

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 Chemicals Co., Ltd.  
948-1 Daechi 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*.<sup>1</sup> The dietary supplement as described in §2.2 contains Wei Ling Xian only in the form of *Clematis mandshurica*.

### 2.2 Product Description

SKI306X is the product name used throughout this document for all information submitted. SKI306X is the product that contains the new dietary ingredient, *Clematis mandshurica*. SKI306X is marketed in Korea as a product called JOINS®. SKI306X 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. The SKI306X is exactly the same as the JOINS® product and both contain the same amount of the new dietary ingredient, *Clematis mandshurica*, that is the subject of this submission.

SKI306X contains three herbal extracts, *Clematis mandshurica*, *Trichosanthes kirilowii* and *Prunella vulgaris*, and additionally silicon dioxide, microcrystalline cellulose (Avicel PH 102), corn starch, sodium starch glycolate and magnesium stearate. SKI306X is a coated tablet and the coating material consists of Hydroxypropyl-methyl-cellulose (HPMC 2910), Polyethylene glycol (PEG 6000), Titanium dioxide, talc, Iron oxide yellow, ethanol and distilled water. See Table 1 for comparison of ingredients for SKI306X and JOINS®.

The active ingredient in the new dietary ingredient *Clematis mandshurica* is oleanolic acid. A second index substance, rosmarinic acid, is also tested and is the active ingredient in *Prunella vulgaris*.

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

**Table 1: Product Comparison Chart**

<b>Ingredient</b>	<b>SKI306X</b>	<b>JOINS®</b>
(TCP Extracts) <i>Clematis mandshurica</i> , <i>Tricosanthes kirilowii</i> , <i>Prunella vulgaris</i> (30% ethanol extract (40:1))	200 mg	200 mg
Silicon dioxide	10 mg	10 mg
Microcrystalline cellulose (Avicel PH 102)	113 mg	113 mg
Corn starch	50 mg	50 mg
Sodium starch glycolate	q.s.	q.s.
Magnesium stearate	q.s.	q.s.
<b>Sub-Total amount</b>	<b>400 mg</b>	<b>400 mg</b>
Hydroxy-propyl-methyl cellulose (HPMC 2910)	20 mg	20 mg
Polyethylene glycol (PEG 6000)	2 mg	2 mg
Titanium dioxide	q.s.	q.s.
Talc	q.s.	q.s.
Iron Oxide yellow	q.s.	q.s.
Ethanol	0.33 mL	0.33 mL
Distilled Water	0.03 mL	0.03 mL
<b>Total Amount</b>	<b>430 mg</b>	<b>430 mg</b>

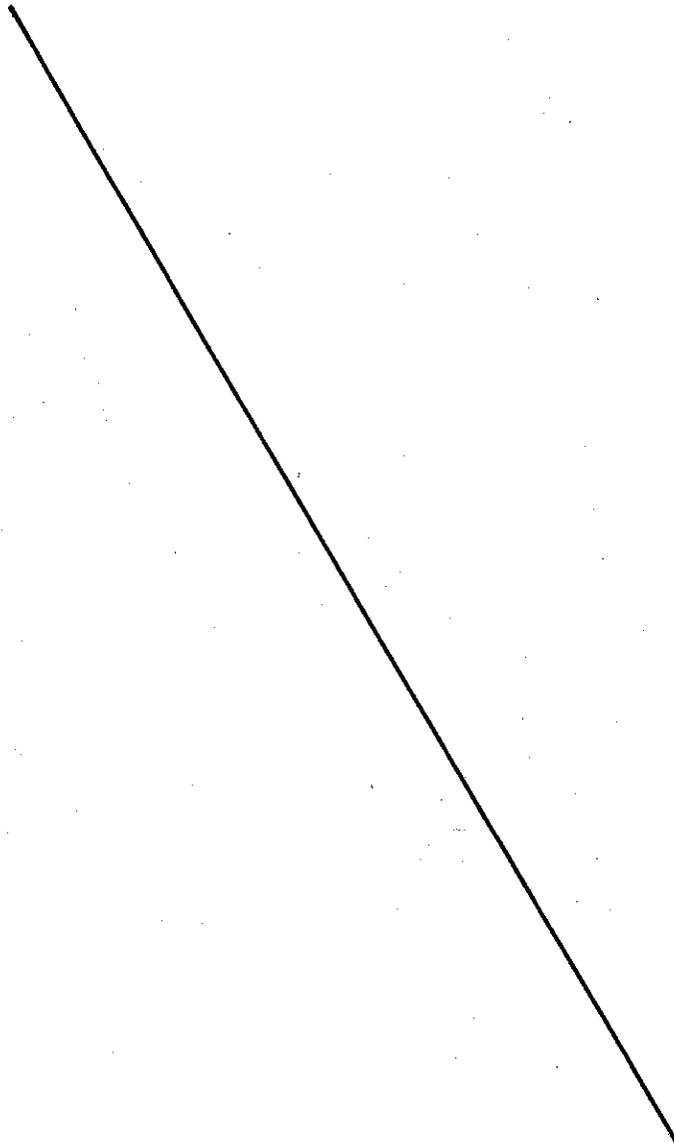
### 2.3 Proposed Product Labeling

- (a) SKI306X is marketed as a tablet containing 200 mg of active ingredient with a total weight of 430 mg. SKI306X contains approximately 100 mg (approximately 25%) of *Clematis mandshurica* extract. The amount is an approximate amount due to the extraction process.
- (b) The conditions of use suggested on the labeling are:

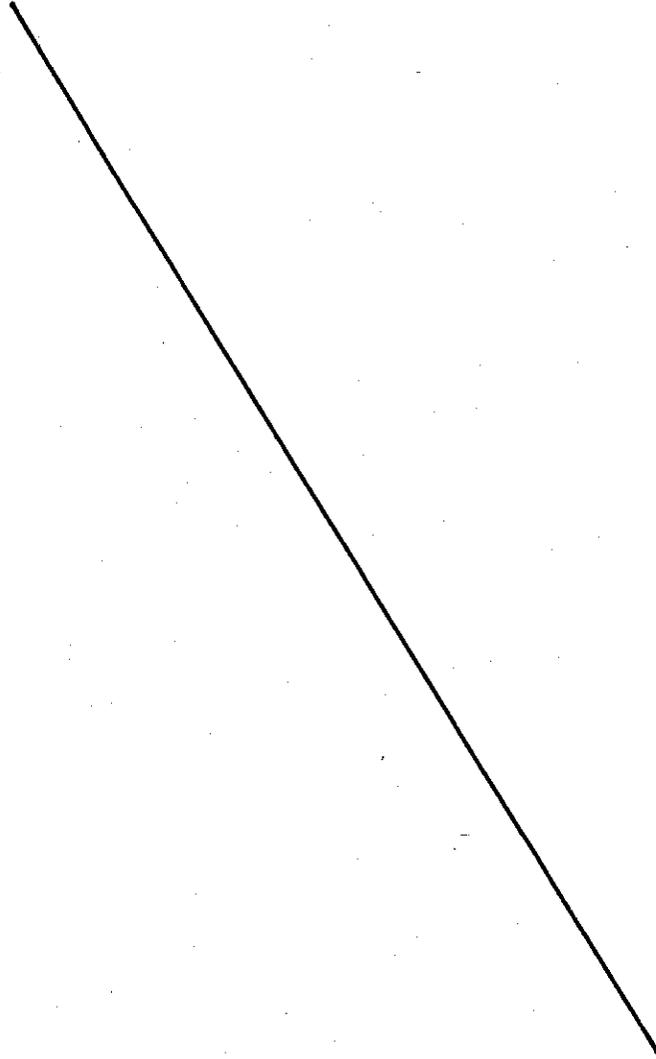
Suggested use: As a dietary supplement to support healthy joints and cartilage, take one tablet, 2-3 times daily.

# Chapter 3: MANUFACTURING PROCESS

## 3.1 Flow Chart



### 3.2 Description



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**3.3 Certificate of Good Manufacturing Practice from Korea Food and Drug Administration**

Enclosed as Exhibit A

**3.4 Certificate of Good Manufacturing Practice and Quality Standards**

Enclosed as Exhibit B

PAGE 10 THROUGH 42

33 PAGES TOTAL

REDACTED IN ITS  
ENTIRETY  
CONTAINS  
TRADE SECRET  
CONFIDENTIAL  
COMMERICAL  
INFORMATION

## Chapter 5: SUMMARY OF ALL TESTING RESULTS

### 5.1 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 SKI306X-treated groups.

The test articles in this study were prepared by extracting a mixture of 3 herbal components, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii*, 1:1:2 (w/w), respectively, in 7 times of 30% (v/v) ethanol-water. The extract solution was filtered and evaporated under vacuum. The residue was partitioned between 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 dark-brown powder. Tablets were made of 200 mg of the resultant product. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively, and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

The dosage in this study was evaluated at 200 mg, 400 mg and 600 mg *ter in die*. This corresponds to a dosage of 600, 1200 and 2400 mg per day. The dosage for the product SKI306X as described in Section 2.2 is 200 mg as active ingredients with a recommended daily use of 2-3 tablets daily. Therefore, the dosage and frequency in this study and in the SKI306X product are qualitatively and quantitatively equivalent.

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. Considering the pharmaco-economical aspect of SKI306X, the dosage of 200 mg *t.i.d.* was deemed the most suitable.

#### 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, urea 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 4<sup>th</sup> week of therapy.

The test articles in this study (identified as the product SKI306X) were prepared by extracting a mixture of 3 herbal components, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii*, 1:1:2 (w/w), respectively, in 7 times of 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 dark-brown powder. Tablets were made of 200 mg of the resultant product. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.3 uses the same three botanical herbs in

the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

The dosage in this study was evaluated at 200 mg as active ingredients of the test article, with patients taking 3 tablets per day. The dosage for the product SKI306X as described in Section 2.2 is 200 mg as active ingredients with a recommended daily use of 2-3 tablets daily. Therefore, the dosage and frequency in this study and in the SKI306X product are qualitatively and quantitatively equivalent.

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.

## 5.2 Toxicology

### A. Acute Toxicity

The test articles in this study (identified as the product SKI306X) were prepared by extracting a mixture of 3 herbal components, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii*, 1:1:2 (w/w), respectively, in 7 times of 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 dark-brown powder. The product was suspended in 0.5% sodium carboxy methyl cellulose. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

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 LD<sub>50</sub> of SKI306X is more than 5.0 g/kg. SKI306X has an extremely large safety margin.

### B. Subacute Toxicity

The test articles in this study (identified as the product SKI306X) were prepared by extracting a mixture of 3 herbal components, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii*, 1:1:2 (w/w), respectively, in 7 times of 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 dark-brown powder. The product was suspended in 0.5% sodium carboxy methyl cellulose. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

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

The test articles in this study (identified as the product SKI306X) were prepared by extracting a mixture of 3 herbal components, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii*, 1:1:2 (w/w), respectively, in 7 times of 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 dark-brown powder. Tablets were made of 200 mg of the resultant product. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Tricosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

This study was conducted to assess the safety of SKI306X for a 26-week repeated dose toxicity in male and female rats. After the discontinuation of the treatment, a 4-week recovery test was conducted. The test item was orally administered at dosages of 0 (control), 500, 1000, and 2000 mg/kg for 26 weeks. The clinical signs, mortality, body weight change, food and water consumption, organ weight analysis, necropsy, and histopathological findings were evaluated.

In the male 500 mg/kg treatment group, one animal died at the 4-week point due to gavage error. Soft feces and diarrhea were observed from the 9-week to 26-weeks in the male and female 1000 and 2000 mg/kg treatment group. Diarrhea was observed from 7-10 hours after administration and was recovered at 16 hours in the male treatment group and at 14 hours in the female group. These were considered to be temporary only when the test item passed the gastrointestinal tract and was not observed after early passage in the case of the daily administration. Soft feces and diarrhea were not observed during the recovery periods.

There was decreasing tendency in mean body weight of the male 2000 mg/kg treatment group. There was an increasing tendency of the food and water consumption. The decreasing tendency of the body weights and increasing tendency of the food and water consumptions were considered to be related to soft feces and diarrhea. During the recovery periods, there were no differences between the treatment group and the control group in body weights, food and water consumption.

There was no treatment-related ophthalmoscopic examination.

At urinalysis, the change of the urine color and the increase of erythrocyte were considered that the urine was mixed with diarrhea and therefore not related to the test item. There were no test item-related effects in the recovery group.

The hematology of the main group, platelet count was increased in the male 1000 and 2000 mg/kg treatment groups, but the value was within the normal range. The blood chemistry in the male 2000 mg/kg treatment group showed a minimal decrease of glucose and a minimal increase of total cholesterol, but these values were within the normal range. There were no differences between the treatment group and the control group in ALT, AST, ALP and total cholesterol related to the liver.

The testis organ weight in the male 1000 mg/kg treatment group was increased. The liver, kidneys and testis organ weights also increased in the male 2000 mg/kg group. The liver weight of the female group was increased. The increasing tendency of the organ weight of the recovery group was similar to the main group but there were no histopathological abnormalities.

At the scheduled necropsy, there were no gross findings of treatment-related issues on the majority of tissues in the main and recovery groups.

Hepatocyte hypertrophy was observed dose-dependently in both sexes of the main group, and it appears that the normal metabolic liver function induces by over dosage of the test item affected the hepatocyte and was not related with the disease status.

The result of the 26-week repeated dose and 4-week recovery study of SKI306X shows that there was no toxic effect in the 500 mg/kg treatment group. Soft feces and diarrhea was induced by physical problems of the test item in the 1000 and 2000 mg/kg treatment groups and the increase of the liver weight was considered to be the effect of the metabolic function of the normal liver. Therefore, the no observed effect level is considered to be 500 mg/kg and the maximum tolerance dose is considered to be 2000 mg/kg in the male and female rats.

### **Summary**

The results from the Phase II Clinical Trials show that SKI306X meets the established safety profile at dosage levels of 200, 400 and 600 mg *t.i.d.* The Phase III Clinical Trial results specify that no statistically significant differences were shown between the two treatment groups, which included an SKI306X-treated group and a diclofenac SR-treated group, in the incidence of any adverse event. Adverse events were mild in severity and the number of patients who had adverse events during the study was slightly greater in the control group than in the active group.

The acute toxicity study established an LD<sub>50</sub> level of more than 5.0 g/kg. Results from the subacute toxicity study showed 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. The 26-week toxicity study results showed a no observed effect level of 500 mg/kg and a maximum tolerance dose of 2000 mg/kg.

The various studies and tests show that SKI306X, with the new dietary ingredient *Clematis mandshurica* is extremely safe, well-tolerated and effective as a dietary supplement to promote joint health.

## Chapter 6: SAFETY DATA

### 6.1 Phase II Clinical Trials<sup>2</sup>

#### A. Patients and Methods

##### *Preparation and Composition of SKI306X*

SKI306X was prepared by extracting a mixture of 3 herbal components (*Clematis mandshurica*, *Prunella vulgaris*, *Trichosanthes kirilowii*,) in a ratio of 1:1:2 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. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Trichosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

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

Tablets were made of 200 mg of a mixture of 3 herbal components with additives for a total tablet weight of 430 mg.

##### *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

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<sup>2</sup> 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 485-91 (2001).

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<sup>3,4,5,6</sup>.

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 medication<sup>7</sup>. 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.

### **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 SKI306X 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.

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

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

<sup>5</sup> Nguyen et al., *Diacherin in the Treatment of Osteoarthritis of the Hip*, Arthritis Rheum. 37:529-560 (1994).

<sup>6</sup> 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).

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

**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	---	---
<b>Total Frequency</b>	5	9	11	4
<b>Total patients</b>	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.

The dosage in this study was evaluated at 200 mg, 400 mg and 600 mg *t.i.d.* by patients. The dosage for the product SKI306X as described in Section 2.3 is 200 mg as active ingredients with a recommended daily use of 2-3 tablets daily. Therefore, the dosage and frequency in this study and in the SKI306X product are qualitatively and quantitatively equivalent.

## **6.2 Phase III Clinical Trial<sup>8</sup>**

### **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.

<sup>8</sup>Jung et al., *A Four-Week, Randomized, Double-Blind Trial of the Efficacy and Safety of SKI306X: A Herbal Anti-arthritis Agent versus Diclofenac in Osteoarthritis of the Knee*, Vol. 32, No. 2, American Journal of Chinese Medicine 291-301 (2004).

The test articles in this study (identified as the product SKI306X) were prepared from the extracts of three medical herbs, *Clematis mandshurica*, *Prunella vulgaris*, and *Trichosanthes kirilowii*. These extracts were combined at a 1:1:2 (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. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Trichosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

### ***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. The dosage in this study was evaluated at 200 mg of the test article, with patients taking 3 tablets per day. The dosage for the product SKI306X as described in Section 2.3 is 200 mg as active ingredients with a recommended daily use of 2-3 tablets daily. Therefore, the dosage and frequency in this study and in the SKI306X product are qualitatively and quantitatively equivalent.

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 <sup>1)</sup>	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) <sup>2)</sup>	44	(35.2)	61	(49.2)	105	(42.2)
Total number of patients experiencing AE <sup>3)</sup>	37	(29.6)	43	(34.7)	80	(32.1)
Total number of drug related AE <sup>2)</sup>	24	(19.2)	46	(37.1)	70	(28.1)
Total number of patients experiencing drug related AE <sup>4)</sup>	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)

<sup>1)</sup> COSTART Classification of Adverse Events; <sup>2)</sup> Some patients had more than one Adverse Event; <sup>3)</sup> p-value was 0.390 by chi-square test; <sup>4)</sup> 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 <sup>1)</sup>
	N(%)	N(%)	N(%)	
<b>Chemistry</b>				
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
<b>Hematology</b>				
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

<sup>1)</sup> p-value by chi-square test or Fisher's exact test

# Chapter 7: TOXICOLOGY

## 7.1 Acute Toxicity<sup>9</sup>

### SUMMARY OF RESULTS

The acute toxicity of the product 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 LD<sub>50</sub> 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:

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

The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Trichosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same ratio. Therefore, the product SKI306X is qualitatively and quantitatively equivalent to the test articles studied herein.

#### 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 with a dose of 20 mg/kg.

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<sup>9</sup> Ahn et al., *Acute Toxicity of SKI306X, an Anti-inflammatory Herbal Extract, in Rats*, 1 Journal of Applied Pharmacology 32 (1996).

- a) Observations and test items:
- b) LD<sub>50</sub>
- c) 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.
- d) Body weight changes: Before the start of drug administration, and at days 4, 7, 11, and 14 (necropsy date).

**E) 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:**

LD<sub>50</sub> 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.

**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
Brain	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

## 7.2 Subacute Toxicity<sup>10</sup>

### SUMMARY OF RESULTS

To evaluate subacute toxicity of the product 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 mammary 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.<sup>11</sup> 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

<sup>10</sup> Kim et al., *Subacute Toxicity of SKI306X, an Anti-inflammatory Herbal Extract, in Rats*, 1 Journal of Applied Pharmacology 19 (1996).

<sup>11</sup> Mosberg and Hayes, *Subchronic Toxicity Testing*, In Principles and Methods of Toxicology, 221-236 (A.W. Hayes ed., Raven Press 1989).

hyperplasia, fibrosis, etc. through gross and histopathological findings. Changes such as these are occasionally found in rats and have no correlation with SKI306X.<sup>12</sup>

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).

### A) Materials and Methods:

The test articles in this study (identified as SKI306X) were prepared by extracting a mixture of 3 herbal components (*Clematis mandshurica*, *Prunella vulgaris*, *Trichosanthes kirilowii*) in a ratio of 1:1:2 in 30% (v/v) ethanol-water. The extract was evaporated *in vacuo*, and partitioned between the water-saturated n-butanol and water. The n-butanol layer was evaporated *in vacuo* and lyophilized. Tablets were made of 200 mg of the resultant product. The test articles in this study used these three botanical herbs, *Clematis mandshurica*, *Prunella vulgaris* and *Trichosanthes kirilowii* in a 1:1:2 (w/w) amount, respectively and the product SKI306X as described in Section 2.2 uses the same three botanical herbs in the same 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 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).

<sup>12</sup> 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)

#### **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 mg/kg. The recovery test was performed by the administration of SKI06X 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 µm. 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:**

- a) 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.

- b) **Body weight:**
  - i) 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.
- c) **Food and water consumption:**
  - i) 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.
- d) **Ophthalmological examinations:**
  - i) No abnormal symptoms were shown during the experimental period.

## **2) Hematology and Blood Biochemical Examinations:**

### **a) Hematology:**

- i) 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-dependent 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).

### **b) Blood Biochemical, 2-Week Administration:**

- i) 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).

### **c) Blood Biochemical, 4-Week Administration:**

- i) 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.
- ii) 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).

**d) Blood Biochemical, 2-Week Recovery Period:**

- i) 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.
- ii) 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:**

- a) 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:**

**a) Absolute Organ Weight:**

- i) 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).

**b) Relative Organ Weight:**

- i) 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:**

**a) Histopathological Findings after the 2-week Repeated Administration:**

- i) Table XVII.

**b) Histopathological Findings after the 4-Week Repeated Administration:**

- i) Table XVIII.

**c) Histopathological Findings after the 2-week Recovery Period:**

- i) Table XIX.

- d) 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<sup>a</sup>**

Sex	Dose (g/kg/day)	Number of animals	Number of animals sacrificed		
			At the end of experimental periods (week)		
			2	4	6 <sup>b</sup>
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

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

<sup>b</sup>At 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.

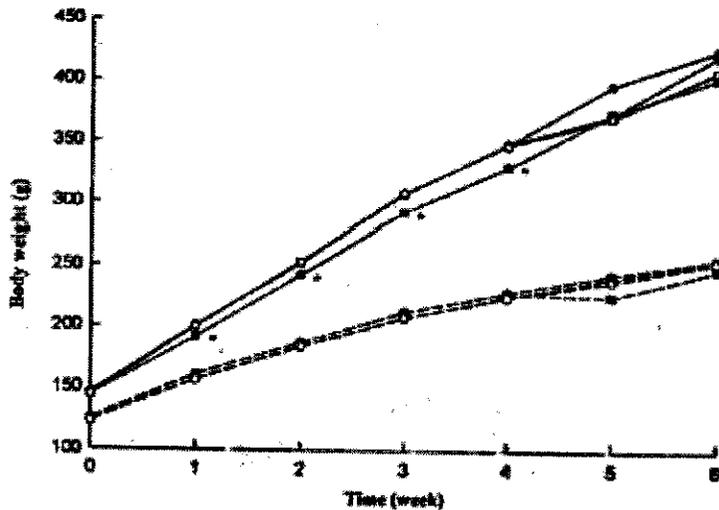


Fig. 1. Body weight changes of rats administered orally with SKI306X. —: Male, —: Female, ○: Control, ●: SKI306X 0.3 g/kg, ▼: SKI306X 1.0 g/kg, ■: SKI306X 3.0 g/kg. \*Significantly different from control ( $p < 0.05$ ).

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	14.04±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	101.84±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	63.3±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 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±67.6	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	311.9±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	230.9±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±63.8	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±29.8	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±9.0	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±0.9*	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±42.9	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	
			-	-	-	-	-	- +	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	
			-	-	-	-	-	-	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	0.53±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	1.00±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 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±0.03	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		
Group	Dose (g/kg/day)	T1	T3	T4	T1	T3	T4
No. of animal		0	1.0	3.0	0	1.0	3.0
		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
Spleen and Lymph node	Fibrosis	0	0	0	0	0	0
	Cysts and Dilatation	0	0	0	0	0	0
	Hemosiderosis	0	0	0	0	0	0
	Extramedullary haemopoiesis	0	0	0	0	0	0
Liver	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
	Lymphocyte infil.	1	1	0	1	0	0
	Foci of altered hepatocytes	0	0	0	0	0	0
Stomach & Intestines	Bile duct hyperplasia	0	1	0	0	0	0
	Fibrosis	0	1	0	0	0	0
	Necrosis	0	0	0	1	0	0
	Inflammation	0	0	0	0	0	0
	Ulcerative changes	0	0	0	0	0	0
Heart	Atrophy	0	0	0	0	0	0
	Hyperplastic changes	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

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

Sex		Male			Female		
Group	Dose (g/kg/day)	T1	T3	T4	T1	T3	T4
No. of animal		0	1.0	3.0	0	1.0	3.0
		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	4	1	1
	Fibrosis	0	0	0	1	0	0
Spleen and Lymph node	Cysts and Dilatation	0	0	0	0	0	0
	Hemosiderosis	0	0	0	0	0	0
	Extramedullary haemopoiesis	0	0	0	0	0	0
	Pigmentation	0	0	0	0	0	0
Liver	Lymphoid changes	0	0	0	0	0	0
	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
Stomach & Intestines	Fibrosis	0	0	0	0	0	0
	Necrosis	1	0	0	0	0	0
	Inflammation	0	0	0	0	0	0
	Ulcerative changes	0	0	0	0	0	0
	Atrophy	0	0	0	0	0	0
Heart	Hyperplastic changes	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	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 SKI306X treatment

	Sex	Sex					
		Male			Female		
		T1	T3	T4	T1	T3	T4
	Group	0	1.0	3.0	0	1.0	3.0
	Dose (g/kg/day)						
	No. of animal	5	5	5	5	5	5
Kidney	Tabular 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
Spleen and Lymph node	Cysts and Dilatation	0	0	0	1	0	1
	Hemosiderosis	0	0	0	0	0	0
	Extramedullary haemopoiesis	0	0	0	0	0	0
	Pigmentation	0	0	0	0	0	0
Liver	Lymphoid changes	0	0	0	0	0	0
	Hydropic and fatty changes	0	0	0	0	0	0
	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
Stomach & Intestines	Necrosis	0	0	0	0	0	0
	Inflammation	0	0	0	0	0	0
	Ulcerative changes	0	0	0	0	0	0
	Atrophy	0	0	0	0	0	0
Heart	Hyperplastic changes	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	Inflammation	0	0	0	0	0	0
	Normal	5	5	5	5	5	5
Nervous system	Normal	5	5	5	5	5	5
	Normal	5	5	5	5	5	5
Reproductive system	Normal	5	5	5	5	5	5

### 8.3 26-Week Toxicity Study (Attached Final Report Study No. B03006)

#### SUMMARY

The study was conducted to assess the safety of the product SKI306X for 26-week repeated dose toxicity in male and female rats. After the discontinuation of the treatment, a 4-week recovery test was conducted. The test item was orally administered to Sprague-Dawley rats at dosages of 0 (control), 500, 1000 and 2000 mg/kg for 26 weeks. The clinical signs, mortality, body weight change, food and water consumption, ophthalmoscopic examination, urinalysis, hematologic examination, blood chemistry examination, organ weight analysis, necropsy, and histopathological findings were evaluated.

In the male 500 mg/kg treatment group, one animal died at the 4-week point due to gavage error. Soft feces and diarrhea were observed from the 9-week to 26-week in the male and female 1000 and 2000 mg/kg treatment group. Soft feces and diarrhea induced by the excess administration of test item were shown, but these were considered to be temporary only when the test item passed the gastrointestinal tract and was not observed after early passage in the case of the daily administration. Diarrhea was observed from 7-10 hours after administration and was recovered at 16 hours in the male treatment group and at 14 hours in the female group. This was not observed during the recovery periods.

There was decreasing tendency in mean body weight of the male 2000 mg/kg treatment group. There was increasing tendency of the food and water consumption. The decreasing tendency of the body weights and increasing tendency of the food and water consumptions were considered to be related to soft feces and diarrhea. During the recovery periods, there were no differences between the treatment group and the control group in body weights, food and water consumption.

There was no treatment-related ophthalmoscopic examination.

At urinalysis, the change of the urine color and the increase of erythrocyte were considered that the urine was mixed with diarrhea and therefore not related to the test item. There were no test item-related effects in the recovery group.

The hematology of the main group, platelet count was increased in the male 1000 and 2000 mg/kg treatment groups, but the value was within the normal range. The blood chemistry in the male 2000 mg/kg treatment group showed a minimal decrease of glucose and a minimal increase of total cholesterol, but these values were within the normal range. There were no differences between the treatment group and the control group in ALT, AST, ALP and total cholesterol related to the liver.

The testis organ weight in the male 1000 mg/kg treatment group was increased. The liver, kidneys and testis organ weights also increased in the male 2000 mg/kg group. The liver weight of the female group was increased. The increasing tendency of the organ weight of the recovery group was similar to the main group but there were no histopathological abnormalities.

At the scheduled necropsy, there were no gross findings of treatment-related issues on the majority of tissues in the main and recovery groups.

Hepatocyte hypertrophy was observed dose-dependently in both sexes of the main group, and it appears that the normal metabolic liver function induced by over dosage of the test item affected the hepatocyte and was not related with the disease status. It was shown that the males tended to recover, while the females were completely recovered in the recovery group.

The result of the 26 week repeated dose and 4 week recovery study of SKI306X shows that there was no toxic effect in the 500 mg/kg treatment group. Soft feces and diarrhea was induced by physical problems of the test item in the 1000 and 2000 mg/kg treatment groups and the increase of the liver weight was considered to be the

effect of the metabolic function of the normal liver. Therefore, the no observed effect level is considered to be 500 mg/kg and the maximum tolerance dose (MTD) is considered to be 2000 mg/kg on the male and female SD rats.

## RESULTS

### Clinical signs and mortality (Table 1, 2, Appendix 1)

In the male 500 mg/kg treatment group, one animal died at the 4-week interval due to gavage error. No dead animals were found in any groups during the treatment period.

In the female 500 mg/kg treatment group, alopecia of chest and wound, edema and inflammation of toe were shown temporarily in 1 case.

In the male 1,000 mg/kg treatment group, soft feces were shown from day 81 to day 182. In the female group, soft feces was shown from day 92 and soft feces and diarrhea were shown till the termination of the administration.

In the male 2,000 mg/kg treatment group, soft feces was shown from day 75. Soft feces and diarrhea were shown from day 91 to the termination of administration in 18 cases. Alopecia of forelimb was present in 1 case from day 118 to the termination of administration. In the female group, soft feces and diarrhea were shown from day 81 and soft feces and diarrhea were present in 18 cases from day 92 to the termination of administration.

In the male and female 2,000 mg/kg treatment group of the recovery group, diarrhea was present in 6 cases at day 1 after the administration was terminated and soft feces was present at day 2 and these were recovered at day 3.

In the male 1,000 mg/kg treatment group, diarrhea was shown in 1 case after 7 – 8 hours from administration, in 4 cases after 9 hours, in 10 cases after 10 – 13 hours, and was recovered after 14 hours. In the male 2,000 mg/kg treatment group, diarrhea was shown in 12 cases after 7 hours from administration, in 13 cases after 8 hours, in 14 cases after 9 hours, in 17 cases after 10 hours, in 11 cases after 11-13 hours and in 1 case after 14-15 hours. Soft feces were shown in 7 cases after 11 – 13 hours from administration, in 5 cases after 14 hours, in 3 cases after 15 hours and was recovered after 16 hours.

In the female 1,000 mg/kg treatment group, diarrhea was shown in 6 cases after 7 – 9 hours from administration, soft feces was shown in 4 cases after 10 – 13 hours and was recovered after 14 hours. In the female 2,000 mg/kg treatment group, diarrhea was shown in 8 cases after 7 hours from administration, in 16 cases after 8 hours, in 17 cases after 9 hours and in 14 cases after 10 hours. Soft feces were shown in 4 cases after 10 hours from administration and in 18 cases after 11 – 13 hours and was recovered after 16 hours.

### Change of body weight (Figure 1 – 2, Table 3 & Appendix 2)

In the male 1,000 mg/kg treatment group, mean body weights were decreased at 26 week ( $p<0.05$ ) but in the female group, increased at the 12 week, 14 week, 18 week, and 26 week ( $p<0.05$ ) compared with those of control group.

In the male 2,000 mg/kg treatment group, mean body weight were decreased at the 18 week ( $p<0.05$ ), 22 week ( $p<0.05$ ) and 26 week ( $p<0.01$ ) compared with those of control group.

During the recovery period, mean body weights were decreased compared with those of the control group ( $p<0.01$ ).

### Food consumption (Table 4, Appendix 3)

During the treatment period, food consumption in the male 2,000 mg/kg treatment group was increased at the 9 week ( $p<0.01$ ) and in the female group increased at the 7 week ( $p<0.05$ ) compared with that of the control group.

### **Water Consumption (Table 5, Appendix 4)**

During the treatment period, in the male 1,000 mg/kg treatment group, water consumption was increased at the 5 week ( $p<0.05$ ) and in the 2,000 mg/kg group increased at the 26 week ( $p<0.01$ ) compared with that of the control group.

### **Ophthalmologic examination (Table 6, Appendix 5)**

In the main and recovery group, there were no treatment related changes.

### **Urinalysis (Table 7, Appendix 6)**

In the male 2,000 mg/kg treatment, the urine color change was shown and there were 3 cases of positive in erythrocyte.

In the recovery group, there were no treatment related changes in the urinalysis parameters.

### **Hematology (Table 8, Appendix 7)**

In the main group, PLT was increased in the male 1,000 and 2,000 mg/kg treatment group compared with the control group ( $p<0.05$  and  $p<0.01$  respectively).

No significant differences in the hematology parameters were noted in the female treatment groups and recovery group compared with the control group.

### **Blood Chemistry (Table 9, Appendix 8)**

In the main group, TP was increased in the male 1,000 mg/kg treatment group compared with the control group ( $p<0.05$ ). In the 2,000 mg/kg group, Glu ( $p<0.05$ ) was decreased but T-Chol ( $p<0.01$ ) was increased.

No significant differences in the blood chemistry parameters were noted in the female treatment groups compared with those of the control group.

In the recovery group, Crea ( $p<0.05$ ) was decreased in the male 2,000 mg/kg treatment group compared with the control group but Alb ( $p<0.05$ ) was increased in the female 2,000 mg/kg group.

## Chapter 9: PROPOSED USE

*Clematis mandshurica* is intended to be used in the dietary supplement SKI306X at a dose of 200 mg (active ingredients) per tablet with a total tablet weight of 430 mg. 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.

## Chapter 10: REFERENCES

- 10.1 Effect of SKI306X, a New Herbal Anti-Arthritic Agent, in Patients with Osteoarthritis of the Knee: a Double-Blinded Placebo Controlled Study

**Enclosed as Exhibit D**

- 10.2 A Four-Week Randomized, Double-Blind Trial of the Efficacy and Safety of SKI306X, an Herbal Anti-Arthritic Agent Versus Diclofenac in Osteoarthritis of the Knee

**Enclosed as Exhibit E**

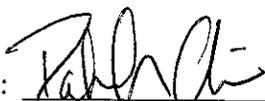
- 10.3 Acute Toxicity of SKI306X, an Anti-inflammatory Herbal Extract, in Rats

**Enclosed as Exhibit F**

- 10.4 Subacute Toxicity of SKI306X, an Anti-inflammatory Herbal Extract, in Rats

**Enclosed as Exhibit G**

Submitted by:

  
SK Chemicals Co., Ltd.

Date: 8-2-2006