

Study Report

Acute Toxicity Test of New Earthworm Powder (HLP) in Rats

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1. Abstract

Acute toxicity test (limit test) of new earthworm powder (HLP) developed by Waki pharmaceutical Co., Ltd. was conducted in rats. A single oral dose of 2000 mg/kg of the sample is given to the test group whereas water for injection is given to the control group. Then it has been observed for 14 days. As a result, no abnormalities or deaths are reported for each sample during the observation period. Consequently, LD50 of single-dose administration of the new earthworm powder (HLP) in both male and female rats is assumed to be over 2000 mg/kg.

2. Title

Acute Toxicity Test of New Earthworm Powder (HLP) in Rats

3. Purpose of the Study

To study the acute toxicity values of HLP in rats.

4. Testing Materials and Methods

4.1 Test sample

New earthworm powder (HLP)

4.2 Negative control sample

Water for injection

4.3 Preparation of test sample solution

Dissolve the sample into water for injection and prepared 100mg/mL of test solution.

4.4 Test animal

5 week old ICR rats (male and female) were purchased from Japan SLC, Inc. Before the test, they were fed for another week to make sure that there's no abnormality in them. Five rats were kept in each polycarbonate animal cage where the room temperature and lighting time were set for $23\text{ }^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 12 hours/ day. They were allowed to take feed [solid feed for mice and rats; Labo MR Stock, Nosan Corporation] and water (tap-water) freely.

4.5 Test method

4.5.1 Group structure

10 rats (5 male and 5 female) are used for test group and control group respectively.

4.5.2 Testing operation

The rats were fasted 4 hours before the administration. After measuring their body weights, 20mL /kg of the sample solution and water for injection are administered via oral gavage using gastric tube to the test group and the control group respectively.

4.5.3 Statistical processing

Compare the each group by t-test at 5% significance level.

5. Results

5.1 Fatal cases

No fatal cases were reported in either group during the observation period.

5.2 General condition

No abnormalities were reported in either group during the observation period.

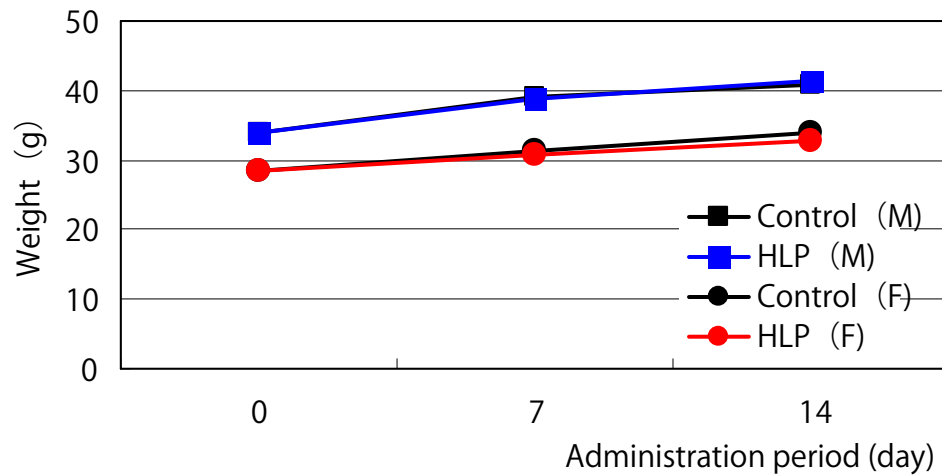
5.3 Body weight changes (as shown in table below)

The weight changes in response to the administration of HLP are indicated in the table below. There's no difference between the body weight of test group and control group after 7 and 14 days of administration.

<Changes in body weight in response to administration of HLP>

Sex	Administration group	Before Administration	After administration (day)	
			7	14
Male	Test group	$34.0 \pm 1.0(5)$	$38.9 \pm 1.3(5)$	$41.3 \pm 1.0(5)$
	Control group	$34.0 \pm 0.9(5)$	$39.2 \pm 0.8(5)$	$40.9 \pm 1.8(5)$
Female	Test group	$28.4 \pm 1.5(5)$	$30.7 \pm 1.9(5)$	$32.7 \pm 2.1(5)$
	Control group	$28.4 \pm 1.7(5)$	$31.3 \pm 1.8(5)$	$33.8 \pm 1.2(5)$

<Changes in body weight in response to administration of HLP (graph)>



6. Autopsy finding

The autopsy after the observation period showed that there were no abnormalities in any of the rats administered HLP.

7. Conclusion

Acute toxicity test (limit test) of new earthworm powder (HLP) was conducted in rats. As a result, no abnormalities or deaths were reported with a single oral dose of 2000mg/kg. Consequently, LD50 of single-dose administration of the new earthworm powder (HLP) in both male and female rats is assumed to be over 2000 mg/kg.