A Study on the Single-dose Oral Toxicity of Super Key in Sprague-Dawley Rats

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Abstract
Objectives: This study was performed to analyze the single-dose oral toxicity of the super key (processed sulfur).

Methods: All experiments were conducted at Medvill, an institution authorized to perform non-clinical studies, under the Good Laboratory Practice (GLP) regulations. In order to investigate the oral toxicity of super key, we administered it orally to Sprague-Dawley (SD) rats. The SD rats were divided into four groups of five male and five female animals per group: group 1 being the control group and groups 2, 3, and 4 being the experimental groups. Doses of super key 500 mg/kg, 1,000 mg/kg and 2,000 mg/kg were administered to the experimental groups, and a dose of normal saline solution, 10 mL/kg, was administered to the control group. We examined the survival rates, weights, clinical signs, gross findings and necropsy findings. This study was conducted under the approval of the Institutional Animal Ethics Committee. (Approval number: A01-14018).

Results: No deaths or abnormalities occurred in any of the four groups. Although slight decreases in the weights of some female rats were noted, no significant changes in weights or differences in the gross findings between the control group and the experimental groups were observed. To check for abnormalities in organs, we used microscopy to examine representative histological sections of each specified organ; the results showed no significant differences in any of the organs.

Conclusion: The results of this research showed that administration of 500 — 2,000 mg/kg of super key did not cause any changes in the weights or in the results of necropsy examinations. Neither did it result in any mortalities. The above findings suggest that treatment with super key is relatively safe. Further studies on this subject are needed to yield more concrete evidence.

1. Introduction

In research and development of herbal formulae, intra-laboratory verification of the effectiveness is an important method to establish evidence based Korean medicine [1].

Despite the frequent use of medicinal plants, few scientific studies have been undertaken to determine the safety of traditional medicinal herbs. If the safety of medicines and plant products intended for human consumption is to be determined, systematic toxicological studies must be performed using various experimental models to predict the toxicity and to set the criteria for selecting a safe dose in humans [2].

Through long practice theories and techniques have been developed to eliminate or reduce the toxicity, as well as the drastic actions and side effects, of some drugs. Drug processing and compatibility are the main...
toxicity removal methods [3]. Sulfur is the third most abundant mineral in the body and is essential for life. Sulfur makes up the vital amino acids used to create proteins for cells and tissues and for hormones, enzymes, and antibodies. Sulfur is one of the mineral drugs traditionally used in herbal medicine. However, direct administered of sulfur to the human body will generally results in strong toxic side effects. Therefore, super key (processed sulfur), the product remaining after sulfur has been processed to remove the poison, was developed for medicinal use. This study was performed to examine the oral toxicity and the lethal dose of super key. The testing methods were visual observation of the general symptoms, body weight changes and necropsy findings in 8-weeks-old Sprague-Dawley (SD) rats. The current research trend for oral toxicity testing of extracts is to study acute and subacute toxicity through Good Laboratory Practice (GLP) regulations. All the experiments for this research were conducted at Medvill, an institution authorized to perform non-clinical studies, under the GLP regulations.

2. Materials and Methods

The super key was prepared in a sterile room at Wonkwang University Gwangju Korean Medical Hospital. The super key was finely ground by using a mortar. After having been weighed by using an electronic scale and mixed with corn oil, it was prepared at normal potency. The animals used in this study were 8-weeks-old SD rats. The reason SD rats were chosen is that they have been widely used in safety tests in the field of medicine, so the results can be easily compared with many other databases. The mean weights of the rats were 285.3 — 314.5 g and 200.5 — 222.9 g, respectively, for the male and the female rats at the time of super key administration. For all animals, a visual inspection was conducted, and all animals were weighed at the beginning of the experiment. During the seven days of acclimatization, the general symptoms of the rats were observed once a day. The weights of the rats were recorded on the last day of acclimatization. No abnormalities were found. The temperature of the lab was 19.7 — 22.6°C, and the humidity was 48.1% — 75.6%. The humidity was set to a low 50.0% ± 20.0%. However, an aberration occurred on July 19, 2014, from 17:30 pm to 18:00 pm when the humidity was 75.6%. The above temporary aberration occurred due to a dysfunction of the air conditioning equipment and the reinstallation of a temperature and humidity sensor. In spite of this, no abnormalities of the general symptoms were observed. Therefore, that aberration did not affect the result of this research.

Enough food (Lab Diet 5053) and ultra violet (UV)-filtered water were provided. Groupings were done after seven days of acclimatization. Animals were selected if their weights and general symptoms were normal. In total, 20 male rats and 20 female rats were selected. The animals were randomly distributed into 4 groups (5 male and 5 female rats per group) as shown in Table 1. In this study, 2,000 mg/kg was set as the high dose, and 1,000 mg/kg and 500 mg/kg were set as the mid and the low doses, respectively. In the control group, a dose of 10 mL/kg of normal saline solution was administered. Super key and normal saline were administered into the mouths of the rats in all groups by using disposable syringes. This study was conducted under the approval of the Institutional Animal Ethics Committee of Medvill Co., Ltd.

From the 1st day to the 14th day after treatment, the general symptoms were examined once a day. On the day of dosing (day 0), the general symptoms (side effects, revealing time, recovery time, etc.), as well as the mortality, were examined at 30 minutes and at 1, 2, 4, and 6 hours after oral administration. The weights were measured immediately before treatment and at 1, 3, 7 and 14 days after treatment. After observations had been terminated, necropsies were conducted on the rats after their abdominal aorta and vein had been cut under CO2 anesthesia, and the organs of all surviving animals were visually inspected and microscopically examined.

The weight data from the experiments were analyzed by using the statistical package for social science (SPSS) program (SPSS 16.0). A Levene test was conducted to evaluate the homogeneity of the variance and the significance. The one-way analysis of variance (ANOVA) test was conducted when the homogeneity of the variance was recognized (significance level: 0.05 on both sides). Also, the Kruskal-Wallis test was conducted when heterogeneity of the variance was recognized (significance level: 0.05 on both sides).

| Group           | Super Key administration (mL/kg) | Number of animals (serial numbers) |
|-----------------|---------------------------------|-----------------------------------|
|                 |                                 | Male                              | Female                           |
| G1: Control group | 0                               | 5 (11001 — 11005)                 | 5 (21001 — 21005)                |
| G2: Low-dose group | 500                             | 5 (12001 — 12005)                 | 5 (22001 — 22005)                |
| G3: Mid-dose group | 1,000                           | 5 (13001 — 13005)                 | 5 (23001 — 23005)                |
| G4: High-dose group | 2,000                           | 5 (14001 — 14005)                 | 5 (24001 — 24005)                |
3. Results

In this study, no deaths or abnormalities occurred in any of the groups. The weight examinations showed slight decreases in the weights of four female rats (two female rats in the 1,000 mg/kg group and two female rats in the 2,000 mg/kg group). However, no significant changes in weight were observed (Table 2). Finally, in both the control and the experimental groups, no meaningful differences in the necropsy findings were noted.

4. Discussion

Hippocrates thought that sulfur was effective in treating the Black Death. Since then, sulfur has been used for various reasons, such as to treat skin diseases, and as an intestinal drug. Sulfur is a bright yellow crystalline solid or powder. Sulfur is the seventh most abundant mineral in the body, and the body of a human weighing approximately 70 kg contains roughly 140 g of sulfur. Sulfur is acquired through the consumption of food primarily in the form of sulfur-containing amino acids (SAAs), such as methionine, cysteine, cystine, and taurine, and in its glucosinolate form, which is found in cruciferous vegetables, such as cabbage and cauliflower [4].

In Dongui Bogam, sulfur is mainly used for the treatments of an abdominal mass and pathogenic Qi in the pit of the stomach, stagnation of cold Qi, chronic cold syndrome in the back and the kidneys, loss of sensation due to cold wind, and coldness, pain, and loss of power in the legs. It fortifies the sinews and bones, tonifies yang Qi, removes balding, malignant furuncles, infantile malnutrition affecting the nose and other conditions in the external genitals, and kills scabies parasites [5].

Sulfur is widely used to detoxify the body, treat scabies [6, 7], heal sores and eliminate itching [8]. Recently, studies have reported that sulfocompounds

| Group & Dose (mg/kg) | Sex   | Mean S.D. N | 0 | 1   | 3   | 7   | 14 | Final weight gain |
|---------------------|-------|-------------|---|-----|-----|-----|----|------------------|
| G1 (0)              | Male  | Mean 299.2  S.D. 11.0 N 5 | 210.2 | 335.0 | 353.4 | 391.8 | 444.3 | 145.1 |
|                     | Female| S.D. 8.0   | N 5 | 9.5  | 11.3 | 17.7 | 14.7 | 9.3  |
| G2 (500)            | Male  | Mean 303.9  S.D. 11.0 N 5 | 206.4 | 339.5 | 363.4 | 408.0 | 470.5 | 166.6 |
|                     | Female| S.D. 4.9   | N 5 | 4.0  | 7.0  | 9.7  | 9.2  | 5.3  |
| G3 (1,000)          | Male  | Mean 299.9  S.D. 8.9 N 5 | 208.4 | 333.5 | 359.7 | 402.4 | 455.0 | 155.1 |
|                     | Female| S.D. 5.1   | N 5 | 4.5  | 9.1  | 9.6  | 8.0  | 8.9  |
| G4 (2,000)          | Male  | Mean 302.0  S.D. 10.3 N 5 | 206.4 | 329.5 | 350.5 | 387.8 | 436.7 | 134.8 |
|                     | Female| S.D. 6.0   | N 5 | 6.1  | 2.8  | 4.1  | 5.8  | 6.2  |

S.D., standard deviation; N, number of animals.
inhibit the growth of cancer cells. Kong et al [9] showed that the treatment of human gastric adenocarcinoma cells (AGS) with extracts from young radishes that had a high organic sulfur glucosinolate content inhibited cancer cell growth. In addition, Bak et al [10] reported that treating HT-29 human colon cancer cells with Kimchi extract made with sulfur treated radishes also inhibited the growth of cancer cells. Choi and Kim [11] reported that, when diverse cancer cells were treated with extracts from a hot water extraction from regular ducks or organic-sulfur-fed ducks, a noticeable effect in proliferation inhibition was seen in the cells treated with the extract from organic-sulfur-fed ducks.

In previous studies, inorganic sulfur has been demonstrated to inhibit the proliferation of breast cancer cells. The study showed that inorganic sulfur reduced cell proliferation by inhibiting the expression and the activation of epidermal growth factor receptor (EGFR) and by increasing the expression of Bcl-2-associated X (Bax) in estrogen-independent breast cancer (MDA-MB-231) human breast cancer cells [12]. Another study investigated the inhibitory effect of sulfur on prostate cancer (PCa) in vivo. In that research, prostate tumors were developed by injecting 22 Rv1 or DU 145 PCa cells into sulfur treated and untreated nude mice. The results showed that sulfur inhibited the growth of androgen independent prostate cancer in vivo [13]. Furthermore, the intake of refined inorganic sulfur has been reported to reduce the clinical side effects of radiotherapy in cancer patients [14].

However, the direct administration of sulfur to the human body will generally result in a strong toxic side effect. Therefore, super key, processed sulfur with the poison removed, was developed for medicinal use.

Although super key has been used in clinics, safety studies on super key are insufficient. Toxicity tests provide important data and are essential for evaluating the safety of test substances in medications [15]. This study was performed to provide objective safety data for super key. Doses of 500 mg/kg, 1,000 mg/kg, and 2,000 mg/kg of super key were administered to the experimental groups, and a dose of 10 mL/kg of normal saline solution was administered to the control group. In all four groups, no deaths occurred, and no abnormalities were found. No significant differences in the clinical signs or weights were noted between the control group and the experimental groups. The necropsy results to check for abnormalities in organs showed no significant findings.

Animal testing is the most fundamental and basic way to assess the safety of materials to be used for medical purposes [16]. For that reason, the Korea Food & Drug Administration of Korea has testing protocol guidelines for the study of toxicity, and all experiments should be conducted following GLP regulations [17]. The results of our study, which followed that protocol and those regulations, showed that the administration of 2,000 mg/kg of super key did not cause any changes in the weights of SD rats or in the results of necropsy examinations. It also did not result in any mortalities, which indicates that super key is safe to use as a treatment. However, the acute and the chronic side effects of super key need to be studied more in order to assess its oral toxicity, and more hematology and blood chemistry studies are required.

5. Conclusion

The results of this research showed that administration of 500—2,000 mg/kg of super key did not cause any changes in the weights of SD rats or in the results of necropsy examinations. Neither did it result in any mortalities, which indicates that the lethal dose of super key is higher than 2,000 mg/kg. The results obtained in this study suggest that administration of super key as treatment may be safe.

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Conflict of interest

The authors declare that there are no conflict of interest.

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