DLBS3233 extract, a novel insulin sensitizer with negligible risk of hypoglycemia: A phase-I study

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Abstract
DLBS3233 is a standardized extract combination of two Indonesian herbals (*Lagerstroemia speciosa* L. and *Cinnamomum burmanii* L.) developed by a patented extraction-technology. DLBS3233 has been proven pre-clinically to have a glucose lowering activity. This Phase 1 clinical study evaluated the safety of DLBS3233 in comparison with the negative-control and pioglitazone 30 mg (active control) in healthy volunteers. In this randomized, open-labeled, Latin-square-designed clinical study consisting of 7 groups of treatment, DLBS3233 capsules were given at doses of 50, 100, 200, 300, 400 mg in each group, respectively. The Active Control group received pioglitazone 30 mg and the Negative Control did not receive any study drug. All study drugs were administered once daily for three days prior to the conduct of 75 g glucose oral loading at each study course. Wash-out period between each course was one week. Safety parameters were blood glucose profile, liver and renal functions, and adverse events. Six healthy volunteers with a mean age of 27.8 ± 6.1 years old were enrolled in the study. The result demonstrated comparable blood glucose profiles between all groups indicating that both DLBS3233 and pioglitazone do not exert a hypoglycemic effect in non-obese healthy subjects with normoglycemia. DLBS3233 treatment did not affect liver and kidney function. Neither did it cause any serious adverse events. Overall, this study demonstrated the low risk of DLBS3233 to induce hypoglycemia and that the extract was safe and well tolerated.

Keywords: *DLBS3233, hypoglycemia, blood glucose, insulin sensitizer.*

Introduction

DLBS3233 is a standardized extract combination of two Indonesian herbals (*Lagerstroemia speciosa* L. and *Cinnamomum burmanii* L.) developed by a patented extraction-technology. Both *Lagerstroemia speciosa* L. and *Cinnamomum burmanii* L. have recently been studied for its anti-diabetic effect.[1-5] Preclinical studies using 3T3-Swiss-Albino pre-adipocyte cells showed that DLBS3233 exerted its glucose lowering activity by increasing the expression of PPARγ and PPARδ at the mRNA level. It also reduced the expression of resistin gene and increased the expression of adiponectin at mRNA level.[6] Furthermore, DLBS3233 was apparently evidenced to induce gene expression of other key enzymes involved in the insulin-signal-transduction pathway, i.e. PI-3 kinase as well as the Akt.[6] Once phosphorylated, Akt is in its active form and phosphorylates other targets that stimulate GLUT-4 translocation to the plasma membrane.[7] Our previous *in vitro* study demonstrated the up-regulation of GLUT-4 expression by DLBS3233 which also corresponded to the increase of GLUT-4 protein levels, and thus, as expected, resulting in an enhancement of glucose uptake in the insulin-resistant 3T3-Swiss-Albino adipocytes. The promotion of glucose uptake activity by DLBS3233 was exerted in a time-dependent manner and with the capacity comparable to that of pioglitazone.[6]

The pharmacological activity of DLBS3233 as a potential insulin sensitizer was also demonstrated in our previous study using insulin-resistant Wistar rats.[8] In the study, the insulin resistance state was developed by administering those rats with high amount of fructose and glucose. Homeostasis model assessment-insulin resistance (HOMA-IR) was used to ensure that the study rats had become insulin-resistant. In the study, insulin-resistant rats showed their HOMA-IR elevated up to 5-fold that of the Control group; and treatment with DLBS3233 afterwards dropped their fasting insulin level and HOMA-IR significantly to the levels of the Control group.[8] In addition, DLBS3233 also lowered the plasma level of LDL-cholesterol, total cholesterol and triglyceride in hyperlipidemic rats, yet increased the HDL-cholesterol level significantly.[8]

The acute toxicity study of DLBS3233 concluded that extract is evidently classified as a practically non-toxic substance with the Lethal Dose $(LD)_{50}$ of more than 15 g/kg body weight (BW) of mice.[9] Subchronic toxicity study of DLBS3233 in rats also demonstrated the safety profile of the extract on long-term use, up to the dose equivalent to 50 mg/kg BW of rat.[10] Teratogenicity study demonstrated the safety of DLBS3233 to the fetus up to the dose equivalent to

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1g/kg BW of rat. No abnormalities in fetal development were found in the study.[11]

To date, DLBS3233 has also been approved by the National Agency of Drug and Food Control, Republic of Indonesia and marketed as a herbal medicine. People who had personally used this product reported that neither hypoglycemic nor other adverse drug reactions were experienced. This collection of individual data was yet to be confirmed through a clinical study which has more robust and reliable level of evidence. This current Phase 1 clinical study was designed to investigate the safety of this extract in healthy subjects which primarily included its effect on the profile of blood glucose levels after an oral loading of 75 g glucose. The safety profile was also evaluated by monitoring the frequency of hypoglycemic as well as other adverse events, and liver and kidney function. The hypothesis of interest of this study was that there were no significant differences in all safety endpoints evaluated between all groups.

Materials and Methods

The protocol, the consent form, and the subject information sheet were reviewed and approved by independent Ethics Committee (University of Udayana, Denpasar, Bali, Indonesia), prior to study initiation. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Consent was obtained from each subject in writing prior to any study-related activities.

Study Design

This was a randomized, open-labeled, controlled and Latin-square-designed clinical study involving 6 healthy volunteers to assess the safety of DLBS3233 at 5 treatment doses compared with negative and active (positive) control, over a study period of 10 weeks.

Subjects with the following criteria were included in the study: 1) male or female healthy volunteer within the age limits of 18-40 years at screening; 2) normal liver and renal functions, as measured by serum glutamate-piruvate transaminase (SGPT) and creatinine levels, respectively; 3) normal fasting blood glucose (FPG < 6.11 mmol/L). The exclusion criteria were participation in any other clinical studies within 30 days prior to screening, pregnancy or breast-feeding mothers, hypersensitivity to study products or other composition in product formulation, the presence of any other disease state or uncontrolled illness, and concurrent use of herbal (alternative) medicines.

Study medication and dosage regimen

The study drugs used were DLBS3233 capsules (Dexa Laboratories of Biomolecular Sciences, PT Dexa Medica, Indonesia) and pioglitazone 30 mg tablets (Actos®, PT Takeda Indonesia). The components of DLBS3233 were extracted from the plants of *Lagerstroemia speciosa* which was obtained from Kerinci, Jambi, Indonesia, and *Cinnamomum burmanii* which was purchased from Cianjur, West Java, Indonesia. Both plants have been identified by Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences with reference no. 1261/IPH.1.02/If.8/XII/2009. DLBS3233 was prepared as a polar extract for which a mixture of dried *L. speciosa* and *C. brumanii* plants at a ratio of 1:3 was made and extracted simultaneously in warm water (1: 8-10) using a percolation technique at a temperature of 50-90°C. The micelles were then filtered and dried using a Rotavapor (Büdhi, Flawil, Switzerland) at a temperature of 40-50°C, and subsequently dissolved in methanol for further study. The detail of phytochemical characterization of DLBS3233, including its identification using both the thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC), was as previously described.[6]

There were 7 groups of treatment in the current study, each consisted of 6 healthy volunteers, i.e.:

1. Treatment I : DLBS3233 50 mg once daily for 3 days;
2. Treatment II : DLBS3233 100 mg oonce daily for 3 days;
3. Treatment III : DLBS3233 200 mg once daily for 3 days;
4. Treatment IV : DLBS3233 300 mg once daily for 3 days;
5. Treatment V : DLBS3233 400 mg once daily for 3 days;
6. Negative-Control : no drug regimen;
7. Active-Control : Pioglitazone 30 mg once daily for 3 days.

All eligible subjects were randomized before initiating the first course of study treatment so that in each course there were 6 subjects distributed in 6 groups running in parallel. Using a Latin-square design, each eligible subject received all those 7 treatments individually over 7 consecutive courses in a total period of 10 weeks. Wash-out period between each course was one week. The following procedure was performed in the same manner in each study course: On day 1, after an overnight (10-hour)-fasting, capillary blood glucose was taken and measured using blood glucometer (OneTouch® Ultra®; Johnson & Johnson Ltd.). The subjects were then instructed to take any of the drug regimens (in accordance with the randomization code received by the subject) for 3 days (Day-1 up to Day-3). On day 4, after an overnight (10-hour) fasting, the blood glucose measurement was first performed at hour-0 (T_0). Right after then, subjects were instructed to take 75 g glucose solution. Subsequent to glucose intake, blood glucose was measured at ½, 1, 2, and 3 hours afterwards ($T_{0.5}$, T_1, T_2, T_3). During fasting as well as blood glucose measurement on day 4 (T_0 until T_3) in each course, volunteers were not allowed to take any meals but limited plain water, and had their physical activity standardized. All subjects were under direct supervision of a medical doctor along the study period. Blood pressure, heart rate, respiratory rate, and body weight were measured at screening (baseline values) and before the measurement of fasting-blood glucose in each study course. SGPT and creatinine levels were measured at screening and at the end of study (after the completion of all 7 courses).

Table 1: Demographic Data of study population

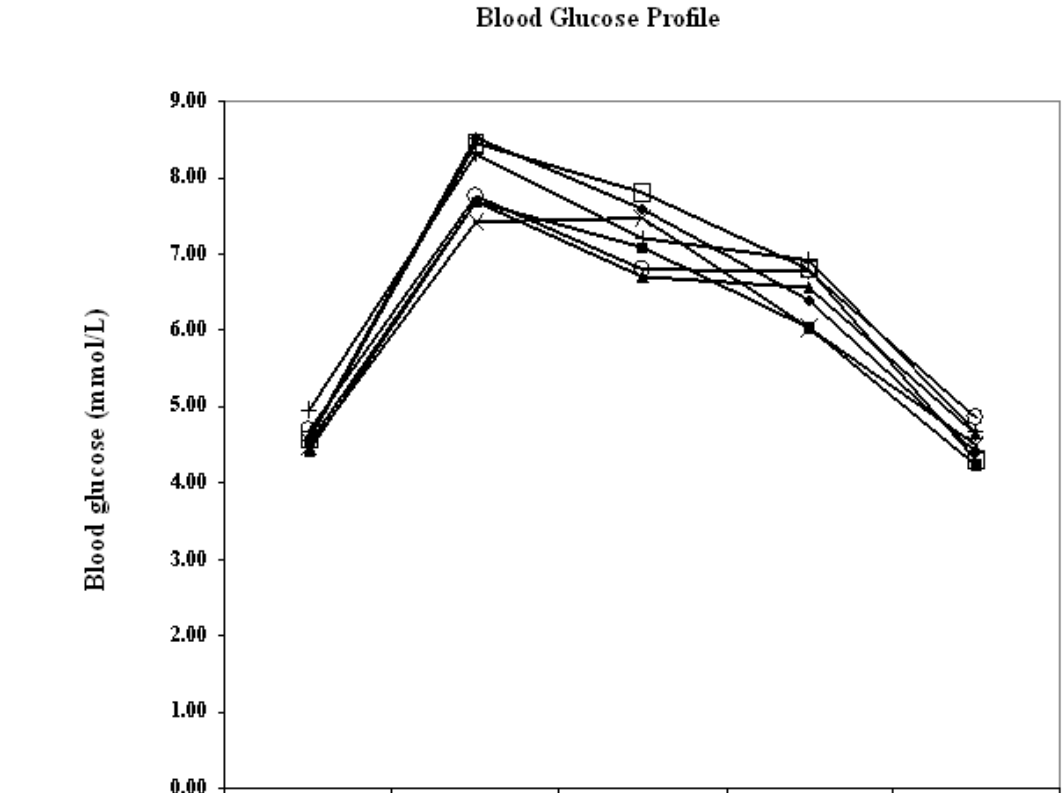
Parameters	**N = 6**	
	Mean (SD)	**range**
Age (year)	27.83 (6.08)	22 – 38
Body Mass Index (kg/m^2)	21.45 (3.12)	18.13 – 27.20
Blood pressure (mmHg)		
• Systolic	110.0 (6.3)	100 – 120
• Diastolic	73.3 (8.1)	60 – 80
Pulse (/min)	82.7 (3.5)	80 – 88
Respiratory rate (/min)	18.3 (1.5)	16 – 20
Fasting Plasma Glucose (mmol/L)	4.96 (0.34)	4.55 – 5.33
SGPT (U/L)	15.4 (5.9)	9 –13
Creatinine (μmol/L)	54.8 (7.1)	44.2 –61.9

N: total number of subjects; SD: standard deviation; SGPT: serum glutamate-piruvate transaminase

Table 2: Endpoints of glucose profile

Variables	**Group (n per group = 6)**	**Mean**	**SD**	**ANOVA p**
AUC_{0-3} – mmol/L.h	(–) Control	19.36	1.88	
	(+) Control	18.43	2.50	
	50 mg	18.87	2.59	
	100 mg	18.70	2.17	0.089
	200 mg	19.69	2.23	
	300 mg	19.48	2.99	
	400 mg	20.75	2.18	
T_0_Tpeak– mmol/L	(–) Control	3.43	1.64	
	(+) Control	3.46	1.43	
	50 mg	3.72	0.96	
	100 mg	3.26	0.64	0.480
	200 mg	4.19	0.59	
	300 mg	4.12	1.29	
	400 mg	3.36	0.89	
Tpeak_T_3– mmol/L	(–) Control	-3.80	2.79	
	(+) Control	-3.75	2.23	
	50 m	-3.50	1.43	
	100 mg	-3.23	1.17	0.688
	200 mg	-4.38	1.57	
	300 mg	-4.37	1.63	
	400 mg	-3.65	1.54	
T_0_T_3– mmol/L	(–) Control	0.16	1.54	
	(+) Control	-0.29	1.33	
	50 mg	0.22	1.05	
	100 mg	0.03	0.96	0.588
	200 mg	-0.18	1.08	
	300 mg	-0.30	0.94	
	400 mg	-0.29	0.96	

n: number of subjects; SD: standard deviation; (–) Control: no treatment; (+) Control: pioglitazone 30 mg; 50, 100, 200, 300, 400 mg are the doses of DLBS3233 in each group, respectively. T_0, T_{peak}, T_3: time-points of glucose level measurement during OGTT-like procedure, i.e. right before glucose loading, at peak level, and 3 hours after glucose loading, respectively. AUC_{0-3}: area under curve from T_0 to T_3; T_0_Tpeak: elevation of blood glucose level from T_0 to Tpeak; Tpeak_T_3: reduction of blood glucose level from Tpeak to T_3 ('–' sign means $T_3 < T_{peak}$); T_0_T_3: change of blood glucose level from T_0 to T_3 ('–' sign means $T_3 < T_0$). The differences between groups are statistically significant when $p < 0.05$.



	0	0.5	1	2	3
(-) control	4.69	7.76	6.80	6.78	4.85
Pioglitazone 30 mg	4.51	7.69	7.10	6.02	4.23
DLBS 50 mg	4.43	7.69	6.71	6.56	4.65
DLBS 100 mg	4.48	7.42	7.46	6.02	4.51
DLBS 200 mg	4.59	8.52	7.58	6.40	4.40
DLBS 300 mg	4.57	8.45	7.80	6.81	4.32
DLBS 400 mg	4.96	8.32	7.21	6.91	4.67

Figure 1: Plasma glucose profile of all subjects after ingestion of 75 g glucose solution with or without DLBS3233. No statistically significant differences between groups (as analyzed by related-anova) were found.

Safety Variables

Safety analyses were performed on all exposed subjects who had received at least one dose of trial products. The safety assessment was based on plasma glucose profile, vital signs, biochemistry safety parameters (SGPT and creatinine for liver and kidney function, respectively) and adverse events.

Statistical Analysis

Evaluation on plasma glucose profile was performed based on per-protocol analyses. The endpoints included: 1) area under the curve of plasma glucose level versus time, from T_0 to T_3 (AUC_{0-3}); 2) change (elevation) of plasma glucose level from T_0 to time when the level peaked (T_{peak}); 3) change (reduction) of plasma glucose level from T_{peak} to T_3; 4) change of plasma glucose level from T_0 to T_3. All endpoints were analyzed between groups by ANOVA. The levels of SGPT and creatinine at the end of study were statistically compared to baseline (before receiving any study treatment) by paired-t-test. All statistical tests were executed at the significance level (α) of 5%. All adverse this study was evaluated descriptively.

Results

Subjects' Characteristics

A total of 6 healthy volunteers were enrolled in the study conducted in August to November 2009. They all completed their participation in the study. Data pertaining to each of them were evaluable for per-protocol safety analyses. Demographic and baseline plasma glucose of the subjects are summarized in Table 1.

Glucose level profile

The effect of DLBS3233 on glucose level profile after an oral loading of 75-gr glucose as compared to that of the negative control and active control (pioglitazone) are elaborated below.

Glucose level profiles were statistically comparable between all groups. ANOVA analyses on plasma glucose levels at each time-point (T_0, $T_{0.5}$, T_1, T_2, and T_3) resulted in no significant difference between all treatments (Figure 1). Elevation of plasma glucose level from baseline to peak(T_0_T_{peak}), reduction of plasma glucose level from peak

Table 3: Laboratory safety parameters before and after treatment

Variable	Baseline		Post Treatment			Paired t-Test 95% CI	
	Mean	SD	Mean	SD	p	Lower Bound	Upper Bound
SGPT (U/L)	15.4	5.90	18.6	4.72	0.22	-9.37	2.97
Serum creatinine (μmol/L)	54.8	7.1	54.8	3.5	1.00	-7.96	7.96

Total number of study subjects, N= 6; SD: standard deviation; CI: confidence interval; SGPT: serum glutamate-piruvate transaminase. Baseline: before the first course of study treatment were initiated; Post-treatment: after the completion of all 7 courses over the 10 weeks of study period. The differences between groups are statistically significant when $p < 0.05$.

Table 4: Anthropometry and Vital Signs at baseline and after each treatment regimen

Group	Mean	SD	ANOVA p	Mean	SD	ANOVA p
n per group = 6	**BW – kg**			**BMI –kg/m^2**		
Baseline	54.33	8.41	0.558	21.45	1.27	0.063
(–) Control	53.33	8.77		21.07	1.37	
(+) Control	53.00	8.77		20.93	1.35	
50 mg	53.00	8.69		20.93	1.35	
100 mg	53.00	8.62		20.93	1.34	
200 mg	53.33	8.78		21.07	1.38	
300 mg	53.42	8.71		21.10	1.36	
400 mg	53.58	9.27		21.16	1.44	
	SBP – mmHg			**DBP – mmHg**		
Baseline	110.00	6.33	0.129	73.33	8.17	0.448
(–) Control	101.67	7.53		71.67	4.08	
(+) Control	106.67	8.17		68.33	4.08	
50 mg	106.67	8.17		71.67	4.08	
100 mg	103.33	5.16		70.00	0.00	
200 mg	106.67	5.16		70.00	0.00	
300 mg	103.33	10.33		73.33	5.16	
400 mg	103.33	5.16		71.67	4.08	
	Pulse – /min			**RR – /min**		
Baseline	82.67*	3.50	0.013	18.33	1.51	0.567
(–) Control	78.67	1.60		18.33	0.82	
(+) Control	78.33	2.34		18.33	0.82	
50 mg	79.00	2.10		18.50	0.83	
100 mg	78.00*	1.79		18.00	1.27	
200 mg	79.33	2.73		19.33	1.03	
300 mg	80.67	2.73		18.67	1.03	
400 mg	82.33	3.88		18.67	1.03	

n: number of subjects; Baseline values were measured at screening, while each group's values were measured right before the conduct of OGTT-like procedure in each study course. SD: standard deviation; CI: confidence interval; BW: body weight; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; RR: respiratory rate; (–) Control: no treatment; (+) Control: pioglitazone 30 mg; 50, 100, 200, 300, 400 mg are the doses of DLBS3233 in each group, respectively; *: the differences between the groups are statistically significant when $p < 0.05$.

to the end of the plasma glucose level profile (T_{peak}_T_3), and reduction of blood glucose level from baseline to T_3 (T_0_T_3) were all not statistically different between groups (Table 2). Despite a statistical difference of area under the curve (AUC_{0-3}) between the groups of "100-mg-DLBS3233" and "400-mg-DLBS3233" (Table 2), all glucose level profiles in resulted from the study demonstrated DLBS3233's safet healthy normoglycemic non-obese subjects.

Table 5: Adverse events

	Frequency of events (no. of subjects exposed)							
Description of AE	**(–) Control**	**(+) Control**	**50 mg**	**100 mg**	**200 mg**	**300 mg**	**400 mg**	**ADR**
Urticaria	-	-	-	-	-	-	1(1)	probable
Muscle discomfort	-	-	1(1)	-	-	-	-	no
Muscle pain	1(1)	-	1(1)	-	-	-	-	no
Dizziness	-	3(1)	3(3)	-	2(1)	5(2)	-	possible
Headache	2(2)	2(2)	2(2)	2(2)	-	-	2(2)	no
Vertigo	-	1(1)	-	-	-	-	-	no
Allergic conjunctivitis	-	1(1)	-	-	-	-	-	no
Blepharitis	-	-	1(1)	-	-	-	-	no
Drowsiness	-	-	-	1(1)	1(1)	-	-	no
Abdominal pain – Epigastric pain not food related	-	1(1)	-	-	-	-	-	no
Diarrhoea	-	-	1(1)	-	-	-	-	no
Flatulence	-	1(1)	-	-	-	-	-	no
Gastritis	-	-	-	-	2(1)	-	-	no
Gingivitis	-	-	-	1(1)	-	-	-	no
Nausea	-	1(1)	3(3)	3(3)	3(3)	1(1)	-	no
Vomiting	-	1(1)	1(1)	-	-	-	-	no
Palpitation	-	1(1)	-	1(1)	-	-	-	possible
Coughing	-	-	1(1)	1(1)	-	-	3(3)	no
Asphyxia	-	-	1(1)	-	-	-	-	no
Pharyngitis	-	-	-	1(1)	-	-	1(1)	no
Fatigue	-	-	1(1)	-	-	-	-	no
Feeling cold	-	-	1(1)	-	1(1)	-	-	possible
Fever	-	-	-	1(1)	-	-	-	no
Influenza like syndrome	-	1(1)	-	1(1)	-	-	2(2)	no
Malaise	-	1(1)	2(2)	-	-	-	-	no
Total number of adverse events (no. of subjects exposed)	3(3)	14(3)	19(6)	12(6)	9(3)	6(2)	9(5)	72 (6)

Total number of subjects enrolled (N) = 6; Each subject might experience more than one adverse event. (–) Control: no treatment; (+) Control: pioglitazone 30 mg; 50, 100, 200, 300, 400 mg are the doses of DLBS3233 in each group, respectively ADR, adverse drug reaction = AE which is judged likely to be attributed to DLBS3233.

Laboratory Safety Parameters and Vital Signs

There were neither statistically nor clinically significant changes from baseline to the end of the study within all groups in terms of biochemistry safety parameters, i.e. liver and renal function Table 3), body weight, body mass index, and vital signs (table 4). Statistical difference found in pulse rate was not clinically significant, with the difference between the highest value (at baseline) and lowest value (at dose of 100 mg) being only 4-5 /minute (Table 4).

None of the subjects was withdrawn from the study due to an adverse event. No serious adverse events were reported either. All adverse events reported (table 5) were mild and had been resolved at the end of study.

Discussion

Hypoglycemia is the most potential and feared adverse reaction of diabetes treatment, both the insulin and oral antidiabetic agents. The threat and incidence of hypoglycemia also increase along the attempts to maintain an intensive glycemic control for diabetic patients as recommended by current treatment guidelines.[12] Fear of developing recurrent hypoglycemia has often led to the reduction of antidiabetic medication dosage by diabetic patients, which in turn brings the glycemic control even to a worse state, in fact.[12,13] Hence, besides providing a proper education to the patients regarding all aspects of diabetes, developing an antidiabetic medication which possesses a low-to-negligible risk for the patients to develop hypgolycemia while maintaining its high potency of controlling blood glucose, is of clinical importance and necessity to prevent the recurrence of hypoglycemia.

This was a phase-1 clinical study of DLBS3233DLBS3233 which primarily aimed to evaluate its safety profile, including its adverse potency of inducing hypoglycemia. Besides with negative control (no regimen), the safety profile of DLBS3233 in this study was also compared to

that of pioglitazone, an FDA-approved oral anti-hyperglycemic agent which is classifed as an insulin sensitizer of the class of thiazolidinediones. Insulin sensitizers improve insulin resistance and increase glucose uptake into peripheral tissues by promoting glucose transporter-4 (GLUT-4) translocation to the cellular plasma membrane, which ends up with decreased plasma insulin level. This agent had been selected to be the active control in the study since the mechanism of action of DLBS3233 in lowering blood glucose resembles that of pioglitazone, i.e. through the regulation of the expression of ppar-(peroxisome proliferator-activated receptors)-γ.[14] PPAR-γ is a member of the nuclear hormone receptor superfamily where its function is to regulate the metabolism of lipids by activating expression of key enzymes. It appears to be primarily involved in adipogenesis and regulation of glut-4 that mediates insulin-stimulated glucose transport. In an *in-vitro* study using 3tc-adipocytes cell culture,[6] DLBS3233 showed the capacity to increase PPAR-γ expression similar to that of thiazolidinediones. The upregulation of GLUT-4 expression by DLBS3233 was also found to be greater than that of pioglitazone and of insulin alone.[6]

To evaluate the safety of DLBS3233, other than laboratory safety parameters and adverse events, we also tried to examine its effect on blood glucose profile through a procedure similar to oral glucose tolerance test (OGTT). DLBS3233 and pioglitazone were administered for 3 days before the oral loading of 75-g glucose solution (or OGTT-like procedure) was performed. The OGTT-like procedure itself was conducted after the subjects had been fasting for 10 hours. In the light of insulin sensitizer's mechanism of action, both products would theoretically not affect blood glucose levels (OGTT profile) if they were taken by normoglycemic non-obese healthy subjects, which also would show their safety in terms of lower risk of hypoglycemia. This would not be the case with an insulin-secretagogue, which inherently (due to its pharmacological action) has more potential risk for subjects to undergo hypoglycemia.[15,16] In non-obese, normoglycemic subjects, insulin sensitizing is unlikely to make a noticeable effect. Insulin-signaling pathway through which translocation of GLUT-4 to the plasma membrane is stimulated also functions well in such subjects so that sensitizing the pathway will not result in a clinically significant effect.

As the evidence to that theoretical hypothesis, in the study we also found statistically comparable plasma glucose profiles between DLBS3233 and the negative control, at all evaluated regimens. Such comparable plasma glucose profiles were also found between pioglitazone (the active control) and the negative control. This result may indirectly corroborate the mechanism of action of DLBS3233 as an insulin sensitizer rather than secretagogue.

Elevation of plasma glucose level from baseline to peak (T_0_T_{peak}), reduction of plasma glucose level from peak to the end of the plasma glucose level profile (T_{peak}_T_3), and reduction of blood glucose level from baseline to T3 (T_0_T_3) were not statistically different between all groups (Table 2). Also since no significant difference was found in the AUC_{0-3} between any doses of DLBS3233 with the negative control as well as with the positive control (Table 2), a significant difference observed in AUC_{0-3} between "100 mg DLBS3233" and "400 mg DLBS3233" consequently has no significant clinical interpretation. In other words, both pioglitazone and DLBS3233 at any evaluated dosage regimens did not exert its effect on blood glucose in healthy normoglycemic and non-obese subjects. Therefore, it is also safe to say that DLBS3233 is less likely to risk subjects for undergoing hypoglycemia.

Such results were also consistent with the result of further analyses on fasting-blood glucose before and after each treatment (Figure 1) which also demonstrated no significant statistical difference between groups. Again, the interpretation that several doses of DLBS3233 (from 50 mg up to 400 mg once daily for 3 days) did not affect fasting blood glucose levels of healthy subjects, demonstrated the negligible risk of DLBS3233 in developing hypoglycemia.

DLBS3233 extract was well tolerated with no serious adverse event related to DLBS3233 consumption was found (Table 5). In terms of weight gain, one of the common adverse events found with certain insulin sensitizers, DLBS3233 did not seem to bring such an undesirable effect. All other vital signs also showed no significant changes along the study period (Table 4).
During the study, all enrolled subjects experienced various adverse events which totted up to 72 events, including dizziness, feeling cold and palpitation which are considered to be associated with hypoglycemia, and thus possibly related to the study product or the active control, pioglitazone. A total of 17 episodes (23.6%) of those symptoms were reported by 3 subjects (13 episodes of dizziness by 3 subjects, 2 episodes of feeling cold by 1 subject, and 2 episodes of palpitation by 1 subject) (Table 5). All those hypoglycemic-associated symptoms occurred in irregular manner (not dose-dependent) a few while after taking either the investigational product or the positive-control (pioglitazone), but were all only mild and transient. Nevertheless, such symptoms were not experienced by the respective subjects while they were being enrolled in negative control group, making the symptoms considered as possibly related to the investigational product. One subject inadvertently took one dose of 550 mg of DLBS3233 which was pretty much higher than the highest regimen in the study, and experienced mild urticaria only on the day the subject took such a high dose, which thus was judged to be attributed to the product. However, the subject did not experience any hypoglycemic event even at the high dose of DLBS3233. All other events reported were unlikely to be related to the product. All adverse events were reported as mild in severity, and at the end of the study they were all already resolved.

Based on the safety profile demonstrated by DLBS3233, the product was safe and well-tolerated by healthy normoglycemic and non-obese subjects. This study result provided a favorable ground for us to conduct the next

clinical studies, particularly in order to evaluate the product's efficacy as well as its safety in type-2 diabetic subjects.

We concluded that this study demonstrated the negligibly low risk of DLBS3233 to induce hypoglycemia and that the extract was safe and well tolerated. The result of this study and previous preclinical studies altogether provide us a firm ground to further conduct efficacy studies of the extract.

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