Clinical Study of DLBS2411, a Mucoprotector and Proton Pump Inhibitor Bioactive Fraction Derived From *Cinnamomum burmanii*, on the Intragastric Acidity

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ABSTRACT

Background: *DLBS2411, a bioactive fraction derived from the bark of Cinnamomum burmanii has been developed to address acid-related gastrointestinal disorders. This study evaluated the pharmacodynamic effect of DLBS2411 on the 24-hour intragastric acidity in healthy adults.*

Methods: *In a 3-arm, parallel, double-blind, randomized, placebo-controlled clinical trial, healthy subjects received a single dose of DLBS2411 (250 mg or 500 mg) or placebo. Gastric pH was monitored, analyzed and profiled over 24 hours.*

Results: *Of a total of 54 enrolled male subjects, 47 subjects (87.04%) were eligible for the analysis. The mean 24-hour intragastric pH for DLBS2411 250 mg and 500 mg was 2.29 ± 0.42 and 2.13 ± 0.50, respectively, both higher than placebo (1.93 ± 0.70). Differences were more pronounced during the first 12 hours (daytime). DLBS2411 250 mg and 500 mg reached a gastric pH > 4 significantly faster (129.9 ± 128.2 and 92.9 ± 106.8 minutes) compared to placebo (196.9 ± 99.7 minutes). No serious adverse events occurred. All adverse events were mild and had been resolved by the end of study, confirming the safety and tolerability of DLBS2411 at the dose of 250 and 500 mg.*

Conclusion: *DLBS2411 effectively suppressed the intragastric acidity and demonstrated a good safety profile in healthy adults. These findings warrant further studies of DLBS2411 in patients with gastric acid-related disorders.*

Keywords: *Alternative medicine, DLBS2411 cinnamomum burmanii, healthy volunteers, intragastric-acidity, proton pump inhibitors*

ABSTRAK

Latar Belakang: *DLBS2411, fraksi bioaktif yang berasal dari kulit kayu manis (Cinnamomum burmanii), telah dikembangkan untuk mengatasi gangguan saluran cerna akibat asam lambung berlebih. Penelitian ini menilai efek farmakodinamik DLBS2411 terhadap keasaman intragastrik selama 24 jam pada relawan sehat.*

Metode: *Dalam uji klinis tersamar ganda, acak, terkontrol plasebo dengan tiga kelompok paralel, subjek dewasa sehat menerima dosis tunggal DLBS2411 (250 mg atau 500 mg) atau plasebo. Profil pH lambung dimonitor dan dianalisis selama 24 jam.*

Hasil: *Dari 54 subjek, 47 memenuhi syarat untuk analisis. Rerata pH intragastrik 24 jam pada DLBS2411 250 mg dan 500 mg masing-masing adalah 2,29 ± 0,42 dan 2,13 ± 0,50, lebih tinggi dibandingkan plasebo (1,93*

± 0,70). Perbedaan antar-kelompok ini lebih nyata lagi selama 12 jam pertama (siang hari). Dalam Kelompok DLBS2411 250 mg dan 500 mg, pH lambung >4 secara signifikan tercapai lebih cepat (129,9 ± 128,2 dan 92,9 ± 106,8 menit) dibandingkan Plasebo (196,9 ± 99,7 menit). Tidak ada Kejadian Tidak Diinginkan (KTD) yang serius yang terjadi. Semua KTD bersifat ringan dan pulih sebelum akhir penelitian, di mana hal ini menunjukkan keamanan dan tolerabilitas DLBS2411.

Simpulan: *DLBS2411 secara efektif menekan keasaman intragastrik dan menunjukkan profil keamanan yang baik pada orang dewasa sehat. Temuan studi ini mendukung penelitian lebih lanjut mengenai DLBS2411 untuk mengatasi gangguan terkait asam lambung.*

Keywords: *Obat alternatif, DLBS 2411 cinnamomum burmannii, relawan sehat, keasaman intragastrik, penghambat pompa proton*

INTRODUCTION

The mainstay of treatment for gastrointestinal disturbances attributable to excessive gastric acid secretion, such as peptic ulcer disease (gastric and duodenal ulcers), Gastro-Esophageal Reflux Disease (GERD), reflux esophagitis, dyspepsia and Zollinger-Ellison syndrome, are proton pump inhibitors (PPIs).[1] This class of drugs is also used as part of the treatment regimen for *Helicobacter pylori* infection,[2] and also appropriately used for prevention of gastro-duodenal mucosal lesions and symptoms in patients at risk for gastrointestinal diseases or under non-steroidal anti-inflammatory drugs (NSAIDs) or antiplatelet therapies. Overall, PPIs are the main stay and irreplaceable drugs in the management of acid-related diseases.[3] However, in the past decade, case control studies and meta-analyses have raised questions about important adverse events associated with the use of long-term PPIs, such as long-bone fractures, pharmacokinetic interaction with clopidogrel, *Clostridium difficile* enteric infections, and hypomagnesaemia. More recently, concerns regarding PPIs and cardiovascular events, kidney disease and dementia have also been resurfaced.[2,4] In attempt to find safer medically active substances particularly for a long-term treatment, a global tendency of exploring the herbal medicines that contribute much in drug research and development has been intensively growing. Indonesia is rich in natural resources which have long been used from generation to generation for treatment of many diseases, including digestive or gastrointestinal diseases. However, traditional experiences only are not scientifically sound to specify and claim the effectiveness of the extract. Current day knowledge about the underlying biochemical mechanism of most of the gastric ulcers deserves appropriate consideration while consolidating the efficacy of a plant extract. Based on thorough preclinical studies, DLBS2411, a bioactive fraction generated from *Cinnamomum burmanii* through a Tandem Chemistry Expression Bioassay System (TCEBS)-based high biotechnological process (model) has been developed and was shown to possess an activity as H+/K+ ATPase inhibitor as well as antioxidant.[5,6]

DLBS2411 is a bioactive fraction of *Cinnamomum burmanii* – locally known as kayu manis, an Indonesian native herbal. The bioactive fraction of DLBS2411 has been proven at cellular and genetic levels to have antiulcer and antioxidant activities. DLBS2411 demonstrated a mechanism of action close to that of proton-pump inhibitors (PPIs). However, it may theoretically be more potential than PPIs in suppressing gastric acid as our previous preclinical studies with DLBS2411 have proven its effects not only to inhibit the activity of the enzyme that regulates proton pump in stomach, H+/K+ ATPase, but it also downregulated its gene expression.[5] In addition, DLBS2411 suppressed the process of hyper-oxidation showing its action as a gastric and colon protector. A previous study on AGS and Caco-2 cell lines demonstrated the mucoprotective property of DLBS2411 through the promotion of a human mucin gene (MUC5AC) and cyclooxygenase-2 (COX-2) transcription, and niticoxide (NO) production. The COX-2 gene affects the metabolism of prostaglandine-E2 (PGE2) that regulates MUC5AC expression. High expression of MUC5AC stimulates gastric and colon mucus synthesis. Elevated NO production will in turn increase the mucosal blood flow.[6] Further, a study of DLBS2411 in Wistar rats showed that it could reduce the number of gastric petechiae and ulceration induced by ethanol and indomethacin proving its anti-ulcerative as well as gastro-protector properties.[5] Its safety was confirmed by the acute[7] and subchronic toxicity,[8] as well as teratogenicity studies.[9] DLBS2411 was classified as a practically non-toxic substance, with the lethal

dose 50 (LD_{50}) of DLBS2411 being beyond 15 gr/kg body weight (BW) of rats (or equivalent to 2.4 gr/kg in human),[7] and the no observed adverse effect level (NOAEL) of greater than 1000 mg/kg BW in rats (or equivalent to 160 mg/kg in human).[8] At the dose up to 200 mg/kg BW in rats, which is equivalent to 8-fold its effective dose in human,[5] DLBS2411 was not teratogenic.[9]

The promising preclinical study results should be followed by further clinical studies to confirm the safety and efficacy of DLBS2411 in human. Since the current study was the first study of DLBS2411 in human, it was aimed to investigate the pharmacodynamics of a single dose of DLBS2411 in healthy adults, through evaluation of its effect on the 24-hour intragastric acidity. The study also observed the safety of DLBS2411 in those subjects.

METHODS

The study was conducted in June until October 2012 at the Gastroenterology Division, Department of Internal Medicine, Faculty of Medicine, University of Indonesia / Dr. Cipto Mangunkusumo Hospital. The protocol, the consent form, and the patient information sheet were reviewed and approved by independent Ethics Committee of Medical Faculty, University of Indonesia, Jakarta, prior to trial initiation, with the Ethical Approval No. 96/PT02.FK/ETIK/2012. The protocol was registered in *ClinicalTrials.gov* with a trial registry number of NCT01573403. The trial was performed in accordance with the Declaration of Helsinki,[10] and the Good Clinical Practice.[11] The subjects were informed of the risks and benefits of the trial and that they could withdraw from the trial at any time for any reason. Consent was obtained in writing prior to any trial-related activities and the Investigator retained the consent forms.

Study Design and Procedure

This was a 3-arm, parallel, double blind, randomized, placebo-controlled clinical study, in which a total of 54 healthy eligible subjects (18 subjects in each group) were allocated to receive a single dose of any of the following treatments: DLBS2411 250 mg, DLBS2411 500 mg, or the Placebo. All of the investigators and enrolled subjects were blind to the allocation. Allocation sequence was generated by an independent research unit of the study funder using the permuted block randomization with a block size of 3 and the Table of Random Numbers. The blinding allocation sequence was disclosed after the study was completed and data entry was locked. Since this was a phase I pharmacodynamic study in healthy volunteers, principally there was no calculation for a minimum sample size required.

The human-equivalent doses of the investigational product used in this study were determined based on the effective dose observed in the previous animal study.[5] As this was the first-in-human study of DLBS2411 bioactive fraction, it involved only healthy adults and the product was administered at two dosages: the minimum effective dose (250 mg) and the escalated (doubling) dose (500 mg). Through such a ranging dose we would also find the optimum effective dose of DLBS2411 in human.

The effect of a single dose administration of the study product on the gastric output pH was monitored for 24 hours, recorded and profiled. The 24-hour pH data were presented as several pharmacodynamic endpoints: 1) mean 24-hour gastric pH; 2) onset of action, defined as time taken to achieve gastric pH of >4 after the dosing; 3) duration (and percentage) of time over 24 hours during which gastric pH is >4; 4) area under the time-pH response curve over 24 hours (AUC_{0-24h}). The healing of acid-related gastrointestinal disorders is directly correlated with the control of intragastric pH above the threshold of 3 (for peptic ulcers) or 4 (for reflux esophagitis (or GERD) and eradication of *Helicobacter pylori*).[12,13] Duration of time with intragastric pH above 4 and mean 24-h gastric pH are powerful surrogate markers in evaluating the pharmacodynamics and efficacy of an acid-suppressive therapy.[12-15]

Safety examination was performed at baseline and the end of study. The safety profile was assessed by vital signs, electrocardiography, and several laboratory parameters, such as liver function (serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transferase (γ--GT), total bilirubin) and renal function (serum creatinine level), routine haematology (haemoglobin level, hematocrit, red blood cell count, white blood cell count, differentiation of WBC and platelet count., and urinalysis. The occurrence of adverse event was observed during the study period.

Subjects had fasted for a minimum of 8 hours prior to baseline examination and instrument installation. At the first day of treatment, at 9.00 am, study subjects' basal acid output was measured by an intragastric pH monitoring instrument, pH catheters connected to the portable digital data recorder. Right after the

instrument installed, subjects were given a single dose of the investigational product, DLBS2411 caplet or the Placebo, orally. Subjects stayed in-ward at the Endoscopy Center at study site for a 24-hour gastric output pH monitoring. At the same hour of the second day, pH monitoring was stopped and the instrument was uninstalled from the patient.

Subjects were provided with standardized meals during the study that were also given at the standardized time. No medications, including any H2–receptor blockers, proton pump inhibitors, antacids, or gastric mucosal protectors (such sucralfate, rebamipide) and any herbal preparations or food supplements, were allowed during the 24-hour pH monitoring. All subjects were under direct supervision of a medical doctor during the study period.

24-hour pH Monitoring

The 24-hour pH monitoring was performed in all subjects, using the pH catheter instrument (Versaflex® Z, Given Imaging Ltd., Israel). The instrument installment in the subjects and pH measurement were performed following the procedure described formerly.[16-18] The pH electrodes were calibrated at 37°C in pH 7 and pH 1 buffer solutions before and after monitoring. The pH probe apparatus was installed to pass trans-nasally to the mouth and swallowed with a minimal amount of water. The pH catheter was then passed to the distal stomach and retracted to the estimated position.

It was thereby located in the proximal stomach, about 5–7 cm distal to the cardia, in the fundic or upper body region. The pH electrodes were connected to the nose and cheek to prevent dislodgement. The pH electrodes were connected to the portable digital data recorder (Digitrapper® pH-Z, Given Imaging Ltd., Israel) worn around the waist, which stored pH data samples every 4 seconds.

Study Subjects

The consecutive sampling was performed to recruit every eligible subject until the target of 54 subjects was achieved. The study recruited male subjects aged between 18 and 45 years old who were clinically healthy as confirmed by normal vital signs and laboratory values for hematology, blood glucose level, liver and renal function, and having normal body mass index (18–25 kg/m2). Male adults were chosen because in general they are emotionally more stable than the females. Emotional state, such as psychological distress, or affective disorders that may be associated with hormonal changes in females, may alter gastrointestinal motility, affect gastric acid secretion and even induce contrary changes of gastric output in different subjects.[19-22] In order to minimize variability due to different genders and not to complicate the interpretation, the study included only male subjects. Nevertheless, the study results can be generalized for both genders since the suppression of the gastric acid secretion by anti-acid-secretory agents is independent of gender.[18,23]

The exclusion criteria were gastric pH $\geq$ 4 at screening confirmed by pH-metry; active smoker, chronic alcoholism, history of or currently active peptic ulcer or any serious abnormality in gastric linings confirmed by endoscopy, clinical diagnosis of Zollinger Ellison syndrome; history of long term treatment with any acid-suppressing agents (H2–receptor blockers, proton pump inhibitors), antacids, or gastric mucosal protectors (such sucralfate, rebamipide), or taking those medicines within 2 weeks prior to screening, or any other medicines, supplements, or herbals within 3 days prior to screening; the presence of any symptomatic chronic diseases, such as chronic kidney disease (CKD), coronary artery disease (CAD), or serious infection(s); and participation in any other clinical studies within 30 days prior to screening. Both cigarette smoking and alcohol are confounding factors for gastric pH evaluation and significantly increase the risk of gastric ulcers as well,[24,25] thus active smokers and subjects with chronic alcoholism were excluded from this study.

The Investigational Product

The investigational products were DLBS2411 bioactive fraction 250 mg caplet or the Placebo. DLBS2411 and its placebo caplets were made identical in appearances to maintain the doubleblinding double dummy fashion. Each enrolled subject received any of the following treatments: a single dose of one caplet of DLBS2411 250 mg and one caplet of the Placebo, a single dose of two caplets of DLBS2411 250 mg, or a single dose of two caplets of the Placebo.

Statistical Analysis

Demography and baseline characteristics of the study population were tabulated and summarized by descriptive statistics. Characteristic comparability between groups at baseline was assessed by ANOVA, for continuous data; or by chi-square test, for categorical data. Pharmacodynamic data were analyzed

between groups using ANOVA, or Kruskal-Wallis test for data that violated normal distribution. Vital signs at each visit were descriptively summarized in the tables of means by group. Laboratory safety parameters at baseline and at end of study were summarized by group in tables of means. Those parameters were statistically analyzed within-group by paired-t test, and betweengroup by one-way ANOVA. Other categorical data were analyzed between-group by chi-square test.

Throughout the analysis, parametric variables might be log transformed in order to meet the underlying distributional assumptions of the statistical models; or, the corresponding nonparametric test was used. All statistical tests were at 5 % significance level. Continuous data were expressed as a mean ± the standard deviation. Adverse events were summarized by system organ class and the number of subjects with events and number of events are tabulated. Statistical program SPSS® version 14.0 was used for the analyses.

RESULTS

Patient Disposition

Of a total of ninety five healthy male subjects screened, 54 subjects were eligible for the study. The overall subject disposition is summarized in **Figure 1**. At the end of study, all of 54 subjects (100.0%) had completed the trial. However, of them, data pertaining to 7 subjects (12.96%) were not eligible for evaluation due to technical problem during installation of the pH monitoring instrument to the subjects and data transfer process from the instrument to the computer. Of the 7 subjects, four, two and one subject(s) were in DLBS2411 250 mg, DLBS2411 500 mg, and Placebo Group, respectively. Hence, of the total of 54 enrolled patients, 47 (87.04%) were evaluated for pharmacodynamics ITT-analysis.

Demography and Baseline Characteristics

Demography and baseline characteristics are described in **Table 1**. Subjects in all groups had comparable demographic data and baseline characteristics. Based on endoscopy findings at Screening, several subjects were found to have esophagitis candidiasis (1 subject in each group), mild gastritis (16 subjects comparably distributed between groups) and moderate gastritis (2 subjects in DLBS2411 250 mg Group) **(Table 1).** Further thorough clinical assessment on the subjects concluded that they were otherwise healthy, their conditions were asymptomatic and not currently active, thus no medications, such as antacids, mucosal protectors, histamine receptor-2 blockers (AH_2) or proton-pump inhibitors, were required. The subjects were individually adjudicated by the Investigator as eligible for the study.

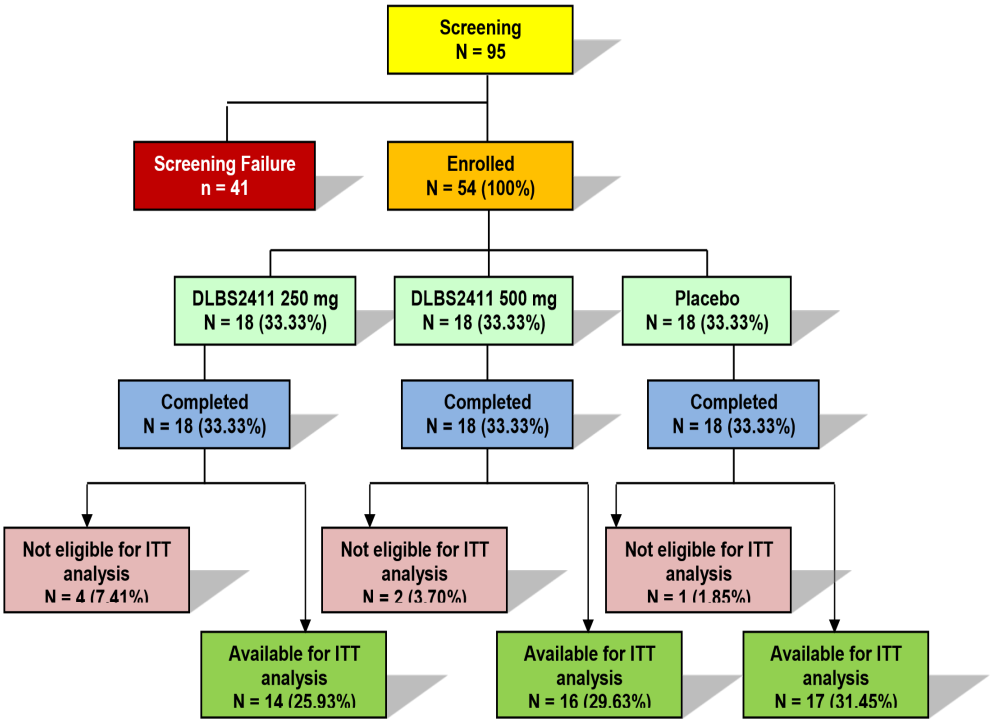

Figure 1. Subject Disposition

ITT = intent-to-treat analysis; 7 subjects (12.96%) were not eligible for ITT-evaluation due to technical problem in installing the pH monitoring instrument to the subject and data transfer from the instrument to the computer

Table 1. Demography and Baseline Characteristics

Variables		DLBS2411 250 mg (n = 14)		DLBS2411 500 mg (n = 16)		Placebo (n = 17)		p [a]
		Mean	SD	Mean	SD	Mean	SD	
Age (year)		29.1	6.5	28.1	7.4	31	7.3	0.476
Weight (kg)		57.9	8.7	58.6	8.7	57.4	9.5	0.932
BMI (kg/m^2)		21	2.2	21.1	2.2	20.8	2	0.917
Systolic Blood Pressure (mmHg)		114.4	5.6	112.4	8.3	117.4	7.1	0.143
Diastolic Blood Pressure (mmHg)		73.2	5.8	73.8	6	75.8	5.2	0.503
Pulse (beats per minute)		83.4	5.1	84.4	4.5	83.8	4.8	0.732
Respiratory rate (per minute)		19.3	2.7	20.1	2	20	1.9	0.625
Gastric pH		1.82	0.55	1.96	0.45	1.82	0.43	0.576
Fasting plasma glucose (mmol/L)		4.69	0.36	4.68	0.40	4.86	0.68	0.407
2-hour post prandial glucose (mmol/L)		5.22	30.90	5.41	1.36	5.12	0.93	0.717
Hematology:								
Haemoglobin (g/dL)		14.5	1.2	14.8	0.9	14.4	0.9	0.62
Hematocrit		0.44	0.03	0.44	0.02	0.43	0.02	0.574
Erythrocyte count (10^6/µL)		5.0	0.4	5.2	0.3	5.0	0.3	0.322
Platelet count (10^3/µL)		285.1	63.5	284.6	53	268.9	65.9	0.682
Leucocyte count (10^3/µL)		9.0	3.0	8.1	1.7	8.1	1.8	0.446
Basophil (%)		0.5	0.3	0.6	0.3	0.5	0.2	0.334
Eosinophil (%)		3.2	1.7	3.9	2.7	4	2.7	0.792
Neutrophil (%)		57.8	6.3	52.2	7.3	56.5	7.6	0.062
Monocyte (%)		5.8	1.5	5.7	1.3	5.3	1.0	0.477
Lymphocyte (%)		30.5	5.6	34.7	6.6	31.1	6.3	0.254
Liver Function:								
Alanine transaminase, ALT (U/L)		21.8	12.5	19.7	9.7	18.8	6.9	0.813
Asparte transaminase, AST (U/L)		21.1	6.4	20.7	4.9	19.9	4.8	0.740
Gamma glutamyl transpeptidase (U/L)		28.5	20.1	29.1	16.3	22.8	9.8	0.416
Total bilirubin (U/L)		0.63	0.23	0.62	0.19	0.65	0.23	0.909
Renal Function:								
Serum Creatinine (mmol/L)		76.02	9.72	77.79	7.96	75.14	10.61	0.553
Urinalysis:								
Urine density (g/cm^3)		1.02	0.01	1.02	0.01	1.02	0.01	0.735
Urine pH		5.88	0.84	5.82	0.5	5.82	0.61	0.952
Smoking status	Active smoker	13	76.5%	16	94.1%	14	82.4%	0.483
	Ex-smoker	1	5.9%	0	0.0%	0	0.0%	
	Non-smoker	3	17.6%	1	5.9%	3	17.6%	
Alcohol status	Ex-drinker	12	70.6%	10	58.8%	7	41.2%	0.219
	Non-drinker	5	29.4%	7	41.2%	10	58.8%	
General Health Status	Good	17	100%	17	100%	17	100%	1.000
Physical examination	Normal	17	100%	17	100%	17	100%	1.000
Urinalysis	Normal	17	100%	17	100%	17	100%	1.000
Endoscopy reading	Normal	10	58.8%	11	64.7%	9	52.9%	0.534
	Esophagitis candidiasis	1	5.9%	1	5.9%	1	5.9%	
	mild gastritis	4	23.5%	5	29.4%	7	41.2%	
	moderate gastritis	2	11.8%	0	0.0%	0	0.0%	

SD, standard deviation; %, percentage of subjects with particular characteristics within the concerned group; [a] , Between-group analyses were conducted using ANOVA (or Kruskal-Wallis Test, if even after being log-transformed, the data were not normally distributed). [b], Between-group analyses were conducted using Chi-square Test.

Pharmacodynamic Evaluation

The pharmacodynamics of DLBS2411 bioactive fraction was measured by its effect on gastric pH over 24 hours after a single dose administration. Pharmacodynamic evaluation was based on the ITT (intent-to-treat)-analysis on the data pertaining to 47 subjects. The profiles of 24-hour intra-gastric pH of each group are shown in **Figure 2.** The pharmacodynamic effect of the bioactive fraction on the 24hour intragastric acidity and the comparison between groups are listed in **Table 2.**

Based on the results presented in **Table 2**, the means of gastric pH over 24 hours observed after a single dose administration of DLBS2411 250 mg, 500 mg (2.29 ± 0.42 and 2.13 ± 0.50, respectively) were greater than that achieved in Placebo Group (1.93 ± 0.70), even though the differences were not statistically significant. The higher dose of DLBS2411 did not seem to suppress the intra-gastric acidity better than the lower dose did. When we included only the gastric pH during daytime (the first 12 hours after dosing), the differences in intra-gastric pH between the respective DLBS2411 groups (DLBS2411 250 and 500 mg) and Placebo were even greater **(Table 2).** The mean intra-gastric pH during night time (the last 12 hours after dosing) was quite similar between groups.

Table 2 also showed that the onset of DLBS2411 250 mg and 500 mg to reach the gastric pH above 4 (129.9 ± 128.2 and 92.9 ± 106.8 minutes, respectively) were significantly faster than that of Placebo (196.9 ± 99.7 minutes). However, the duration of time during which the intragastric pH was above 4 did not differ between groups. The AUC of pH response over 24 hours ($AUC_{0\text{-}24h}$) tended to be higher with DLBS2411 250 mg and 500 mg than with the Placebo, and the difference in $AUC_{0\text{-}24h}$ between DLBS2411 250 mg and Placebo groups was significant over the first 12 hours ($AUC_{0\text{-}12h}$). However, administration of DLBS2411 500 mg resulted in a slightly reduced AUC of pH over 12 and 24 hours from those of DLBS2411 250 mg, and they were not significantly different with those of the Placebo **(Table 2).**

There were slightly more subjects in DLBS2411 250 mg Group (94.1%) and 500 mg Group (82.3%) than that of Placebo Group (76.5%) with intragastric pH reaching over 4 within 12 hours after the initial dosing, yet the rates were not different between groups (p=0.263). Two subjects (11.8%) in DLBS2411 500 mg Group and 4 subjects (23.5%) in Placebo Group reached the intragastric pH over 4 in between 12 and 24 hours after dosing. However, there was one subject (5.9%) in DLBS2411 250 mg and 500 mg Group, respectively, did not reach the intragastric pH over 4 over the 24 hours.

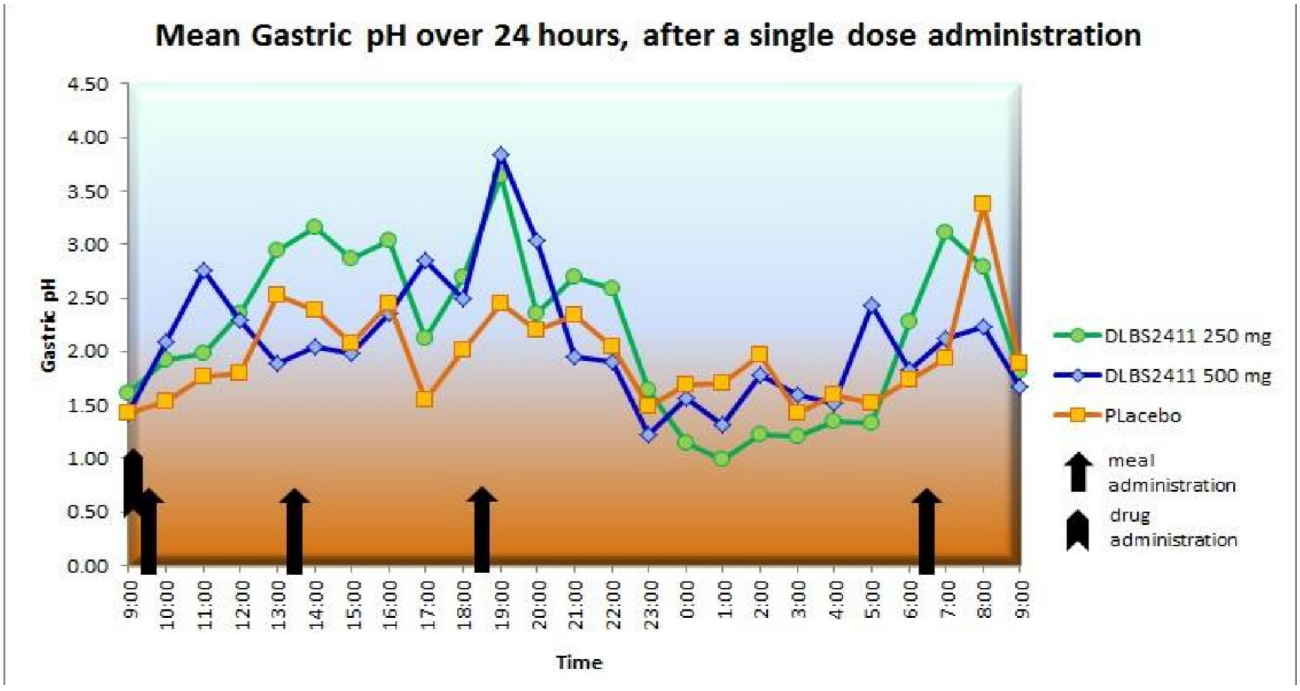

Figure 2. 24-hour intragastric acidity profile after a single dose of DLBS2411 and Placebo

Table 2. Pharmacodynamic Effect of DLBS2411 and Placebo on 24-h intra-gastric pH following a single dose administration

Variables	DLBS2411 250 mg (n = 14)		DLBS2411 500 mg (n = 16)		Placebo (n = 17)		p
	Mean	SD	Mean	SD	Mean	SD	
Gastric pH at Baseline	1.82	0.57	1.93	0.46	1.82	0.43	0.713
Mean Gastric pH over 24 hours	2.29	0.42	2.13	0.50	1.93	0.70	0.294
Mean Day-time-Gastric pH (H0 - H12)	**2.66**	**0.68**	**2.52**	**0.73**	**2.05**	**0.76**	**0.053**
Mean Night-time-Gastric pH (H13 - H24)	1.91	0.48	1.73	0.68	1.81	0.83	0.800
AUC Gastric pH over 24 hours (AUC_{0-24h})	3,296.37	609.31	3,063.74	727.20	2,774.19	1,007.00	0.197
AUC Day-time-Gastric pH (H0 - H12) (AUC_{0-12h})	**1,920.81***	**494.82**	**1,820.60**	**531.37**	**1,474.19**	**546.96**	**0.052**
AUC Night-time-Gastric pH (H13 - H24) (AUC_{13-24h})	1,375.55	347.20	1,243.15	495.59	1,300.00	599.27	0.770
Time to reach pH> 4 (minutes)	**129.9**	128.2	**92.9***	106.8	**196.9**	99.7	**0.007**
Duration of gastric pH > 4 (minutes), over 24 hours	248.5	171.1	254.0	149.2	232.6	179.5	0.889
Duration of gastric pH > 4 (%), over 24 hours	17.3	11.9	17.6	10.4	16.1	12.5	0.889

SD, standard deviation; Between-group analyses were conducted using ANOVA, or Kruskal-Wallis Test for data that violated normal distribution. Time to reach pH>4 was measured as time taken to achieve gastric pH of > 4 (onset of action) at the first time after the dosing. * statistically significant ($p < 0.05$) as compared to Placebo.

Safety Evaluation

The safety analyses were based on all exposed patients who had received at least one dose of trial products. Clinical and laboratory safety parameters were evaluated statistically based on before and after treatment data analyses (within-group analyses) and between-group analyses at the end of study **(Table 3).** Number of adverse events observed and subjects exposed to such events were tabulated in **Table 4**. While the adverse events occurred during the study conduct were descriptively presented in **Table 5.**

At the end of study, there were statistically significant changes from baseline found with hematocrit, erythrocyte count, eosinophil percentage as well as systolic blood pressure in DLBS2411 250 mg Group; and in haemoglobin, hematocrit, erythrocyte count as well as systolic blood pressure in Placebo Group. A reduction of AST level in DLBS2411 500 mg Group was also found. However, all the changes were quantitatively too small, thus clinically negligible. At the end of study, there was a statistically significant difference in basophil percentage between groups, which was also clinically insignificant. There were no significant changes of all other clinical and laboratory safety parameters within each group and between groups **(Table 3).**

Table 3. Safety Evaluation

Variables	Visit	DLBS2411 250 mg (n = 14)		DLBS2411 500 mg (n = 16)		Placebo (n = 17)		p[b]
		Mean	SD	Mean	SD	Mean	SD	
Liver Function:								
Alanine transaminase, ALT (U/L)	Baseline	21.8	12.5	19.7	9.7	18.8	6.9	0.813
	End	23.6	15.4	19.4	10.0	19.2	11.0	0.673
	p[a]	0.350		0.169		0.776		
Aspartate transaminase, AST (U/L)	Baseline	21.1	6.4	**20.7**	**4.9**	19.9	4.8	0.740
	End	24.5	13.8	**19.7**	**6.0**	19.5	6.0	0.643
	p[a]	0.393		**0.040***		0.601		
Gamma glutamyl transpeptidase (U/L)	Baseline	28.5	20.1	29.1	16.3	22.8	9.8	0.416
	End	30.5	21.5	28.6	17.8	23.6	10.1	0.700
	p[a]	0.359		0.468		0.338		
Total bilirubin (U/L)	Baseline	0.63	0.23	0.62	0.19	0.65	0.23	0.909
	End	0.66	0.23	0.63	0.17	0.72	0.25	0.694
	p[a]	0.245		0.962		0.201		
Renal Function:								
Serum Creatinine (mmol/L)	Baseline	76.02	9.72	77.79	7.96	75.14	10.61	0.553
	End	74.26	12.38	76.02	7.96	74.26	7.96	0.709
	p[a]	0.374		0.211		0.569		
Hematological Function:								
Haemoglobin, Hb (g/dL)	Baseline	14.5	1.2	14.8	0.9	**14.4**	**0.9**	0.620
	End	14.9	1.3	14.7	1.0	**14.8**	**0.9**	0.864
	p[a]	0.165		0.893		**0.029***		

Variables	Visit	DLBS2411 250 mg (n = 14)		DLBS2411 500 mg (n = 16)		Placebo (n = 17)		p[b]
		Mean	SD	Mean	SD	Mean	SD	
Hematocrit, Ht (%)	Baseline	43.9	3.4	44.3	2.4	**43.3**	**2.3**	0.574
	End	44.9	3.5	43.9	2.9	**44.4**	**1.9**	0.479
	p[a]	0.070		0.433		**0.037***		
Erythrocyte count (10^6/µL)	Baseline	**5.0**	**0.4**	5.2	0.3	**5.0**	**0.3**	0.322
	End	**5.2**	**0.4**	5.1	0.3	**5.1**	**0.3**	0.343
	p[a]	**0.016***		0.351		**0.032***		
Platelet count (10^3/µL)	Baseline	285.1	63.5	284.6	53.0	268.9	65.9	0.682
	End	288.1	62.2	277.8	57.4	266.2	62.8	0.523
	p[a]	0.569		0.495		0.582		
Leucocyte count (10^3/µL)	Baseline	8.96	2.95	8.08	1.68	8.14	1.83	0.446
	End	8.75	2.10	8.08	2.20	7.62	1.92	0.283
	p[a]	0.647		0.985		0.27		
Vital Signs:								
Systolic Blood Pressure, SBP (mmHg)	Baseline	**114.4**	**5.6**	112.4	8.3	**117.4**	**7.1**	0.143
	End	**112.9**	**4.7**	114.7	5.1	**114.1**	**6.2**	0.570
	p[a]	**0.017***		0.537		**0.007***		
Diastolic Blood Pressure, DBP (mmHg)	Baseline	73.2	5.8	73.8	6.0	75.8	5.2	0.503
	End	73.2	4.3	75.0	5.0	75.3	4.8	0.403
	p[a]	0.253		0.511		0.780		
Pulse (beats per minute)	Baseline	**83.4**	**5.1**	84.4	4.5	83.8	4.8	0.732
	End	**85.6**	**5.3**	84.0	6.1	83.8	5.0	0.453
	p[a]	**0.011***		0.741		0.202		
Other variables at the End of Study		**n**	**%**	**n**	**%**	**n**	**%**	**p[c]**
General Health Status - End of study	Good	17	100%	17	100%	17	100%	1.000
Physical examination - End of Study	Normal	17	100%	17	100%	17	100%	1.000
Urinalysis - End of Study	Normal	17	100%	17	100%	17	100%	1.000

SD, standard deviation; [a] , Within-group analyses were conducted using paired-t Test (or Wilcoxon-signed-rank Test, if even after being log-transformed, the data were not normally distributed). [b] , Between-group analyses were conducted using ANOVA (or Kruskal-Wallis Test, if even after being log-transformed, the data were not normally distributed). [c], Between-group analyses were conducted using Chi-square Test. * statistically significant ($p < 0.05$) as compared to its baseline value.

Table 4. Number of subjects exposed to adverse events

Adverse events	Total (N = 54)	Within group		
		DLBS2411 250 mg (n = 18)	DLBS2411 500 mg (n = 18)	Placebo (n = 18)
Number of subjects exposed to AE – n (%)	6 (11.1)	4 (22.2)	0 (0)	2 (11.1)
Number of adverse events – n (%)	6 (100)	4 (66.7)	0 (0)	2 (33.3)
Serious adverse events (SAE)	0 (0)	0 (0)	0 (0)	0 (0)
Other adverse events	6 (100)	4 (66.7)	0 (0)	2 (33.3)

Table 5. Adverse Events

Adverse events (AE)	DLBS2411 250 mg (n = 18)			DLBS2411 500 mg (n = 18)			Placebo (n = 18)			ADR
	AEn (nE)	AEn (%)	nE (%)	AEn (nE)	AEn (%)	nE (%)	AEn (nE)	AEn (%)	nE (%)	
Convulsion seizure	1 (1)	9.1%	5.6%	0	0.0%	0.0%	0	0.0%	0.0%	unlikely
Dizziness	1 (1)	9.1%	5.6%	0	0.0%	0.0%	1 (1)	9.1%	5.6%	unlikely
Abdominal discomfort	2 (2)	18.2%	11.1%	0	0.0%	0.0%	1 (1)	9.1%	5.6%	unlikely
Total number of AE	**4**	**66.7%**		**0**	**0.0%**		**2**	**33.3%**		**6 (100%)**
Total number of subjects with AE	**4**		**22.2%**	**0**		**0.0%**	**2**		**11.1%**	**6 (11.11%)**

AEn, number of event; nE, number of subjects with event. One subject might have more than one event. All subjects who took the study medication of at least one dose were subject to adverse event evaluation. By the end of study, all adverse events had already been resolved.

There were no serious adverse events occurred during the study conduct **(Table 4**). One subject experienced a brief episode of mild convulsion seizure during the study conduct that was regarded as strongly associated with the subject's history of seizure which had been present since the subject's childhood. The subject's consciousness was fully recovered just a few minutes after the event, and completed his participation in the study. All adverse events reported were mild, unlikely to have a causal relationship with the study product **(Table 5)** and had been resolved by the end of study. Those data demonstrated a good safety profile of DLBS2411 at the dose of 250 and 500 mg.

DISCUSSION

The clinical study investigated the pharmacodynamics of a single dose of DLBS2411 Cinnamomum burmanii through the evaluation of its effect on the 24-hour intragastric acidity and its safety in healthy adults. The study demonstrated that the pharmacodynamic effect did not differ across the doses of DLBS2411, but was better than that of the Placebo (Table 2), suggesting that DLBS2411 250 mg is the optimal dose of the bioactive fraction to control the intragastric pH in human.

We realized that the absence of active control groups for direct comparison of DLBS2411 activity in this study limits the interpretation of the results. However, indirect comparison of the findings with those of other similar studies on currently available proton pump inhibitors in healthy volunteers indicates that the degree of acid suppression by a single dose of DLBS2411 250 and 500 mg (with a mean 24-h intragastric pH of 2.29 ± 0.42 and 2.13 ± 0.50, respectively) was approximately between that of a single dose of omeprazole 20 mg (with a mean pH of 1.8),[26] and that of a single dose of pantoprazole 40 mg (with a mean pH of 2.9).[26,27]

The elevated mean of the 24-hour-intragastric pH by DLBS2411 as compared to the Placebo observed in this study was associated with its mechanism of action as demonstrated in the previous in-vitro study of DLBS241 conducted upon HEK293 cell line and gastric parietal cells, where the treatment with the bioactive fraction downregulated the H+/K+ ATPase mRNA expression as well as inhibited the H+/K+ ATPase activity. The in-vitro study also indicated that such an inhibition of proton pump activity by DLBS2411 might partly be due to the reduced expression of the gene.[5]

A further analysis dividing the intragastric pH during the 12-hour day-period (the first 12 hours) and 12-hour night-period (the last 12 hours) demonstrated that the means of gastric pH and AUC of pHover-time in all groups were higher during the day-time, and DLBS2411, particularly at the investigated dose of 250 mg provided higher mean gastric pH and greater AUC of pH-over-time than the Placebo (Table 2). However, during the night-time, the mean intragastric pH and the AUC were not different between groups. In this study, DLBS2411 and the placebo were administered in the morning before breakfast. The study indicated that the acid suppression by DLBS2411 was noticeable particularly during 12 hours after dosing; and that will practically determine the appropriate timing of DLBS2411 administration in order to provide effective acid suppression according to the gastrointestinal symptom pattern of individual patients. In this study, DLBS2411 morning-dosing sufficiently increased daytime intragastric pH and could overcome meal-stimulated acid secretion. It is also suggesting that it may require a twice-daily dosing for DLBS2411 to sustain its optimal control on gastric pH for 24 hours; or, an evening-daily dosing may be appropriate when nocturnal acid suppression is required. In an early study, esomeprazole 40 mg was also reported to provide better pH control during the initial four hours after a single dose administration,[28] which also influenced the timing and regimen of esomeprazole. It was reported that esomeprazole 20 mg or 40 mg twice daily was associated with superior acid inhibition compared to either 20 mg or 40 mg once daily regimens. Once daily dosing of esomeprazole 20 mg or 40 mg before breakfast improved the day-time acid inhibition; while once daily esomeprazole 40 mg administered before dinner or at bedtime improved the night-time acid inhibition.[29] Another former study reported that the H2-receptor antagonists (H2RAs), such as ranitidine 150 mg given twice daily provided no significant difference with the regimen of 300 mg once daily at night in reducing the 24-h intragastric acidity. However, the basal nocturnal acid secretion was controlled significantly better with once-daily evening-dosing ranitidine.[30]

Table 2 showed that a pH above 4 was reached for the first time after 129.9 ± 128.2 and 92.9 ± 106.8 minutes with DLBS2411 250 mg and 500 mg, respectively; and they were significantly earlier than that of Placebo (196.9 ± 99.7 minutes). The onset to reach pH > 4 showed by DLBS2411 in this study was close to that showed by lansoprazole 30

mg in a former similar study with lansoprazole and omeperazole in healthy adults.[31] The former study reported that the pH of more than 4 was reached for the first time after 130 minutes with a single oral dose of lansoprazole 30 mg, and after 250 minutes with omeprazole 20 mg.[31] In another study of H2RAs, a single oral administration of lafutidine 10 mg was reported to reach the intragastric pH > 4 about 210 min after administration; while famotidine 20 mg increased the intragastric pH at 60 min after administration and remained at approximately pH 3.[32]

The percentage of time with intragastric pH was above 4 did not differ much across the groups (Table 2) and the values (around 17.3% and 17.6% referring to DLBS2411 250 mg and 500 mg, respectively) were considerably lower compared to those previously reported for proton pump inhibitors at a single dose administration in healthy volunteers.[26] A single dose of omeprazole 20 mg, esomeprazole 20 mg, pantoprazole 40 mg, lansoprazole 15 mg and rabeprazole 10 mg, in healthy volunteers were reported to provide duration of time with intragastric pH> 4 of 30.4%, 32.5%, 29.2%, 28.1%, 29.8%, respectively.[26]

The suppression of intragastric acidity is dynamically correlated with the healing of peptic ulcer and erosive oesophagitis and control of acid-related symptoms.[30] Taken all the results together, the acid suppression effect by DLBS2411 should be further compared directly with other anti-secretory agents, particularly to confirm its efficacy in patients with excessive gastric acid disorders.

CONCLUSION

This pharmacodynamic study concluded that DLBS2411 suppressed the intragastric acidity and was safe and tolerable in healthy volunteers. The effect on the 24-hour-intra-gastric pH provided by a single dose of DLBS2411 250 mg was similar to that of DLBS2411 500 mg, and the acid-suppression was more noticeable during the first twelve hours after administration. The study provided the ground to conduct further studies of DLBS2411 to evaluate its acid-suppressing efficacy in acid-related disorders, such as dyspepsia, peptic ulcer or GERD.

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Author Contributions

MA and RRT were responsible for the study concept and design, interpreted the study data, and critically reviewed the intellectual content of the manuscript. MA and MS supervised study conduct, and assume responsibility for the integrity and completeness of the whole data. KR recruited and enrolled subjects, conducted the study and acquired the study data, completed the CRFs, and resolved all study-related queries. LWS was responsible for data analysis and interpretation, and preparation of the draft manuscript. The authors and Sponsor agreed to submit the manuscript for publication and maintain confidentiality of the data. All authors had access to the data and approved the final version of the submitted manuscript.

Disclosure

This work was supported by Dexa Medica. The authors have nothing else to declare.

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