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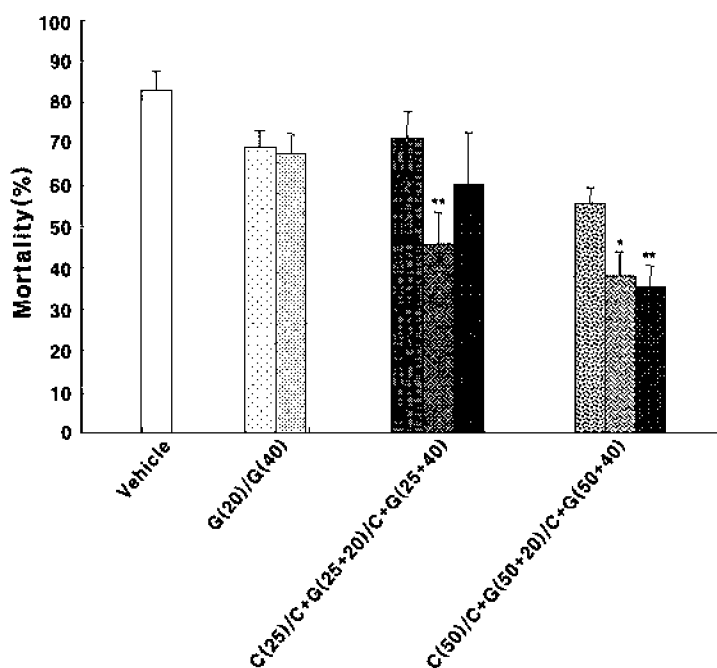
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(54) Title: COMBINATION DRUG OF CILOSTAZOL AND GINKGO BILOBA EXTRACTS



(57) Abstract: The present invention relates to a pharmaceutical composition comprising cilostazol and ginkgo biloba extract, and particularly to a pharmaceutical composition where cilostazol is contained as an anti-thrombotic agent in combination with a ginkgo biloba extract with PAF- inhibitory and antioxidant activities, thereby enabling to increase anti-thrombotic efficacy while decreasing side effects such as headache and cardiovascular disorders by inhibiting platelet aggregation.



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Description

COMBINATION DRUG OF CILOSTAZOL AND GINKGO BILOBA EXTRACTS

Technical Field

- [1] The present invention relates to a pharmaceutical composition comprising cilostazol and ginkgo biloba extract.

Background Art

- [2] Cilostazol is a quinolinone-based compound, which inhibits platelet aggregation and promotes vasorelaxation by hampering phosphodiesterase type III. It is known that cilostazol inhibits the ADP- or epinephrine-induced primary aggregation of platelet separated from a mouse, a rat, a rabbit, a dog or a man, and dissociates platelet aggregate. Further, it inhibits ADP- or collagen-induced platelet aggregation when orally administered to a beagle dog, and also suppresses the platelet aggregation induced by ADP, collagen, arachidonic acid and epinephrine when orally administered to a patient suffering from chronic arterial occlusive disease. The inhibitory activity of cilostazol is immediately expressed to human, and maintained even after repeated administration. When the administration of cilostazol is stopped, the platelet aggregation increases to pre-administration level due to the decrease in the concentration of cilostazol without showing any rebound phenomenon.
- [3] It also shows an anti-thrombotic activity, i.e., suppresses the death caused by pulmonary embolism when ADP or collagen is intravenously injected into a mouse. Cilostazol inhibits the progress of thrombotic stunning induced by the administration of sodium laurate in femoral artery of a dog, and also suppresses thrombotic occlusion on the surgery region when femoral artery of a dog is replaced with artificial blood vessel.
- [4] With regard to vasodilatory activity, cilostazol relaxes an isolated femoral artery of a dog, which is contracted by potassium chloride or prostaglandin F₂ alpha, and increases blood flow in femoral artery of an anesthetize dog. It increases the tissue blood flow in the foot joint and the calf of the leg of a patient suffering from chronic arterial occlusive disease as ascertained by a plethysmography, and also causes the increase in temperature of cutaneous limb and skin blood flow as ascertained by thermography.
- [5] The mechanism of action of cilostazol is follows. It inhibits the release of serotonin in platelet of a rabbit without affecting the entrance of serotonin and adenosine into platelet. It inhibits the platelet aggregation caused by TXA₂ (thromboxane A₂) without affecting the metabolism of arachidonic acid in platelet. This is because it inhibits the

activity of cAMP-PDE(cyclic AMP phosphodiesterase) in platelet and vascular smooth muscle, thereby inducing anti-platelet activity and vasodilatory activity. However, cilostazol is limited in the clinical use because it is relatively weaker than ticlopidine or clopidogrel in anti-platelet activity and cilostazol causes side effects of a serious headache probably due to its vasodilatory activity. Further, it also affects cardiovascular system in an animal test. That is, when intravenously injected or orally administered, it induces cardiac burden by decreasing the blood pressure and increasing heart rate, contractile force, oxygen consumption of cardiac muscle and tidal rate, thereby causing or deteriorating heart problems. Thus, there have been needs for resolving the aforementioned problems.

- [6] Ginkgo biloba extract have been used for centuries for the treatment of bronchial asthma or bronchitis in China. In Western Europe including Germany and France, clinical studies have reported positive outcomes when used in patients suffering from peripheral blood disorders and brain failure. It is also reported that there is almost no side effect although used for a long period of time, and the mechanism of action is also well known. Ginkgo biloba extract comprises 24% flavonoid and 6% terpenoid.

[7]

Disclosure of Invention

Technical Problem

- [8] Therefore, the present inventors have exerted extensive researches to overcome the aforementioned problems of a single therapy of cilostazol such as low anti-platelet activity, headache and other side effects on heart. As a result, they have developed an anti-thrombotic pharmaceutical composition, which comprises cilostazol and ginkgo biloba extract in a predetermined ratio and may maximize platelet aggregation, while improving one of major side effects of cilostazol (headache), thereby finally completing the present invention.

- [9] Accordingly, the present invention aims to provide an anti-thrombotic pharmaceutical composition comprising cilostazol and ginkgo biloba extract.

Technical Solution

- [10] The present invention relates to an anti-thrombotic pharmaceutical composition comprising cilostazol and ginkgo biloba extract.

Advantageous Effects

- [11] A pharmaceutical composition of the present invention employs a combinatory therapy of cilostazol and ginkgo biloba extract in a predetermined ratio, thereby enabling to maximize the inhibition of platelet aggregation and decrease the side effects of cilostazol. Thus, a pharmaceutical composition herein is expected as very useful as an anti-thrombotic agent.

Brief Description of the Drawings

- [12] Figure 1 shows the inhibition of platelet aggregation by the addition of cilostazol and ginkgo biloba extract in human blood.
- [13] Figure 2 shows the decrease in fatality rate (dead or paralyzed population/the total number of mice administered) by the addition of cilostazol and ginkgo biloba extract in an ICR mouse model, where a thrombus was induced by collagen.
- [14] Figure 3 shows the inhibition of shear-induced platelet aggregation (SIPA) [* : significant difference from the cilostazol administration group ($P < 0.05$); #: significant different from the group of ginkgo biloba extract ($P < 0.05$); C: cilostazol (μM), G: ginkgo biloba extract ($\mu\text{m/mL}$)].
- [15] Figure 4 show the inhibition of SIPA by the combination drug of cilostazol and ginkgo biloba extract ($n=3$).
- [16] Figure 5 shows the influence on the PS exposure induced by shear stress.
- [17] Figure 6 shows the inhibition of arterial thrombosis by cilostazol ($n=4$) and ginkgo biloba extract ($n=4$) [* : significant difference from the cilostazol administration group ($P < 0.05$); #: significant difference from the group of cilostazol or ginkgo biloba extract ($P < 0.05$). C: cilostazol (μM), G: ginkgo biloba extract ($\mu\text{m/mL}$)].
- [18] Figure 7 shows the inhibition of an arterial thrombosis in a rat.
- [19] Figure 8 shows the inhibition of collagen-induced platelet aggregation in a beagle dog [N.S.: no significant difference from the vehicle group; (a): significant difference from the vehicle group ($P < 0.05$); (b): significant difference from the sum of G(4)+C(5) ($P < 0.05$)].
- [20] Figure 9 shows the effect of drugs on bleeding time in an ICR mouse [$P < 0.05$ as compared to the vehicle group; no significant difference in BT between a single administration group of cilostazol and a combination administration group of cilostazol and ginkgo biloba extract].
- [21] Figure 10 shows a profile of headache outbreak as a function of time in a single administration group of cilostazol (CIL) and a combination administration group of cilostazol and ginkgo biloba extract (GETC).
- [22]
- [23]

Mode for the Invention

- [24] Hereunder is provided a detailed description of the present invention.
- [25] The present invention relates to a pharmaceutical composition comprising cilostazol and ginkgo biloba extract, and particularly to a pharmaceutical composition where cilostazol is contained as an anti-thrombotic agent in combination with ginkgo biloba extract with PAF-inhibitory and antioxidant activities, thereby enabling to increase

anti-thrombotic efficacy while decreasing side effects such as headache and cardiovascular disorders by inhibiting platelet aggregation.

- [26] Ginkgo biloba extract used in the present invention may be prepared according to various known processes such as, for example, (i) the conventional solvent extraction using a mixed solvent of water and lower alcohol or lower ketone, or (ii) a combination extracting process comprising the steps of extracting with alkaline aqueous solution and extracting with a mixed solvent of lower alcohol and lower acetate or lower ketone, or (iii) a supercritical fluid extraction.
- [27] It is preferred that thus prepared ginkgo biloba extract comprise more than 15 wt% of flavone glycoside, and more preferably they contain particularly flavone glycoside, terpene lactone and alkylphenol compounds.
- [28] Ginkgo biloba extract generally contain flavone glycoside, and are reported to have an antioxidant and cell-protective activities. Further, they are also known to have the activity of inhibiting the PAF- and ADP-induced platelet aggregation due to the terpene lactone along with cardiovascular activity such as a vasorelaxation activity.
- [29] It is assumed that, in the present invention, ginkgo biloba extract decrease the side effects of cilostazol by maintaining vasotonia and showing an antioxidant activity, while reinforcing the inhibition platelet aggregation induced by cilostazol.
- [30] In the present invention, it is preferred that ginkgo biloba extract is contained in the amount of 10-480 weight parts, more preferably 10-200 weight parts relative to 100 weight parts of cilostazol. When the amount is less than 10 weight parts, the effect may not be sufficient. When the amount is more than 480 weight parts, various side effects may be observed.
- [31] The present invention also include a combinational therapy of cilostazol and ginkgo biloba extract, which enhances an anti-thrombotic activity by increasing the activity of inhibiting the platelet aggregation, for the prevention or treatment of various thrombus-induced disorders such as ischemic cerebrovascular disorders, coronary artery disease, chronic arterial occlusive disease, an embolus, intermittent claudication and a thrombus after vascular surgery and retinal hemorrhage surgery.
- [32] A pharmaceutical composition of the present invention may be formulated into a dosage form suitable for via-intestine administration, i.e., oral administration, in particular a tablet, an effervescent tablet, granules and a capsule. The pharmaceutical composition is preferred to be a unit dosage form, which may comprise a pre-determined amount of cilostazol and ginkgo biloba extract. Diluents, carriers or other excipients may also be contained according to the conventional method. For example, a tablet may comprise any conventional granulating agent such as diluents, binding agents, disintegrants, lubricants, stabilizers, coloring agents, light-proofing agents, sweetening agents and flavors besides an active filler. Further, other formulating

methods may be utilized for preparing tablets with separated layers when drugs may be unstable due to the interaction between drugs.

[33]

[34] *Preferable content of cilostazol in the formulated unit dosage form is 50-100 mg. Preferable content of ginkgo biloba extract is 40-80 mg. Although daily dosage may vary depending on the administration method or the treatment conditions, administration of 2-4 times daily is preferred for an adult.

[35]

[36] **Example 1: Measurement of anti-platelet activity of combination drug in human blood (platelet rich plasma)**

[37] Blood sample was taken from a donor in the morning of the test day, and treated with 0.38% sodium citrate to prevent blood coagulation. PRP layer was obtained by centrifuging the sample, and blood coagulation was observed by tracing transmittance for 4 minutes with a platelet aggregation analyzer 3 minutes after vehicle and test drug were added, followed by the addition of collagen (an agonist). Figure 1 shows the degree of aggregation inhibition when two kinds of combinational therapy comprising 20 $\mu\text{m/mL}$ or 40 $\mu\text{m/mL}$ ginkgo biloba extract [Ginexin, SK Chemicals] along with a fixed concentration of cilostazol (4 μM).

[38] It may be ascertained that blood coagulation was significantly decreased by the combinational therapy of cilostazol and ginkgo biloba extract as compared to the cilostazol single treatment.

[39]

[40]

[41] ***Example 2: Measurement of anti-thrombotic activity of combination drug in an ICR mouse**

[42] <Preparation of combination drug and administration>

[43] Combination drug was prepared by suspending ginkgo biloba extract (Ginexin) and cilostazol in 0.5% carboxymethyl cellulose (CMC) just before the administration.

[44] A mixture of collagen and epinephrine was used as a thrombus-inducing material. A collagen solution (5% glucose isotonic solution, pH 2.7) and an epinephrine solution (diluted in a physiologic saline injection) was mixed in a volumetric ratio of 1:1. The final concentrations of collagen and epinephrine were 50 $\mu\text{m/kg}$ and 5 $\mu\text{m/kg}$, respectively, at administration. The solution was mixed by a soft shaking, and used within 2 hours from the preparation.

[45] <Measurement of thrombus>

[46] ICR 6.5-week-old male mice were selected as a test subject, and used in the test after adaptation to test conditions for 5 days.

[47] The mice were fasted for 15 hours, and divided into two groups, i.e., a negative

control group and a test control group. The two groups were administered once with 0.5% carboxymethyl cellulose (CMC) solution and a test solution (a solution prepared by dissolving test drugs in 0.5% CMC), respectively. An hour after the administration, the mice were immobilized in a holder, and a thrombus-inducing mixture (collagen+epinephrine) was intravenously into their tails. They were observed until 15 minutes after the intravenous injection, and the state of death or paralysis (no movement only with heartbeat) was considered as fatal. Resulting fatality rate (fatal population/the total number of mice administered) and survival rate (the number of survivors/the total number of mice administered) are provided in Table 1 below.

[48] Table 1

Drug	Dose of administration (mg/kg)	Collagen		
		Fatality rate (%)	Survival rate (%)	Increase in survival rate (%)
Vehicle	-	82.9 ± 10.7	17.1 ± 10.7	-
Ginkgo biloba extract	20	69.4 ± 7.9	30.6 ± 7.9	13.5
	40	67.6 ± 10.3	32.4 ± 10.3	15.3
Cilostazol+	25	71.2 ± 15.0	28.8 ± 15.0	11.7
Ginkgo biloba extract	25 + 20	45.8 ± 17.2 **	54.2 ± 17.2	37.1
	25 + 40	60.3 ± 27.9	39.7 ± 27.9	22.6
Cilostazol+	50	55.8 ± 8.2	44.2 ± 8.2	27.1
Ginkgo biloba extract	50 + 20	38.0 ± 12.9 *	62.0 ± 12.9	44.8
	50 + 40	35.3 ± 11.8 **	64.7 ± 11.8 **	47.6

[49] As shown in Table 1, when a mixture of collagen and epinephrine was intravenously injected into a mouse, a thrombus is induced and the mouse dies or is paralyzed within 15 minutes after the injection. A single therapy of cilostazol or ginkgo biloba extract inhibited a thrombus induced death although the efficacy was not satisfactory. However, a combination therapy of cilostazol and ginkgo biloba extract significantly reduced fatality rate, thus ascertaining that a thrombus was effectively inhibited. Further, when compared to the survival rate of the negative control group (17.1%) as a baseline, all the combination therapies showed higher efficacy than the simple sum of single therapies of cilostazol and ginkgo biloba extract, except one combination therapy, i.e., cilostazol (25 mg/kg) and ginkgo biloba extract (40 mg/kg). Furthermore, the combination therapies showed higher efficacy even than a single therapy of cilostazol of twice amount (50 mg/kg).

[50] As described above, although anti-platelet activity of a combination drug herein comprising cilostazol and ginkgo biloba extract may vary in human blood (platelet rich plasma) depending on the test conditions, the combination drug showed remarkably

higher inhibition of blood coagulation than the addition of the effects of the cilostazol single group and the ginkgo biloba extract single group. In particular, the combination therapy of cilostazol and ginkgo biloba extract enhances anti-platelet activity more than the cilostazol single group of twice the concentration. Therefore, the combination therapy of cilostazol and ginkgo biloba extract is expected to have superior efficacy while decreasing side effects by supplementing the comparatively weak activity of inhibiting platelet aggregation.

[51]

[52] **Example 4: Effect of inhibiting SIPA (shear-induced platelet aggregation) and anti-thrombotic activity**

[53] Effect of combination therapy of cilostazol (C) and ginkgo biloba extract (G) on SIPA was observed. The combination therapy used in the test was the following two sets: (i) cilostazol 4 μ M + ginkgo biloba extract 40 μ m/mL and (ii) cilostazol 8 μ M + ginkgo biloba extract 80 μ m/mL. As compared to the single group of cilostazol or the single group of ginkgo biloba extract, the effect of aggregation inhibition increased in both the sets of combination therapy [Figures 3 and 4].

[54]

[55] **Example 5: Effect on exposure of PS**

[56] Effect of cilostazol (C) and ginkgo biloba extract (G) on the exposure of PS (phosphatidyl inositol) induced by shear stress was observed [Figure 5]. It was ascertained that neither cilostazol nor ginkgo biloba extract affects the exposure of PS. The exposure of PS was not influenced by combination of the two drugs either.

[57]

[58] **Example 6: Effect of inhibiting arterial thrombosis**

[59] Efficacy of cilostazol (C) and ginkgo biloba extract (G) was investigated in an arterial thrombosis animal model. When cilostazol was administered in the amount of 25, 50 and 75 mg/kg, respectively, a concentration-dependent inhibition of a thrombus was observed. When ginkgo biloba extract were administered in the amount of 20 and 40 mg/kg, respectively, the generation of a thrombus was significantly inhibited, thereby extending occlusion time [Figure 6].

[60]

The effect of the combination therapy of cilostazol and ginkgo biloba extract on arterial thrombosis was studied based on the aforementioned results. Three sets of combination therapy was used (cilostazol 25 mg/kg + ginkgo biloba extract 20 mg/kg; cilostazol 50 mg/kg + ginkgo biloba extract 40 mg/kg; and cilostazol 50 mg/kg + ginkgo biloba extract 20 mg/kg) to investigate optimum effect of the combination administration depending on the concentration variation. A combination therapy (cilostazol 25 mg/kg + ginkgo biloba extract 20 mg/kg) significantly decreased the generation of thrombus while two therapies of each single drug have no such efficacy.

Another combination therapy (cilostazol 50 mg/kg + ginkgo biloba extract 40 mg/kg) synergistically increased the effect of inhibiting thrombus, although two therapies of each single drug show such efficacy. Still another combination therapy (cilostazol 50 mg/kg + ginkgo biloba extract 20 mg/kg) showed remarkably higher effect of inhibiting thrombus than a single therapy of cilostazol [Figure 7]. Therefore, it was ascertained that the aforementioned three sets of combination therapy increase the effect of inhibiting arterial thrombosis.

[61]

[62] **Example 7: *Ex vivo* test of inhibition of platelet aggregation using beagle dog**

[63] A beagle dog was administered with each drug, and blood was separated at pre-determined intervals after the administration.

[64] Thus obtained PRP was treated with collagen and aggregation rate was measured

[65] Drug activity of inhibiting platelet aggregation was calculated by considering the platelet aggregation rate before the administration as 100%.

[66] Collagen-inducing platelet aggregation rate an hour after the administration was measured by using each of single therapies of cilostazol (C) and ginkgo biloba extract (G) and a combination therapy of the two drugs, and the results are presented in Figure 8.

[67] An hour after the administration, the aggregation rate of a combination administration group (cilostazol 5 mg/kg and ginkgo biloba extract 4 mg/kg) was 58.9%, which is significantly increased (ANOVA) as compared to the aggregation rate of a vehicle administration group (110.5%), and a single administration group of ginkgo biloba extract (96.4%) and a single administration group of cilostazol (86.1%). In contrast, the aggregation rates of the two single administration groups of ginkgo biloba extract (4 mg/kg) and cilostazol (5 mg/kg) were 96.4% and 86.1%, respectively, which are not significantly increased as compared to the aggregation rate of a vehicle administration (110.5%) although the inhibition of platelet aggregation was observed.

[68]

[69] **Example 8: Change in bleeding time**

[70] The effect of the combination drug of ginkgo biloba extract and cilostazol on BT (bleeding time) was investigated. Each drug was orally administered to C57BL/6 mice once, and the end of a tail was cut an hour after the administration.

[71] The effect of drugs on bleeding was evaluated by measuring how long it takes bleeding to stop due to the platelet aggregation.

[72] As compared to the vehicle administration group, a combination administration of cilostazol (C) 25 mg/kg and ginkgo biloba extract (G) 20 mg/kg showed an increase in BT by 157% (from 311 seconds to 490 seconds), which is not statistically significant. Another combination administration (cilostazol 25 mg/kg and ginkgo biloba extract 40

mg/kg), where the amount of ginkgo biloba extract was doubled, showed no significant increase in BT, either (150% increase, from 311 seconds to 469 seconds). Further, still another combination administration (cilostazol 50 mg/kg and ginkgo biloba extract 40 mg/kg), where the amounts of both the drugs were doubled, showed no significant increase in BT, either (156% increase, from 311 seconds to 487 seconds). However, when Aspirin 30 mg/kg was administered as a positive control, BT was ascertained to be significantly increased by 219% (from 311 seconds to 683 seconds) as compared to the vehicle control [Figure 9].

[73] As described above, a combination therapy of ginkgo biloba extract and cilostazol was statistically similar to the positive control group in the BT increase as compared to the vehicle group. Thus, it is expected that the combination therapy will cause no bleeding as a side effect.

[74]

[75] Example 9: Effect on APTT and PT

[76] In this test the effect of a combination therapy on blood coagulation cascade is evaluated with regard to the bleeding side effect. A combination drug used herein is GETC 45 comprising ginkgo biloba extract and cilostazol, which are known as an anti-platelet agent and an inhibitor of platelet aggregation, respectively, in the ratio of 4:5.

[77] The drugs were orally administered to SD rats and blood was taken. Plasma was separated from the blood and the measurement of APTT (activated partial thromboplastin time) and PT (prothrombin time) was performed.

[78] GETC 45 (each dose: 100, 300 and 900 mg/kg) showed no significant difference in APTT and PT as compared to the negative control group.

[79] The findings showed that the administration of GETC 45 does not affect the APTT and PT indicates that GETC 45 is a safe drug without affecting blood coagulation and having bleeding side effect

[80]

[81] **Example 10: Effect on hepatic metabolizing enzyme**

[82] Cilostazol is metabolized by cytochrome P 450 (CYP) enzyme system, which is composed with various enzymes such as CYP1A2, 2C8, 2C19, 3A4 and 2D6, through hydroxylation and dehydration.

[83] Therefore, the generation of any metabolite is not completely inhibited by each isozyme selective inhibitor, and most selective inhibitors are ascertained to affect the generation of cilostazol metabolites (M1-M11).

[84] In general, ginkgo biloba extract inhibit CYP isozymes, and in particular the inhibitory activity against enzymes in CYP2C family was relatively high.

[85] The production of cilostazol metabolites was observed after the treatment of ginkgo biloba extract (G) and cilostazol (C). As a result, the production of all the metabolites

was inhibited in a similar manner. The inhibitory activity of ginkgo biloba extract on the cilostazol metabolism showed relatively high IC_{50} value of about 40-50 μ M/mL.

[86] Ginkgo biloba extract contains about 10% of quercetin and about 2% of ginkgolide B, which is calculated based on plasma concentration of the indicator materials of ginkgo biloba extract. It is reported that, when an onion containing 68 mg of quercetin and 80 mg of ginkgo biloba extract were intaken, the maximum plasma concentration of quercetins increases to 200 ng/mL and about 20 ng/mL, respectively.

[87] When ginkgo biloba extract 80 mg is administered in combination with cilostazol, the calculated maximum plasma concentration is assumed to be about 240 ng/mL on the supposition that ginkgo biloba extract contains only quercetin. The calculated maximum plasma concentration is about 1,000 ng/mL on the supposition that ginkgo biloba extract contains only ginkgolide B.

[88] It is assumed that there is no possibility of interaction between drugs when an IC_{50} value is higher than 10 μ M in *in vitro* test. The concentration that corresponds to 10 μ M is 5 μ M/mL even when considering conservatively that average molecular weight of ginkgo biloba extract is 500. Thus, it may be assumed that there is little possibility of interaction between drugs when ginkgo biloba extract is administered in combination with cilostazol.

[89]

[90] **Example 11: Pharmacokinetic Behavior in a body of a beagle dog**

[91] To study the interaction between cilostazol and ginkgo biloba extract, the drugs were administered into a beagle dog in combination, and the pharmacokinetic change of cilostazol was investigated.

[92] A method of analyzing the plasma concentration of cilostazol in a beagle dog was established and validated by using LC-MS/MS. According to the Latin square design, drugs were administered three times with twice washing-out periods. After a single administration of cilostazol, a combination administration with lower-concentration ginkgo biloba extract (8 mg/kg) and a combination administration with higher-concentration ginkgo biloba extract (16 mg/kg), plasma was analyzed. It was assumed that there is no carryover effect by the previous administration because, just before the administration after washing-out period, plasma concentration of cilostazol was lower than limit of quantification in all cases.

[93] A single administration group, a combination administration group of lower-concentration ginkgo biloba extract and a combination administration group of higher-concentration ginkgo biloba extract were similar in average maximum plasma concentration (C_{max}), i.e., 417, 418 and 499 ng/mL, respectively. The aforementioned three groups were also similar in time to average maximum, i.e., 1.75, 1.67 and 1.67 h.

There was no significant different among the groups in $t_{1/2}$ (2.55, 2.21 and 2.29 h) and AUC (1610, 1410 and 1660 ng h/mL). Apparent oral clearances (CL/F) of a single administration group of cilostazol, a combination group of lower-concentration ginkgo biloba extract and a combination group of higher-concentration ginkgo biloba extract were 131, 146 and 119 mL/min/kg, respectively. There was no significant difference in all the pharmacokinetic efficient of cilostazol among a single administration group of cilostazol, a combination group of lower-concentration ginkgo biloba extract and a combination group of higher-concentration ginkgo biloba extract [Table 2].

[94] Therefore, two doses (8 mg/kg and 16 mg/kg) of ginkgo biloba extract does not affect the pharmacokinetics of cilostazol (dose: 10 mg/kg) in a beagle dog, which indicates that a combination administration causes no interaction between drugs.

[95] Table 2

Classification	A cilostazol single administration group	Lower-concentration ginkgo biloba extract administration group (8 mg/kg)	Higher-concentration ginkgo biloba extract administration group (16 mg/kg)
C _{max} (ng/mL)	417 ± 290	418 ± 168	499 ± 280
T _{max} (h)	1.75 ± 1.25	1.67 ± 0.82	1.67 ± 0.516
T _{1/2} (h)	2.55 ± 1.10	2.21 ± 0.860	2.29 ± 1.28
CL/F	131 ± 62.7	146 ± 95.6	119 ± 45.6
AUC(ng)	1610 ± 972	1410 ± 508	1660 ± 841

[96] **Example 12: Effect of mitigating headache by cilostazol**

[97] One of the side effects of cilostazol is headache, which is considered as a result of the vasorelaxation activity of cilostazol. It was assumed that ginkgo biloba extract may alleviate the side effect of cilostazol by maintaining cerebrovascular contractile force, considering that ginkgo biloba extract has an activity of maintaining vasotonia.

[98] A double blind test and a cross over test were conducted as a pilot test with healthy adults (20-40 years old). Either of cilostazol 100 mg (CIL) or a combination drug (cilostazol 100 mg + ginkgo biloba extract 80 mg; GETC) was orally administered once, and the washing-out period was a week long. Two test subjects among 28 volunteers failed to proceed further, and the results of the remaining 26 subjects were analyzed [Figure 10].

[99] The cilostazol administration group showed 54% of grade 2, while it was 35% in the combination drug administration group. Cilostazol caused no headache in 4% subjects in comparison to 23% in the combination drug.

[100] These results show that the cilostazol-induced headache may be mitigated by the combination therapy with ginkgo biloba extract, which is most likely to be ascertained

in a clinical test.

[101]

[102] **Example 13: Toxicity test**

[103] A) Acute toxicity test

[104] To investigate the toxicity, a combination drug of cilostazol and ginkgo biloba extract was orally administered once into SD (Sprague-Dawley) female-male rat. The tested drug comprises cilostazol and ginkgo biloba extract in the weight ratio of 5:4, and administered at dose of 500, 1000 or 2000 mg/kg. MC (0.5%) was administered to an excipient control group.

[105] Drug administration caused no test subject to die in any group during the entire test period. No abnormal findings were found to be caused by the test drugs in general symptom, weight change and autopsy findings. Thus, it was ascertained that a combination drug of cilostazol and ginkgo biloba extract (5:4) has a minimum lethal dose exceeding 2,000 mg/kg in both male and female rats.

[106]

[107] B) Toxicity test in an ICR mouse by using a composition comprising cilostazol and ginkgo biloba extract

[108] Repeated dose toxicity was conducted by using a composition (1 g) comprising 100 weight parts of cilostazol and 80 weight parts of ginkgo biloba extract. ICR mice (4-5 weeks old, 5 mice in a group) were used. A combination composition (1 g) comprising cilostazol and ginkgo biloba extract dissolved in 0.5% carboxymethyl cellulose (CMC) was orally administered repeatedly for 5 days. No subject died, and no abnormal findings such as organ damage were found.

[109]

[110] C) One-week repeated dose toxicity test

[111] GETC 45 (ginkgo biloba extract : cilostazol = 4:5 weight ratio) was orally administered into SD female and male rats repeatedly for a week for the investigation of brief toxicity. The test material was administered at dose of 250, 500 or 1000 mg/kg/day into 10 rats per group (5 female and 5 male rats). Death rate, general symptom, weight change, feed and water intake, urine test, hematological and serum biochemical test, autopsy findings, organ weight and histopathological findings were investigated, and the following results were obtained.

[112] 1. No death related to the administration of the test material was observed during the entire test period.

[113] 2. No general symptom related to the administration of the test material was observed.

[114] 3. With regard to weight change, no toxicologically harmful change was observed.

[115] 4. With regard to feed and water intake, no significant change related to the admin-

istration of the test material was observed.

[116] 5. With regard to urine test, no toxicological abnormal findings related to the administration of the test material were observed.

[117] 6. With regard to autopsy findings with the naked eye, no abnormal change related to the administration of the test material abnormal was observed.

[118] 7. As a result of hematological test, no change related to the administration of the test material was observed.

[119] 8. As a result of serum biochemical test, the increase in BUN and the decrease in BIL were observed in male and female subject of the 1,000 mg/kg/day administration group due to the administration of the test material, which is concluded as not toxicologically harmful.

[120] 9. As a result of organ weight measurement, absolute and relative weight of intestines was observed in a male group administered at a dose of 250 mg/kg/day or higher and a female group administered at a dose of 500 mg/kg/day or higher.

[121] 10. As a result of the histopathological test, a dose-dependent increase in centrilobular hepatocellular hypertrophy was observed in a group administered at a dose of 500 mg/kg or higher, which was concluded as the adaptive response to extraneous material.

[122]

[123] As described above, when GETC 45 is orally administered into male or female SD rats repeatedly for a week, the increase in the weight of intestines (in a male group administered at a dose of 250 mg/kg/day or higher and a female group administered at a dose of 500 mg/kg/day or higher), the increase in BUN and the decrease in BIL (in male and female subject of the 1,000 mg/kg/day administration group) and centrilobular hepatocellular hypertrophy (in a group administered at a dose of 500 mg/kg/day or higher) were observed, all of which were not toxicologically harmful change.

[124] Therefore, NOEL (no observed adverse effect level) of the test material of the present invention is concluded as 1,000 mg/kg/day for both male and female under the test conditions herein.

[125]

[126] D) Irwin test

[127] For the general pharmacological test, a combination drug of cilostazol and ginkgo biloba extract was orally administered into a mouse. The test material was so prepared that the weight ratio of cilostazol to ginkgo biloba extract is 5:4. Dose of the test material was 180, 540 and 1800 mg/kg, respectively, and an excipient control group was administered with 0.5% MC.

[128] Effect on the test drug on the general behavior and body temperature was investigated. As a result, a temporary decrease in voluntary movement was observed in

the male animal group administered with 1,800 mg/kg of the test material 60 minutes and 120 minutes after the administration. Significant decrease in body temperature was observed 60 minutes and 120 minutes after the administration as compared to the excipient group in the female and male animal group administered with 1,800 mg/kg of the test material. Voluntary movement and body temperature were restored to normal.

[129] However, it is expected that these symptoms will not be observed because the maximum dose of 1,800 mg/kg is about 350 times the prearranged amount in a clinical test (180-360 mg/human/day) and no change in body temperature was not affected by the administration of 540 mg/kg (about 100 times the prearranged amount in a clinical test?).

[130]

[131] E) Effect on respiration

[132] To investigate the effect of a combination drug of cilostazol and ginkgo biloba extract on respiration, rats were orally administered with the combination drug. The test material was so prepared that the weight ratio of cilostazol to ginkgo biloba extract is 5:4. Dose of the test material was 180, 540 and 1,800 mg/kg, respectively. An excipient control group was administered with 0.5% MC.

[133] As a result of the study of the effect on tidal rate and tidal air in a rat, no statistically significant change due to the administration of the test material was observed as compared to the excipient control group.

[134] Considering the aforementioned results, it may be concluded that the test material, i.e., a combination drug of cilostazol and ginkgo biloba extract does not affect the respiratory system even at maximum dose of 1,800 mg/kg.

[135]

[136] **Formulation Example 1: Preparation of powders and capsules**

[137] A composite composition 100 mg comprising cilostazol 100 weight parts and ginkgo biloba extract 80 weight parts was mixed with lactose 14.8 mg, crystalline cellulose 3 mg and magnesium stearate 0.2 mg. The mixture was filled in a gelatin capsule (No. 5) by using an appropriate device.

[138]

[139] **Formulation Example 2: Preparation of ointments**

[140] Ointments comprising the following ingredients were prepared by using a composite composition comprising cilostazol 100 weight parts and ginkgo biloba extract 80 weight parts:

[141] Active ingredient 5 g, cetyl palmitate 20 g, cetanol 40 g, stearyl alcohol 40 g, isopropyl myristate 80 g, sorbitan monostearate 20 g, polysorbate 60 g, propyl paraoxybenzoic acid 1 g, methyl paraoxybenzoic acid 1 g, phosphoric acid and an appropriate amount of distilled water.

[142]

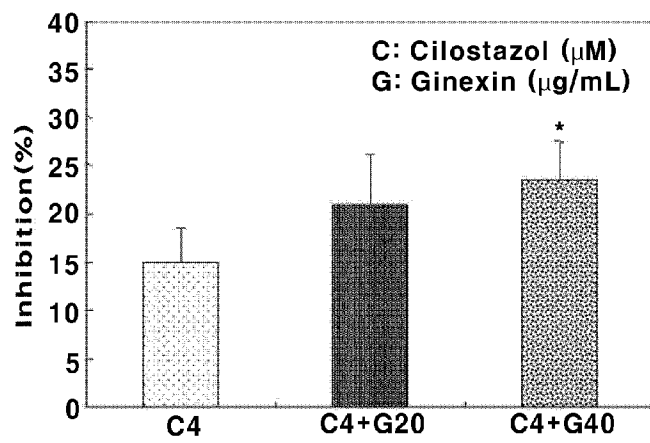
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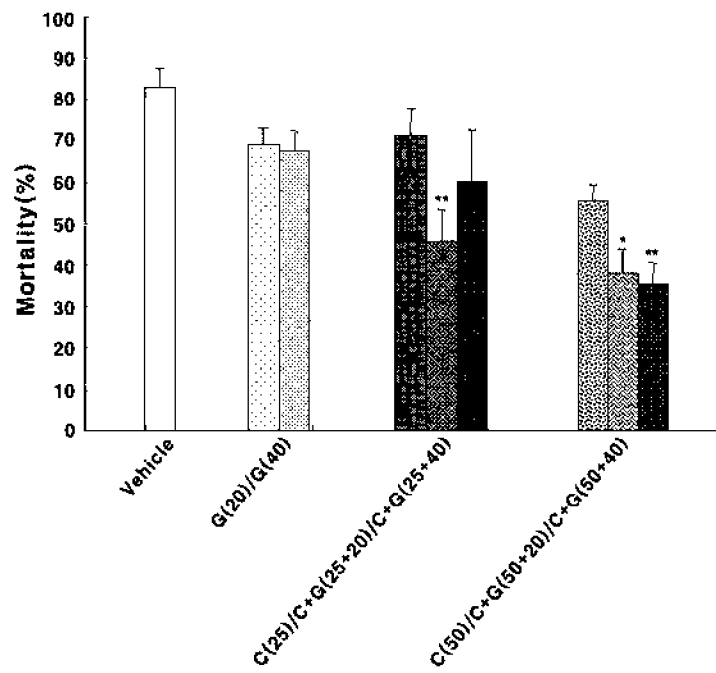
Claims

- [1] An anti-thrombotic composition comprising cilostazol and a ginkgo biloba extract as active ingredients.
- [2] The composition of claim 1, wherein the ginkgo biloba extract is contained in the amount of 10-480 weight parts relative to 100 weight parts of cilostazol.
- [3] The composition of claim 1, wherein the composition is formulated into a unit dosage form selected from the group consisting of tablets, effervescent tablets, granules and capsules.
- [4] The composition of claim 3, wherein the unit dosage form comprises 50-100 mg of cilostazol and 40-80 mg of a ginkgo biloba extract.
- [5] An anti-thrombotic agent for the prevention and treatment of a disease caused by thrombus, the agent comprising cilostazol and a ginkgo biloba extract as active ingredients.
- [6] The agent of claim 5, wherein the disease is selected from the group consisting of ischemic cerebrovascular disease, coronary artery disease, chronic arterial occlusive disease and a thrombus or an embolus after vascular surgery and retinal hemorrhage surgery.

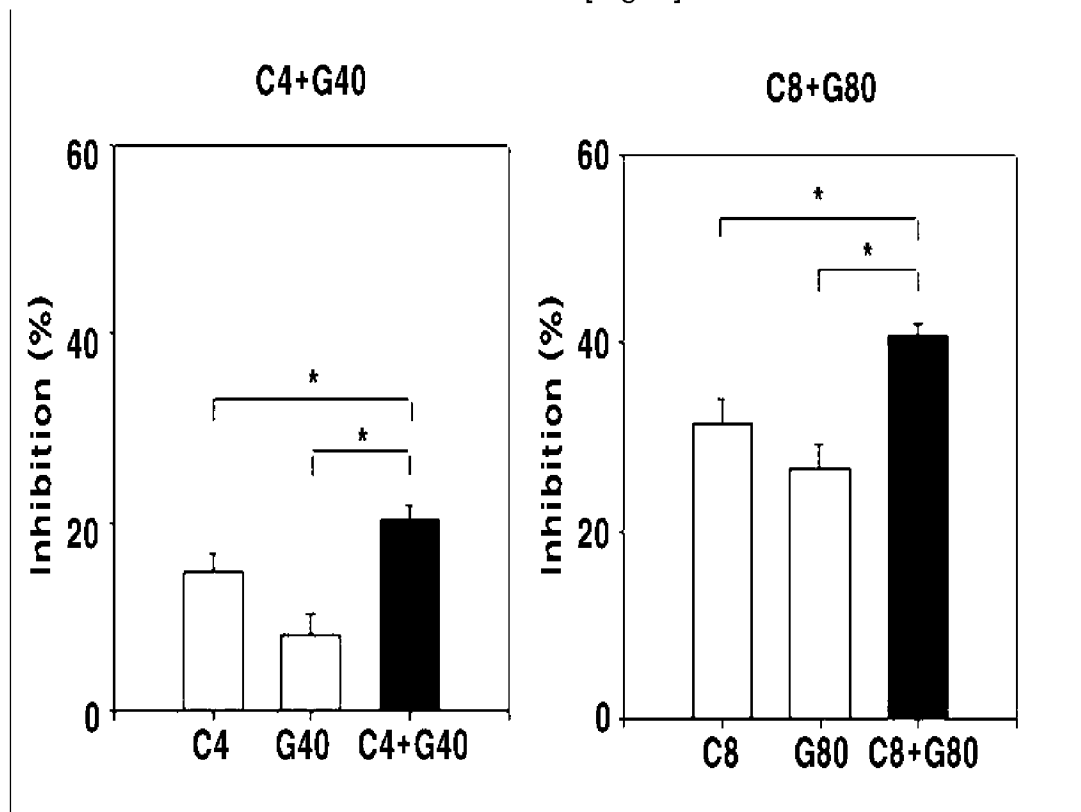
[Fig. 1]



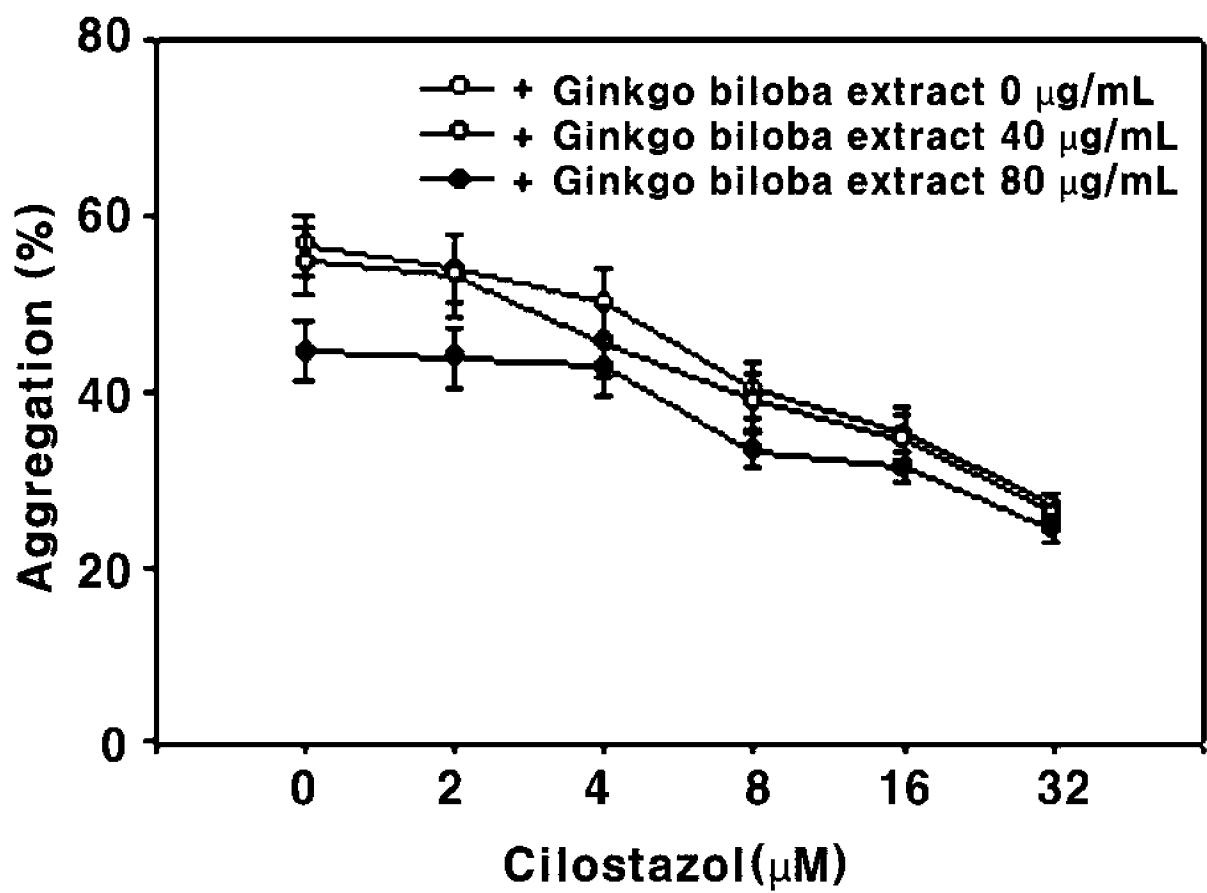
[Fig. 2]



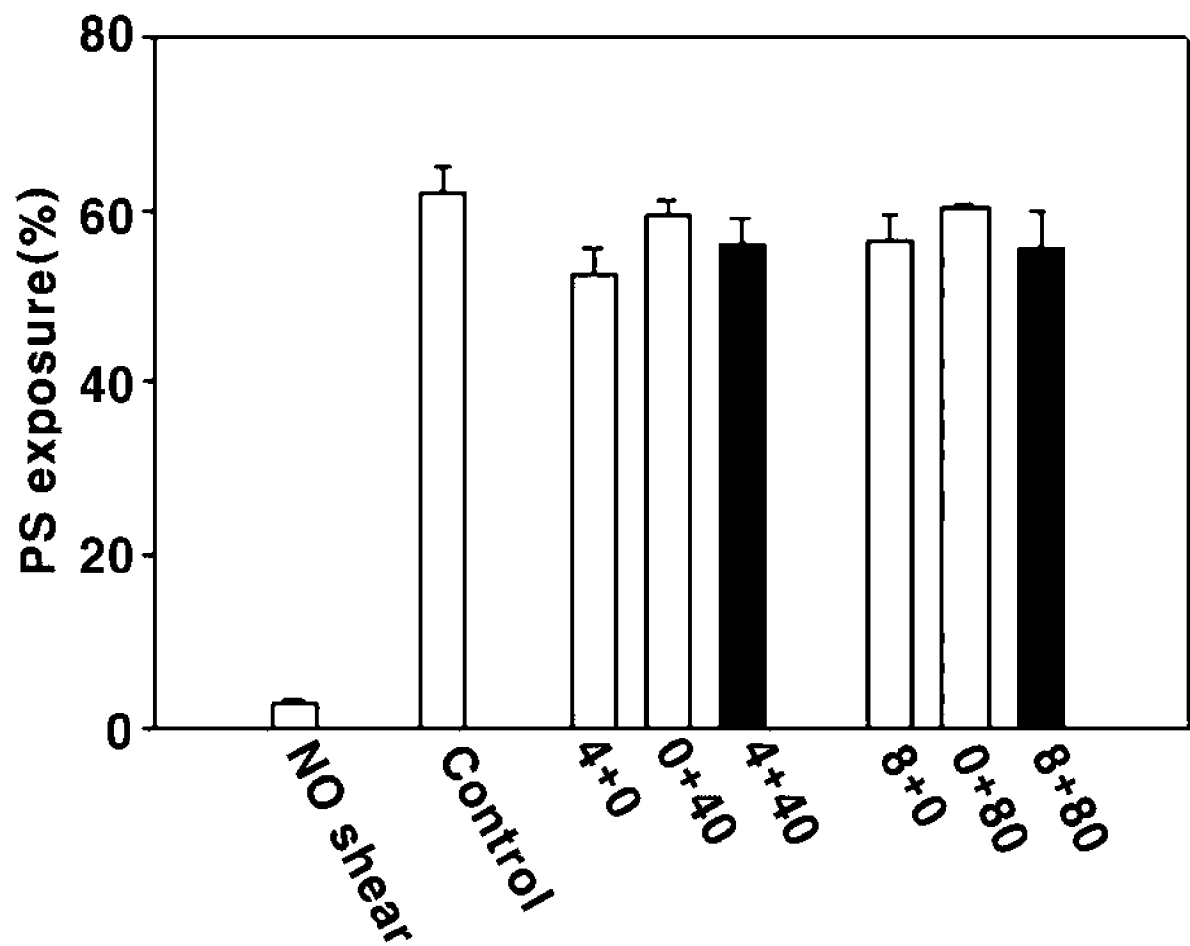
[Fig. 3]



[Fig. 4]

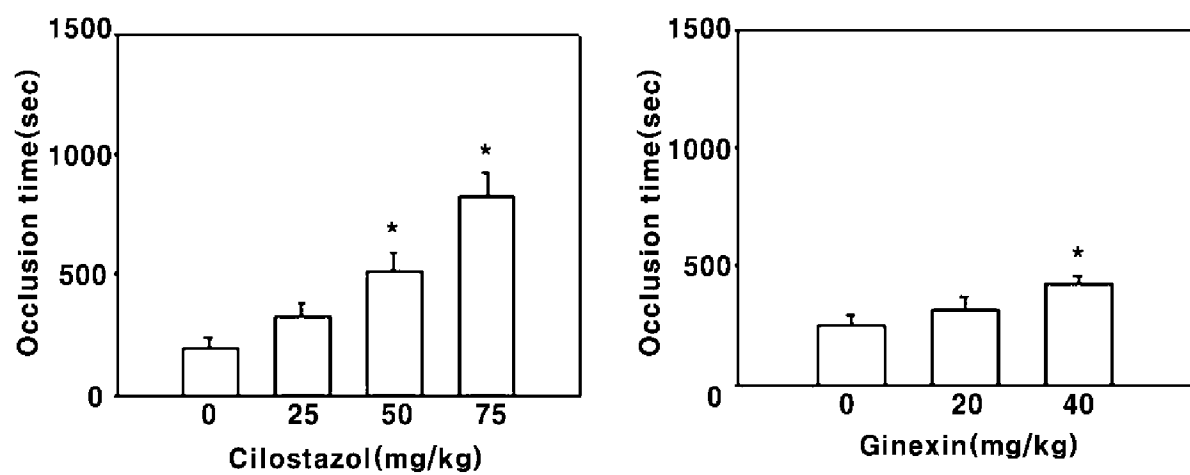


[Fig. 5]

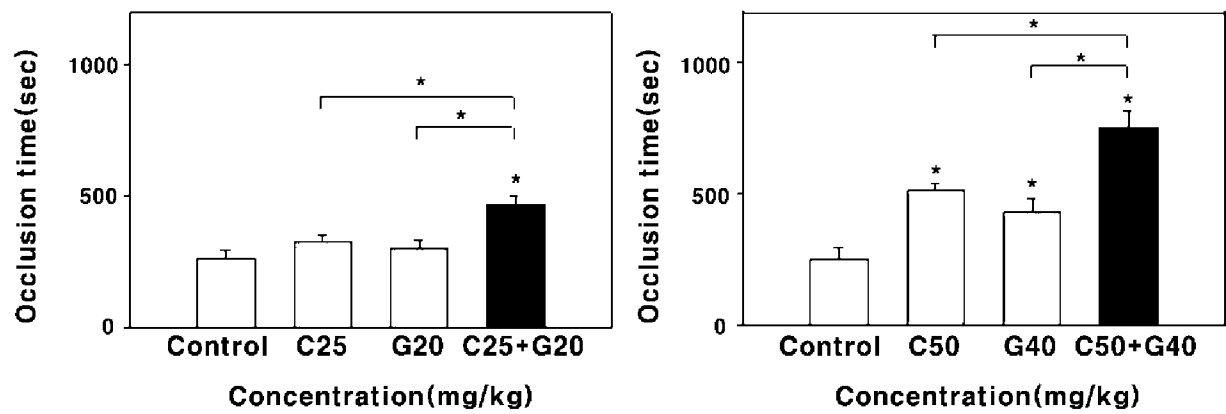


Cilostazol (μM) + Ginexin ($\mu\text{g/ml}$)

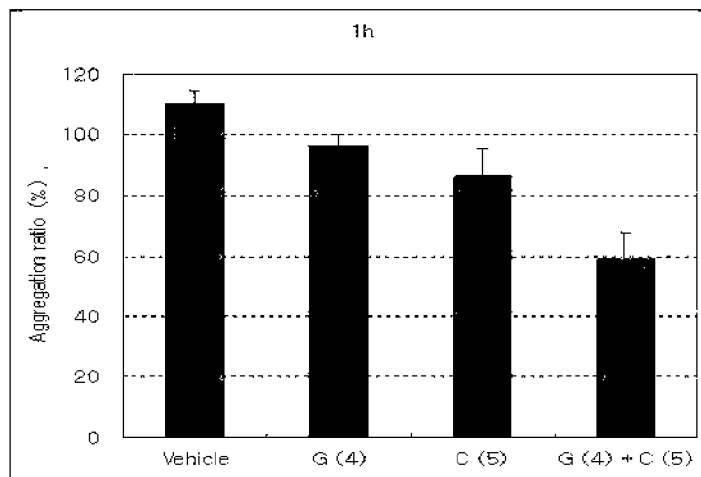
[Fig. 6]



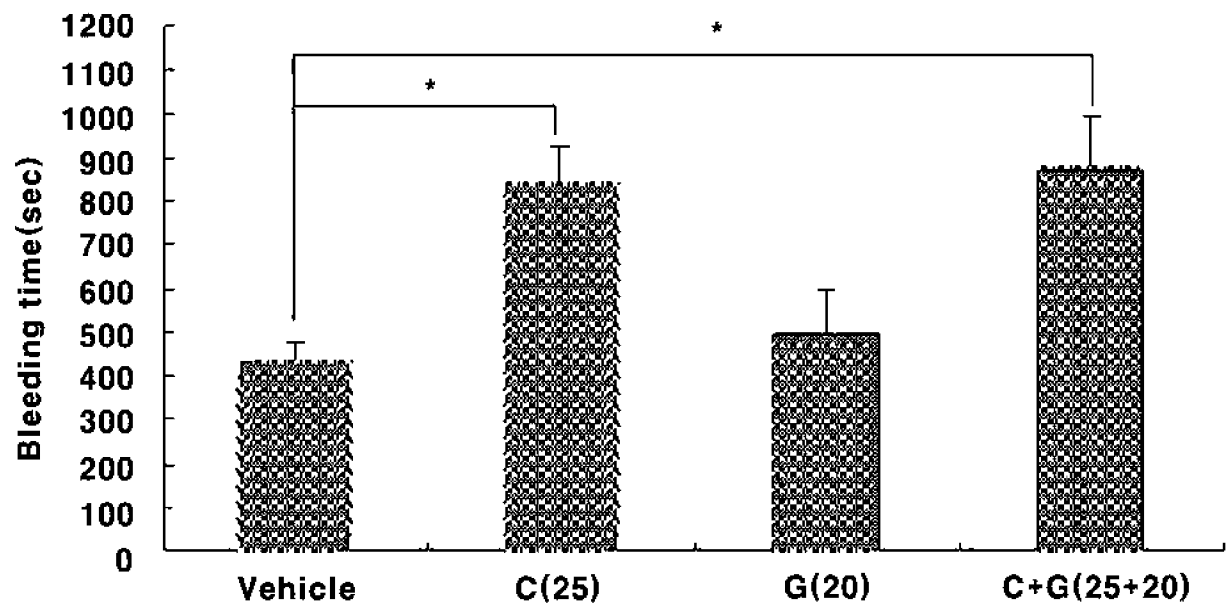
[Fig. 7]



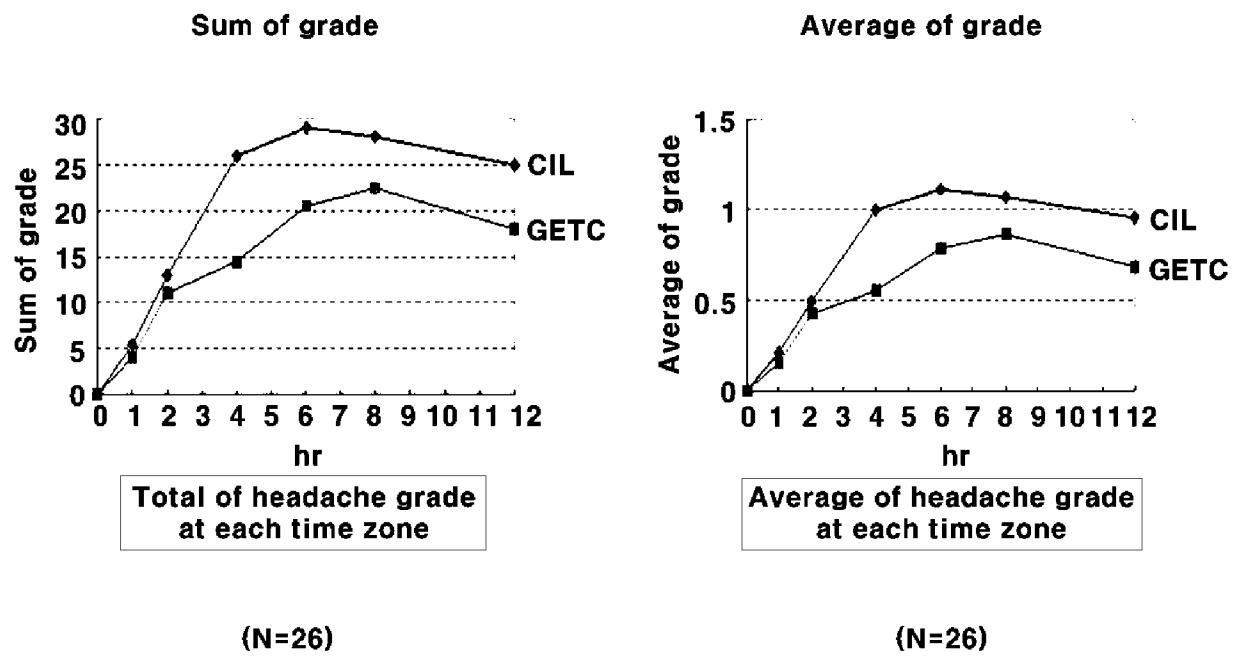
[Fig. 8]



[Fig. 9]



[Fig. 10]



A. CLASSIFICATION OF SUBJECT MATTER*A61K 31/47(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS, PUBMED

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 2006-52208 A (DAI ICHI SEIYAKU CO., LTD.) 23 Feb. 2006 See paragraphs 1-7.	1-6
Y	KR 10-1999-0053247 A (YU YU INDUSTRIAL CO., LTD.) 15 Jul. 1999 See pages 1-3.	1-6



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

30 JANUARY 2008 (30.01.2008)

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2007/005923

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP18052208	23.02.2006	None	
KR1019990053247	15.07.1999	CN1159010C CN1283119 DE69823226C0 DE69823226T2 EP01039917A1 EP01039917B1 JP2001526237T2 JP3868209B2 KR100258018B1 US6383528B1 W09932131A1	28.07.2004 07.02.2001 19.05.2004 14.04.2005 04.10.2000 14.04.2004 18.12.2001 17.01.2007 01.07.2000 07.05.2002 01.07.1999