

**Acute Toxicity Test of
OSTEOKING
Orally Administrated to Mice**

**Kunming Medical College
Pharmacology Dept.**

June 28, 1998

Test Reporter:

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Manufacturer:

**Yunnan Crystal Natural Drugs
Pharmaceutical Factory
Kunming China**

1. Materials:

1.1 Drug to be tested:

OSTEOKING, sepia solution, 1 ml of the solution equivalent to 0.17g of crude drug, provided by Yunnan Crystal Natural Drug Pharmaceutical Factory (Lot NO: 98051)

1.2 Test animals:

ICR mice were provided by the Animal Feeding Room, the Key Laboratory of Pharmacology of Natural Drugs, Yunnan Province. (Animal quality Certificate: No.: 0000265).

2. Methods:

2.1 Tested drug:

Concentrated OSTEOKING was dispensed for experimental use at three times the clinical concentration.

2.2 Division of test animals:

60 healthy mice (body weights: 18-22g) were selected and divided randomly into three groups: 20 mice in each group, half of them male and half female.

2.2 Dosage design and drug administration:

The three groups were designated as a high-dosage group (50ml/kg of OSTEOKING, equivalent to 25.5g/kg of crude drug), a medium-dosage group (35ml/kg, equivalent to 17.9g/kg of crude drug) and a low-dosage group (25ml/kg, equivalent to 12.8g/kg of crude drug), respectively. The animals fasted for 12 hours before drug administration. The mice in each group were given the drug at the dosages specified above, through one-time perfusion into the animal's stomachs. The reaction of the animals was observed every day after drug administration and continued for 7 days.

3. Results:

No toxic reactions or deaths occurred following one-time perfusion of OSTEOKING into the animal's stomachs. All of the tested animals' conditions were fine, their activities were normal and their hair looked smooth. Therefore, even when the dosage of OSTEOKING solution reached 50ml/kg, equivalent to 25.5g of crude drug at 1 time (converted accordingly to surface area), equivalent to 21.5 times the adult daily clinical dosage and 180 times the dosage calculated according to body weight, we could not assay the half lethal dose. The following is the assay of the maximum tolerated dosage.

4. Assay of maximum tolerated dosage (MTD)

20 qualified mice (described above), were selected for the assay, half male and half female. Their stomachs were perfused with 50ml/kg of OSTEOKING solution (equivalent to three times the clinical concentration) once after 12 hours of fasting and then once every 8 hours (three times a day). The daily dosages were 150ml/kg (equivalent to 76.5g/kg of crude drug, according to surface area conversion). This dosage was equivalent to 64.5 times the adult daily clinical dosage. If converted according to body weight, the dosage would be 540 times the adult daily clinical dosage. But even at this dosage, no animals died. We observed the animals' reaction every day, continuing for 14 days. All the animals survived in good health. Their hair was smooth, and their diets and activities were normal. We did not find any apparent toxic reactions. Therefore the MTD is greater than 150ml/kg (three times the clinical concentration).

5. Evaluation

We could not assay the half lethal dose in mice (LD_{50}) even after perfusion into the animals' stomachs of three times the clinical concentration of OSTEOKING and a dosage as high as 50ml/kg (equivalent to 25.5g/kg of crude drug). The test of MTD showed that the daily dosage reached 150ml/kg (equivalent to 76.5g/kg of crude drug). According to surface area conversion, it would be 64.5 times the adult daily clinical dosage and 540 times the adult daily clinical dosage if converted according to body weight. But even at this dosage, no animals died.. The results indicate that the MTD of OSTEOKING is greater than 150ml/kg, (three times the clinical concentration). The clinical application dosage of OSTEOKING is therefore safe.

(The original data of the test is kept in Pharmacology Teaching and Research Section, Kunming Medical College).