Tropical Journal of Pharmaceutical Research January 2023; 22 (1): 15-22
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i1.3

Original Research Article

Galangin protects gastric mucosa from indomethacin-induced injury via scavenging ROS and inhibition of PKC, Akt, and NF-κB protein expressions

Jingwen Gong, Yu Xie, Yinfen Tan, Hailong Li, Junqing Zhang*
Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Provincial Key Laboratory for Research and Development of Tropical Herbs, Haikou Key Laboratory of Li Nationality Medicine, School of Pharmacy, Hainan Medical University, Haikou 571199, China

For correspondence:* ***Email:* *jqzhang2011@163.com;* ***Tel:*** *+86-898-66895337*

Sent for review: 15 August 2022 *Revised accepted: 21 December 2022*

Abstract

Purpose: *To investigate the mechanism of action of galangin (GAL) against indomethacin (Indo)-induced gastric injury.*
Methods: *Ten micromolar GAL with 2 mM Indo or 0.5 mM L-NAME or 2 mM propargylglycine were co-administered to human gastric mucosal epithelial cells GES-1 for 24 h. Cell viability assay and reactive oxygen species (ROS) test were conducted with commercial kits. Protein expressions of constitutive and inducible nitric oxide synthase (cNOS and iNOS, respectively), and cystathionine-γ-lyase (CSE) in GSE-1 cells were investigated by western blot. After 100 - 300 mg/kg, GAL was daily administered to rats for 5 days, and 30 mg/kg Indo was administered to induce gastric injury. Protein expressions of endothelial NOS (eNOS), iNOS, CSE, nuclear factor (NF)-κB, PKC, Akt, phospho-eNOS (Ser-1177), and phospho-eNOS (Thr-495) were investigated by western blot.*
Results: *Galangin ameliorated the proliferative inhibitions of Indo, propargylglycine, and L-NAME on GSE-1 cells ($p < 0.05$), rapidly scavenged ROS, and increased cNOS protein expressions ($p < 0.05$). Galangin (300 mg/kg) inhibited protein expressions of iNOS ($p < 0.01$), NF-κB, Akt, and PKC ($p < 0.05$), inhibited the Ser-1177 and Thr 495 phosphorylation of eNOS, elevated eNOS and CSE protein expressions ($p < 0.001$).*
Conclusion: *Galangin protects gastric mucosa from Indo by scavenging ROS and inhibiting protein expressions of Akt and PKC. The inhibition leads to Ser-1177 and Thr 495 dephosphorylation of eNOS, thereby prolonging eNOS activity. Besides, GAL inhibits iNOS-produced NO by inhibiting NF-κB to ameliorate the inflammatory reaction induced by Indo.*

Keywords: *Galangin, Non-steroidal anti-inflammatory drug, cNOS, Gastric injury*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Gastric injury induced by non-steroidal anti-inflammatory drugs (NSAIDs) has been studied for years due to their significant social impact [1]. In addition to commercial drugs, some natural plant extracts have been found to have significant potentials to protect gastric mucosa

against NSAIDs [2]. Galangin (GAL), one of the major flavonoids of *Alpinia officinarum* Hance, has been reported to possess many pharmaceutical effects, like antioxidant and anti-inflammatory activity [3,4]. In preliminary reports, GAL significantly increased gastric mucosal blood flow and VEGF levels in an indomethacin (Indo)-induced gastric injury rat model [5]. Besides, ELISA tests showed that GAL had the potential in triggering constitutive nitric oxide synthase (cNOS) and cystathionine-γ-cleavage enzyme (CSE) expressions [5]. The cNOS and CSE proteins are key enzymes in nitric oxide (NO) and sulfuretted hydrogen (H_2S) synthetic signaling pathways [6]. The NO and H_2S help to promote mucus secretion, improve gastric mucosal microcirculation and strengthen the gastric mucosal barrier [6]. Furthermore, NO and H_2S both exert a proangiogenic effect [7], and NO stimulates prostaglandin synthesis [8]. Therefore, a hypothesis was raised that NO and H_2S synthetic signaling pathways may be implicated in the antiulcer mechanism of GAL.

Three NOS isoforms have been identified, including cNOS (containing neuronal NOS (nNOS) and endothelial NOS (eNOS)) and inducible NOS (iNOS) [7]. The central mechanisms controlling iNOS expression appear to be the nuclear factor (NF)-κB pathway [9]. The eNOS is subject to several overlapping modes of post-translational regulatory modifications, like phosphorylation of Ser 1117 and Thr 495, kinase Akt, protein kinase Cα (PKC), and so on [10]. It should be noted that oxidative stress and $ONOO^-$ production are related to eNOS "uncoupling" (self-generation of O_2^- rather than NO) and nNOS excitotoxicity [11]. Plant flavonoids have been shown to regulate NO bioavailability at the cellular level by acting on the expression and/or activity of the NOS enzymes [12]. By scavenging O_2^-, flavonoids not only protect against NO but also prevent $ONOO^-$ formation. Furthermore, many flavonoids have been found to regulate the expressions of PI3K/Akt, NF-κB, and cNOS/NO [12]. Thus, cellular and animal models were employed in this research to further investigate the protective mechanisms of GAL against indomethacin-induced gastric injury. The level of reactive oxygen species (ROS) and protein expressions of cNOS, iNOS, CSE, NF-κB, PKC, Akt, phospho-eNOS (Ser-1177), and phospho-eNOS (Thr-495) were also investigated.

EXPERIMENTAL

Materials

Indomethacin was purchased from Sigma-Aldrich. Galangin was purchased from Pufei De Biotech Co., Ltd (China). L-NAME was purchased from Multi Sciences Co., Ltd (China). The PPG was purchased from Aladdin. GES-1 cells were purchased from Procell Life Science and Technology Co., Ltd (China).

Cell culture and cytotoxicity assay

Human gastric mucosal epithelial cells GES-1 were grown in RPMI 1640 medium supplemented with 10 % fetal calf serum at 37 °C in a humidified incubator with 5 % CO_2. Then 5×10^4 cells/mL GES-1 cells were plated in sterile 96-well plates in triplicates. After 12 h, cells were cultured in serum-free medium containing 1 and 2 mM of PPG, respectively, 0.1, 0.5, and 1 mM L-NAME, 0.5, 5, and 10 μM GAL, 1, and 2 mM Indo for another 24 h. Cell counting kit-8 (CCK-8) (Bios harp BS350B) was then used and the absorbance was read at 450 nm with a full wavelength microplate reader.

Drug treatment in GES-1 cells

The GES-1 cells were seeded in a 96-well plate with 2×10^3 cells/well for 12 h, then treated with the conditions presented in Table 1. Consistent volume was ensured in each well. The treatment lasted for 24 h.

Table 1: Additions of drugs by each group

Group	Drugs				
	serum-free RPMI 1640 medium	10μm GAL	2 mM Indo	2 mM PPG	0.5 mM L-NAME
CON	√				
GAL	√	√			
Indo	√		√		
Indo + GAL	√	√	√		
PPG	√			√	
L-NAME	√				√
PPG + GAL	√	√		√	
L-NAME + GAL	√	√			√
PPG + L-NAME	√			√	√
PPG + L-NAME + GAL	√	√		√	√

Cell viability assay

After 24 h, the CCK-8 reagent was added to the solution and the plates were incubated for 2 h. The absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated using (experimental group absorbance value/control group absorbance value) × 100 %.

Intracellular ROS level measurement

The intracellular ROS levels were measured using a Reactive Oxygen Species Assay Kit (Beyotime Biotechnology, China). After 24 h of drug treatment, the cells were incubated with 2', 7'-dichlorofluorescein-diacetate (DCFH-DA) for 20 min at room temperature and measured at 488 nm excitation and 525 nm emission with a fluorescence microplate reader.

Animals

Sprague Dawley (SD) rats (six weeks old, purchased from Tianqing Biotechnology, China) were housed in seven cages at six rats per cage and allowed *ad libitum* access to water and food and given two weeks for acclimatization. All the animal experiments were performed according to the guidelines for the Care and Use of Laboratory Animals [13], with the approval of the animal ethics committee of Hainan Medical University (approval no. HYLL-2021-150).

Indo-induced gastric injury rat model

Forty-two SD rats were randomly selected to six groups. Each group was daily administered GAL (100 mg/kg, GAL-L group; 200 mg/kg, GAL-M group; 300 mg/kg, GAL-H group), 200 μg/kg misoprostol (positive group, POS) and an equivalent volume of vehicle solvent (control group (CON) and model group (MOD)), respectively. After 5 days of the administration, 30 mg/kg Indo was intragastrically administered to all groups, except the CON group which was administered with vehicle solvent. The vehicle solvent was 1 % (w/v) sodium carboxymethyl cellulose containing 2 % (w/v) glycerol. The histological index and gastric ulcer index (GUI) were calculated using the reported methods [5].

Western blot analysis for rats and GES-1 cells

After 24 h of drug treatment, the cells were collected and homogenized in RIPA buffer (Biosharp, BL504A), then centrifuged at 12000 rpm for 5 min. The supernatants were diluted to uniform concentration and then boiled with 5 x loading buffer. The following protocol was reported previously [5]. The blots were probed overnight at 4 ^{o}C with the following primary antibodies: CSE (diluted 1:2000, Abcam, ab136604), iNOS (diluted 1:1000, Proteintech, 18985-1-AP), cNOS (diluted 1:1000, Abcam, ab1376) and β-actin (diluted 1:4000, Abcam, ab6276).

The protocol of western blot of rat gastric mucosa was reported previously [11]. The blots were probed overnight at 4 ℃ with the following primary antibodies: CSE (diluted 1:2000, No.ab80643), iNOS (diluted 1:1000, No.18985-1-AP), cNOS (diluted 1:1000, No. ab199956), p-eNOS-S1177 (diluted 1:2000, No. ab215717), p-eNOS-T495 (diluted 1:2000, No. ab138430), Akt (diluted 1:2000, No. ab179463) , NF-κB (diluted 1:2000, No. ab207297), PKC (diluted 1:3000, No. ab32376) and β-actin (diluted 1:4000, No. ab6276). Those primary antibodies were purchased from Abcam (USA), except for iNOS which was purchased from Proteintech Group, Inc (UK). Chemidoc XRS^{+} high-sensitivity chemiluminescence imaging system was used for image analysis.

Statistical analysis

The statistical analysis of data was evaluated by carrying out one-way analysis of variance (ANOVA) or Kruskal-Wallis *H*-test using SPSS 21. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

As shown in Table 2, 10 μM GAL exhibited the best cell viability when compared with that of the CON group; while 1-, and 2-mM Indo showed a significant cytotoxic effect ($p < 0.05$ vs CON). A 0.5 mM L-NAME and 2 mM PPG exhibited significant cytotoxic effects on GES-1 cells ($p < 0.05$), and the viability values were suitable for the experiment. Thus 2 mM Indo, 10 μM GAL, 2 mM PPG, and 0.5 mM L-NAME were chosen for GSE-1 cell experiments.

GAL promoted the proliferation of GES-1 cells against Indo, PPG or L-NAME

Table 3 showed that Indo and L-NAME + PPG groups significantly inhibited the cell proliferation of GES-1 cells ($p < 0.001$). The cell proliferation of the Indo + GAL group was significantly higher than that in the Indo group ($p < 0.05$), suggesting that GAL could promote the proliferation of GSE-1 cells against Indo. GAL increased the cell proliferation of the L-NAME and PPG groups to some extent. Also, GAL significantly enhanced the proliferation of the L-NAME + PPG + GAL group when compared with the L-NAME + PPG

group ($p < 0.05$); indicating that the mechanism of GAL in protecting GSE-1 cell may be related to the H_2S and NO synthetic signaling pathways.

Table 2: The drug effects of Indo, GAL, and misoprostol on GES-1 cell viability

Group	Dose	Cell viability (%)
CON		100.00 ± 0.10
PPG	1 mM	78.21 ± 0.03
	2 mM	74.88 ± 0.02*
L-NAME	0.1 mM	88.34 ± 0.03
	0.5 mM	67.06 ± 0.02***
	1 mM	64.51 ± 0.02***
GAL	0.5 μM	90.17 ± 0.06
	5 μM	97.76 ± 0.09
	10 μM	102.60 ± 0.07
Indo	0.5 mM	95.55 ± 0.10
	1 mM	69.20 ± 0.07***
	2 mM	55.67 ± 0.06***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs CON

Table 3: The proliferation effects of drugs on GES-1 cells

Group	Cell viability (%)
CON	100.00 ± 0.17
Indo	31.86 ± 4.94$^{\#\#\#}$
Indo+GAL	73.20 ± 4.99*
L-NAMA	75.30 ± 2.22
L-NAMA+GAL	88.56 ± 4.21
PPG	72.50 ± 4.95
PPG+GAL	80.64 ± 6.52
L-NAMA+PPG	42.74 ± 5.00$^{\#\#\#}$
L-NAMA+PPG+GAL	61.44 ± 5.58^{T}

$^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs CON: *$p < 0.05$ vs Indo; ^{T} $p < 0.05$ vs L-NNMA+PPG

Galangin rapidly increased cNOS protein expressions in GSE-1 cells

Decreased protein expressions of CSE and cNOS ($p < 0.05$) in the Indo group were observed in Figure 1, indicating that GES-1 cells were injured by Indo and the NO and H_2S synthesis pathways were impaired. PPG and L-NAME also inhibited the protein expressions of CSE and cNOS compared to the CON group. The protein expression of iNOS was significantly increased in the Indo group ($p < 0.05$) compared with the CON group (Figure 1 A and B). However, GAL only exhibited an inhibited potential of iNOS in those drug groups. The GAL significantly increased protein expression of cNOS ($p < 0.05$), while it showed no significant influence on protein expression of CSE (Figure 1 A and B). Therefore, it was inferred that acute administration (24 h) of GAL in GES-1 cells only triggered cNOS protein expression.

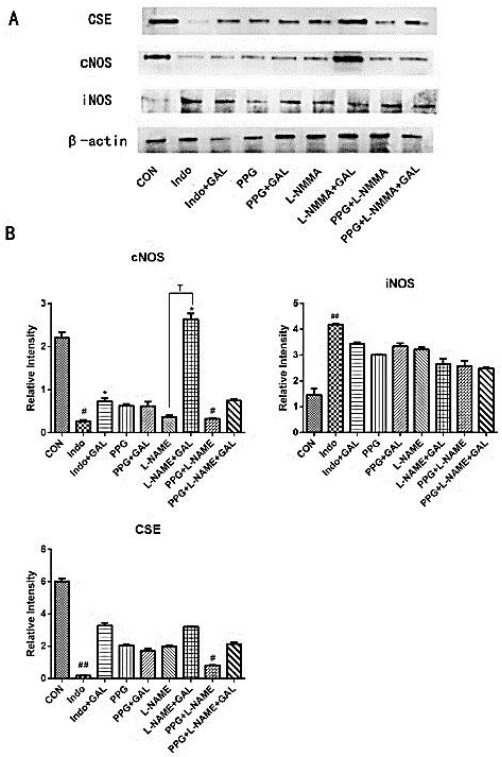

Figure 1: Representative Western blots (A) and statistical analysis (B) of the protein expressions of CSE, cNOS, and iNOS in GES-1 cells after drug treatment. $^{\#}P < 0.05$, $^{\#\#}p < 0.01$ vs CON; $^{T}p < 0.05$ vs L-NAME

GAL decreased ROS generation in GES-1 cells

As Figure 2 showed, Indo, PPG, and L-NAME significantly increased the intracellular level of ROS ($p < 0.001$, $p < 0.01$, $p < 0.01$, respectively). Concomitant use of GAL significantly inhibited the elevated intracellular concentration of ROS by Indo, PPG or L-NAME.

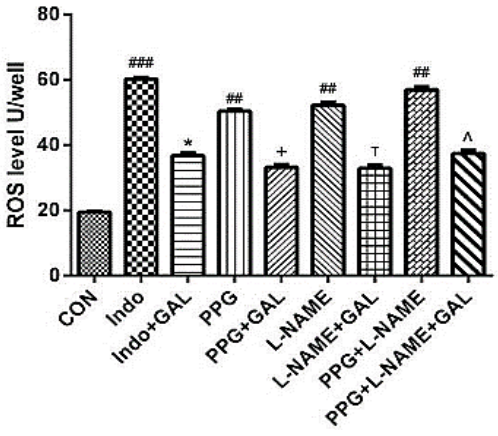

Figure 2: Effects of drug treatment on the intracellular ROS production of GES-1 cells. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs CON; *$p < 0.05$ vs Indo, +$p < 0.05$ vs PPG, $^{T}p < 0.05$ vs L-NAME, $^{\wedge}p < 0.05$ vs PPG+L-NAME

GAL dose-dependently protects rat gastric mucosa from Indo-induced injury

As shown in Figure 3, after 5 days of continuous protective administration, GAL and misoprostol significantly protected the gastric mucosa from Indo-induced damage: the gastric ulcer index decreased from 20.23 ± 0.5 (Figure 3 A(b)) to 1.29 ± 0.10 (Figure 3 A(c)) and 1.75 ± 0.05 (Figure 3 A(f)), with a 93.62 % ($p < 0.001$) and 91.34 % ($p < 0.001$) reduction, respectively. The H & E index was reduced from 5.86 ± 0.40 (Figure 3 B(b)) to 0.57 ± 0.20 (Figure 3 B(c)) and 0.71 ± 0.36 (Figure 3 B(f)), with a 90.27 % ($p < 0.001$) and 87.88 % ($p < 0.01$) reduction in injury, respectively. The protective efficiency of 300 mg/kg GAL was similar to that of misoprostol.

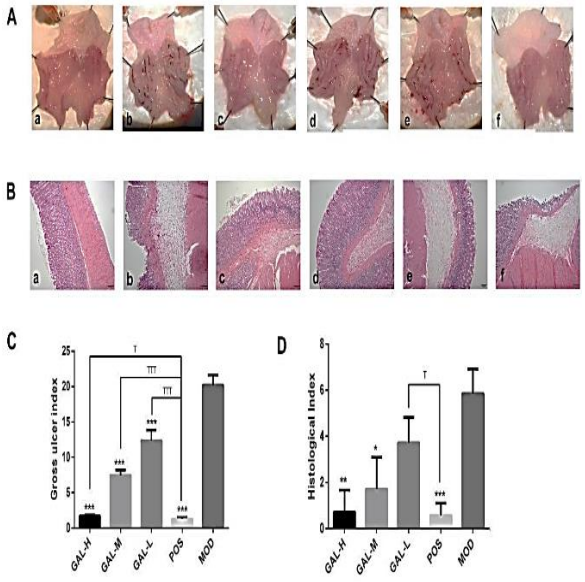

Figure 3: Gross findings (A), histological findings (B), GUI indices (C), and H & E indices (D) of the indomethacin-induced gastric damage in the rat stomach. a: CON group, b: MOD group, c: POS group, d: GAL-L group, e: GAL-M group, f: GAL-H group. Healthy gastric mucosa data of CON rats were not shown in C, D. $^{T}p < 0.05$, $^{TTT}p < 0.001$ vs POS; $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs MOD

GAL inhibited the phosphorylations of eNOS (S1177) and eNOS (Thr495)

By comparing the ratio of phosphorylated eNOS and non-phosphorylated eNOS expressions, the results showed that Indo elevated the phosphorylation of eNOS at Ser-1177 (Figure 4, $p < 0.05$), and exhibited stronger phosphorylation of eNOS at Thr-495 (Figure 4, $p < 0.05$). 300 mg/kg GAL significantly inhibited the phosphorylations of eNOS at Thr-495 and Ser-1177($p < 0.05$) respectively.

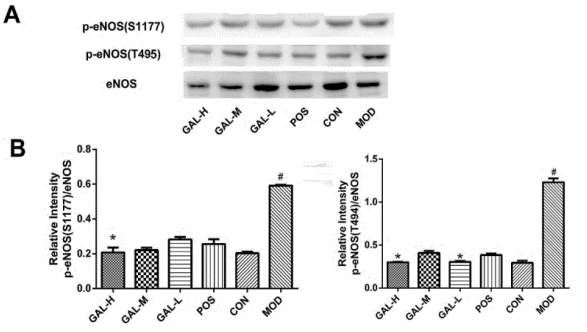

Figure 4: Representative Western blots (A) and statistical analysis (B) of the protein expressions of p eNOS (S1177), and p-eNOS(t494) in gastric mucosal tissues of Indo-induced gastric injury rats. (Mean ± SEM). #p < 0.05 vs CON;*p < 0.05 vs MOD" should corrected into "Figure 4: Representative Western blots (A) and statistical analysis (B) of the protein expressions of peNOS (S1177), and p-eNOS(T495) in gastric mucosal tissues of Indo-induced gastric injury rats. (Mean ± SEM). #p < 0.05 vs CON;*p < 0.05 vs MOD"

GAL reversed the effects of Indo on iNOS, cNOS, and NF-κB proteins, and inhibited Akt and PKC protein expressions

As shown in Figure 5, Indo significantly increased the expressions of iNOS, PKC, NF-κB and Akt proteins ($p < 0.001$, $p < 0.05$, $p < 0.05$, $p < 0.05$, respectively) and decreased the expressions of CSE and eNOS proteins ($p < 0.01$) but 300 mg/kg GAL significantly reversed the effects of Indo on those proteins, resulting in decreased protein expressions of iNOS, PKC, NF-κB and Akt ($p < 0.01$, $p < 0.05$, $p < 0.05$, $p < 0.05$, respectively), and increased protein expression of eNOS ($p < 0.001$). In addition, all three concentrations of GAL significantly increased the protein expressions of CSE ($p < 0.001$, $p < 0.01$, respectively).

DISCUSSION

Signaling molecules such as NO and H_2S are proven to act in conjunction with prostaglandins to maintain gastric mucosal defense and induce healing [7]. They can increase gastric mucosal blood flow, stimulate mucus secretion, inhibit neutrophil adhesion, and maintain the integrity of gastric mucosa. NO, H_2S and prostaglandins can dynamically complement each other, and when one of them is suppressed, there is a compensatory increase in the others[14]. Besides, NO is able to stimulate prostaglandin synthesis [8]. However, the dynamic compensation mechanism of NO, H_2S, and prostaglandins in long-term NSAIDs user is damaged. NSAIDs reduce the synthesis of H_2S, and NO by inhibiting CSE and cNOS enzymes

[7]. Therefore, drug development that promotes the synthesis of NO and H_2S or directly provides these two molecules has become a new method to protect gastric mucosa from NSAID damage.

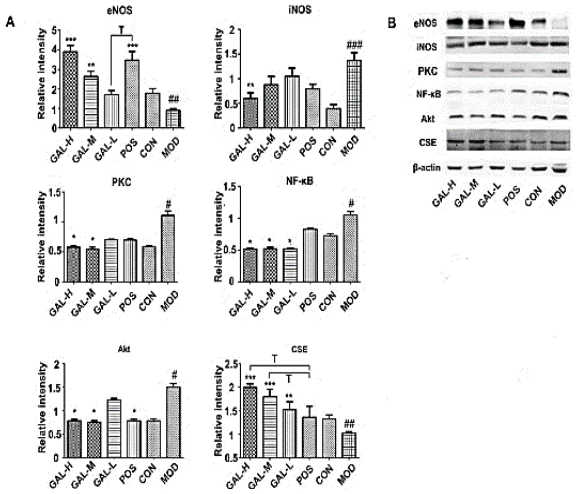

Figure 5: Representative Western blots (B) and statistical analysis (A)of the effects of GAL on iNOS, eNOS, CSE, NF-κB, Akt, and PKC proteins in gastric mucosal tissues of Indo-induced gastric injury rats. (Mean ± SEM). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs CON; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs MOD; $^{T}p < 0.05$ vs POS

Low amounts of NO produced by NOS exert effects such as mucosal repair and ulcer healing by increasing mucosal blood flow and promoting angiogenesis (stimulating VEGF factors) [6]. When an inflammatory response occurs in a lesion, iNOS is synthesized to promote ulcer healing (by promoting mucosal blood flow) or conversely promote mucosal damage (by enhancing apoptosis) [7]. Therefore, the regulation of NO is a very complex process. In the periphery, The NO-sensitive guanylate cyclase is stimulated by the NO from nNOS in nitrogenergic nerves, which results in a decrease in the vascular and gastrointestinal tone. In contrast, oxidative stress mediated by $ONOO^-$ can lead to excitotoxic effects of nNOS, resulting in excess NO. The eNOS is normally expressed in endothelial cells while NO from eNOS is an important homeostatic regulator of cardiovascular function. However, eNOS generate O_2^- instead of NO under oxidative stress conditions, which is known as eNOS "uncoupling". The direct scavenging effect of flavonoids on ROS has been extensively studied, and by scavenging O_2^-, flavonoids not only protect NO but also prevent the formation of $ONOO^-$, thereby preventing eNOS "uncoupling" [12]. In this investigation, the ROS experiment showed that GAL had a significant antioxidant effect. The direct scavenging effect of GAL on ROS suggested that GAL could inhibit the reaction of O_2^- with NO, reduce the excitotoxic effects of nNOS and the eNOS "uncoupling", thereby promoting the efficiency of NO production by eNOS, increase the bioavailability of NO, and improve the blood flow of the gastric mucosa.

The cellular experiment also exhibited the increased effect of GAL on cNOS protein expression. To further investigate the regulation mechanism of GAL, an indo-induced gastric injury rat model was utilized in this investigation. The activity of eNOS is mainly regulated through the post-translational modifications of enzymes (phosphorylation, acetylation, and S-nitrosylation) by complex mechanisms and diverse pathways. It is worthy of note that Ser-1177 phosphorylation of eNOS is catalyzed by Akt and Thr-495 phosphorylation of eNOS is dependent on PKC [15,16]. Phosphorylation at Ser-1177 is stimulatory while phosphorylation at Thr-495 is inhibitory. Meanwhile, GAL was shown to have some effects on PKC, Akt, and other protein kinases [17]. Thus, by investigating PKC and Akt protein expressions, Indo was found to significantly promote PKC and AKt protein expressions, which led to Ser-1177 and Thr-495 phosphorylation, indicating the dramatic activation and inactivation of eNOS. In contrast, GAL inhibited both Akt-mediated Ser-1177 phosphorylation and PKC-mediated Thr-495 phosphorylation. Two dephosphorylations suggested that the activity of eNOS will neither increase nor decrease. Prolonged eNOS activity resulted in sustaining NO production. Combined with the direct scavenging effect of GAL on ROS, the efficiency of NO production by eNOS would increase. This finding also explains the increased NO content in gastric tissues observed in previous experiments [18]. As eNOS is essential to the cardiovascular system, GAL may have the potential to be used in the treatment of cardiovascular disease.

iNOS can be expressed in any cell or tissue after stimulation with LPS, cytokines, or other agents. Despite the controversy, excessive NO produced by iNOS remains a major factor mediating the inflammatory response in many diseases. The central mechanisms controlling iNOS expression appear to be the NF-κB pathway [9]. Animal experiments showed that GAL inhibited the protein expressions of NF-κB and iNOS, indicating that excessive NO produced by iNOS was inhibited, and the mucosal damage was ameliorated. This is consistent with the previous results [18].

H_2S is mainly converted from L-cysteine via CSE enzymes and, unlike NO, H_2S promotes

angiogenesis in a VEGF-independent manner [7]. In this paper, Indo reduced the synthesis of H_2S by inhibiting CSE protein expression in rats, However, in cellular assays, no significant enhancement of CSE protein was observed in acute GAL-treated GES-1 cells. It has been noted that NO can mediate the synthesis of H_2S [8], therefore more experiments are needed to verify whether the increased expression of CSE detected in rats is due to the effect of GAL or the mediation of NO.

CONCLUSION

This study demonstrates that GAL protects the gastric mucosa from Indo by promoting the synthesis of endogenous NO, through prolonging the NOS activity via scavenging the ROS and inhibiting the protein expressions of Akt, PKC, which led to dephosphorylations of Ser-1177 and Thr 495 of eNOS. Besides, GAL inhibits iNOS-produced NO via inhibiting NF-κB to ameliorate the inflammatory reaction induced by Indo. This finding show that the gastroprotective mechanism of GAL against indomethacin-induced gastric injury involves a combination of multiple targets, and may provide detailed data for the possible clinical application of galangin in treating gastric mucosal injury induced by NSAIDs.

DECLARATIONS

Acknowledgements

This work was supported by the Natural Science Foundation of Hainan Province (no. 819QN233) and the National Natural Science Foundation of China (no. 81860749).

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by Jingwen Gong, Xie Yu, Yingfeng Tan, Hailong Li and Junqing Zhang and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Junqing Zhang concived and designed the study. Jing-wen Gong collected, analysed the data and wrote the manuscript. Xie Yu and Yinfeng Tan conducted some of the experiments. Hai-long Li participated in the study design and revised the manuscript.

REFERENCES

1. *Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. Biochem Pharmacol 2020, 180: 114147.*
2. *Kim YS, Nam Y, Song J. Gastroprotective and healing effects of polygonum cuspidatum root on experimentally induced gastric ulcers in rats. Nutrients 2020, 12(8): 2241.*
3. *Rajendra S, Nalban N, Alavala S, Rajendran V, Kumar J, Sistla R. Protective effect of galangin against dextran sulfate sodium (DSS)-induced ulcerative colitis in Balb/c mice. Inflam Res 2019, 68(8): 691-704.*
4. *Aloud AA, Veeramani C, Govindasamy C, Alsaif MA, El Newehy AS, Al-Numair KS. Galangin, a dietary flavonoid, improves antioxidant status and reduces hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. Redox Report 2017, 22(6): 290-300.*
5. *Gong J, Zhang Z, Zhang X, Chen F, Tan Y, Li H, Jiang J, Zhang J. Effects and possible mechanisms of Alpinia officinarum ethanol extract on indomethacin-induced gastric injury in rats. Pharm Biol 2018, 56(1): 294-301.*
6. *Cirino G, Vellecco V, Bucci M. Nitric oxide and hydrogen sulfide: the gasotransmitter paradigm of the vascular system. Brit J Pharmacol 2017, 174(22): 4021-4031.*
7. *Musumba C, Pritchard DM, Pirmohamed M. Review article: cellular and molecular mechanisms of NSAID-induced peptic ulcers. Aliment Pharmacol Ther 2009, 30(6): 517-531.*

8. Kim SF. The Nitric Oxide-mediated regulation of prostaglandin signaling in medicine. Vitam Horm 2014, 96: 211-245.
9. Dai R, Phillips RA, Karpuzoglu E, Khan D, Ahmed SA. Estrogen regulates transcription factors STAT-1 and NF-kappaB to promote inducible nitric oxide synthase and inflammatory responses. J Immunol 2009, 183(11): 6998-7005.
10. Dudzinski DM, Michel T. Life history of eNOS: partners and pathways. Cardiovascular Res 2007, 75(2): 247-260.
11. Li H, Förstermann U. Chapter 9 - Uncoupling of eNOS in cardiovascular disease, in Nitric Oxide (Third Edition), Louis J. Ignarro and Bruce A. Freeman, Editors. 2017, Academic Press. p. 117-124.
12. Duarte J, Francisco V, Perez-Vizcaino F. Modulation of nitric oxide by flavonoids. Food Funct 2014, 5(8): 1653-16568.
13. Clark Jd GG, Gonder JC, Keeling ME, Kohn DF. The 1996 guide for the care and use of laboratory animals. Ilar J 1997, 38(1): 41-48.
14. Kolluru GK, Shen X, Kevil CG. A tale of two gases: NO and H2S, foes or friends for life? Redox Biol 2013, 1(1): 313-318.
15. Takahashi S, Mendelsohn ME. Synergistic activation of endothelial nitric-oxide synthase (eNOS) by HSP90 and Akt: calcium-independent eNOS activation involves formation of an HSP90-Akt-CaM-bound eNOS complex. J Biol Chem 2003, 278(33): 30821-30827.
16. Chen F, Kumar S, Yu Y, Aggarwal S, Gross C, Wang Y, Chakraborty T, Verin AD, Catravas JD, Lucas R, et al. PKC-dependent phosphorylation of eNOS at T495 regulates eNOS coupling and endothelial barrier function in response to G+ -toxins. PLoS One 2014; 9(7): e99823.
17. Wang HB, Huang SH, Xu M, Yang J, Yang J, Liu MX, Wan CX, Liao HH, Fan D, Tang QZ. Galangin ameliorates cardiac remodeling via the MEK1/2-ERK1/2 and PI3K-AKT pathways. J cell Physiol 2019; 234(9): 15654-67.
18. Lin K, Wang Y, Gong J, Tan Y, Deng T, Wei N. Protective effects of total flavonoids from Alpinia officinarum rhizoma against ethanol-induced gastric ulcer in vivo and in vitro. Pharm Biol 2020; 58(1): 854-862.