

Premarket Notification for New Dietary Ingredient:
Clematis mandshurica

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Chapter 1: *Clematis mandshurica*, AN HERBAL INGREDIENT FOR DIETARY SUPPLEMENTS

1.1 Name and address of the manufacturer or distributor:

SK Pharma Co., Ltd.
948-1 Daechei 3-dong, Gangnam-gu, Seoul 135-283, Korea

1.2 Name of the Dietary Ingredient for Dietary Supplements

Scientific Name: Radix Clematidis
Latin Binomial Name: *Clematis mandshurica*

Chapter 2: DESCRIPTION OF *Clematis Mandshurica*, THE DIETARY INGREDIENT

2.1 General Description

Clematis mandshurica is listed in the Chinese pharmacopoeia by its Pinyin name, Wei Ling Xian. The Chinese pharmacopoeia states that Wei Ling Xian is the dry root and rootstalk of *Clematis chinensis* Osbeck, *Clematis mandshurica* Rupr. or *Clematis hexapetala*.¹ The dietary supplement as described in §2.2 contains Wei Ling Xian only in the form of *Clematis mandshurica*.

2.2 Product Description

SKI306X, marketed in Korea and Australia as JOINS[®] tablet and Cararthron[®], respectively, is a purified extract from a mixture of three oriental herbal medicines that have been widely used to support healthy joints and cartilage in far East Asia.

2.3 Product Labeling

(a) SKI306X (Joins) is marketed as a 200 mg tablet and contains about 100 mg of *Clematis mandshurica* extract.

(b) The conditions of use suggested on the labeling are:

Suggested use: As a dietary supplement to support healthy joints and cartilage, take one tablet, 2-3 times daily.

¹ Ministry of Health P.R. China, Pharmacopoeia Committee, Pharmacopoeia of P.R. China, Volume 1, 1990, Beijing, People's Medical Publishing House, 222

Chapter 3: SUMMARY OF ALL TESTING RESULTS

Clinical Trials

A. Phase II Clinical Trials

From the Phase II clinical studies, *Effect of SKI306X, a New Herbal Anti-Arthritic Agent, in Patients with Osteoarthritis of the Knee a Double-Blind Placebo Controlled Study*, patients with clinically and radiographically confirmed osteoarthritis of the knee were evaluated. Other criteria used included moderate to severe pain in the affected knee joint, more than a score of 35 mm as assessed by a visual analogue scale (VAS) ranging from 0 mm (no pain) to 100 mm (unbearable pain) and normal hepatic and renal function. Safety was assessed in both the placebo and treatment groups, with the adverse events in all groups being classified as mild in severity. No significant difference in severity was observed among the placebo and SKI306-treated groups.

Considering the safety and tolerability in this study, SKI306X was very safe and well-tolerated. Results from this study indicated that SKI306X had a good efficacy and excellent safety profile when administered at doses of 200, 400 and 600 mg to patients with osteoarthritis of the knee.

B. Phase III Clinical Trials

The Phase III clinical trials evaluated patients who had osteoarthritis of the knee and had both clinical and radiological evidence of osteoarthritis. Assessments were performed at 0, 2 and 4 weeks. In this randomized, double-blind study, patients were assigned to one of two study groups; a study group treated with SKI306X and a control group treated with Diclofenac sustained release (SR), a well-known NSAIDS (non-steroidal anti-inflammatory drug). Adverse events and vital signs were recorded throughout the study. Laboratory investigations, including hematology (complete blood cell count with differential), chemistry (glucose, electrolyte, urica nitrogen, creatinine, total protein, albumin, creatine kinase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, total bilirubin levels) and urinalysis (pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, and nitrite) were performed at the screening and after the 4th week of therapy.

Throughout the study, there were no statistically significant differences between the two treatment groups in the incidence of any adverse event. Drug-related adverse events were significantly less frequent in SKI306X than in the diclofenac SR group even though both SKI306X and diclofenac in general were well tolerated. Moreover, reported severe adverse events were less frequently reported in the SKI306X group than in the diclofenac SR group. There were more frequent laboratory adverse events in the diclofenac SR group than in the SKI306X group. Elevations of alanine aminotransferase values in the diclofenac SR group were especially more frequent than in the SKI306X group and was a statistically significant difference. There was a more frequent elevation in aspartate aminotransferase values in the diclofenac SR group than in the SKI306X group, although there were no statistically significant differences.

Toxicology

A. Acute Toxicity

The acute toxicity of SKI306X was evaluated in rats by a single oral administration. After oral administration of SKI306X with several doses (5.0 g/kg, 3.3 g/kg, 2.2 g/kg, 1.5 g/kg, and 1.0 g/kg), mortality, clinical signs, body weight, and macroscopical observations in organs were examined. No toxic effect was shown in terms of mortality, clinical signs, body weight changes and macroscopical observations. The suggested LD₅₀ of SKI306X is more than 5.0 g/kg. SKI306X has an extremely large safety margin.

B. Subacute Toxicity

To evaluate the subacute toxicity of SKI306X, SKI306X was administered orally to rats once a day for 4 weeks at doses of 0.3, 1.0, and 3.0 g/kg/day. Two subgroups were included for interim study of the 2-week administration and 2-week recovery test.

During the study, general symptoms were observed (anorexia, salivation, diarrhea, vomiting, polyuria, and fecal change) and their severities were recorded daily, and during the 2-week recovery period, body weights were measured once daily. Food and water intake was measured two times a day during the administration period and once daily during the recovery period. Ophthalmological examinations on all animals were performed at 2-week intervals during the course of the study. Urinalysis was performed on all animals at the final week of administration and the animals were housed in cages for 24 hours to collect urine, noting its appearance and measuring the amount. Immediately thereafter, pH, protein, nitrite, urobilinogen, bilirubin, glucose and blood were measured using a urinalysis paper.

At the end of the study, all animals were anesthetized with ether and major organs and tissues were examined for weight change, including: liver, kidney, spleen, heart, lung, brain, thyroid gland, stomach, prostate gland, uterus, ovary, testes, etc. Hematological parameters included red blood cell count, white blood cell count, hematocrit, hemoglobin, and Plt. Blood biochemistry parameters included alanine transaminase, total cholesterol, blood glucose, creatinine, albumin, Na, Cl, K, etc. Histopathological examinations were made of the organs and tissues.

All rats survived and no adverse clinical symptoms were observed. Although male rats treated with high dose (3.0 g/kg) of SKI306X showed slight loss of body weight in comparison with the control animals during the administration period, their body weight was normally restored during the recovery period. Food consumption did not show any differences during the experimental period; male rats treated with low dose (0.3 g/kg) and intermediate dose (1.0 g/kg) of SKI306X showed differences in water intake compared with those of the control group at day 7, but no more abnormal differences were found.

No significant change was found in all hematological parameters of SKI306X-treated groups except for the decreased number of red blood cells in all female groups at the interim study. SKI306X is not involved in inducing any reduction of red blood cells as: 1) there were different red blood cell levels between males and females; 2) the red blood cell changes were in the normal level and reversible; 3) the red blood cell count did not show any dose-or-administration period-dependent mammary changes; and 4) some anemia-associated parameters such as HGB, HCT, etc. were normally measured.

Blood biochemical results showed that the SKI306X-treated groups showed differences over the control group, however, since most of these increases were within the normal range and reversible without more toxicity, the results show that SKI306X does not induce any abnormal change due to its toxicity. It is considered that the increase of ALP, AST and ALT in male rats treated with an intermediate dose of SKI306X and in female rats treated with a low dose of SKI306X for two weeks may be induced by organ damages associated with bile duct hyperplasia, fibrosis, etc. through gross and histopathological findings. Changes such as these are occasionally found in rats and have no correlation with SKI306X.

In SKI306X-treated groups, the absolute and relative weights of some organs showed changes over the control group. Although the absolute and relative weight of the heart in male rats treated with an intermediate dose and high dose of SKI306X was reduced over the control group after the 4-week administration of SKI306X, these differences disappeared in all SKI306X-treated groups at the end of the recovery period and abnormal histopathological findings were not observed. The weight of the stomach in male rats treated with the high dose was increased, but abnormal proliferation was not found histopathologically. In the case of the liver or heart, dose-dependent response or time-dependent severe toxicity was not observed.

In histopathological findings, local lymphocyte infiltration in the kidney and some lesions in the liver were found in both the SKI306X-treated groups as well as the control group.

The results of subacute toxicity of SKI306X indicated that some parameters were significantly different from those of the control group; however, these parameters did not show dose-or administration-period related response and more severe toxicity was not found in proportion to prolongation of the administration period.

Based on these results, it is concluded that the non-toxic dose of SKI306X was estimated to be between 0.3 and 1.0 g/kg/day and the maximum tolerated dose of SKI306X was higher than 3.0/g/kg/day.

C. 26-Week Toxicity Study

PAGES 7 AND 8

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INFORMATION

4.2 Phase II Clinical Trials²

A. Patients and Methods

Preparation and Composition of SKI306X

SKI306X was prepared by extracting a mixture of 3 herbal components (*Clematis mandshurica*, *Trichosanthes kirilowii*, *Prunella vulgaris*) in a ratio of 1:2:1 in 30% (v/v) ethanol-water. The extract solution was filtered and evaporated under vacuum. The residue was partitioned between n-butanol and water. The n-butanol layer was evaporated under vacuum for complete removal of residual solvent, and the resulting residue was lyophilized to yield 2.5% (w/w) of a dark-brown powder.

SKI306X was standardized conforming to the regulations imposed by the Korean Food and Drug Administration (KFDA).

Tablets were made of 200 mg of SKI306X with additives.

Patients and Treatment

Patients were enrolled in two university hospitals in Seoul, Korea and 96 patients with classical osteoarthritic of the knee were allocated in this study. The study was approved by the Institutional Review Boards for all investigational sites and the Korea FDA and carried out in accordance with revised Declaration of Helsinki guidelines. Patients with clinically and radiographically confirmed osteoarthritis of the knee were eligible for this study. Their age ranged from 35-75 years. Other criteria included: moderate to severe pain in the affected knee joint, more than a score of 35 mm as assessed by a visual analogue scale (VAS) ranging from 0mm (no pain) to 100mm (unbearable pain) and normal hepatic and renal function.

Patients were excluded if they had rheumatoid arthritis, lymphoma and any concomitant clinically unstable disease, or clinically relevant laboratory test abnormalities or if they were pregnant, lactating or of childbearing potential or on any medicine or procedures that might influence or obscure the action of the SKI306X.

Following a wash-out period of more than 1 week for patients currently receiving treatment with NSAIDS (non-steroidal anti-inflammatory drugs), all the patients who met the eligibility criteria were randomly assigned double-blind to treatment with SKI306X (200, 400, and 600 mg *ter in die, t.i.d.*) and placebo for 4 weeks. 4 groups of 24 patients per group were administered the dosage 30 minutes after each meal, one SKI306X tablet and two placebo tablets, two SKI306X tablets and one placebo tablet, three SKI306X tablets or three placebo tablets.

Patients were assessed after 2 weeks and 4 weeks of the treatment and 1 week after the end of treatment. The primary endpoint was measured by overall pain in the affected knee joint during the treatment period as assessed by the patient on a VAS ranging from 0mm which corresponds to "no pain", to 100mm "unbearable pain". Secondary endpoints included the Lequesne index which was measured by the investigator-administered questionnaire concerning the patient's daily activities (scored ranging from 0 to 24), and patients' and investigators' opinions of therapeutic efficacy rated as 1, very good; 2, good; 3, fair; 4, unchanged; and 5, deterioration^{3 4 5 6}.

Safety variables were recorded including assessment by the patients, tolerability of the treatment at each visit and the occurrence of any adverse events with an indication of severity, duration period and probability of self medication⁷. Serum biochemistry, hematology and urinalysis were performed before and after the treatment. A physical examination was performed before the treatment and at each visit after the start of the treatment. Compliance was assessed by counting the amount of returned trial medication.

² Jung et al., *Effect of SKI306X, a New Herbal Anti-Arthritic Agent, in Patients with Osteoarthritis of the Knee: A Double-Blind Placebo Controlled Study*, 29 American Journal of Chinese Medicine 485 (2001)

³ Kircheimer et al., *Diclofenac sodium (Voltaren) in Rheumatoid arthritis: A Double-Blind Comparison with Indomethacin and Placebo* Int. J. Pharmacol. 13:292-7 (1976).

⁴ Lequesne et al., *Indexes of Severity for Osteoarthritis of the Hip and Knee*, Scand. J. Rheumatol. 65 (suppl):85-89 (1987)

⁵ Nguyen et al., *Diacherein in the Treatment of Osteoarthritis of the Hip*, Arthritis Rheum. 37:529-560 (1994).

⁶ Scali et al., *Double-Blind Cross-over Study of Indoprofen Versus Ibuprofen and Placebo Rheumatoid Arthritis Patients*, Eur J Rheumatol Inflamm 4:93-96 (1981).

⁷ Spilker, B. *Collecting Adverse Event and Adverse Reaction Data in Clinical Trials*, In: Guide to Clinical Trials Philadelphia, 196-201 (1st ed. Lippincott-Raven Publishers 1996)

Statistical Methods

Statistical analyses were carried out to assess the effects of the treatments on all the patients entering the study (intent-to-treat analysis). One-way ANOVA with multiple comparison using the Duncan test was performed on assessments of the pain and the Lequesne index, and a Fisher's exact test was performed on patients' and investigators' rating of therapeutic efficacy. P-Values less than 0.05 were considered statistically significant. Incidences of adverse events were tabulated by the World Health Organization (WHO) body systems organ class.

The adequacy of the sample size was estimated by the Chi-square approximation method on the basis of demonstrating therapeutic efficacy among placebo and SKI306X-treated groups. A sample of 72 patients, 18 in each treatment group, were analyzed.

B. Results

Among 96 patients who were randomized to treatment with either SKI306X or placebo (n=24 in each group), one patient from the SKI 306X 400 mg, one from the 600 mg treatment groups and one from the placebo group were not included in the intent-to-treat analysis because these patients were not assessed from the first administration of drug or placebo.

C. Safety and Tolerability

Safety was assessed in all 93 patients who were included in the intent-to-treat analysis. Adverse events were reported from 5/23 patients (21.7%) in the placebo group and from 5/24 patients (20.8%), 6/23 patients (26.1%) and 3/23 patients (13.0%) in the SKI306X 200, 400, and 600 mg *t.i.d.* groups, respectively. The spectrum and occurrence rates of adverse events observed from patients treated with SKI306X were similar to those of the placebo group (Table I). The adverse events in all groups were classified as mild in severity. There was no significant difference in severity among placebo and SKI306X-treated groups.

Table I

Frequency				
SKI306X Treated Groups (<i>t.i.d.</i>)				
Organ Class	Placebo (n=23)	200 mg (n=24)	400 mg (n=23)	600 mg (n=23)
Body as a Whole	---	1	2	1
Gastrointestinal	3	6	9	3
Kidney	---	1	---	---
Psychiatric	2	1	---	---
Total Frequency	5	9	11	4
Total patients	5	5	6	3
p-value*	0.7410			

*p-value by Chi-square test

There were no clinically observable changes in either blood pressure or pulse rate from all groups during this study. As was expected, there was no significant difference in laboratory parameters of serum biochemistry, urinalysis and hematology among all the groups.

D. Summary

This study confirmed the therapeutic value of SKI306X based on improvement of all efficacy parameters in the SKI306X-treated groups compared with the placebo group after 4 weeks of treatment.

Taking into account safety and tolerability, SKI306X was safe and well-tolerated. There were no significant gastrointestinal adverse events in patients treated with SKI306X compared with the placebo group. In the percentage of patients who reported a gastrointestinal adverse event, the major adverse event during treatment was lower than oral NSAIDS treatment (Coles et al., 1983). There was no dose-dependency of the observed gastrointestinal adverse events in the SKI306X-treated groups.

Results from this study indicated that SKI306X had a good efficacy and safety profile when administered 200, 400 and 600 mg *t.i.d.* to patients with osteoarthritis of the knee.

4.3 Phase III Clinical Trial

A. Materials and Methods

This multi-center, double blind, randomized, phase III clinical trial was performed in 5 university hospitals (Seoul National University Hospital, Chung-Ang University Medical Center, The Catholic University of Korea, Kangnam St. Mary's Hospital, Ewha Womans University Tongdaemun Hospital). This study was approved by the Institutional Review Boards for all investigational sites and the Korea FDA about the ethical and scientific aspects of the study protocol, and was conducted in accordance with the Declaration of Helsinki as amended in 1989 and according to Good Clinical Practice guidance.

SKI306X was prepared from the extracts of three medical herbs, *Clematis mandshurica*, *Trichosanthes Root* and *Prunella Spike*. These extracts were combined at a 1:2:1 (w/w) ratio with 30% (v/v) ethanol-water. After the extracted solution was filtered and evaporated *in vacuo*, the residue was partitioned between *n*-butanol and water. The *n*-butanol layer was evaporated *in vacuo* and freeze-dried for complete removal of the residual solvent, yielding the final product in powder form.

Subjects

A total of 249 adults were enrolled and randomly assigned to one of two study groups, 125 subjects in one study group (SKI306X) and 124 subjects in the control group (Diclofenac SR). Stratified block randomization with stratification by study site using 4 or 6 block size was used. The SAS program (version 6.12) was used for random number generation following uniform distribution. Eligible patients were 35 to 75 years old with osteoarthritis (OA) of the knee and had both clinical and radiographic evidence of OA. Radiographic criteria for OA of the knee were narrowness of joint space and the presence of osteophytes. Patients were enrolled in this study if they had at least moderate pain in the affected knee joint, larger than the scores of 35 mm as assessed by a visual analogue scale (VAS) ranging from 0 mm (no pain) to 100 mm (unbearable pain) and normal hepatic and renal function. Patients were excluded if they had significant renal or hepatic impairment, clinically significant abnormalities on physical or laboratory examinations at the screening visit, and active infection to antibiotics. Patients were excluded if they had a previous sensitivity to NSAIDs or required corticosteroids or other NSAIDs. Women were not eligible if they were pregnant or had childbearing potential or were lactating. Massages and exercises remained unchanged. All patients experienced a washout period during which all prior medications were withdrawn.

Study design

In this randomized, double blind study, 249 patients were recruited from 5 institutes in Korea from February 28, 2000 and August 28, 2000. Patients were screened to ensure eligibility after receiving informed consent. After confirmation of eligibility, patients were randomized to receive either SKI306X 200mg 3 times daily or diclofenac sustained release 100mg once daily. Blinding was maintained by using double dummy technique. Assessments of OA variables were repeated after 0, 2, 4 weeks, and laboratory variables were assessed at screening and at the 4th week. Concomitant pharmacotherapy for conditions unrelated to OA was permitted if it didn't interfere with the study medication.

Safety assessments

Spontaneously reported adverse events and vital signs were recorded throughout the study. Laboratory investigations, including hematology (complete blood cell count with differential), chemistry (glucose, electrolyte, urea nitrogen, creatinine, total protein, albumin, creatine kinase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, total bilirubin levels), urinalysis (pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite) were performed at screening and 4th week of therapy.

For all clinical adverse events, the investigators recorded the intensity, the relation to the test drug ("definitely not" and "probably not" related were scored as "not drug-related" adverse events: "possibly", "probably" and "definitely" related were scored as "drug-related" adverse events), the outcome, and any action taken. Vital signs were monitored at every visit. The compliance was also assessed by counting the amount of returned test drugs at the 2nd and 4th weeks.

B. Safety and tolerability assessment

During the study, there was no statistically significant difference between the 2 treatment groups (0.35 cases per subjects in SKI306X and 0.49 cases per subjects in Diclofenac) in the incidence of any adverse event. Moreover, the number of patients who had adverse events during the study was slightly greater in the control treatment group than in the active group (37 subjects in SKI306X group and 43 subjects in diclofenac group).

Adverse events were mild in severity and were more frequent in diclofenac SR group than in SKI306X group (24 cases (19.2%) vs. 46 cases (37.1%)). The most common clinical adverse events reported were from the digestive system, which accounted for 22.4% in SKI306X group and 25.8% in the diclofenac group (Table I). One serious adverse event was reported in diclofenac treatment group, which was due to intracranial hemorrhage from a patient with history of hypertension. This event was considered unrelated to the drug.

There were more frequent laboratory adverse events in diclofenac group than in SKI 306X group, largely due to the greater incidence of increased serum aminotransferase levels in the former group. Table II shows the result of laboratory tests [hematology, biochemistry, urinalysis, alanine aminotransferase(ALT)], in which the numbers represent the number of patients who had normal laboratory values at baseline and ended with abnormal termination values at the end of the study. There were no statistically significant differences between two groups in any of the laboratory indices obtained in this study. Elevations of ALT in diclofenac SR was more frequent than that in SKI306X group (2 vs.11), which was a statistically significant difference (p=0.01). And also with regard to aspartate aminotransferase (AST), there is more frequent elevation in diclofenac group than in the SKI306X group, although there was no statistically significant difference (p=0.10). Regarding vital signs including heart rate and blood pressure, there were no clinically or statistically significant changes in time for either treatment.

Table I
Comparison of incidence of adverse events by body system and drug relatedness

Body system ¹⁾	SKI 306X (N=125)		Diclofenac (N=124)		Total (N=249)	
	N	%	N	%	N	%
Allergy	0	(0.0)	1	(0.8)	1	(0.4)
Cardiovascular	3	(2.4)	3	(2.4)	6	(2.4)
Dermatological	2	(1.6)	0	(0.0)	2	(0.8)
Digestive	28	(22.4)	32	(25.8)	60	(24.1)
Musculoskeletal	0	(0.0)	1	(0.8)	1	(0.4)
Neurology	1	(0.8)	5	(4.0)	6	(2.4)
Respiratory	6	(4.8)	2	(1.6)	8	(3.2)
Renal/Genitourinary	1	(0.8)	5	(4.0)	6	(2.4)
Others	3	(2.4)	12	(9.7)	15	(6.0)
Total number of adverse event (AE) ²⁾	44	(35.2)	61	(49.2)	105	(42.2)
Total number of patients experiencing AE ³⁾	37	(29.6)	43	(34.7)	80	(32.1)
Total number of drug related AE ²⁾	24	(19.2)	46	(37.1)	70	(28.1)
Total number of patients experiencing drug related AE ⁴⁾	22	(17.6)	36	(29.0)	58	(23.3)
Total number of patients with serious AE	0	(0.0)	1	(0.8)	1	(0.4)

¹⁾ COSTART Classification of Adverse Events; ²⁾ Some patients had more than one Adverse Event, ³⁾ p-value was 0.390 by chi-square test; ⁴⁾ p-value was 0.033 by chi-square test

Table II
Number of patients with abnormal termination values in laboratory tests

Laboratory Measure	SKI 306X (N=125)	Diclofenac (N=124)	Total (N=249)	p-value ¹⁾
	N(%)	N(%)	N(%)	
Chemistry				
Glucose	14(11.2)	16(12.9)	30(12.1)	0.68
BUN	9(7.2)	13(10.5)	22(8.8)	0.36
Creatinine	5(4.0)	3(2.4)	8(3.2)	0.72
Protein, total	5(4.0)	4(3.2)	9(3.6)	1.00
Albumin	1(0.8)	0(0.0)	1(0.4)	1.00
T. Bilirubin	2(1.6)	1(0.8)	3(1.2)	1.00
ALP	2(1.6)	2(1.6)	4(1.6)	1.00
ALT	2(1.6)	7(5.7)	9(3.6)	0.10
AST	2(1.6)	11(8.9)	13(5.2)	0.01
GGT	2(1.6)	6(4.8)	8(3.2)	0.17
Hematology				
WBC	5(4.0)	5(4.0)	10(4.0)	1.00
RBC	6(4.8)	11(8.9)	17(6.8)	0.20
Hemoglobin	2(1.6)	6(4.8)	8(3.2)	0.17
Hematocrit	5(4.0)	11(8.9)	16(6.4)	0.12

¹⁾ p-value by chi-square test or Fisher's exact test

Chapter 5: Toxicology

5.1. Acute Toxicity⁸

SUMMARY OF RESULTS

The acute toxicity of SKI306X was evaluated in rats by a single oral administration. After oral administration of SKI306X with several doses (5.0 g/kg, 3.3 g/kg, 2.2 g/kg, 1.5 g/kg, and 1.0 g/kg), mortality, clinical signs, body weight, and macroscopical observations in organs were examined. No toxic effect was shown in terms of mortality, clinical signs, body weight changes and macroscopical observations. It is therefore suggested that the LD₅₀ of SKI306X would be more than 5.0 g/kg.

STUDY DESIGN

The study, Acute Toxicity of SKI306X, an Anti-inflammatory Herbal Extract, in Rats, was performed in accordance with the Guideline for Toxicity Test of Drug, etc. (Notification No. 94-3 of National Institute of Safety Research dated April 14, 1994).

A. Materials

SKI306X was prepared by extracting a mixture of 3 herbal components (*Clematis mandshurica*, *Trichosanthes kirilowii*, *Prunella vulgaris*) in a ratio of 1:2:1 in 30% (v/v) ethanol-water. The extract was evaporated *in vacuo*, and partitioned between the water-saturated butanol and water. The butanol layer was evaporated *in vacuo* and lyophilized. The typical index substances include oleanolic acid and rosmarinic acid in a certain ratio

B. Animals and breeding conditions

Experimental animals were pathogen-free Sprague Dowley rats at 4 weeks of age. The rats were housed in an environmental safety cabinet for 7 days and healthy subjects were chosen after observation during this acclimation period. 5 animals were housed per cage, fed tap water and laboratory feed; the animals were fasted for 18 hours prior to administration of the test substance.

C. Dose levels and treatment groups

The highest dose level was determined as 5 g/kg and all rats were divided into 5 SKI306X-treated groups with the same ratio X 0.66. Each group consisted of 5 males and 5 females.

D. Administration of test substance

A yellowish, brown SKI306X brown was suspended in 0.5% sodium carboxy methyl cellulose and administered orally to rats daily at a dose of 20 ml/kg.

E. Observations and test items

1. LD₅₀
2. Observation of clinical signs and lethality: For all experimental animals, the general signs, toxic symptoms and death were observed per hour up to 6 hours during day 0 of administration and once a day to days 14 as a termination of observation.
3. Body weight changes: Before the start of drug administration, and at days 4, 7, 11, and 14 (necropsy date)
4. Necropsy All survivors were necropsied at the termination date of observation, and a macroscopic examination was carefully made on the external or internal organs.

F. Statistical analyses

LD₅₀ were calculated using Litchfield-Wilcoxon method, and statistical analyses were performed with Duncan's multiple range test of one-way analysis of variance (ANOVA) for measuring the significance level.

⁸ Ahn et al. *Acute Toxicity of SKI306X, an Anti-inflammatory Herbal Extract, in Rats*, 1 Journal of Applied Pharmacology 32 (1996)

RESULTS

1. Lethality: No death of animals was observed during the experimental period (Table I).
2. Clinical signs: All experimental animals did not show any abnormal clinical signs during the experimental period (Table II).
3. Body weight changes: The significant body weight changes were not observed between SKI306X-treated groups and control group during test period (Table III).
4. Macroscopical observations: After termination, all survivors were anesthetized with ether and sacrificed by exsanguinations. The internal organs were carefully investigated macroscopically. No abnormal findings were observed on organs associated with drug administration (Table IV)

Table I. Mortality of SD Rats Treated Orally with SKI306X

Sex	Dose (g/kg)	Hours After Treatment						Days After Treatment						Final Morality	
		1	2	3	4	5	6	1	2	3	12	13	14		
male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	5.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	1.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5
	5.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

Table II. Clinical Signs of SD Rats Treated Orally with SKI306X

Sex	Dose (g/kg)	Clinical Sign	Hours After Treatment						Days After Treatment						
			1	2	3	4	5	6	1	2	3	12	13	14	
male	0	NAD*	--	--	--	--	--	--	--	--	--	--	--	--	--
	1.0	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	1.5	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	2.2	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	3.3	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	5.0	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
female	0	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	1.0	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	1.5	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	2.2	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	3.3	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--
	5.0	NAD	--	--	--	--	--	--	--	--	--	--	--	--	--

*NAD, -- : no abnormality detected

Table III. Body Weight Changes of SD Rats Treated Orally with SKI306X

Sex	Dose (g/kg)	Days After Treatment				
		0	4	7	11	14
Male	0	119.08±3.73	162.55±4.21	189.20±4.74	215.57±3.33	237.30±3.89
	1.0	116.00±2.38	157.00±4.80	182.79±6.93	210.00±9.04	231.24±8.78
	1.5	118.35±6.21	160.35±9.81	187.25±10.92	212.12±11.30	235.56±12.86
	2.2	117.97±2.93	160.58±3.43	187.44±4.42	218.39±5.35	240.47±5.50
	3.3	115.33±3.93	157.27±5.95	185.02±6.90	212.11±8.11	235.81±7.86
	5.0	116.35±3.57	156.49±4.19	186.68±5.36	212.66±5.83	235.75±7.43
Female	0	104.26±2.18	140.04±5.46	155.01±5.58	168.22±8.21	176.74±10.42
	1.0	105.06±2.96	139.36±7.22	158.15±9.09	172.88±11.16	182.41±12.61
	1.5	102.73±5.49	137.96±5.72	157.63±4.16	173.15±5.43	184.75±5.12
	2.2	103.92±3.07	138.18±3.88	156.11±4.89	170.23±4.48	181.06±7.70
	3.3	102.24±4.91	134.11±4.68	152.07±5.56	167.59±5.01	175.78±6.84
	5.0	103.00±4.54	136.17±8.09	154.32±8.20	172.88±8.18	185.32±9.53

Table IV. Macroscopical Observations in Organs of SD Rats Treated Orally with SKI306X

Organ	Male						Female					
	Dose (g/kg)						Dose (g/kg)					
	0	1.0	1.5	2.2	3.3	5.0	0	1.0	1.5	2.2	3.3	5.0
Braim	0/5*	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Heart	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Liver	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Lung	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Stomach	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Spleen	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Kidney, L	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Kidney, R	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Adrenal Gland, L	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Adrenal Gland, R	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis, L	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis, R	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Ovary	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

*the number of animals with abnormality/the number of animals per group

5.2 Subacute Toxicity⁹

SUMMARY OF RESULTS

To evaluate subacute toxicity of SKI306X, SKI306X was administered orally to rats once a day for 4 weeks at doses of 0.3, 1.0, and 3.0 g/kg/day, followed by a 2-week recovery test. For interim study, a 2-week repeated dosing group was also included.

All rats survived and no adverse clinical symptoms were observed. Although male rats treated with high dose (3.0 g/kg) of SKI306X showed slight loss of body weight (approximately 5%) in comparison with control animals during the administration period, their body weight was normally restored during the recovery period. Food consumption did not show any differences during the experimental period; male rats treated with low dose (0.3 g/kg) and intermediate dose (1.0 g/kg) of SKI306X showed differences in water intake compared with those of the control group at day 7, but showed no abnormal signs thereafter.

No significant change was found in all hematological parameters of SKI306X-treated groups except for the decreased number of red blood cells in all female groups at the interim study. It was reasoned that SKI306X is not involved in inducing any reduction of red blood cells as 1) there were different red blood cell levels between males and females; 2) the red blood cell changes were in the normal level and reversible; 3) the red blood cell count did not show any dose- or administration period-dependent changes; and 4) some anemia-associated parameters including HGB, HCT, etc. were normally measured.

Blood biochemical results showed that the SKI306X-treated groups showed differences over the control group, however, since most of these increases were within the normal range and reversible without more toxicity, the results show that SKI306X does not induce any abnormal change due to its toxicity.¹⁰ It is considered that the increase of ALP, AST and ALT in male rats treated with an intermediate dose of SKI306X and in female rats treated with a low dose of SKI306X for two weeks may be induced by organ damages associated with bile duct hyperplasia, fibrosis, etc. through gross and histopathological findings. Changes such as these are occasionally found in rats and have no correlation with SKI306X.¹¹

In SKI306X-treated groups, the absolute and relative weights of some organs showed changes over the control group. Although the absolute and relative weight of the heart in male rats treated with an intermediate dose and high dose of SKI306X was reduced over control group after the 4-week administration of SKI306X, these differences disappeared in all SKI306X-treated groups at the end of the recovery period and abnormal histopathological findings were not observed. The weight of the stomach in male rats treated with the high dose was increased, but abnormal proliferation was not found histopathologically. In the case of liver or heart, dose-dependent response or time-dependent severe toxicity was not observed.

In histopathological findings, local lymphocyte infiltration in the kidney and some lesions in the liver were found both in SKI306X-treated groups. But, these signs were also found in the control group.

The results of acute toxicity of SKI306X indicated that some parameters were significantly different from those of the control group; however, these parameters did not show dose- or administration period-related response and more severe toxicity was not found in proportion to prolongation of the administration period.)

In consideration of the fact that weight loss was observed in male rats treated with high dose of SKI306X it is concluded that the non-toxic dose of SKI306X was estimated to be between 0.3 and 1.0 g/kg/day and the maximum tolerated dose of SKI306X was higher than 3.0 g/kg/day.

STUDY DESIGN

This study was performed in accordance with the Guideline for Toxicity Test of Drug, etc. (Notification No. 94-3 of National Institute of Safety Research dated April 14, 1994).

⁹ Kim et al., *Subacute Toxicity of SKI306X, an Anti-inflammatory Herbal Extract, in Rats*, 1 Journal of Applied Pharmacology 19 (1996).

¹⁰ Mosberg and Hayes, *Subchronic Toxicity Testing*, In Principles and Methods of Toxicology, 221-236 (A.W. Hayes ed. Raven Press 1989).

¹¹ Eustis et al., *Liver*, In Pathology of the Fisher Rat, 71-94 (G.A. Boorman, S.L. Eustis, M.R. Elwell, C.A. Montgomery, Jr., W.F. Mackenzie eds. 1990)

A. Materials and Methods

SKI306X was prepared by extracting a mixture of 3 herbal components (*Clematis mandshurica*, *Trichosanthes kirilowii*, *Prunella vulgaris*) in a ratio of 1:2:1 in 30% (v/v) ethanol-water. The extract was evaporated *in vacuo*, and partitioned between the water-saturated butanol and water. The butanol layer was evaporated *in vacuo* and lyophilized.

B. Animals and Breeding Conditions

Experimental animals were pathogen-free Sprague Dowley rats at 4 weeks of age. The rats were housed in an environmental safety cabinet for 7 days and healthy subjects were chosen after observation during this acclimation period. 5 animals were housed per cage, fed tap water and laboratory feed; the animals were fasted for 18 hours prior to administration of the test substance.

C. Dose and Treatment Groups

Based on preliminary results, all rats were divided into 3 groups; high dose (3.0 g/kg), intermediate dose (1.0 g/kg) and low dose (0.3 g/kg). Animals that had similar body weights were allocated for this experiment. Animals were organized into the following groups: 5 animals for a repeated oral administration of SKI306X for 2 weeks, 10 animals for a repeated oral administration of SKI306X for 4 weeks, and 5 animals for a recovery test for 2 weeks. Each group consisted of 20 males and 20 females (Table I).

D. Administration of Test Substance

SKI306X was suspended in 0.5% sodium carboxy methyl cellulose (CMC) and administered orally. Control was treated with 0.5% CMC solution in a dose of 10 ml/kg. The recovery test was performed by the administration of SKI306X for 4 weeks and then a washout period was established for 2 weeks of 5 males and 5 females each.

E. General Signs and Observation Items

During the study, the general symptoms (anorexia, salivation, diarrhea, vomiting, polyuria, and fecal change) and their severities were recorded daily, and during the 2-week recovery period, body weights were measured once daily. Food and water intake was measured two times a day during the administration period and once daily during the recovery period. Ophthalmological examinations on all animals were performed at 2-week intervals during the course of the study. Urinalysis was performed on all animals and at the final week of administration, animals were housed in cages for 24 hours to collect urine, noting its appearance and measuring the amount. Immediately thereafter, pH, protein, nitrite, urobilinogen, bilirubin, glucose and blood were measured using a urinalysis paper (Ames Co., N-multistix).

F. Necropsy and organ weight measurement

No dead animals were observed during the administration period. At the end of the study, all animals were anesthetized with ether and exsanguinated to perform a macroscopic organ weight measurement. Major organs and tissues were examined for weight change, including: liver, kidney, spleen, heart, lung, brain, thyroid gland, stomach, prostate gland, uterus, ovary, testes, etc. The relative organ weight over body weight was measured.

G. Hematological and Blood Biochemistry Examination

Survivors were anesthetized with ether and their blood samples were collected through abdominal binder vein. The hematological parameters included red blood cell count, white blood cell count, hematocrit, hemoglobin, and Plt. The blood biochemistry examination were performed in such a manner that blood samples were collected at room temperature for 1 hour and centrifuged at 3000 rpm X 15 minutes to obtain a serum. The blood biochemistry parameters using a serum biochemistry automatic analyzer (550 Express, Ciba-Corning, USA) included alanine transaminase, total cholesterol, blood glucose, creatinine, albumin, Na, Cl, K, etc.

H. Histopathological Examination

All survivors were sufficiently exsanguinated and after the organ weights were measured, all organs and tissues were fixed with 10% neutral formalin. The organs and tissues, with a sufficient fixation for over 2 weeks, were embedded using a paraffin embedding device (Tissue Embedding Center, Germany) to make a fragment of 4-5 μ m. The fragment was observed by Hematoxylin & Eosin staining.

I. Statistical Analyses

Statistical analyses were performed with Duncan's multiple range test of one-way analysis of variance (ANOVA) and statistical analysis system (SAS) for measuring the significance level.

RESULTS

1. **General Signs and Observations:** There were no abnormal symptoms in SKI306X-treated groups with high dose, intermediate dose and low dose and the control (Table II). Animals that appeared to be dead or moribund during the experimental period were not observed.

Body weight: Increasing body weight between SKI306X-treated groups and control groups during the experimental period were noted (Fig. 1). The body weight gains of male rats treated with the high dose showed differences over the control from 1-week administration to termination of the administration. During the 2-week recovery period, no significant body weight changes were observed between the SKI306X-treated groups and the control group.

Food and water consumption: No significant differences were observed between the SKI306X-treated groups and the control groups. The male rats treated with the low dose and intermediate dose during the experimental period showed differences of water consumption over control at 7 days after the commencement of the administration but thereafter, no statistically significant differences were observed.

Ophthalmological examinations: No abnormal symptoms were shown during the experimental period.

2. Hematology and Blood Biochemical Examinations:

Hematology: The hematological results on blood samples of rats collected from the repeated 2-week administration of SKI306X showed that the red blood cell count in all SKI306X-treated groups were different over control, but in the case of the repeated 4-week administration of SKI306X, no significant differences were observed between all SKI306X-treated groups and the control groups. Therefore, it is reasoned that SKI306X is not involved in inducing any reduction of the red blood cell count as: 1) there were different red blood cell levels between the males and females; 2) the red blood cell count changes were in the normal level and reversible; 3) the red blood cell count did not show any dose- or administration period-dependant changes; and 4) some anemia-associated parameters including HGB, HCT, etc. were normally measured. Other parameters did not show any significant differences in rats treated with SKI306X (Tables III-V).

Blood Biochemical, 2-Week Administration: The blood biochemical results conducted after the 2-week repeated oral administration of SKI306X showed an increase in ALP in the female rats treated with the low dose and intermediate dose while some parameters such as ALT, BUN and Cl were increased in the low dose group. BUN in male rats treated with the high dose was increased over the control group (Table VI)

Blood Biochemical, 4-Week Administration: The blood biochemical results conducted after the 4-week repeated administration of SKI306X showed that Cl and Na were reduced over control in male rats treated with the intermediate dose. the BUN in male rats treated with the low dose was increased over the control group, and the glucose and Na were reduced over the control group.

Cl in female rats treated with the high dose was increased over the control, and creatinine in female rats treated with high and intermediate doses were increased over the control. Glucose in the female rats treated with the low dose group was reduced. Ca in all female SKI306X-treated groups was reduced over the control. Unlike the repeated 2-week administration, no increased ALT levels were observed during the repeated 4-week administration (Table VII).

Blood Biochemical, 2-Week Recovery Period: AST in male rats treated with intermediate dose was increased over the control, while Cl and Na in the low dose group were reduced over the control. Creatinine and Na in female rats treated with intermediate dose were increased over the control, while bilirubin in the low dose group was reduced over the control.

The blood biochemistry results showed that several parameters indicating statistical differences in SKI306X-treated groups were found over the control group, but these changes were within the normal ranges without any dose-dependent patterns and the severity of toxicity associated with the long-term treatment was not observed (Table VIII).

3. **Urinalysis:** No specific changes were observed in SKI306X-treated groups orally administered for the repeated 4-week period and followed by the recovery period (Tables IX, X).

4. Organ weight changes:

Absolute Organ Weight: The absolute organ weight measured after the 2-week administration showed an increase of liver in male rats treated with intermediate dose and stomach in female rats treated with the high dose (Table XI). The absolute weight measured after the 4-week administration showed an increased heart weight in male rats treated with the intermediate and high dose and stomach weight in male rats treated with the high dose SKI306X (Table XII). There was no difference of absolute organ weight measured at the termination of the recovery period (Table XIII).

Relative Organ Weight: The relative organ weight measured after the 2-week oral administration showed an increase in the liver weight in male rats treated with the intermediate and high doses and female rats treated with the low dose (Tables XIV, XV). However, during the 4-week administration, the relative weight of the liver was increased only in male rats treated with the low dose. The relative weight of the stomach during the administration period showed an increasing tendency, while the weight of the heart after the 4-week administration was reduced in male rats treated with the intermediate and high doses. The relative weight of the brain after the 4-week administration showed an increasing tendency in male rats treated with the high dose. There was no difference of organ weight when the relative organ weights were measured at the termination of the recovery period (Table XVI).

5. Histopathological Findings:

Histopathological Findings after the 2-week Repeated Administration: Table XVII.

Histopathological Findings after the 4-Week Repeated Administration: Table XVIII.

Histopathological Findings after the 2-week Recovery Period: Table XIX.

The histopathological findings indicate that main lesions were limited to the kidney and liver. These lesions were different from toxicopathological findings such as cellular swelling, and organostructural necrosis, proliferation, hypertrophy and atrophy. These lesions were commonly observed both in SKI306X-treated groups and the control and the number of histopathological findings was few. Further, since bile duct hyperplasia and periductular fibrosis was not significantly related to nor more significant in SKI306X-treated groups, the lesions were not induced by SKI306X.

Table I. Experimental design of subacute toxicity study of SKI306X in rats*

Sex	Dose (g/kg/day)	Number of animals	Number of animals sacrificed		
			At the end of experimental periods (week)		
			2	4	6 ^b
Male	Control (T1)	20	5	10	5
	0.3 (T2)	20	5	10	5
	1.0 (T3)	20	5	10	5
	3.0 (T4)	20	5	10	5
Female	Control (T1)	20	5	10	5
	0.3 (T2)	20	5	10	5
	1.0 (T3)	20	5	10	5
	3.0 (T4)	20	5	10	5

*SKI306X was administered orally to rats once daily for 28 days, followed by a recovery period of 2 weeks.

^bAt the end of two-week recovery period.

Table II. Mortality in rats treated orally with SKI306X

Sex	Dose (g/kg/day)	Experimental period (week)								
		Administration period (week)					Recovery period (week)			
		1	2	3	4	Mortality	5	6	Mortality	
Male	Control	0	0	0	0	0/15	0	0	0/5	
	0.3	0	0	0	0	0/15	0	0	0/5	
	1.0	0	0	0	0	0/15	0	0	0/5	
	3.0	0	0	0	0	0/15	0	0	0/5	
Female	Control	0	0	0	0	0/15	0	0	0/5	
	0.3	0	0	0	0	0/15	0	0	0/5	
	1.0	0	0	0	0	0/15	0	0	0/5	
	3.0	0	0	0	0	0/15	0	0	0/5	

*Number of dead animal

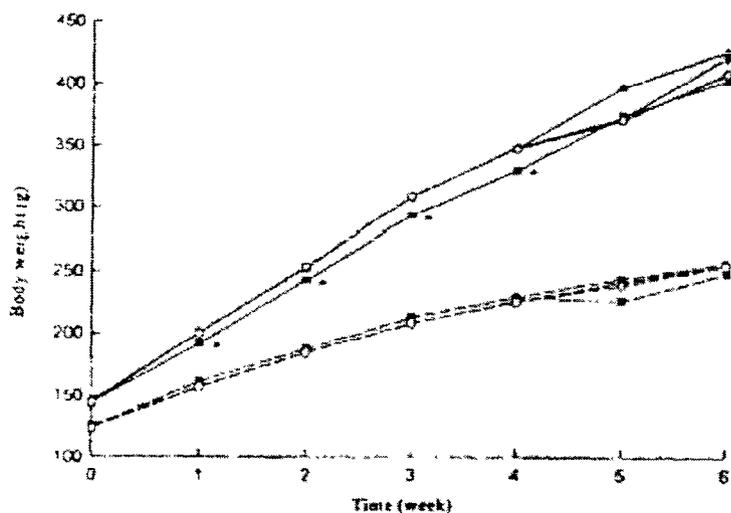


Fig. 1. Body weight changes of rats administered orally with SKI306X. —: Male, - - : Female, ○: Control, ●: SKI306X 0.3 g/kg, ▼: SKI306X 1.0 g/kg, ■: SKI306X 3.0 g/kg. *Significantly different from control ($p < 0.05$).

Table III. Hematological results of rats treated orally with SKI306X for two weeks

Sex	Dose (g/kg)	WBC ($10^3/mm^3$)	Lymph (%)	Mono. (%)	Granul (%)	RBC ($10^6/mm^3$)	HGB (g/dl)	HCT (%)	PLT ($10^3/mm^3$)
Male	Control	13.06±3.29	90.02±2.53	8.52±2.27	1.46±0.34	6.29±0.55	6.29±1.02	40.56±2.82	128.64±22.77
	0.3	8.86±2.81	89.94±1.44	8.64±1.10	1.42±0.53	6.04±0.42	12.60±0.50	39.20±2.19	121.80±10.70
	1.0	10.78±2.76	89.10±2.62	9.26±2.02	1.64±0.67	6.09±0.39	12.80±0.45	39.88±1.92	133.24±9.08
	3.0	11.70±3.38	90.54±1.99	8.32±1.51	1.04±0.30	5.95±0.40	11.88±0.41	37.44±1.03	117.76±19.31
Female	Control	8.10±1.79	93.20±1.48	5.92±1.06	0.88±0.43	6.28±0.17	12.6±0.38	38.94±1.38	127.28±14.51
	0.3	8.50±3.89	88.72±5.81	9.62±5.46	1.66±0.88	5.89±0.18*	12.24±0.36	37.26±0.46	111.60±29.36
	1.0	12.04±3.66	93.02±1.78	5.98±1.42	1.00±0.43	5.90±0.21*	12.44±0.42	37.32±1.23	126.60±14.53
	3.0	8.90±1.71	94.40±1.41	4.98±1.23	0.63±0.22	5.85±0.14*	12.25±0.13	36.95±1.16	137.65±7.86

Each value represents the mean±S.D. of 5 rats *Significantly different from control ($p < 0.05$)

Table IV. Hematological results of rats treated orally with SKI306X for four weeks

Sex	Dose (g/kg)	WBC ($10^3/mm^3$)	Lymph (%)	Mono. (%)	Granul (%)	RBC ($10^6/mm^3$)	HGB (g/dl)	HCT (%)	PLT ($10^3/mm^3$)
Male	Control	14.27±5.25	89.30±2.43	9.28±2.07	1.42±0.48	7.17±0.38	14.09±0.79	43.88±2.00	124.78±12.91
	0.3	14.89±5.40	89.30±2.14	9.17±1.81	1.53±0.83	7.05±0.33	14.20±0.63	43.28±1.71	110.94±10.82
	1.0	14.87±4.32	90.14±1.50	8.19±1.06	1.67±0.72	7.07±0.35	14.05±0.56	43.35±1.75	121.18±12.85
	3.0	14.75±5.94	88.95±2.33	9.42±1.61	1.63±0.87	7.21±0.44	14.29±0.70	44.69±2.34	113.34±16.10
Female	Control	10.89±3.96	94.01±0.72	5.44±0.65	0.55±0.21	6.88±0.38	13.53±0.71	41.62±1.92	110.94±10.37
	0.3	12.98±4.37	92.89±2.13	6.20±1.67	0.91±0.52	6.62±0.31	13.72±0.77	39.62±2.66	115.48±10.64
	1.0	12.61±3.05	92.73±1.21	6.52±1.19	0.75±0.26	6.88±0.24	13.21±0.41	40.49±1.28	119.80±15.08
	3.0	12.77±3.33	92.11±2.07	7.02±1.78	0.87±0.41	6.72±0.25	13.27±0.23	40.64±1.33	113.90±10.70

Each value represents the mean±S.D. of 10 rats

Table V. Hematological results of rats at the end of two-week recovery period after SKI306X treatment

Sex	Dose (g/kg)	WBC ($10^3/mm^3$)	Lymph (%)	Mono. (%)	Granul (%)	RBC ($10^6/mm^3$)	HGB (g/dl)	HCT (%)	PLT ($10^3/mm^3$)
Male	Control	15.70±4.37	93.46±2.26	6.02±2.05	0.52±0.23	7.45±0.29	13.84±0.72	43.24±1.86	111.32±15.20
	0.3	16.56±0.99	93.44±0.71	5.98±0.63	0.58±0.13	7.60±0.26	14.20±0.30	44.84±0.85	116.68±10.21
	1.0	16.06±5.52	92.73±2.19	6.64±2.04	0.62±0.26	7.53±0.29	13.90±0.59	43.84±1.78	106.36±8.31
	3.0	14.35±1.87	92.77±1.64	6.68±1.38	0.55±0.26	7.38±0.16	14.20±0.45	44.33±0.72	98.95±20.43
Female	Control	11.86±2.66	93.66±1.24	5.84±1.14	0.50±0.12	7.05±0.32	13.42±0.54	41.08±2.21	108.00±10.29
	0.3	10.96±2.95	94.32±1.37	5.16±1.24	0.52±0.18	6.79±0.13	13.02±0.36	39.52±0.62	105.40±3.68
	1.0	9.86±1.53	95.12±0.62	4.42±0.67	0.46±0.13	7.24±0.23	13.60±0.32	41.56±0.96	103.84±5.93
	3.0	13.58±8.59	92.74±3.12	6.56±2.56	0.70±0.57	7.15±0.34	13.58±0.83	41.50±2.31	108.72±17.71

Each value represents the mean±S.D. of 5 rats.

Table VI. Blood biochemistry of rats treated orally with SKI306X for two weeks

Sex	Dose (g/kg)	Albumin (g/dl)	ALP (U/L)	AST (U/L)	ALT (U/L)	Bilirubin (mg/dl)	Chol (mg/dl)	BUN (mg/dl)	Glucose (mg/dl)	Protein (g/dl)	Creatinine (mg/dl)	Tg (mg/dl)	Ca (mg/dl)	Cl (meq/L)	Na (meq/L)	K (meq/L)
M	Control	3.9±0.3	272.3±83.5	88.2±6.7	26.1±5.4	0.2±0.0	63.1±8.4	13.6±1.7	197.3±41.5	6.3±0.5	0.5±0.0	119.8±52.3	11.5±0.6	109.8±0.8	154.2±3.9	6.0±0.9
	0.3	3.8±0.2	216.7±43.6	121.3±21.2*	28.3±2.9	0.2±0.0	69.4±9.5	14.9±1.3	169.7±16.2	6.3±0.3	0.5±0.1	139.6±55.0	11.2±0.5	110.6±1.7	153.9±2.0	5.3±0.3
	1.0	3.9±0.2	396.0±107.9	95.7±11.3	31.5±3.0	0.2±0.0	63.3±5.1	16.2±2.1	215.9±37.8	6.5±0.3	0.5±0.0	133.1±66.1	11.8±0.7	111.0±1.9	157.1±1.5	6.7±1.1
	3.0	3.5±0.1	342.5±91.9	94.4±12.8	28.5±4.9	0.1±0.0	58.6±8.1	16.6±1.2*	160.9±11.8	5.9±0.3	0.4±0.1	130.2±57.9	10.9±0.4	108.8±3.4	151.5±5.2	5.1±0.4
F	Control	4.0±0.3	161.0±72.7	97.5±13.6	23.0±5.1	0.1±0.0	63.6±6.1	14.4±2.9	168.5±19.0	6.5±0.3	0.5±0.0	66.3±28.9	11.0±0.2	108.8±0.8	151.6±0.8	151.6±0.8
	0.3	4.0±0.1	353.1±56.9*	113.7±15.9	37.7±6.2*	0.1±0.0	65.9±9.0	19.8±3.2*	145.9±3.1	6.6±0.2	0.5±0.0	85.1±26.9	10.7±0.3	111.2±0.8*	111.2±0.8*	153.0±1.0
	1.0	4.0±0.3	334.3±75.6*	90.6±20.6	31.2±3.7	0.1±0.0	60.5±1.8	16.5±2.2	157.4±19.6	6.6±0.2	0.5±0.0	85.2±31.7	11.1±0.4	109.6±1.5	109.6±1.5	151.2±1.7
	3.0	3.9±0.2	191.9±78.0	94.2±10.4	24.0±4.2	0.2±0.0	63.1±10.7	14.6±2.4	148.7±14.1	6.5±0.2	0.5±0.0	56.5±9.8	10.9±0.0	109.5±1.3	109.5±1.3	151.0±1.9

Each value represents the mean±S.D. of 5 rats *Significantly different from control (p < 0.05).

Table VII. Blood biochemistry of rats treated orally with SKI306X for ^{four} two weeks

Sex	Dose (g/kg)	Albumin (g/dl)	ALP (U/L)	AST (U/L)	ALT (U/L)	Bilirubin (mg/dl)	Chol (mg/dl)	BUN (mg/dl)	Glucose (mg/dl)	Protein (g/dl)	Creatinine (mg/dl)	Tg (mg/dl)	Ca (mg/dl)	Cl (meq/L)	Na (meq/L)	K (meq/L)
M	Control	3.8±0.2	320.8±61.1	77.9±7.2	33.6±4.4	0.1±0.0	69.4±11.0	15.0±1.8	235.4±46.1	6.9±0.4	0.5±0.0	201.1±128.3	11.7±0.7	110.2±1.4	154.9±2.3	5.8±1.2
	0.3	3.9±0.2	369.1±94.7	85.5±23.6	36.6±6.7	0.1±0.0	66.7±10.7	18.7±2.4*	185.9±22.2*	6.8±0.3	0.5±0.0	158.4±87.6	11.5±0.6	108.3±2.5	151.8±3.3*	5.7±0.9
	1.0	3.8±0.2	368.7±57.6	84.2±12.6	33.3±3.9	0.1±0.0	70.7±5.6	17.0±1.5	197.5±31.1	6.7±0.3	0.5±0.0	164.9±61.1	11.1±0.7	108.8±1.2*	151.5±2.1*	5.6±1.2
	3.0	3.9±0.3	371.9±69.2	86.5±9.5	31.7±4.5	0.1±0.0	55.4±6.8*	15.3±1.4	212.7±30.4	6.8±0.3	0.5±0.0	84.5±36.6*	11.2±0.5	111.3±1.3	155.0±1.9	6.1±1.2
F	Control	4.2±0.3	206.5±61.1	73.6±8.1	30.9±3.4	0.2±0.0	63.8±11.2	18.9±2.5	194.7±34.3	7.6±0.3	0.5±0.0	111.4±69.5	12.1±0.7	109.6±2.1	153.5±2.5	5.3±0.7
	0.3	4.1±0.2	210.9±41.6	85.2±13.9	32.4±5.7	0.2±0.0	62.0±7.5	18.7±3.2	156.6±18.0*	7.2±0.3	0.6±0.0	58.4±39.8	11.0±0.4*	111.0±1.2	152.3±1.6	4.6±0.3
	1.0	4.2±0.2	210.2±74.6	83.8±19.4	30.6±5.3	0.2±0.0	62.9±9.7	18.4±3.3	173.5±15.0	7.3±0.3	0.6±0.0*	120.7±63.6	10.9±0.5*	110.6±2.2	152.5±1.4	4.8±0.8
	3.0	4.1±0.2	194.6±65.8	86.1±16.7	26.1±3.6*	0.2±0.0	61.1±7.7	19.9±2.9	175.6±15.3	7.2±0.4	0.6±0.0*	84.4±53.2	10.8±0.3*	112.2±1.1*	152.7±2.5	4.8±0.6

Each value represents the mean±S.D. of 10 rats. *Significantly different from control (p < 0.05).

Table VIII. Blood biochemistry of rats at the end of two-week recovery period after SKI306X treatment

Sex	Dose (g/kg)	Albumin (g/dl)	ALP (U/L)	AST (U/L)	ALT (U/L)	Bilirubin (mg/dl)	Chol (mg/dl)	BUN (mg/dl)	Glucose (mg/dl)	Protein (g/dl)	Creatinine (mg/dl)	Tg (mg/dl)	Ca (mg/dl)	Cl (meq/L)	Na (meq/L)	K (meq/L)
M	Control	3.7±0.2	313.6±61.0	80.5±4.3	30.8±5.2	0.2±0.0	67.2±5.8	18.9±1.5	172.7±15.2	6.9±0.2	0.5±0.1	153.6±61.9	10.6±0.1	109.2±1.3	152.0±1.9	4.5±0.3
	0.3	3.7±0.2	249.1±52.8	81.3±9.6	34.3±5.7	0.2±0.0	63.3±12.1	20.3±2.8	164.7±8.2	6.9±0.3	0.6±0.0	191.7±126.4	10.4±0.2	105.8±0.8*	146.7±1.1*	4.5±0.2
	1.0	3.7±0.2	254.0±68.0	107.5±22.1*	31.8±3.1	0.2±0.0	69.3±10.7	20.6±2.3	169.4±19.4	7.0±0.3	0.6±0.0	208.9±174.4	10.7±0.5	107.6±1.1	149.3±2.4	5.3±1.8
	3.0	3.7±0.1	242.8±84.8	83.4±8.5	27.9±2.1	0.2±0.0	57.8±6.5	18.3±2.4	176.1±18.6	6.9±0.1	0.5±0.0	150.3±20.8	10.6±0.3	108.2±1.1	150.1±1.8	5.4±1.0
F	Control	4.1±0.3	182.3±59.9	97.6±39.2	40.9±20.4	0.2±0.0	78.2±16.4	22.5±3.2	163.1±14.3	7.4±0.4	0.6±0.0	127.3±83.5	10.4±0.1	107.8±1.3	146.9±0.5	3.9±0.3
	0.3	4.2±0.1	179.1±40.1	67.8±5.0	33.9±7.5	0.1±0.0*	77.3±9.4	23.7±1.0	157.5±6.3	7.5±0.5	0.6±0.0	250.1±197.9	10.5±0.4	107.6±1.7	147.5±1.5	4.1±0.3
	1.0	4.4±0.4	195.5±72.8	84.9±11.0	29.4±4.5	0.2±0.0	65.2±12.6	23.7±1.8	135.7±16.5	7.8±0.6	0.7±0.0*	162.2±55.4	10.7±0.3	108.2±1.6	149.9±3.9*	4.0±0.3
	3.0	4.0±0.3	195.5±72.8	88.9±18.2	31.0±6.4	0.2±0.0	73.9±10.8	22.6±6.3	149.3±16.7	7.2±0.3	0.6±0.0	107.7±47.9	10.4±0.2	107.0±1.9	147.2±2.3	4.1±0.2

Each value represents the mean±S.D. of 5 rats. *Significantly different from control (p < 0.05).

Table IX. Urine analysis of rats treated orally with SKI306X for four weeks

Sex	Dose (g/kg)	n*	Urobil-	Occult	Bilirubin	Ketone	Glucose	Protein	pH				Nitrite
			inogen	blood						6	6-7	7	7-8
Male	Control	5	5	5	5	5	5	4 1	0	2	1	2	5
	0.3	5	5	5	5	5	5	4 1	0	2	3	0	5
	1.0	5	5	5	5	5	5	4 1	0	2	1	2	5
	3.0	5	5	5	5	5	5	4 1	0	1	2	2	5
Female	Control	5	5	5	5	5	5	5 0	2	2	1	0	5
	0.3	5	5	5	5	5	5	4 1	2	1	1	1	5
	1.0	5	5	5	5	5	5	5 0	3	1	0	1	5
	3.0	5	5	5	5	5	5	5 0	2	1	0	2	5

*Number of animals used. -: Negative, +: Slight Each value represents the number of animals under grade

Table X. Urine analysis of rats at the end of two week recovery period after SKI306X treatment

Sex	Dose (g/kg)	n*	Urobil-	Occult	Bilirubin	Ketone	Glucose	Protein	pH				Nitrite
			inogen	blood						6	6-7	7	7-8
Male	Control	5	5	5	5	5	5	5	1	0	3	1	5
	0.3	5	5	5	5	5	5	5	1	0	4	0	5
	1.0	5	5	5	5	5	5	5	1	0	3	1	5
	3.0	5	5	5	5	5	5	5	0	1	4	0	5
Female	Control	5	5	5	5	5	5	5	1	1	3	0	5
	0.3	5	5	5	5	5	5	4	0	3	2	0	5
	1.0	5	5	5	5	5	5	5	0	2	3	0	5
	3.0	5	5	5	5	5	5	5	0	3	2	0	5

*Number of animals used. -: Negative, +: Slight Each value represents the number of animals under grade.

Table XI. Absolute organ weight of rats treated orally with SKI306X for two weeks

Sex	Dose (g/kg)	Brain (g)	Thymus (g)	Lung (g)	Heart (g)	Liver (g)	Stomach (g)	Spleen (g)	Kidney (g)	Prostate (g) /Uterus (g)	Testes (g) /Ovaries (g)
Male	Control	1.93±0.13	0.67±0.08	1.14±0.06	0.88±0.03	10.72±0.81	1.32±0.11	0.68±0.07	2.17±0.13	0.35±0.11	2.37±0.16
	0.3	1.89±0.05	0.74±0.13	1.20±0.10	0.88±0.06	10.78±1.07	1.32±0.09	0.66±0.11	2.19±0.41	0.25±0.05	2.27±0.07
	1.0	1.98±0.09	0.76±0.09	1.28±0.12	0.88±0.02	12.91±1.18*	1.43±0.06	0.62±0.09	2.49±0.33	0.33±0.03	2.31±0.10
	3.0	1.87±0.07	0.63±0.12	1.19±0.12	0.84±0.04	11.46±0.69	1.34±0.13	0.61±0.11	2.09±0.09	0.32±0.08	2.31±0.23
Female	Control	1.81±0.08	0.55±0.05	0.94±0.09	0.69±0.05	8.09±0.89	1.10±0.13	0.46±0.05	1.51±0.08	0.40±0.19	0.09±0.01
	0.3	1.84±0.09	0.58±0.08	0.97±0.05	0.74±0.06	9.84±1.20	1.06±0.05	0.56±0.08	1.87±0.55	0.35±0.05	0.09±0.02
	1.0	1.82±0.09	0.54±0.10	0.93±0.07	0.71±0.04	8.91±0.75	1.15±0.09	0.50±0.05	1.57±0.21	0.38±0.09	0.10±0.02
	3.0	1.82±0.09	0.57±0.05	1.04±0.08	0.74±0.07	9.83±0.66	1.33±0.12*	0.53±0.05	1.73±0.16	0.38±0.06	0.11±0.02

Each value represents the mean±S.D. of 5 rats. *Significantly different from control (p < 0.05).

Table XII. Absolute organ weight of rats treated orally with SKI306X for four weeks

Sex	Dose (g/kg)	Brain (g)	Thymus (g)	Lung (g)	Heart (g)	Liver (g)	Stomach (g)	Spleen (g)	Kidney (g)	Prostate (g) /Uterus (g)	Testes (g) /Ovaries (g)
Male	Control	1.99±0.08	0.61±0.08	1.31±0.10	1.28±0.06	14.97±1.54	1.49±0.13	0.95±0.21	2.79±0.18	0.49±0.09	2.94±0.17
	0.3	2.05±0.09	0.61±0.07	1.35±0.14	1.21±0.12	16.24±1.88	1.44±0.16	0.88±0.15	2.66±0.28	0.39±0.05	2.87±0.10
	1.0	2.05±0.05	0.64±0.07	1.37±0.12	1.14±0.09*	16.00±1.44	1.58±0.14	0.88±0.11	2.68±0.29	0.40±0.06	3.01±0.31
	3.0	2.04±0.09	0.62±0.09	1.29±0.12	1.08±0.07*	14.80±0.79	1.65±0.07*	0.84±0.10	2.54±0.24	0.40±0.09	2.94±0.23
Female	Control	1.89±0.07	0.55±0.09	1.02±0.08	0.80±0.07	9.43±0.85	1.17±0.10	0.62±0.09	1.78±0.13	0.43±0.06	0.09±0.02
	0.3	1.94±0.09	0.50±0.09	0.98±0.06	0.78±0.04	9.64±0.65	1.17±0.05	0.64±0.08	0.50±0.13	0.50±0.13	0.09±0.02
	1.0	1.90±0.06	0.54±0.08	1.08±0.15	0.75±0.07	9.69±0.87	1.19±0.10	0.64±0.15	0.45±0.10	0.45±0.10	0.09±0.02
	3.0	1.87±0.06	0.48±0.06	1.08±0.18	0.80±0.06	10.51±0.66	1.28±0.10	0.57±0.11	0.45±0.05	0.45±0.05	0.08±0.01

Each value represents the mean±S.D. of 10 rats. *Significantly different from control (p < 0.05).

Table XIII. Absolute organ weight of rats at the end of two-week recovery period after SKI306X treatment

Sex	Dose (g/kg)	Brain (g)	Thymus (g)	Lung (g)	Heart (g)	Liver (g)	Stomach (g)	Spleen (g)	Kidney (g)	Prostate (g) /Uterus (g)	Testes (g) /Ovaries (g)
Male	Control	2.08±0.09	0.56±0.08	1.28±0.17	1.24±0.18	14.91±1.21	1.48±0.11	0.76±0.04	2.73±0.19	0.63±0.19	2.99±0.17
	0.3	2.13±0.11	0.53±0.08	1.40±0.19	1.18±0.09	16.51±1.81	1.46±0.09	0.75±0.08	2.83±0.17	0.65±0.19	2.99±0.15
	1.0	2.08±0.10	0.61±0.13	1.36±0.09	1.31±0.07	16.14±2.43	1.46±0.25	0.81±0.19	2.85±0.39	0.65±0.04	3.00±0.07
	3.0	2.08±0.09	0.51±0.07	1.29±0.07	1.22±0.06	15.57±0.69	1.49±0.11	0.74±0.04	2.91±0.20	0.62±0.12	3.15±0.21
Female	Control	1.89±0.07	0.47±0.10	1.00±0.09	0.79±0.04	9.81±1.27	1.11±0.10	0.59±0.09	1.71±0.05	0.65±0.24	0.08±0.01
	0.3	1.88±0.01	0.45±0.01	1.09±0.16	0.80±0.08	9.98±0.97	1.20±0.10	0.56±0.05	1.65±0.12	0.56±0.21	0.06±0.01
	1.0	1.94±0.03	0.42±0.07	0.98±0.11	0.82±0.07	9.58±1.02	1.12±0.06	0.54±0.04	1.73±0.18	0.39±0.03	0.08±0.01
	3.0	1.92±0.05	0.41±0.05	0.93±0.06	0.80±0.07	9.78±1.95	1.24±0.07	0.61±0.11	1.67±0.09	0.62±0.13	0.08±0.01

Each value represents the mean±S.D. of 5 rats

Table XIV. Relative organ weight (%) of rats treated orally with SKI306X for two weeks

Sex	Dose (g/kg)	Brain (g)	Thymus (g)	Lung (g)	Heart (g)	Liver (g)	Stomach (g)	Spleen (g)	Kidney (g)	Prostate (g) /Uterus (g)	Testes (g) /Ovaries (g)
Male	Control	0.79±0.04	0.27±0.03	0.44±0.05	0.36±0.01	4.34±0.21	0.53±0.04	0.28±0.03	0.88±0.05	0.14±0.04	0.96±0.08
	0.3	0.77±0.03	0.30±0.05	0.48±0.03	0.35±0.03	4.36±0.32	0.54±0.03	0.27±0.03	0.81±0.06	0.10±0.02	0.92±0.06
	1.0	0.76±0.03	0.29±0.03	0.49±0.03	0.34±0.02	4.98±0.31*	0.55±0.04	0.24±0.03	0.96±0.10	0.13±0.01	0.89±0.04
	3.0	0.79±0.03	0.27±0.05	0.51±0.04	0.36±0.01	4.86±0.30*	0.57±0.03	0.26±0.04	0.89±0.05	0.14±0.03	0.98±0.10
Female	Control	1.00±0.06	0.30±0.03	0.52±0.04	0.38±0.02	4.44±0.42	0.61±0.05	0.25±0.03	0.83±0.06	0.22±0.09	0.05±0.01
	0.3	0.99±0.08	0.31±0.03	0.52±0.03	0.40±0.01	5.27±0.47*	0.57±0.04	0.30±0.04	0.90±0.28	0.18±0.03	0.05±0.01
	1.0	0.99±0.05	0.30±0.06	0.51±0.05	0.39±0.02	4.85±0.23	0.63±0.05	0.27±0.03	0.86±0.09	0.21±0.06	0.05±0.01
	3.0	0.95±0.04	0.30±0.03	0.54±0.05	0.39±0.04	5.12±0.34	0.69±0.05*	0.28±0.03	0.90±0.07	0.20±0.04	0.06±0.01

Each value represents the mean ± S.D. of 5 rats. *Significantly different from control (p<0.05).

Table XV. Relative organ weight (%) of rats treated orally with SKI306X for ^{four} ~~two~~ weeks

Sex	Dose (g/kg)	Brain (g)	Thymus (g)	Lung (g)	Heart (g)	Liver (g)	Stomach (g)	Spleen (g)	Kidney (g)	Prostate (g) /Uterus (g)	Testes (g) /Ovaries (g)
Male	Control	0.57±0.05	0.18±0.03	0.37±0.03	0.36±0.03	4.26±0.45	0.42±0.03	0.27±0.05	0.80±0.05	0.14±0.02	0.84±0.05
	0.3	0.60±0.03	0.18±0.02	0.39±0.03	0.35±0.02	4.70±0.33*	0.42±0.05	0.26±0.04	0.77±0.04	0.12±0.01	0.83±0.05
	1.0	0.59±0.04	0.18±0.02	0.39±0.04	0.33±0.03*	4.57±0.28	0.45±0.03	0.25±0.02	0.77±0.05	0.12±0.02	0.86±0.08
	3.0	0.63±0.04*	0.19±0.03	0.40±0.03	0.33±0.02*	4.53±0.20	0.50±0.02*	0.26±0.03	0.78±0.07	0.13±0.03	0.90±0.07
Female	Control	0.84±0.06	0.24±0.03	0.45±0.02	0.35±0.02	4.18±0.25	0.52±0.05	0.27±0.03	0.79±0.03	0.19±0.04	0.04±0.01
	0.3	0.85±0.05	0.22±0.04	0.43±0.03	0.34±0.02	4.22±0.31	0.51±0.04	0.28±0.03	0.77±0.06	0.22±0.06	0.04±0.01
	1.0	0.85±0.06	0.24±0.04	0.48±0.08	0.34±0.02	4.34±0.29	0.53±0.04	0.29±0.07	0.75±0.08	0.20±0.05	0.04±0.01
	3.0	0.81±0.05	0.21±0.03	0.47±0.07	0.35±0.03	4.54±0.34	0.55±0.04	0.25±0.05	0.76±0.06	0.20±0.02	0.04±0.01

Each value represents the mean ± S.D. of 10 rats. *Significantly different from control (p<0.05).

Table XVI. Relative organ weight (%) of rats at the end of two-week recovery period after SKI306X treatment

Sex	Dose (g/kg)	Brain (g)	Thymus (g)	Lung (g)	Heart (g)	Liver (g)	Stomach (g)	Spleen (g)	Kidney (g)	Prostate (g) /Uterus (g)	Testes (g) /Ovaries (g)
Male	Control	0.51±0.03	0.14±0.01	0.31±0.04	0.30±0.04	3.64±0.18	0.36±0.03	0.18±0.02	0.67±0.05	0.16±0.05	0.73±0.06
	0.3	0.50±0.04	0.13±0.02	0.33±0.05	0.28±0.02	3.87±0.23	0.34±0.02	0.18±0.03	0.67±0.02	0.15±0.05	0.70±0.04
	1.0	0.50±0.03	0.15±0.03	0.32±0.04	0.31±0.00	3.82±0.40	0.35±0.05	0.19±0.04	0.68±0.06	0.15±0.02	0.71±0.03
	3.0	0.52±0.02	0.13±0.02	0.32±0.02	0.31±0.01	3.87±0.11	0.37±0.03	0.18±0.01	0.72±0.04	0.16±0.03	0.78±0.06
Female	Control	0.75±0.05	0.18±0.03	0.39±0.02	0.31±0.02	3.88±0.54	0.44±0.03	0.23±0.04	0.68±0.04	0.26±0.10	0.03±0.01
	0.3	0.74±0.05	0.18±0.01	0.43±0.05	0.31±0.02	3.89±0.21	0.47±0.02	0.22±0.01	0.64±0.02	0.22±0.09	0.03±0.01
	1.0	0.76±0.04	0.16±0.02	0.38±0.04	0.32±0.03	3.75±0.29	0.44±0.02	0.21±0.01	0.68±0.06	0.15±0.02	0.03±0.00
	3.0	0.78±0.06	0.17±0.02	0.38±0.01	0.32±0.03	3.95±0.67	0.50±0.02	0.25±0.04	0.67±0.02	0.25±0.06	0.03±0.00

Each value represents the mean ± S.D. of 5 rats.

Table XVII. Histopathological findings on rats treated with SKI306X for two weeks

Sex		Male			Female		
		T1	T3	T4	T1	T3	T4
Group	Dose (g/kg/day)	0	1.0	3.0	0	1.0	3.0
No of animal		5	5	5	5	5	5
Kidney	Tubular degeneration	0	0	0	0	0	0
	Urinary cast	1	0	0	0	0	0
	Lymphocyte infil.	1	1	2	0	0	0
	Fibrosis	0	0	0	0	0	0
	Cysts and Dilatation	0	0	0	0	0	0
Spleen and Lymph node	Hemosiderosis	0	0	0	0	0	0
	Extramedullary haemopoiesis	0	0	0	0	0	0
	Pigmentation	0	0	0	0	0	0
	Lymphoid changes	0	0	0	0	0	0
Liver	Hydropic and fatty changes	0	0	0	0	0	0
	Lymphocyte infil.	1	1	0	1	0	0
	Foci of altered hepatocytes	0	0	0	0	0	0
	Bile duct hyperplasia	0	1	0	0	0	0
	Fibrosis	0	1	0	0	0	0
	Necrosis	0	0	0	1	0	0
Stomach & Intestines	Inflammation	0	0	0	0	0	0
	Ulcerative changes	0	0	0	0	0	0
	Atropy	0	0	0	0	0	0
	Hyperplastic changes	0	0	0	0	0	0
Heart	Myocardial changes	0	0	0	0	0	0
	Inflammation	0	0	0	0	0	0
Lung	Myocardial changes	0	0	0	0	0	0
	Inflammation	0	0	0	0	0	0
Adrenal gland	Normal	5	5	5	5	5	5
Nervous system	Normal	5	5	5	5	5	5
Reproductive system	Normal	5	5	5	5	5	5

Table XVIII. Histopathological findings on rats treated with SKI306X for four weeks

Sex		Male			Female		
		T1	T3	T4	T1	T3	T4
Group	Dose (g/kg/day)	0	1.0	3.0	0	1.0	3.0
No of animal		5	5	5	5	5	5
Kidney	Tubular degeneration	0	0	0	1	0	0
	Urinary cast	2	1	1	2	1	1
	Lymphocyte infil.	1	2	3	2	1	1
	Fibrosis	0	0	0	1	0	0
	Cysts and Dilatation	0	0	0	0	0	0
Spleen and Lymph node	Hemosiderosis	0	0	0	0	0	0
	Extramedullary haemopoiesis	0	0	0	0	0	0
	Pigmentation	0	0	0	0	0	0
	Lymphoid changes	0	0	0	0	0	0
Liver	Hydropic and fatty changes	0	0	0	0	0	0
	Lymphocyte infil.	1	0	1	0	0	0
	Foci of altered hepatocytes	0	0	0	0	0	1
	Bile duct hyperplasia	0	0	0	0	0	0
	Fibrosis	0	0	0	0	0	0
	Necrosis	1	0	0	0	0	0
Stomach & Intestines	Inflammation	0	0	0	0	0	0
	Ulcerative changes	0	0	0	0	0	0
	Atropy	0	0	0	0	0	0
	Hyperplastic changes	0	0	0	0	0	0
Heart	Myocardial changes	0	0	0	0	0	0
	Inflammation	0	0	0	0	0	0
Lung	Myocardial changes	0	0	0	0	0	0
	Inflammation	0	0	0	0	0	0
Adrenal gland	Normal	10	10	10	10	10	10
Nervous system	Normal	10	10	10	10	10	10
Reproductive system	Normal	10	10	10	10	10	10

Table XIX. Histopathological findings on rats at the end of 2 weeks recovery period after SK1306X treatment

Sex		Male			Female		
		T1	T3	T4	T1	T3	T4
Group	Dose (g/kg/day)	0	1.0	3.0	0	1.0	3.0
No. of animal		5	5	5	5	5	5
Kidney	Tubular degeneration	0	0	0	1	0	1
	Urinary cast	1	1	2	1	0	1
	Lymphocyte infil	1	1	0	1	1	1
	Fibrosis	0	0	0	1	0	1
	Cysts and Dilatation	0	0	0	1	0	1
Spleen and Lymph node	Hemosiderosis	0	0	0	0	0	0
	Extramedullary haemopoiesis	0	0	0	0	0	0
	Pigmentation	0	0	0	0	0	0
	Lymphoid changes	0	0	0	0	0	0
	Hydropic and fatty changes	0	0	0	0	0	0
Liver	Lymphocyte infil	1	1	0	1	1	1
	Foci of altered hepatocytes	0	0	0	0	0	0
	Bile duct hyperplasia	0	0	0	1	0	1
	Fibrosis	0	0	0	1	0	1
	Necrosis	0	0	0	0	0	0
Stomach & Intestines	Inflammation	0	0	0	0	0	0
	Ulcerative changes	0	0	0	0	0	0
	Atrophy	0	0	0	0	0	0
	Hyperplastic changes	0	0	0	0	0	0
	Myocardial changes	0	0	0	0	0	0
Heart	Inflammation	0	0	0	0	0	0
	Myocardial changes	0	0	0	0	0	0
Lung	Inflammation	0	0	0	0	0	0
	Myocardial changes	0	0	0	0	0	0
Adrenal gland	Normal	5	5	5	5	5	5
Nervous system	Normal	5	5	5	5	5	5
Reproductive system	Normal	5	5	5	5	5	5

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Chapter 6: Proposed Use

Clematis mandshurica is intended to be used in the dietary supplement SKI306X at a dose of 200 mg per tablet. The recommended use is 400-600 mg per day. The amount of *Clematis mandshurica* in each 200 mg tablet is about 100 mg. The recommended use of 400-600 mg would therefore translate to about 200-300 mg of *Clematis mandshurica* per day.

SKI306X is a dietary supplement intended to be used to support healthy joints and cartilage. This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, mitigate or prevent any disease.

Submitted by: Ann-Yung Chiang
SK Pharma Co., Ltd.

Date: 7-19-06

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