

Original Article

BIOACTIVE FRACTION DLBS2411 FROM *CINNAMOMUM BURMANNII*, (NEES AND T. NEES) BLUME AS COLON AND GASTROPROTECTOR BY STIMULATING MUC5AC AND CYCLOOXYGENASE-2 GENE EXPRESSION

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ABSTRACT

Objective: Mucus therapy is one of the therapies for gastric ulcer management aside from proton pump inhibitor (PPI) and H₂-blocker medication. Bioactive fraction DLBS2411 which comes from *Cinnamomum burmannii* has been identified as a gastric acid anti-secretory agent by inhibiting the activity of hydrogen-potassium adenosine triphosphate (H⁺/K⁺ATPase). The study was aimed to evaluate the effect of DLBS2411 as a neuroprotective agent in gastric and colon by investigating its regulation on mucus related pathway.

Methods: Total RNA was extracted from gastric and colon cells followed by quantitative real-time polymerase chain reaction (qPCR) analysis for mucus synthesis and mucosal blood flow gene expression. Protein expression of prostaglandin E₂ (PGE₂) and phosphorylation of IκB kinase subunit alpha (IKKα) was analyzed with enzyme-linked immunosorbent assay (ELISA) kit and western blot. Measurement of nitric oxide (NO), which is related to mucosal blood flow, was also analyzed.

Results: Treatment of DLBS2411 elevated phosphorylation of IKKα and activated nuclear factor-κB (NF-κB) which in turn stimulated mucus synthesis and mucosal blood flow. High level of NF-κB increased mucus synthesis pathway by promoting cyclooxygenase-2 (COX-2) and PGE₂ expression, which increased the MUC5AC gene. Activation of NF-κB also increased production of NO, which stimulated mucosal blood flow.

Conclusion: DLBS2411 is a promising candidate for gastric and colon mucus protection by increasing mucus synthesis and stimulating mucosal blood flow.

Keywords: DLBS2411, Mucus production, MUC5AC, Prostaglandin, *Cinnamomum burmannii*

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INTRODUCTION

Epithelial surface of the gastrointestinal tract is covered with the mucosal layer. This mucosal layer can be injured by endogenous and exogenous substances. These include hydrochloric acid, pepsin, *Helicobacter pylori*, alcohol, and non-steroidal anti-inflammatory drugs (NSAIDs) [1]. Excessive exposure to these substances can lead to multiple complications such as gastritis and gastric ulcers.

One of the main pharmacotherapeutic options for these conditions is proton pump inhibitor (PPI) or H₂ receptor blocker medications which help decrease hyperacidity in the stomach [2]. Several experiments suggest that most factors that trigger hyperacidity are mediated through reactive oxygen species (ROS). ROS is defined as a chemically reactive molecule that contains one or more free electron in their outer orbital. Aside from causing hyperacidity, ROS also causes oxidative damage in the gastric and colon mucosa which leads to peptic ulcer. Mucosal layer damage might be caused by a low level of endogenous antioxidant or impairment of antioxidative enzyme activity of the cells [3, 4].

Our laboratory has investigated numerous pharmacological effects of a natural product for chronic internal diseases [5-8]. Previously, bioactive fraction DLBS2411 was reported to decrease the level of H⁺/K⁺ATPase messenger RNA expression in human embryonic kidney and rat gastric parietal cells. DLBS2411 also inhibited gastric H⁺/K⁺ATPase activity at various pH levels. Furthermore, DLBS2411 showed free radical scavenging activity and reducing power ability. This indicates that DLBS2411 has the potential to be an antioxidative agent that can be used to alleviate gastric ulcer problems [2].

Mechanism of gastric mucosa protection is complex. Several factors that contribute to this mechanism are mucus secretion, bicarbonate secretion, and mucosal blood flow. *In vitro* studies have reported

that prostaglandin E₂ (PGE₂) and nitric oxide (NO) stimulates mucus secretion [9, 10]. In regards to these previous findings, the effect of DLBS2411 on mucus secretion was further evaluated in this study.

MATERIALS AND METHODS

Preparation of bioactive fraction of DLBS2411

Preparation of bioactive fraction DLBS2411 was done according to Tjandrawinata, *et al.* (2013) [2]. The bark of *Cinnamomum burmannii* (*C. burmannii*) was purchased from West Sumatra, Indonesia. This plant has been identified by the Herbarium Bogoriense, Research Center of Biology, Indonesian Institute of Sciences with certificate 1261/IPH. 1.02/If.8/XII/2009. The extraction process of *C. burmannii* started with maceration of the bark in different solvents. Maceration was done in hot water at temperatures around 60 °C–90 °C for 1–2 h. Miscella was collected during the filtration process and evaporated by vacuum using a rotary evaporator (EandE, Warendorf, Germany) at a temperature around 60 °C–80 °C to obtain its concentrate. The concentrate was further processed through liquid-liquid extraction (LLE) using dichloromethane at a ratio of 1:2 (v/v) to remove any organic components. Subsequently, the water phase was collected and then evaporated using a rotary evaporator (EandE, Warendorf, Germany) at temperature around 50 °C–120 °C to obtain the dry extract. This dry extract is referred to as bioactive fraction DLBS2411 which is used in this study.

Maintenance of AGS and Caco-2 cell culture

AGS (ATCC® CRL-1739) and Caco-2 (ATCC® HTB-37™) cell lines were purchased from American Type Culture Collection (Manassas, VA, United States). The AGS cell was propagated in F-12 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin (Thermo Fisher Scientific, Waltham, Massachusetts, United States);

while Caco-2 cell was propagated in minimum essential medium with the same composition of serum and antibiotic. For *in vitro* molecular assay, the cells were plated in 6-well plates (BD Falcon, New Jersey, United States). All cells were cultured in the same growth medium containing 1% penicillin/streptomycin without fetal bovine serum and incubated at 37 °C, 5% CO₂ for 4 h prior to treatment. The cells were treated with DLBS2411 in concentrations of 10 µg/ml and 25 µg/ml and incubated in a CO₂ incubator (New Brunswick Scientific, New Jersey, United States) for 24 h at 37 °C, 5% CO₂.

Effect of DLBS2411 on MUC5AC, nuclear factor-κB (NF-κB), cyclooxygenase-2 (COX-2) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) gene expression

Total RNA was extracted using TRIzol® reagent (Thermo Fisher Scientific, Waltham, Massachusetts, United States) according to the manufacturer's protocol. The RNA concentration was quantified using NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States). The expression of several target genes were determined using a quantitative real-time polymerase chain reaction (qPCR) assay in 25 µl reaction that consisted of 12.5 µl iQ™ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 1 µM of each targeted primer, and 0.1 µM of each internal control primer Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The quantitative real-time PCR was performed using a Mini Opticon MJ Mini™ (Bio-Rad Laboratories, Hercules, CA, USA) in optimum conditions for each primer. The primers used in this study were (F, forward; R, reverse): MUC5AC (F: AGTGGTTCGACGTGGACTTC; R: CTGTCAACCCCTCTGACCAC); NF-κB (F: GGGGATCAACCTGAAGATTCT; R: TCCGGAACACAATGGCATACTGT); COX-2 (F: CCGCTCCGGACTAGATGATA; R: GACT CCTTTCTCCGCAACAG); 15-PGDH (F: TGCTTCAAAGCATGGCATAG; R: AATCAATG GTGGTCCAAAA).

Protein preparation from AGS and Caco-2 cells

AGS and Caco-2 cells were washed in ice-cold PBS and were extracted using RIPA buffer containing 50 mM Tris (pH 6.8), 0.5% (w/vol) sodium dodecyl sulfate, 150 mM sodium chloride, 1% NP-40, 2 mM ethylenediaminetetraacetic acid (EDTA) and 1x protease inhibitor. Supernatant of the cells were collected by centrifugation at 1,400 rpm for 15 min at 4 °C using Hettich Mikro 200 centrifuge (Hettich, Massachusetts, USA) and stored at -20 °C.

In vitro PGE₂ and phosphorylation of IκB kinase subunit alpha (IKKα) Inhibitor assay

PGE₂ and phosphorylation of IKKα were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (PGE₂, GE Life Sciences, United Kingdom) and (IKKα, Cell Signaling Technologies, Massachusetts, United States) according to the manufacturer's protocol as described previously [11, 12].

Western Blot of IKKα/β and pIKKα/β

Protein concentration was quantified using Lowry method [13]. Total cell extracts were electrophorized by the sodium dodecyl sulfate-polyacrylamide gel. Proteins were electrotransferred onto polyvinylidene difluoride membranes in a semi-dry blotting apparatus Transblot Turbo (Bio-Rad Laboratories, California, USA). Blots were blocked with 5% (w/vol) dried skim milk in TBS-t for 2 h at room temperature and then incubated for 16 h at 4 °C with a specific monoclonal mouse anti-human β-actin (diluted 1/1000), polyclonal rabbit anti-human IKKα/β and p-IKKα/β (diluted 1/500) (Santa Cruz, Texas, United States).

Blots were then incubated with a horseradish peroxidase-conjugated anti-mouse and anti-rabbit secondary antibody (diluted 1/1,000) for 2 h at 25 °C. Immunodetection was performed with enhanced chemiluminescence using Chemidoc XRS (Bio-Rad Laboratories, California, USA).

NO production from AGS and caco-2 cells

AGS and Caco-2 cells were treated with 10-25 µg/ml of DLBS2411 and 20 µg/ml of sucralfate. After 24 h of treatment, the supernatant of the cell was aspirated and put into a 96-well plate. Production of NO was measured using the Griess reagent (Sigma-Aldrich, Saint Louis, USA) with equal volume to supernatant as described previously [14]. The

reagent was allowed to stand for 15 min at room temperature in dark condition. The absorbance was measured at 520 nm wavelength.

Statistical analysis of result

The statistical differences between treatment and control samples were determined using Student's t-test using the Stat-View software package (Abacus Concepts, Piscataway, NJ, USA). Values are expressed as mean±standard deviation for two independent experiments.

RESULTS

DLBS2411 increases expression of mucin

Mucus consists of mucin which provides an important protective barrier against acid and pepsin. One of the methods of treating gastric and colonic ulcer is by increasing its mucus production. Mucus gel is secreted by surface epithelial cells and is formed by a large amount of water (about 95%) and various kinds of mucin glycoproteins (i.e. MUC2, MUC5AC, MUC5B and MUC6). Our study revealed that DLBS2411 significantly stimulated MUC5AC (human mucin gene) in transcription level, both in AGS and Caco-2 cells [fig. 1A, 1B]. DLBS2411 with a dose of 25 µg/ml was shown to elevate MUC5AC gene expression significantly compared to control.

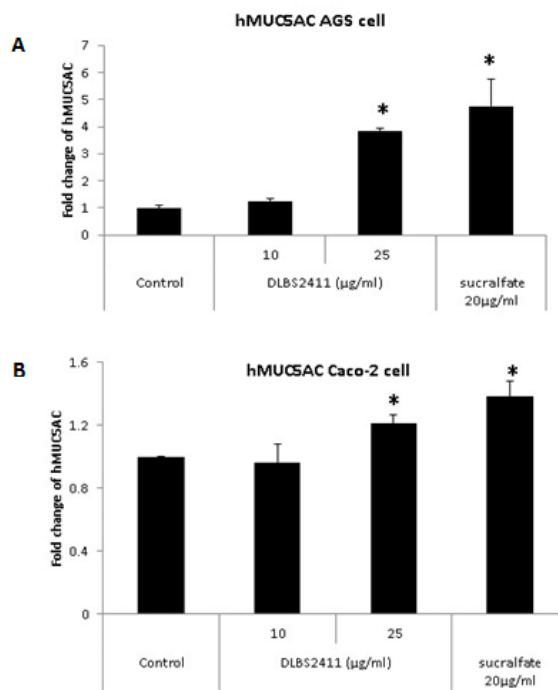


Fig. 1: Expression of MUC5AC gene following treatment of (A) AGS cell and (B) Caco-2 cells with sucralfate or DLBS2411 for 24 h. Values are expressed as the means±standard deviation of two independent experiments. Asterisk (*) indicates significant changes as compared with the control group. Note: *P<0.05

Stimulation of DLBS2411 on PGE₂ production *in vitro*

Prostaglandin, particularly PGE₂, has an important role in modulating mucosal integrity. PGE₂ has been recognized as an agent that can induce the synthesis and secretion of mucus to prevent any damage in the gastric mucosa surface. The effect of DLBS2411 on PGE₂ production was investigated by ELISA. The result showed that DLBS2411 stimulated the production of PGE₂ in AGS cells (table 1). DLBS2411 was shown to be more effective on Caco-2 cells compared to AGS cells (table 2). Specifically at dose 25 µg/ml, DLBS2411 was more effective in comparison with the control.

Table 1: PGE₂ in AGS cells after 24 h treatment with sucralfate and DLBS2411.

Sample	PGE ₂ [pg/ml]	SD	P value
Control	109.84	2.12	0.000
DLBS2411 10 µg/ml	118.32	6.80	0.059
DLBS2411 25 µg/ml	116.11	2.11	0.076
Sucralfate 20 µg/ml	121.08	2.39	0.055

Values are expressed as mean±standard deviation of three independent experiments. Asterisk (*) indicates significant changes as compared with the control group. Notes: *P<0.05

Table 2: PGE₂ in Caco-2 cells after 24 h treatment with sucralfate and DLBS2411.

Sample	PGE ₂ [pg/ml]	SD	P value
Control	33.66	1.3	0.000
DLBS2411 10 µg/ml	38.10	0.4	0.0446*
DLBS2411 25 µg/ml	51.76	4.0	0.0260*
Sucralfate 20 µg/ml	82.17	4.3	0.0099*

Values are expressed as mean±standard deviation of two independent experiments. Asterisk (*) indicates significant changes as compared with the control group. Notes: *P<0.05

DLBS2411 regulates COX-2 and 15-PGDH through NF-κB pathway

In order to investigate the MUC5AC upregulation pathways, the effect of DLBS2411 on COX-2 and NF-κB gene was studied. Many studies revealed that NF-κB-based transcriptional mechanism is involved in MUC5AC regulation. The ultimate step leading to MUC5AC gene upregulation is the activation of several transcription factors including NF-κB. NF-κB is a crucial regulator of many cellular

processes, including immune response, inflammation and apoptosis. DLBS2411 was administered to AGS and Caco-2 cells at a concentration of 10 µg/ml and 25 µg/ml.

After being treated for 24 h, RNA was isolated and used for real-time PCR analysis. The result revealed that NF-κB expression was increased along with the increase of DLBS2411 concentration, both in AGS and Caco-2 cells (fig. 2A, 2B). The activation of NF-κB subsequently is the cause of increased MUC5A gene expression.

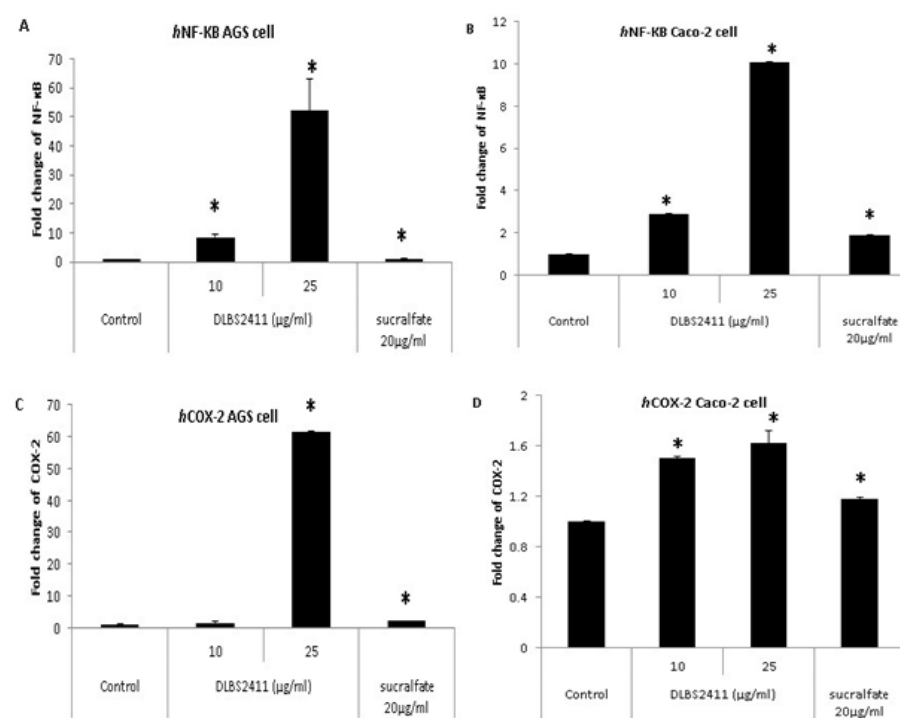


Fig. 2: (A-D) Effect of DLBS2411 on NF-κB and COX-2 gene expression. (A) Expression of NF-κB gene in AGS and (B) in Caco-2 cells with sucralfate or DLBS2411 treatment for 24 h. Expression of COX-2 gene in (C) AGS cells and (D) in Caco-2 cells with sucralfate or DLBS2411 treatment for 24 h. Values are expressed as mean±standard deviation of two independent experiments. Asterisk (*) indicates significant changes as compared with the control group. Note: *P<0.05

Following the discovery of two isoforms of COX, COX-2 is the enzyme that triggers pain. It is an inducible pro-inflammatory enzyme which is essential in inflammatory cascades where arachidonic acid is involved as its substrate and is positively regulated by NF-κB activation [15]. Selective COX-2 inhibitors were clinically developed as novel anti-inflammatory drugs characterized by increased viability of the gastric cell.

NF-κB acts as a transcription factor for the COX-2 gene. Therefore a high level of NF-κB gene expression indicated that COX-2 gene will be expressed at a high level. In the present study, COX-2 gene expression was increased after treatment of DLBS2411 (fig. 2C, 2D). Therefore, it can be concluded that DLBS2411 stimulates PGE₂ production through the COX-2 pathway. Aside from COX-2 gene expression, the observation was continued in other pathways that

might be affected by DLBS2411. Hence, 15-PGDH gene expression which is responsible for PGE₂ catabolism in AGS cells was observed.

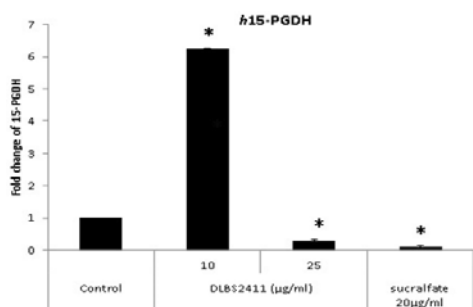


Fig. 3: Expression of the 15-PGDH gene in AGS cells treated with sucralfate or DLBS2411 for 24 h.

Values are expressed as the mean \pm standard deviation of two independent experiments. An asterisk (*) indicates significant changes as compared with the control group. Note: * $P < 0.05$

The result showed that the expression decreased at a dose of 25 $\mu\text{g/ml}$ (fig. 3). Caco-2 cells do not express the 15-PGDH gene. Therefore analysis was only done on AGS cell. The observation was conducted using the same experimental procedure as above.

DLBS2411 induces phosphorylation of IKK α

In order to affirm the activity of DLBS2411 as NF- κ B upregulation, measurement of IKK α phosphorylation was conducted. The phosphorylation was evaluated using ELISA and western blot assays. It was demonstrated that phosphorylation of IKK α increased with DLBS2411 treatment (fig. 4A, 4B). The phosphorylation of IKK α was also displayed in western blot technique in Caco-2 cells (fig. 4C).

DLBS2411 increases mucosal blood flow through NO production

Recent research has highlighted the fact that, other than prostaglandin, gastric mucosal protection can be accomplished through other mediators, with particular regard to the gaseous mediators NO. NO is formed enzymatically from its precursor L-arginine. It stimulates mucosal blood flow via cyclic 3', 5'-monophosphate (cGMP) dependent pathway. Production of cGMP is stimulated by activation of guanylate cyclase. Guanylate cyclase is activated by NO diffusion to the vascular smooth muscle cells. Production of NO after treatment with DLBS2411 was observed and compared to those affected by sucralfate in AGS and Caco-2 cells (fig. 5A, 5B). The production of NO exhibited a similar pattern in both cells. At a dose of 10 $\mu\text{g/ml}$, DLBS2411 was able to upregulate NO compared to sucralfate. As shown in fig. 5A and 5B, a higher dose of DLBS2411 was able to increase NO production.

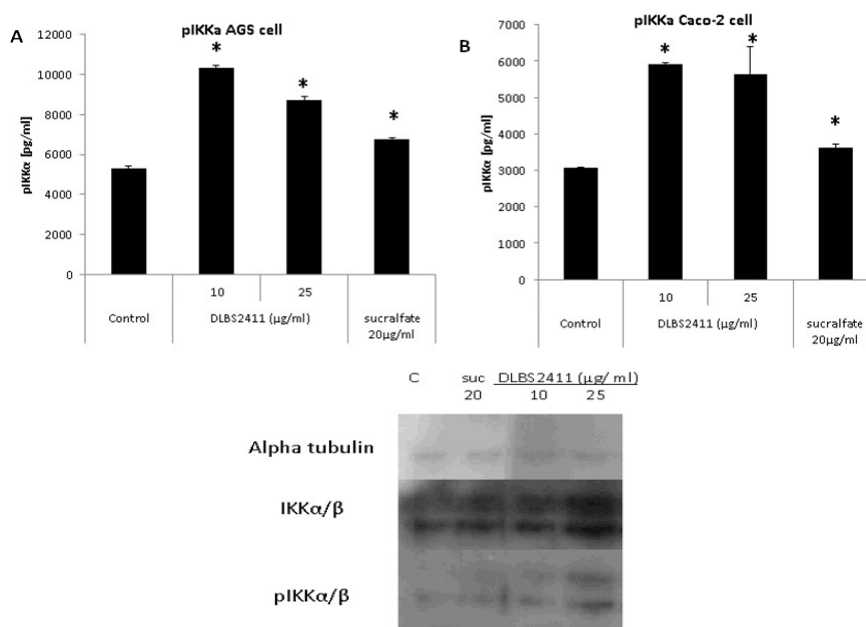


Fig. 4: (A-C) Phosphorylation of IKK α protein with the treatment of sucralfate or DLBS2411 added for 24 h.

Phosphorylation of IKK α in (A) AGS cells and (B) Caco-2 cells were analyzed by ELISA. Values are expressed as mean \pm standard deviation of two independent experiments. Asterisk (*) indicates significant changes as compared with the control group. Note: * $P < 0.05$ (C) Phosphorylation of IKK α in Caco-2 cells with western blot

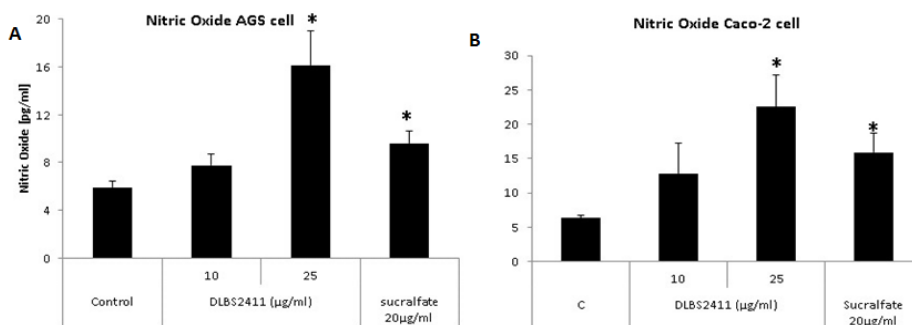


Fig. 5: DLBS2411 elevated NO production in (A) AGS cells and (B) Caco-2 cells.

Values are expressed as mean \pm standard deviation of two independent experiments. Asterisk (*) indicates significant changes as compared with the control group. Note: * $P < 0.05$

DISCUSSION

The development of a plant-based drug for treatment of chronic diseases, including gastric ulcer treatment, has become more popular in recent years. The previous study by Alqasoumi (2012) exhibits how the treatment of *Cinnamomum zeylanicum* extract in rats had the capacity to stimulate mucus secretion, making it potential to be developed as an antiulcer treatment [16]. Bioactive fraction DLBS2411, which is derived from *C. burmannii*, has been proven to confer its effect as gastric acid antisecretory agent [2]. Although the effect of DLBS2411 on the expression of H⁺/K⁺ATPase has previously been evaluated [2], there is still no study on the effect of DLBS2411 on mucus synthesis.

Gastric mucosal layer acts as a barrier that restricts the exposure of gastric cells to various injurious agents of both exogenous and endogenous origin. Therefore, stimulation of mucus production in gaster tissue is one of the central factors in ulcer healing by protecting the damaged tissue against aggressors such as harmful drugs and oxidants. In fact, secretion of mucus is an important contributor in gastric epithelial tissue recovery after acute injury through the formation of a mucoïd cover. In this study, DLBS2411 was tested to verify its role in protecting the mucus layer in gaster and colon.

Mucus is comprised of glycoproteins, water, and mucin, which is regarded as the major structural protein of the mucus. One mucin protein, MUC5AC, is located in the pit cells of gastric pit extending to part of the surface epithelium [17, 18]. MUC5AC is coded for MUC5AC protein that is responsible as secretory mucin. In this study, AGS and Caco-2 cells were used to study MUC5AC gene expression. AGS is a part of the gastric adenocarcinoma cells that expressed MUC5AC gene, while Caco-2 has the highest expression of MUC5AC gene compared to other colon cancer cells [19]. DLBS2411 was shown to enhance MUC5AC gene expression in AGS and Caco-2 cells.

One of the stimulators that induce mucus production is PGE₂. PGE₂ is a major component of the protective factors that maintain the gastrointestinal mucosal integrity and mucosal microcirculation. It could reduce damage caused by necrotizing agents (such as alcohol, aspirin and bile salts) in the gastric mucosal barriers and inhibit gastric acid secretion [20]. PGE₂ analog was reported to increase the thickness of mucus gel in rat gastric mucosa [21, 22]. In rat and human, prostaglandin stimulates synthesis of mucus in gastric, elevates the mucus gel layer and maintains the pH gradient as well as increasing mucus viscosity [23]. Therefore, prostaglandin was also examined in order to confirm the effect of prostaglandin production to mucus synthesis. The present study shows that DLBS2411 was able to elevate PGE₂ production in Caco-2 cells. However, elevation in AGS cell was not significant (table 1, 2). This result is in accordance with the study of Shindo *et al.* (2006) which stated that prostaglandin exerts a stimulatory effect on mucus production [23].

Expression of MUC5AC gene is regulated by NF-κB gene expression as its transcription factor. Similar to sucralfate, DLBS2411 was shown to up-regulate the expression of NF-κB gene (fig. 2A, 2B). Sucralfate has been known to have potassium sucrose octa sulfate (SOS) as the major soluble functional element. SOS is known for its anti-apoptotic mechanism and works through activation of NF-κB pathway [24]. NF-κB signaling pathway is an important innate defense mechanism in human [25], yet it is also responsible for the inflammatory reaction in the body. Some harmful stimuli can cause inflammatory responses by activation of NF-κB pathway. However, some conditions might disrupt inflammatory responses and repressed mucin production [26, 27].

Since NF-κB gene regulates COX-2 gene expression, the COX-2 level was also examined in this study. COX-2 is known to stimulate prostaglandin production which mediated the inflammation process [28]. However, the COX-2 expression is also related to gastric protection. Several studies have shown that COX-2 inhibitor can elicit gastric damage [20, 29]. The up-regulation of COX-2 expression in intestinal epithelial cells after treatment of sucralfate was evident in the study by Shindo, *et al.* (2006) (fig. 2C, 2D) [24].

Prostaglandin catabolism is catalyzed by NAD⁺-dependent 15-PGDH [28]. Expression of 15-PGDH was identified in an SGC7901 cell with

COX-2/siRNA treatment [22]. DLBS2411 was shown to down-regulate the 15-PGDH gene expression at a dose of 25μg/ml in the gastric cell (fig. 3). Similar mechanism was also demonstrated by rebamipide as a mucoprotective drug. The drug was able to downregulate 15-PGDH expression, yet increase COX-2 and PGE₂ synthesis [30].

In resting cells, NF-κB is bound to the cytoplasmic NF-κB inhibitors (IκBα) in its inactive form. Therefore, NF-κB activation requires phosphorylation of IκBα. Stimulation such as up-regulation of cytokines results in the activation of specific intracellular signaling pathways with subsequent activation of the IKK complex. This complex comprises of two catalytic subunits (IKKα and IKKβ) and the regulatory subunit (IKKγ), which then phosphorylates IκBα in their active form. Phosphorylation leads to ubiquitination and degradation of IκBα, thereby releasing NF-κB from the complex where it translocates to the nucleus and activates gene that are responsible for the gastric mucosal integrity, such as COX-2 [25]. In this experiment, it was shown that IKKα was phosphorylated with DLBS2411 treatment (fig. 4A-4C), while control cell remained unphosphorylated. Another possible mechanism of DLBS2411 was also evaluated, for example, the ability of DLBS2411 to protect gastric and colon cells through NO elevation [10]. It is shown that DLBS2411 up-regulated the production of NO (fig. 5A, 5B), which increased gastric blood flow and maintained gastric microcirculation. Therefore, it can be inferred that DLBS2411 was able to increase the mucosal blood flow through NO production.

Another study suggests that DLBS2411 has antioxidative activity. DLBS2411 was proven to possess free radical scavenging activity and reducing power ability [2]. It was also shown that DLBS2411 was able to reduce the number of petechiae in a dose-dependent manner in rats [2]. Antioxidant activity is suggested to have a positive correlation with mucus healing property [23, 30].

In this study, sucralfate as an approved gastro protective drug was used as positive control. In several experiments with sucralfate, it is shown that sucralfate stimulated prostaglandin and mucus secretion and elevated the mucosal blood flow [31, 32]. The result of this study proved that DLBS2411 works similarly to sucralfate. Since DLBS2411 exhibits similar activity to sucralfate, DLBS2411 can be deemed as a promising candidate for gastric ulcer treatment.

CONCLUSION

DLBS2411 has gastric and colon protective effect through several pathways (fig. 6). DLBS2411 elevates phosphorylation of IKKα and activate NF-κB. High level of NF-κB affects several pathways; promotes transcription of MUC5AC and COX-2, and increases production of NO. High expression of COX-2 has a reverse effect on expression of 15-PGDH. The COX-2 gene affects the metabolism of PGE₂ which will regulate MUC5AC expression. High expression of MUC5AC stimulates gastric and colon mucus synthesis. High production of NO by NF-κB expression increases mucosal blood flow. Therefore, it is evident that DLBS2411 promotes mucus healing by stimulating gastric mucus synthesis and secretion.

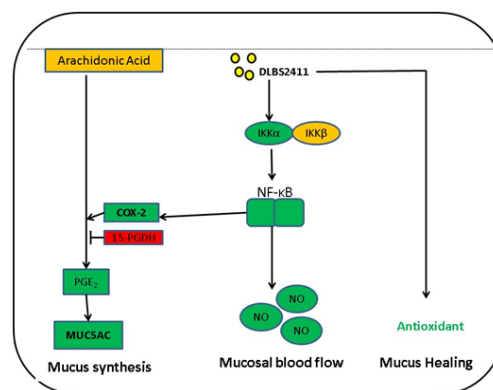


Fig. 6: Proposed mechanism of action of DLBS2411

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CONFLICTS OF INTERESTS

The authors report no conflicts of interest in this work.

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