34]. Moreover, indomethacin can impair the stomach mucosa epithelial cells' mitochondria from undergoing oxidative phosphorylation. This causes the cells to generate internal ROS. The numerous harmful effects of ROS cause damage to the stomach's mucosa and enhance the permeability of gastric epithelial cells [33].

By raising SOD activity and lowering MDA levels, Baic dramatically reduced the oxidative stress brought on by IND. The current study's findings concurred with those of earlier research [35], which highlights the compound's capacity to scavenge free radicals and have an antioxidant impact. Additionally, studies have shown that Baic can enhance antioxidant qualities by boosting the gene expression of antioxidant enzymes [36]. Furthermore, via raising Nrf2, Baic's antioxidant qualities help to avoid mitochondrial dysfunction [37].

Strong evidence suggests that one of the features of stomach ulcers is the disruption of the inflammatory response. Parallel to other findings, the current study showed that the rats with indomethacin ulcers had a higher degree of gastric inflammation, as evidenced by noticeably higher levels of gastric TNF-α and IL-1β as well as an increase of NF-kB gene expression in the stomach [38].Furthermore, IND triggers NF-κB, which in turn increases the production of genes for pro-inflammatory cytokines. These molecules are essential because they enter the nucleus of the gastric epithelial cells and promote the production of several target genes, including proinflammatory

cytokines, which can worsen the damage to the stomach mucosa [33].

According to earlier research, Baic greatly reduced the inflammatory state that IND caused in the stomach, as shown by a considerable drop in pro-inflammatory cytokines and a downregulation of NF-kB gene expression [16]. By blocking the TLR4/MyD88/NF-κB signaling pathway, baicalein treatment decreased the glial cell activation and the generation of pro-inflammatory chemicals in previous study [35].

By controlling the main inflammatory cytokines, the PI3K/AKT pathway appears to trigger immune cell activation. One of the PI3Ks' primary downstream targets for controlling cell migration and proliferation is the AKT [39].GSK3β, which is active in cells at rest, is phosphorylated as a result of AKT activity, which inactivates it. A group of transcription factors, including NF-κB, have been found to be regulated by GSK3β [40]. Proinflammatory expression is also inhibited when PI3K is activated. Furthermore, it appears that the PI3K/AKT/GSK3β pathways suppress proinflammatory cytokines and increase anti-inflammatory cytokines [41].

Our results revealed that IND induced downregulation in PI3K and Akt genes with dramatically upregulated NF-kB in gastric mucosa compared to control. A previous study suggested a key role of the PI3K-Akt pathway in gastric ulcers [42].There is a crosstalk between the PI3K/Akt pathway and the NF-*κ*B pathway [43].

Actually, cell migration and proliferation—two critical processes for ulcer healing and reepithelialization—depend on the PI3K/AKT pathway being activated [42].

Ulcer +Baic dramatically upregulated PI3K/Akt and downregulated NF-kB compared to Ulcer. Baic impact in PI3K/AKT/NF-κB pathway was previously documented [44].Our study revealed the Baic gastroprotective impact on IND induced ulcer by modulation of PI3K/AKT/NF-κB pathway

Furthermore, the findings of PAS staining which identified mucopolysaccharides in the stomach mucosa and indicated its integrity [45] supported the standard H&E stain and showed a reduction in PAS reactivity in the ulcer group, which is in line with earlier research [20].In agreement with other researchers, the Ulcer+Baic group showed robust PAS-reactive mucosal cells following treatment with gastroprotective agents [46].

Conclusion

Baicalein exhibits gastroprotective benefits by modifying the Akt/Pi3k/NF-kB signaling pathway and having anti-inflammatory and antioxidant qualities, baicalein helps rats with IND-induced ulcers recover. Baic may therefore be a natural substance that works through many, frequently complementary methods to prevent and cure stomach ulcers. The study's findings lay the groundwork for the practical use of Baic in the management of stomach ulcers.

Conflicts of Interest

The authors declare no conflict of interest.

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References

1- **Serafim, C., Araruna, M.E., Júnior, E.A., Diniz, M., Hiruma-Lima, C. & Batista, L. A. (2020).** Review of the role

of flavonoids in peptic ulcer (2010–2020). *Molecules*, *25*, 5431.

2- **Mohamed, M. M., &Farid, O. (2024).** Gastro-Protective, Antioxidant, and Anti-Inflammatory Potential of Three Vegetable Oils Against Indomethacin-Induced Gastric Ulceration in Rats: in Vivo and in Vitro Study. *Egyptian Journal of Chemistry*, *67*(7), 341-359.

3- **El-Ashmawy NE, Khedr EG, El-Bahrawy HA, et al.** Nebivolol prevents indomethacin-induced gastric ulcer in rats. J immunotoxicol 2016;13:580e9.

4- **Danisman B, Cicek B, Yildirim S, et al.** Carnosic acid ameliorates indomethacin-induced gastric ulceration in rats by alleviating oxidative stress and inflammation. Biomedicines 2023;11:829.

5- **Suzuki, H., Nishizawa, T., Tsugawa, H., Mogami, S., &Hibi, T. (2012).** Roles of oxidative stress in stomach disorders. *Journal of ClinicalBiochemistry and Nutrition*, *50*(1), 35–39.

6- **Aziz, R. S., Siddiqua, A., Shahzad, M., Shabbir, A., &Naseem, N. (2019).** Oxyresveratrol ameliorates ethanol-induced gastric ulcer via downregulation of IL-6, TNF-*α*, NF-κB, and COX-2 levels, and upregulation of TFF-2 levels. *Biomedicine&Pharmacotherapy*, *110*, 554–560.

7- **He, M., Zhang, Y., Xie, F., Dou, X., Han, M., & Zhang, H. (2018).** Role of PI3K/Akt/NF-κB and GSK-3β pathways in the rat model of cardiopulmonary bypass-related lung injury. *Biomedicine & Pharmacotherapy*, *106*, 747-754.

8- ***Zeng B, Liu L, Liao X, et al.*** *Thyroid hormone protects cardiomyocytes from H2O2-induced oxidative stress via the PI3K-AKT signaling pathway. Exp Cell Res. 2019;380(2):205–215.*

9- **Ajzashokouhi, A. H., Rezaee, R., Omidkhoda, N., &Karimi, G. (2023).** Natural compounds regulate the PI3K/Akt/GSK3β pathway in myocardial ischemia-reperfusion injury. *Cell Cycle*, *22*(7), 741-757.

10- **Al-Wajeeh,N. S., Hajrezaie, M.,Al-Henhena,N.,Kamran, S., Bagheri, E., Zahedifard, M., Saremi, K., Noor, S. M., & Abdulla, M. A. (2017).** The antiulcer effect of *Cibotiumbarometz*leaves in rats with experimentally induced acute gastric ulcer. *Drug Design, Development and Therapy*, *11*, 995–1009.

11- **Boeing, T., Costa, P., Venzon, L., Meurer, M., Mariano, L. N. B., França, T. C. S., Gouveia, L., de Bassi, A. C., Steimbach, V., de Souza, P., de Almeida, M. O., Arruda, C., de Andrade, S. F., Bastos, J. K., & da Silva, L. M. (2021).** Gastric healing effect of p-coumaric acid isolated from Baccharisdracunculifolia DC on animal model. *Naunyn-Schmiedeberg's archives ofpharmacology*, *394*(1), 49–57.

12- **Li, J., Zhou, Y., Du, G. et al. (2019a).** 'Integration of transcriptomics and network analysis deciphers the mechanisms of baicalein in improving learning and memory impairment in senescence-accelerated mouse prone 8

(SAMP8) '.*Eur J. Pharmacol*. 865, 172789.

13- **Yang, H., Lu, Y., Zeng, X. F., Li, L., Zhang, R. P., Ren, Z. K., & Liu, X. (2018).** Antichronic Gastric Ulcer Effect of Zinc-Baicalin Complex on the Acetic Acid-Induced Chronic Gastric Ulcer Rat Model. *Gastroenterology Research and Practice*, *2018*(1), 1275486.

14- **Shaik RA, Eid BG.** Piceatannol affects gastric ulcers induced by indomethacin: association of antioxidant, anti-inflammatory, and angiogenesis mechanisms in rats. Life 2022;12:356.

15- **Song, J., Li, M., Kang, N. et al. (2024).** 'Baicalein ameliorates cognitive impairment of vascular dementia rats via suppressing neuroinflammation and regulating intestinal microbiota'. *Brain Research Bulletin*, 208, 110888.

16- **Nasif, E., Shalaby, R., El-Khalik, A., Ragab, S., &AbdEllatif, R. (2022).** The Potential Anti-ulcerogenic Effects of Baicalein and/or Empagliflozin in Induced Gastric Ulcer in Rats: Modulating HO-1/SIRT1/HMGB1 signaling Pathway. *Bulletin of Egyptian Society for Physiological Sciences*, *42*(3), 246-264.

17- **Choo BK, Roh SS.** Berberine protects against esophageal mucosal damage in reflux esophagitis by suppressing proinflammatory cytokines. ExpTher Med 2013;6:663e70

18- **Peskar BM, Ehrlich K, Peskar BA**. Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in the rat. J pharmacolExpTher 2002;301:969e74.

19- **Aeffner F, Wilson K, Martin NT, Black JC, Hendriks CLL, Bolon B, Rudmann DG, Gianani R, Koegler SR, and Krueger J:** The gold standard paradox in digital image analysis: manual versus automated scoring as ground truth. Archives of pathology & laboratory medicine 141: 1267-1275, 2017.

20- **Khodir, S. A., Abd-Elhafiz, H. I., Sweed, E. M., Gaafar, A. M., El-aziz, N. M. A., Salman, N. A. A., ... &Sheref, A. A. (2024).** Luteolin ameliorates Indomethacin-Induced Gastric Ulcer in Rats via Activation of Nrf2/HO-1 Pathway. *Menoufia Medical Journal*, *37*(1), 17

21- **Manrique-Moreno, M., Garidel, P., Suwalsky, M., Howe, J. & Brandenburg, K. (2009).** The membrane-activity of Ibuprofen, Diclofenac, and Naproxen: a physico-chemical study with lecithin phospholipids. *Biochimicaetbiophysicaacta*, 1788, 1296–1303,

22- **Tarnawski A, Ahluwalia A, Jones MK.** Gastric cytoprotection beyond prostaglandins: cellular and molecular mechanisms of gastroprotective and ulcer healing actions of antacids. CurrPharmaceut Des 2013;19:126e32.

23- **Ribeiro AR, do NascimentoValença JD, da Silva Santos J, Boeing T, da Silva LM, de Andrade SF, et al.** The effects of baicalein on gastric mucosal ulcerations in mice: Protective pathways and anti-

secretory mechanisms. ChemBiol Interact. 2016; 260:33-41.

24- **García-Rayado, G., Navarro, M., &Lanas, A. (2018).** NSAID-induced gastrointestinal damage and designing GI-sparing NSAIDs. *Expert review of clinical pharmacology*, 11(10), 1031–1043.

25- **Jackson, L.M., Wu, K.C., Mahida, Y.R., Jenkins, D., &Hawkey, C.J. (2000).** Cyclooxygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. *Gut* 47:762–770.

26- **Fazalda, A., &Quraisiah, A. (2018).** NurAzlina, M.F. Antiulcer effect of honey in nonsteroidal anti-inflammatory drugs-induced gastric ulcer model in rats: A systematic review. *Evid.-Based Complement. Altern. Med.*, 2018, 1–12.

27- **Ruiz-Hurtado, P.A., Garduño-Siciliano, L., Dominguez-Verano, P., Martinez-Galero, E., Canales-Martinez, M.M., & Rodriguez- Monroy, M.A. (2021).** Evaluation of the gastroprotective effects of Chihuahua propolis on indomethacininduced gastric ulcers in mouse. *Biomed.Pharmacother.*,*137*, 111345.

28- **Shahin NN, Abdelkader NF, Safar MM:** A novel role of irbesartan in gastroprotection against indomethacin-induced gastric injury in rats: targeting DDAH/ADMA and EGFR/ERK signaling. Scientific reports 8: 1-12, 2018.

29- **Ajani E, Sabiu S, Bamisaye F, et al.** Ethanolic leaf extract of Langenariabreviflora (bitter gourd) inhibits gastric onslaught in indomethacininduced ulcerated rats. J ApplBiosci 2015;85: 7871e80.

30- **Sabiu S, Garuba T, Sunmonu T, et al.** Indomethacin-induced gastric ulceration in rats: Protective roles of Spondiasmombin and Ficusexasperata. Toxicol Rep 2015;2:261e7.

31- **Bjarnason I, Scarpignato C, Holmgren E, et al.** Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. Gastroenterol 2018;154:500e14

32- **Sidahmed HMA, Vadivelu J, Loke MF, Arbab IA, Abdul B, Sukari MA, et al.** Anti-ulcerogenic activity of dentatin from clausenaexcavataBurm.f. against ethanol-induced gastric ulcer in rats: Possible role of mucus and anti-oxidant effect. Phytomedicine. 2019; 55:31-9.

33- **Katary,M., &Salahuddin, A. (2017).** Gastroprotective effect of vanillin on indomethacin induced gastric ulcer in rats: Protective pahways and anti-secretory mechanism. *Clin. Exp. Pharmacol.*, *7*, 232.

34- **Abdel-Raheem, I.T. (2010).** Gastroprotective effect of rutin against indomethacin-induced ulcers in rats. *Basic Clin. Pharmacol. Toxicol.*,107: 742–750. doi: 10.1111/j.1742-7843.2010.00568.x.

35- **Jadhav, R. and Kulkarni, Y. A. (2023).** 'Effects of baicalein with memantine on aluminium chloride-induced neurotoxicity in Wistar rats'. *Frontiers in Pharmacology*, *14*, 1034620

36- **Kang, K. A., Zhang, R., Piao, M. J. et al. (2012).** **'**Baicalein inhibits oxidative

stress-induced cellular damage via antioxidant effects. *Toxicology and industrial health*, *28*(5),p.p. 412-421.

37- **Hu, Z. Guan, Y. Hu,W. et al. (2022).** 'An Overview of Pharmacological Activities of Baicalin and Its AglyconeBaicalein: New Insights into Molecular Mechanisms and Signaling Pathways'. *Iranian Journal of Basic Medical Sciences*, *25*(1),p.p. 14.

38- **El-Hussieny, E.A., Mohamed, E.F., Attala, N.R., &Abd El-Rahman F.A. (2017).** Gastroprotective effect of a new formulated milk tablet on ethanolinduced gastric mucosal injury in rats. *Int J Adv Res*., 5(3):2374–88.

39- **Kitagishi, Y., Kobayashi, M., Kikuta, K., & Matsuda, S. (2012).** Roles of PI3K/AKT/GSK3/mTOR pathway in cell signaling of mental illnesses. *Depression research and treatment*, *2012*(1), 752563.

40- ***Die L, Yan P, Jiang ZJ, Min Hua T, Cai W, Xing L.*** *Glycogen synthase kinase-3 beta inhibitor suppresses Porphyromonasgingivalis lipopolysaccharide-induced CD40 expression by inhibiting nuclear factor-kappa B activation in mouse osteoblasts. Molecular Immunology. 2012;52(1):38–49. doi: 10.1016/j.molimm.2012.04.005.*

41- **Leonard, B., &Maes, M. (2012).** Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neuroscience &Biobehavioral Reviews*, *36*(2), 764-785

42- **Arab, H. H., Salama, S. A., Eid, A. H., Kabel, A. M., &Shahin, N. N. (2019).** Targeting MAPKs, NF-*κ*B, and PI3K/AKT pathways by methyl palmitate ameliorates ethanol-induced gastric mucosal injury in rats. *Journal of Cellular Physiology*, *234*(12), 22424–22438.

43- **Gong, M., Li, Q., Guo, H., Cui, B., Liu, Y., Wang, P., ...& Wang, Z. (2022).** Protective effect of active components of Eucommiaulmoides leaves on gastric ulcers in rats: involvement of the PI3K/Akt/NF-κB pathway. *Journal of Food Science*, *87*(7), 3207-3222.

44- **Liu, Z., Xiang, H., Deng, Q., Fu, W., Li, Y., Yu, Z., ...& Xu, L. (2023).** Baicalin and baicalein attenuate hyperuricemic nephropathy via inhibiting PI3K/AKT/NF-κBsignalling pathway. *Nephrology*, *28*(6), 315-327

45- **Tarnawski A, Szabo I, Husain S, Soreghan B:** Regeneration of gastric mucosa during ulcer healing is triggered by growth factors and signal transduction pathways. Journal of Physiology-Paris 95: 337-344, 2001.

46- **Hajrezaie M, Salehen N, Karimian H, Zahedifard M, Shams K, Batran RA, Majid NA, Khalifa SA, Ali HM, and El-Seedi H:** Biochanin a gastroprotective effects in ethanol-induced gastric mucosal ulceration in rats. PloS one 10: e0121529, 2015.