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General Pharmacological Study of GCSB-5, a Herbal Formulation

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Abstract – The general pharmacological properties of GCSB-5, a herbal formulation consisting of 6 Oriental herbs (Ledebouriellae Radix, Achyranthis Radix, Acanthopanacis Cortex, Cibotii Rhizoma, Glycine Semen and Eucommiae Cortex), were investigated in mice, rats, guinea pigs and rabbits. The administration of GCSB-5 had no effect on general behavior, and did not influence the central nervous system. Mean blood pressure, heart and respiratory rate and contractile response of the isolated guinea pig atrium were unaffected by the treatment of GCSB-5. Addition of GCSB-5 did not cause spontaneous relaxation and contraction of the isolated guinea pig ileum and rat uterus. And also, GCSB-5 had no effect on the gastrointestinal system and the blood system of the animals examined in this study. GCSB-5, at higher doses (1,000 and 3,000 mg/kg), increased the urinary excretion of electrolytes, however, the urine volume and pH in rats were unaffected. Taken together, these results indicate that GCSB-5 does not induce any adverse effects in experimental animals and is expected to have no significant general pharmacological activities.

Key words □ GCSB-5, General pharmacology

INTRODUCTION

Arthritis and associated chronic inflammatory conditions are more prevalent in women than in men, and the chances of developing arthritis increases with age (Elliott *et al.*, 1999). Arthritis is the leading cause of disability over the age of 65, and the enormous economic and social burden of osteoarthritis (OA) and rheumatoid arthritis (RA) includes increased costs of healthcare, lost productivity and a decreased quality of life. This burden can only be expected to increases as the population ages and more individuals are affected.

Analgesics are widely used in the treatment of OA and RA for the management of pain. Non-steroidal anti-inflammatory drugs (NSAIDs), which nonspecifically inhibit both cyclooxygenase (COX) isoenzymes, COX-1 and COX-2, are effective in the management of arthritis. However, the major emphasis of adverse events with NSAIDs has centered on upper gastrointestinal tolerability (Silverstein *et al.*, 2000). Therefore, efforts are being made to seek the role of alternative natural products for the treatment of arthritis. Developing therapeutics from herbal sources may reduce the risk of toxicity when the drug is clinically used.

GCSB-5 is a purified extract from a mixture of 6 Oriental herbs (Ledebouriellae Radix, Achyranthis Radix, Acanthopanacis Cortex, Cibotii Rhizoma, Glycine Semen and Eucommiae Cortex) that have been used in traditional medicine to treat various bone disorders. Our previous studies have confirmed strong antinociceptive and anti-inflammatory properties of GCSB-5 (Lee *et al.*, 2005; Kim *et al.*, 2005). The analgesic activity of GCSB-5 was suggested to be mediated via peripheral mechanisms and it showed effective suppression of acute inflammatory responses, in the previous study. As a part of the preclinical evaluation of GCSB-5, this article provides an overview of the general pharmacological effects of GCSB-5 on general behavior, central nervous system, autonomic nervous system and cardiovascular, respiratory, gastrointestinal, urinary and blood system.

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MATERIALS AND METHODS

Animals

Male ICR mice (24-26 g), male Hartley guinea pigs (300-320 g), male New Zealand White rabbits (2-2.5 kg) and male and female Sprague-Dawley rats (200-300 g) were acclimatized to laboratory conditions at Sungkyunkwan University for at least one week. Animals were kept in a temperature and humidity controlled room (25 ± 1°C and 55 ± 5%, respectively) under a 12 h light-dark cycle. All animals were treated humanely according to the guidelines issued by the Sungkyunkwan University Animal Care Committee.

Chemicals

Unless otherwise noted, all reagents were obtained from Sigma Chemicals Co. (St. Louis, MO, USA).

Preparation and dose determination of GCSB-5

GCSB-5 was prepared at the Hanpoong Pharmaceutical Co., Ltd., Jeonju, Korea. 6 Herbs (Ledebouriellae Radix, Achyranthis Radix, Acanthopanacis Cortex, Cibotii Rhizoma, Glycine Semen and Eucommiae Cortex) were blended and extracted with distilled water. The resulting extract was subjected to ultrafiltration and the components with molecular weight over 10,000 were excluded. The filtrate was lyophilized as powder and kept at 4°C until use. GCSB-5 dissolved in physiological saline was administered orally at the doses of 300, 1,000 and 3,000 mg/kg, respectively. The range of doses was selected up to approximately 10 times of the pharmacological dose, as GCSB-5 had showed the anti-inflammatory and antinociceptive effects at the doses of 300 mg/kg (Kim *et al.*, 2005; Lee *et al.*, 2005).

Effect on general behavior

The methods used were based on the procedures described by Irwin (1968). Mice were fasted 12 h and then administered orally with GCSB-5 and the twenty three general behaviors were observed at 30, 60, 120 and 240 min after the drug administration.

Effect on central nervous system

Effect on spontaneous locomotor activity

Each mouse was fasted 12 h and then placed in an activity cage with a computer-based video tracking system (NeuroVision, Busan, Korea), and after 5 min adaption, activity for 10 min was recorded at 30, 60, 120 and 240 min after the GCSB-5 administration. Chlorpromazine (10 mg/kg) was used as positive control.

Effect on pentobarbital sodium-induced sleeping time

By the method of Rang (1964), mice were fasted 12 h. 1 h after the GCSB-5 administration, pentobarbital sodium (32 mg/kg) was injected intraperitoneally into the mice. The onset time of sleeping and the duration of sleeping time of each mouse were recorded. Chlorpromazine (10 mg/kg) was used as positive control.

Rota-rod test

According to Dunham (1957), mice were fasted 12 h and then orally administered with GCSB-5 and were subjected to the rota-rod tests at 30, 60, 120 and 240 min after the GCSB-5 administration. The mice were placed on a 3 cm diameter rod rotating at 8 rpm, and the rota-rod deficit was obtained by counting the number of animals fallen from the rotating rod within 1 min. Chlorpromazine (10 mg/kg) was used as positive control.

Anticonvulsant activity

Effect on pentylenetetrazole-induced convulsion

The test was conducted according to the method of Swinyard (1952). Mice were fasted 12 h and then injected with pentylenetetrazole (85 mg/kg) subcutaneously 30 min after the GCSB-5 administration. The induction time of the first generalized clonic seizure with loss of righting reflexes was measured for 1 h. The incidence of convulsion and mortality were also determined.

Effect on strychnine-induced convulsion

The test was conducted according to the method of Araki and Ueki (1972). Mice were fasted 12 h and then injected with strychnine (2.5 mg/kg) subcutaneously 30 min after the GCSB-5 administration. The induction time of the first generalized convulsion with loss of righting reflexes was measured for 1 h. The incidence of convulsion and mortality were also determined.

Effect on body temperature

Body temperature was measured rectally using a Thermistor thermometer (MCA-III 2A331, Shibauta, Japan), and the male rats which has normal temperature (37-38°C) were fasted 18 h. GCSB-5 was administered orally and rectal temperatures were measured at 30, 60, 120 and 240 min after the GCSB-5 administration. Chlorpromazine (20 mg/kg) was used as positive control.

Effect on cardiovascular system

Effect on mean blood pressure and heart rate

The male rats were fasted 18 h and then anesthetized with urethane (1 g/kg, i.p.), and the catheter was inserted into the

carotid artery for recording mean arterial pressure and heart rate. The animals were allowed 1 h to recover and stabilize. The catheter was connected to a pressure transducer (MLT0380, ADInstruments, Inc., CO, USA) coupled to a chart recorder (PowerLab/400, ADInstruments, Inc., CO, USA). Mean arterial pressure and heart rate were monitored and recorded at the time of 30, 60, 120, 180, 240 and 300 min after oral administration of GCSB-5.

Effect on the isolated heart of guinea pig

Guinea pigs were fasted 18 h and then stunned by a blow on the head and bled from the carotid arteries. The longitudinal muscle preparations were isolated from segments of the heart. The preparations were suspended in a 10 mL water-jacked organ bath containing Krebs-Henseleit bicarbonate buffer aerated continuously with 95% O_2 and 5% CO_2 at 33°C and their responses were recorded through an isotonic transducer (FT03, Grass-Telefactor, Astro-Med. Inc, RI, USA) and the result was recorded in a computerized physiograph. The GCSB-5 was applied by 6, 60 and 600 mg/mL. After 1 h of recovery, the contractile responses of drug alone were observed.

Effect on respiratory system

Guinea pigs were fasted 18 h by method of Takagi and Lee (1972). GCSB-5 was given orally before the intraperitoneal administration of urethane (1 g/kg) for the induction of anesthesia. Respiration belt transducer (MLT1132, ADInstruments, Inc., CO, USA) was attached to the abdomen of anesthetized guinea pig, and the respiration was recorded in a computerized physiograph. The number of respiration was measured at the time of 30, 60, 120, 180, 240 and 300 min after the oral administration of GCSB-5.

Effect on the autonomic nerve

Effect on the isolated guinea pig ileum

Guinea pigs were fasted 24 h and then stunned by a blow on the head and bled from the carotid arteries. The longitudinal muscle preparations (about 1 cm in length) were isolated from segments of the terminal ileum. The preparations were suspended in a 10 mL water-jacked organ bath containing Tyrode solution aerated continuously with 95% O_2 and 5% CO_2 at 37°C and their responses were recorded through an isotonic transducer. The result was recorded in a computerized physiograph. The GCSB-5 was applied by 6, 60 and 600 mg/mL. After 1 h of recovery, the contractile responses of drug alone and responses to acetylcholine were expressed as a percentage of the maximal response to acetylcholine (1×10^{-6} M).

Effect on the isolated rat uterus

β-Estradiol-3-benzoate (0.5 mg) suspended in cotton seed oil was given to female rats (200-220 g) and after 6 h, rats were fasted. After fasting for 18 h, the rats were stunned by a blow on the head and bled from the carotid arteries. The longitudinal muscle preparations (about 1 cm in length) were isolated from segments of the uterus. The preparations were suspended in a 10 mL water-jacked organ bath containing Tyrode solution aerated continuously with 95% O_2 and 5% CO_2 at 33°C and their responses were recorded through an isotonic transducer. The GCSB-5 was applied by 6, 60 and 600 μg/mL. After 1 h of recovery, the contractile responses of drug alone and responses to oxytocin were expressed as a percentage of the maximal response to oxytocin (1×10^{-4} U/mL).

Effect on gastrointestinal system

Effect on intestinal transport

According to the method of Takemori *et al.* (1969), mice were fasted 24 h and then administered orally with GCSB-5. 30 min after the administration of GCSB-5, each mouse received orally 3% charcoal suspension (0.1 mL/10 g). 30 min after the administration of charcoal meal, the mice were sacrificed. The intestine was isolated and distance which charcoal meal covered was measured. The distance between the pylorus and the top of the charcoal and the total length of the intestine measured. The transport rate was defined as a percentage of the former to the latter. Atropine (10 mg/kg) was used as a reference drug.

Effect on gastric secretion

The male rats were fasted for 24 h. Under anesthesia and with rats in the supine position, their abdomen was opened along the midline. The pylous was ligated and then GCSB-5 was orally given. At 4 h after closing the abdomen, the rats were sacrificed and gastric juice was collected from stomach (Shay *et al.*, 1945).

Effect on urinary system

Effect on urine volume and electrolyte excretion

Male rats were acclimated to respective metabolic cages. After deprivation of food and water and measurement of body weight, water of 2.5 mL/100 g was administered orally and after 18 h, water administration of 2.5 mL/100 g was repeated again. 3 h after of water administration, GCSB-5 and physiological saline (2.5 mL/100 g) were administered orally and urine volume, pH and urinary electrolytes (Na^+, K^+ and Cl^-) were measured from rat urine collected for 5 h.

Table I. The composition of GCSB-5

Crude Medicinal Plants	Ratio (g)
Root of *Saposhnikovia divaricata* Schiskin	2.143
Root of *Achyranthes bidentata* Blume	2.143
Cortex of *Acanthopanax sessiliflorum* Seeman	2.143
Rhizome of *Cibotium barometz* J. Smith	1.429
Semen of *Glycine max* Merrill	1.429
Cortex of *Eucommia ulmoides* Oliver	0.174

GCSB-5 was prepared by blending the above medicinal plants at the ratio indicated in the table.

Effect on blood system

Effect on coagulation

Male rats were fasted 18 h and then administered orally with GCSB-5. The blood was drawn through abdominal aorta 30 min after the administration of GCSB-5 and the activated partial thromboplastin time (aPTT) and prothrombin time (PT) were measured.

Effect on platelet aggregation

The rabbits were administered orally with GCSB-5. 30 min after the administration of GCSB-5, washed rabbit platelets were prepared. Platelet aggregation was induced by the addition of adenosine 5'-diphosphate (ADP, 1×10^{-6} - 1×10^{-3} M) into the platelet 5×10^{5}/mL and then percentage of aggregation was measured with aggregometer (Hemavet 850, Drew Scientific Group, Inc., CT, USA).

Statistical analysis

Each value represents the mean ± S.E.M. Two-way analysis of variance (ANOVA) followed by Dunnett's *t*-test or Chi-square were used to determine the statistical significance of differences between the experimental groups. Results were considered significant at the $p<0.05$ level.

RESULTS

Effect on general behavior

Oral administration of GCSB-5 at doses of 300, 1,000 and 3,000 mg/kg showed no observable changes in behavioral, neurological and autonomic profiles in mice during 4 h periods (Table II).

Table II. Effect of GCSB-5 on the general behavior in mice

Group	Control				GCSB-5											
Dose (mg/kg)	0				300				1,000				3,000			
Time (min)	30	60	120	240	30	60	120	240	30	60	120	240	30	60	120	240
Pinna reflex	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pupil reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Traction	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Motor incoordination	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Catalepsy	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Analgesia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streotyped behavior	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Exopthalmos	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Piloelection	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Righting reflex	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tail elevation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ptosis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abdominal tone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscle tone	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
Skin color	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salivation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urination	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Each value is the number of abnormalities on general behavior out of 9 mice used.

Effect on central nervous system

Effect on spontaneous locomotor activity

GCSB-5 at the doses of 300, 1,000 and 3,000 mg/kg did not show any significant change in spontaneous locomotor activity for 4 h, when compared with control group (Table III).

Effect on pentobarbital sodium-induced sleeping time

Orally administered GCSB-5 (300, 1,000 and 3,000 mg/kg) did not show any significant change of the pentobarbital-induced sleeping time in mice (Table IV).

Rota-rod test

GCSB-5 (300, 1,000 and 3,000 mg/kg) did not affect the motor coordination in mice during 4 h periods (Table V).

Anticonvulsant activity

Effect on pentylenetetrazole-induced convulsion

No alterations in the pentylenetetrazole-induced clonic seizure observed in mice following oral administration of GCSB-5 at the doses of 300, 1,000 and 3,000 mg/kg (Table VI).

Effect on strychnine-induced convulsion

A single oral administration of GCSB-5 at the doses of 300, 1,000 and 3,000 mg/kg produced no proconvulsant and anticonvulsant activities in mice (Table VI).

Effect on body temperature

As shown in Table VII, GCSB-5 (300, 1,000 and 3,000 mg/kg) did not affect the body temperature in mice for 4 h.

Effect on cardiovascular system

Effect on mean blood pressure and heart rate

As shown in Table VIII, orally administered GCSB-5 (300, 1,000 and 3,000 mg/kg) did not produce any significant changes in mean arterial pressure and heart rate for 5 h after the drug administration.

Effect on the isolated heart of Guinea Pig

The additions of 6, 60 and 600 μg/mL of GCSB-5 did not cause the contraction rate of isolated right atrium (Table IX).

Effect on respiratory system

Table IV. Effect of GCSB-5 on the pentobarbital sodium-induced sleeping time in mice

Group	Dose (mg/kg)	Sleeping time (sec)	
		Onset	Duration
Control	0	438.0 ± 34.5	2,353.6 ± 291.0
GCSB-5	300	469.5 ± 30.5	2,289.7 ± 282.9
	1,000	348.8 ± 31.6	1,956.0 ± 164.8
	3,000	433.3 ± 44.7	2,201.8 ± 253.1
Chlorpromazine	10	190.5 ± 10.5**	5,515.7 ± 489.5**

Each value represents the mean ± S.E.M. from 6-7 mice per each group.
**Significantly different ($p<0.01$) from control group.

Table V. Effect of GCSB-5 on the motor coordination in mice

Group	Dose (mg/kg)	Number of incidence			
		30 min	60 min	120 min	240 min
Control	0	0/8	0/8	0/8	0/8
GCSB-5	300	0/8	0/8	0/8	0/8
	1,000	0/8	0/8	0/8	0/8
	3,000	1/8	0/8	0/8	0/8
Chlorpromazine	10	7/10*	7/10*	8/10**	8/10**

Each value represents the number of fallen mice/tested mice from 8-10 mice per each group.
*,** Significantly different ($p<0.05$, $p<0.01$) from control group.

Table VI. Effect of GCSB-5 on the pentylenetetrazole-induced convulsion in mice

Group	Dose (mg/kg)	Pentylenetetrazole- induced convulsion (Number of incidence)	
		Convulsion	Death
Control	0	10/10	6/10
GCSB-5	300	8/8	4/8
	1,000	8/8	6/8
	3,000	8/8	7/8
		Strychnine-induced convulsion (Number of incidence)	
		Convulsion	Death
Control	0	8/8	8/8
GCSB-5	300	8/8	8/8
	1,000	8/8	8/8
	3,000	8/8	8/8

Each value represents the number of positive/tested.

Table III. Effect of GCSB-5 on the spontaneous locomotor activity in mice

Group	Dose (mg/kg)	Spontaneous locomotor activity (cm/10 min)			
		30 min	60 min	120 min	240 min
Control	0	2,062.4 ± 183.5	1,442.1 ± 239.9	1,689.3 ± 295.7	1,549.7 ± 302.6
GCSB-5	300	2,170.8 ± 524.0	1,351.3 ± 487.1	1,522.6 ± 570.5	1,567.6 ± 218.3
	1,000	2,271.5 ± 187.2	1,477.5 ± 291.4	1,567.1 ± 386.2	1,581.0 ± 169.8
	3,000	2,179.7 ± 221.4	1,506.9 ± 288.8	1,517.0 ± 283.4	1,511.7 ± 131.9
Chlorpromazine	10	292.8 ± 85.2**	260.1 ± 125.0**	213.5 ± 46.8**	345.7 ± 84.3**

Each value represents the mean ± S.E.M. from 8-11 mice per each group.
** Significantly different ($p<0.01$) from control group.

Table VII. Effect of GCSB-5 on the rectal temperature in rats

Group	Dose (mg/kg)	Rectal temperature (°C)			
		30 min	60 min	120 min	240 min
Control	0	37.5 ± 0.2	37.3 ± 0.2	37.0 ± 0.2	36.9 ± 0.2
GCSB-5	300	37.8 ± 0.1	37.3 ± 0.2	37.2 ± 0.1	37.0 ± 0.1
	1,000	37.9 ± 0.1	36.9 ± 0.2	37.1 ± 0.1	37.0 ± 0.1
	3,000	37.8 ± 0.2	37.4 ± 0.3	37.2 ± 0.1	37.1 ± 0.2
Chlorpromazine	20	37.1 ± 0.1	36.1 ± 0.1**	35.1 ± 0.3**	34.9 ± 0.2**

Each value represents the mean ± S.E.M. from 6-7 rats per each group.
**Significantly different ($p<0.01$) from control group.

Table VIII. Effect of GCSB-5 on the mean arterial pressure and heart rate in rats

Group	Dose (mg/kg)	Mean arterial pressure (mmHg)					
		30 min	60 min	120 min	180 min	240 min	300 min
Control	0	89.7 ± 2.3	84.7 ± 3.6	92.1 ± 2.7	85.5 ± 4.5	85.1 ± 2.4	90.9 ± 3.2
GCSB-5	300	86.6 ± 5.8	84.4 ± 6.3	85.3 ± 5.1	86.0 ± 4.8	86.5 ± 4.9	85.6 ± 4.5
	1,000	84.0 ± 4.5	87.3 ± 3.6	88.8 ± 2.6	89.6 ± 2.8	90.2 ± 3.0	90.9 ± 2.5
	3,000	86.1 ± 4.3	90.3 ± 4.9	89.6 ± 4.3	89.2 ± 4.1	90.3 ± 3.8	90.9 ± 4.2
		Heart rate (beats/min)					
		30 min	60 min	120 min	180 min	240 min	300min
Control	0	343.0 ± 22.6	345.0 ± 28.5	363.0 ± 22.3	368.0 ± 32.0	358.0 ± 26.7	378.0 ± 23.0
GCSB-5	300	349.0 ± 15.8	356.0 ± 14.1	353.0 ± 18.0	375.0 ± 22.5	377.0 ± 23.5	362.0 ± 18.4
	1,000	370.8 ± 24.0	381.6 ± 21.9	388.8 ± 23.0	400.8 ± 23.2	405.6 ± 19.0	418.8 ± 22.2
	3,000	364.0 ± 17.7	387.0 ± 18.4	407.0 ± 13.5	397.0 ± 17.5	393.0 ± 19.9	392.0 ± 22.4

Each value represents the mean ± S.E.M. from 5-6 rats per each group.

There were no significant difference on the respiratory rate in guinea pig administered with GCSB-5 at the doses of 300, 1,000 and 3,000 mg/kg, when compared with control (Table X).

Effect on the autonomic nerve

Effect on the isolated guinea pig ileum

The additions of 6, 60 and 600 μg/mL of GCSB-5 did not cause the spontaneous relaxation and contraction of the ileum (data not shown). GCSB-5 at concentrations of 6, 60 and 600 did not affect acetylcholine-induced contraction of ileum (Table XI).

Effect on the isolated rat uterus

GCSB-5 at concentrations of 6, 60 and 600 μg/mL had no effect on the spontaneous relaxation and contraction (data not shown) and oxytocin-induced contraction of uterus (Table XII).

Table IX. Effect of GCSB-5 on the isolated right atrium of guinea pig

Group	Dose (μg/mL)	Contraction Rate (beats/min)
Control	0	181.5 ± 5.1
GCSB-5	6	177.0 ± 5.2
	60	171.0 ± 3.0
	600	171.0 ± 3.9

Each value represents the mean ± S.E.M. from 5 guinea pigs per each group.

Effect on gastrointestinal system

Effect on intestinal transport

As shown in Table XIII, GCSB-5 (300, 1,000 and 3,000 mg/kg) caused no observable effects on the intestinal motility in mice.

Table X. Effect of GCSB-5 on the respiration rate in guinea pigs

Group	Dose (mg/kg)	Respiration rate (counts/min)					
		30 min	60 min	120 min	180 min	240 min	300min
Control	0	82.5 ± 3.1	67.0 ± 1.7	62.5 ± 8.2	67.5 ± 6.3	59.8 ± 2.8	66.8 ± 4.0
GCSB-5	300	82.0 ± 12.1	73.0 ± 7.7	83.5 ± 10.4	74.5 ± 11.8	79.0 ± 13.7	76.0 ± 11.9
	1,000	82.5 ± 5.4	75.5 ± 7.6	84.0 ± 7.9	70.5 ± 2.1	75.5 ± 6.8	70.0 ± 6.4
	3,000	72.5 ± 7.2	58.0 ± 4.7	64.5 ± 8.0	62.0 ± 6.5	57.0 ± 3.1	53.0 ± 2.6

Each value represents the mean ± S.E.M. from 5 guinea pigs per each group.

Effect on gastric secretion

No significant changes in gastric fluid volume, pH and acidity and total acid secretion of the gastric juice were observed in rats following oral administration of GCSB-5 at the doses of 300, 1,000 and 3,000 mg/kg (Table XIV).

Table XI. Effect of GCSB-5 on the acetylcholine-induced contractions in the isolated ileum of guinea pig

Group	Dose (μg/mL)	Relative contraction (%)
Control	0	105.0 ± 4.1
GCSB-5	6	100.2 ± 3.9
	60	106.5 ± 5.4
	600	104.2 ± 2.0

Each value represents the mean ± S.E.M. from 5 guinea pigs per each group.

Table XII. Effect of GCSB-5 on the oxytocin-induced contractions in isolated rat uterus

Group	Dose (μg/mL)	Relative contraction (%)
Control	0	102.9 ± 3.8
GCSB-5	6	105.2 ± 3.4
	60	104.3 ± 3.7
	600	105.7 ± 5.9

Each value represents the mean ± S.E.M. from 5-6 rats per each group.

Table XIII. Effect of GCSB-5 on the intestinal propulsion in mice

Group	Dose (mg/kg)	Intestinal propulsion (%)
Control	0	50.6 ± 2.0
GCSB-5	300	48.9 ± 1.2
	1,000	54.9 ± 1.8
	3,000	55.9 ± 1.9
Atropine	10	36.0 ± 1.6**

Each value represents the mean ± S.E.M. from 7-8 mice per each group.
**Significantly different ($p<0.01$) from control group.

Effect on urinary system

Effect on urine volume and electrolyte excretion

There were no significant changes in urine volume and pH in rats following oral administration of GCSB-5 at the doses of 300, 1,000 and 3,000 mg/kg. However, at the dose of 3,000 mg/kg, GCSB-5 significantly increased urinary excretion of electrolytes (Na^+, K^+ and Cl^-) (Table XV).

Effect on blood system

Effect on coagulation

As shown in Table XVI, GCSB-5 (300, 1,000 and 3,000 mg/kg) caused no observable effects on the prothrombin time and activated partial thromboplastin time in rats.

Effect on platelet aggregation

Oral administered GCSB-5 (300, 1,000 and 3,000 mg/kg) did not affect the platelet aggregation induced by ADP (Table XVII).

DISCUSSION

The purpose of the present study was to examine the pharmacological properties of GCSB-5 to get some insight into the potential side effects on the central nervous, cardiovascular, respiratory, gastrointestinal, urinary and other organ systems, resulting from the secondary pharmacological activity of high

Table XIV. Effect of GCSB-5 on the gastric secretion in rats

Group	Dose (mg/kg)	Gastric fluid volume (mL)	pH	Acidity (μEq/mL)	Total acid secretion (μEq/4 h/100 g)
Control	0	8.3 ± 1.0	1.35 ± 0.03	104.1 ± 3.3	71.1 ± 9.1
GCSB-5	300	7.7 ± 1.4	1.34 ± 0.11	110.2 ± 3.8	66.5 ± 12.0
	1,000	8.1 ± 0.5	1.35 ± 0.08	104.1 ± 4.5	66.0 ± 5.8
	3,000	8.5 ± 0.5	1.61 ± 0.11	97.8 ± 6.0	65.5 ± 5.8
Ranitidine	20	4.2 ± 0.6**	1.86 ± 0.22*	81.3 ± 5.5**	28.7 ± 2.9**

Each value represents the mean ± S.E.M. from 6-7 rats per each group.
*,**Significantly different ($p<0.05$, $p<0.01$) from control group.

Table XV. Effect of GCSB-5 on urine volume, pH and urinary excretion of electrolytes in rats

Group	Dose (mg/kg)	Urine volume (mL)	pH	Na^+ (mM)	K^+ (mM)	Cl^- (mM)
Control	0	6.2 ± 0.5	6.4 ± 0.1	69.8 ± 8.5	32.5 ± 3.6	81.2 ± 9.9
GCSB-5	300	7.2 ± 0.6	6.2 ± 0.2	55.0 ± 8.8	29.3 ± 7.4	71.8 ± 14.6
	1,000	6.5 ± 0.6	6.4 ± 0.1	72.2 ± 4.8	56.3 ± 6.9*	103.8 ± 10.8
	3,000	6.9 ± 0.9	6.8 ± 0.1	122.8 ± 10.9**	105.2 ± 8.1**	161.7 ± 12.8**

Each value represents the mean ± S.E.M. from 6-11 rats per each group.
*,**Significantly different ($p<0.05$, $p<0.01$) from control group.

Table XVI. Effect of GCSB-5 on the coagulation in rats

Group	Dose (mg/kg)	PT (sec)	aPTT (sec)
Control	0	16.9 ± 0.5	22.0 ± 1.9
GCSB-5	300	16.9 ± 0.5	24.7 ± 1.2
	1,000	16.8 ± 0.5	24.8 ± 2.3
	3,000	17.5 ± 0.3	23.6 ± 0.9

Each value represents the mean ± S.E.M. from 7-9 rats per each group.
PT, prothrombin time; aPTT, activated partial thromboplastin time.

Table XVII. Effect of GCSB-5 on platelet aggregation induced by ADP in rabbit plasma

Group	Dose (mg/kg)	Platelet aggregation (%)			
		1×10^{-6}†	1×10^{-5}†	1×10^{-4}†	1×10^{-3}†
Control	0	20.8 ± 4.4	43.7 ± 6.1	51.8 ± 6.3	35.5 ± 7.2
GCSB-5	300	22.4 ± 2.9	49.1 ± 4.0	60.3 ± 6.2	36.0 ± 4.1
	1,000	20.8 ± 2.2	45.5 ± 2.6	50.6 ± 2.8	25.4 ± 2.8
	3,000	20.9 ± 2.3	45.3 ± 2.9	54.7 ± 3.5	32.1 ± 2.4

Each value represents the mean ± S.E.M. from 5 rabbits per each group.
†ADP concentration (mol/L).

doses of the agent.

At doses approximately 10 times higher than the anti-inflammatory and antinociceptive dose of GCSB-5, there were no obvious effects on the central nervous system, cardiovascular, respiratory, gastrointestinal and the other organ systems. Urinary excretion of electrolytes was not affected by anti-inflammatory and antinociceptive dose of GCSB-5 (300 mg/kg), whereas high doses (1,000 and 3,000 mg/kg) of GCSB-5 increased urinary excretion of electrolytes. Previous studies showed that Achyranthis Radix has the diuretic activity (Ahn *et al.*, 1978). Therefore, we suggest that its use in high doses should be approached with caution to the urinary excretion of electrolytes.

Based on these results, it was concluded that GCSB-5 is a safe compound and it does not appear to have significant general pharmacological effects.

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