

## 【Original Article】

## Acute Oral Toxicity Test of Hot Water Extract of *Coix lacryma-jobi* L. var. *ma-yuen* Stapf in Rats

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### [ABSTRACT]

*Coix lacryma-jobi* L. var. *ma-yuen* Stapf (Coix seed) is a grass crop that has long been used in traditional medicine as a nourishing food. However, high-intake safety of the extract of the husks, pellicles and astringent skin of Coix seed has rarely been evaluated. We performed a safety test of hot water extract of all parts of Coix seed (CRD extract) in rats. CRD extract showed no significant toxicity on body weight, blood analyses, urinalysis and histopathological examination in acute toxicity tests.

### [Key words]

*Coix lacryma-jobi* L. var. *ma-yuen* Stapf, Coix seed, adlay, acute oral safety test, rat

### 1. INTRODUCTION

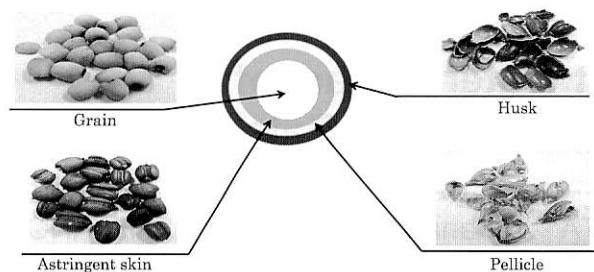
A grain with the husk [scientific name: seed] of a Coix seed (adlay, Job's tears, pearl barley) belonging to the genus coix of the family Poaceae [scientific name: *Coix lacryma-jobi* L. var. *ma-yuen* (Roman.) Stapf] is composed of, from the outermost to innermost layer: a husk [scientific name: involucre], a pellicle [scientific name: glume, palea and lemma], an astringent skin (bran) [scientific name: pericarp], and a grain (sarcocarp) [scientific name: caryopsis] (Fig. 1). We invented a composition for foods or medicaments containing hot water extracts of the husks, pellicles and astringent skin of Coix seed (Japan patent No 3590042). The pharmaceutical composition is expected to have an anti-neoplastic effect, anti-human papillomaviral disease effect, and effect against various cutaneous diseases (or the like), such that it is useful for chemoprophylaxis or therapy against tumors, and for preventing or treating human papillomaviral diseases such as condyloma acuminatum, verruca vulgaris, adolescent verruca plana, senile verruca or laryngeal papillomatosis.

In the preliminary toxicity test of dehulled Coix seed (yokuinin), it was reported that the administration of a powder diet containing 5% yokuinin to pregnant rats revealed no treatment-related effects on birth index, viability indices, sex

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**Fig. 1** Structure of *Coix lacryma-jobi* L. var. *ma-yuen* Stapf (Coix seed)

ratio, body weight or gross pathological findings<sup>1)</sup>. However, high-intake safety tests of the extract of the husks, pellicles and astringent skin of Coix seed has rarely been evaluated. Therefore, we performed a safety test of hot water extract of all parts of Coix seed (CRD extract) on rats.

## 2. MATERIALS AND METHODS

### 2.1 Production of extract of husks, pellicles and astringent skin of Coix seed (CRD extract)

Hot water extract of husks, pellicles, astringent skin and grains of Coix seed produced by CRD Co., Ltd (Ishikawa, Japan) was used for the test article. Hot water extract of husks, pellicles and astringent skin of Coix seed can be obtained through various methods. Specifically, Coix seed with husks (grains with husks) are washed well with distilled water and dried. Husks are then lightly ground with a rice-milling machine. After dehulling, Coix seed grains are separated into undehulled and dehulled grains using a sieve with a mesh size of approximately 3.5 (5.6 mm). Undehulled grains are remilled to obtaining husks, pellicles or astringent skin. At this point, it is necessary to adjust the strength of the milling machine so that the grains are not broken. 1 kg of grain, husks, pellicles and astringent skin are then immersed in 7 liters of distilled water for 1 hour. Next, this solution was gradually heated for 60 minutes to boiling point, followed by further boiling for 60 minutes. Subsequently, the solution is concentrated by vacuum centrifugation for 60 minutes while heating at 40–50°C. The product is then cooled, sterilized at 98°C for 30 minutes, and dried by spray-dry method.

### 2.2 Test animal and housing conditions

Experimental procedures were approved by the Committee on Animal Experimentation at Kanazawa University, Ishikawa, Japan.

Male (n=21) and female (n=21) 8-week old Wister rats were obtained from Charles River Japan Inc., (Shizuoka, Japan). Rats were kept in a room maintained at controlled temperature (23±2°C), humidity (55±10%), and lighting (9:00 to 21:00 hours), with 3–4 rats per cage. Animals had free access to solid feed (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water.

### 2.3 Group condition and dose levels

When rats were 9-weeks old, the test article was administered at doses of 0 (male n=7, female n=7), 500 (male n=7, female n=7), and 2,000 mg/kg (male n=7, female n=7). The test article was dissolved in distilled water and injected intragastrically by direct stomach intubation. At 14 days after administration, animals were sacrificed under anesthesia, blood collected from the vena cava inferior, and necropsies performed.

### 2.4 Observation of general condition and body weight

General condition was monitored twice daily (morning and afternoon) for 14 days after administration. Body weight was measured every second day for 14 days after administration.

### 2.5 Necropsy and organ weight measurement

On day 14, all animals were sacrificed and subjected to necropsies. The following organs were removed and weighed: brain, thyroids, heart, lungs, thymus, liver, spleen, kidneys, testes, seminal vesicle, epididymis, ovaries and uterus.

### 2.6 Histopathological examination

In the control and high-dose groups (2,000 mg/kg), histopathological examination were made of the following organs: brain, pituitary, accessory thyroid, thyroid, heart, thymus, lung, liver, spleen, kidney, adrenal gland, testes, gonocyst, epididymis, uterus and ovaries. All samples were fixed in 10% neutral buffer formalin and stained with hematoxylin-eosin. Manufacture of specimens was performed by the Department of Pathology, Kanazawa University Hospital.

### 2.7 Hematology

Blood was collected into a tube with EDTA-2K and was examined for white blood cell count (WBC), red blood cell count (RBC), hemoglobin content, hematocrit value, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration

(MCHC), blood platelet, neutrophil, eosinophil, basophil, monocyte and lymphocyte. All measurements were performed by SRL Co., Ltd (Tokyo, Japan).

### 2.8 Blood biochemistry

The following analyses were also performed by SRL Co., Ltd.: total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),  $\gamma$ -GTP, total bilirubin, total cholesterol, neutral lipid, HDL-cholesterol, LDL-cholesterol, blood urea nitrogen (BUN), creatinine, Na, Cl, K and glucose (blood serum).

### 2.9 Urinalysis

Urine was collected before euthanasia. Hidden blood, glucose, and protein levels in urea were qualitatively analyzed using urine test paper obtained from WAKO, Co., Ltd., (Osaka, Japan).

### 2.10 Statistical analysis

Data obtained from the acute oral toxicity tests were statistically analyzed using Student's *t*-test. One-way parametric ANOVA with Tukey's test was used to examine body weight, organ weight, hematological and blood biochemistry data.

## 3. RESULTS

### 3.1 Observation of general condition and body weight

Administration of the test article revealed no clinical signs, adverse effects or death during the 14-day observation period.

Body weight gain was unaffected by the test article during the test period (Fig. 2).

### 3.2 Necropsy findings, organ weight measurement and histopathological examination

No significant changes were observed in any organs at necropsy on day 14. Liver weight of male rats in the 500 mg/kg group was higher than control and liver weight of female rats in the 500 mg/kg group was lower than control. However no concentration dependence or histopathological abnormalities was found in these groups. There were no significant differences in other organs (Table 1).

Pathological examinations of each organelle revealed no abnormal findings.

### 3.3 Hematology

The MCV value of female rats in the 2,000 mg/kg group was lower than control and the MCHC of female rats in the 2,000 mg/kg group was higher than control ( $p < 0.05$ ) (Table 2). However there were no significant differences in concentration dependence in these results.

### 3.4 Blood biochemistry

AST values of both male and female rats in the 500 and 2,000 mg/kg groups were lower than control ( $p < 0.05$ ) (Table 3). ALT values of females in the 500 and 2,000 mg/kg groups were lower than control ( $p < 0.05$ ) (Table 3). ALP values of females in the 2,000 mg/kg group were lower than control ( $p < 0.05$ ) (Table 3). Finally, Cl values of females in the 500 mg/kg group were lower than control ( $p < 0.05$ ) (Table 3). However

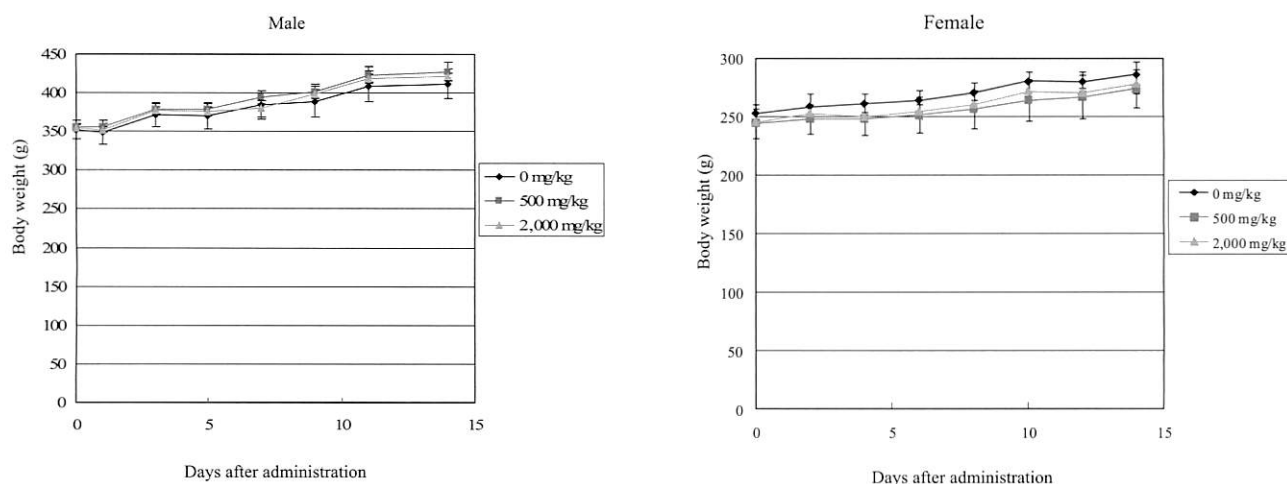


Fig. 2 Body weight of rats over 14 days after single oral administration of *Coix lacryma-jobi* L. var. *ma-yuen* Stapf. 0 mg/kg (male n=7, female n=7), 500 mg/kg (male n=7, female n=7), 2,000 mg/kg (male n=7, female n=7)

**Table 1** Organ weights (mean±S.D.) in rats at 14-days after administration of *Coix lacryma-jobi* L. var. *ma-yuen* Stapf extract

	Male			Female		
	Control (n=7)	500 mg/kg (n=7)	2,000 mg/kg (n=7)	Control (n=7)	500 mg/kg (n=7)	2,000 mg/kg (n=7)
Brain (g)	1.320±0.180	1.402±0.091	1.467±0.079	1.314±0.092	1.234±0.113	1.287±0.031
Thyroids (mg)	0.756±0.069	0.827±0.155	0.890±0.261	0.756±0.069	0.827±0.155	0.890±0.261
Heart (g)	1.333±0.127	1.258±0.072	1.348±0.072	0.940±0.085	0.814±0.083	0.840±0.022
Lungs (g)	1.724±0.097	1.635±0.260	1.813±0.091	1.379±0.096	1.260±0.073	1.383±0.146
Thymus (g)	0.680±0.175	0.635±0.119	0.663±0.093	0.523±0.100	0.477±0.096	0.447±0.077
Liver (g)	16.359±1.430	19.012±1.076*	17.258±1.231	11.644±1.091	10.403±0.092*	11.097±0.391
Spleen (g)	1.098±0.124	1.248±0.156	1.187±0.092	0.771±0.081	0.797±0.114	0.757±0.057
Kidneys (g)	2.860±0.223	3.038±0.175	2.968±0.146	2.029±0.150	1.851±0.144	1.880±0.185
Testes (g)	3.825±0.332	3.888±0.193	3.888±0.395	—	—	—
Seminal vesicle (g)	1.171±0.179	1.087±0.138	1.250±0.116	—	—	—
Epididymis (g)	0.458±0.058	0.447±0.047	0.457±0.023	—	—	—
Ovaries (g)	—	—	—	0.610±0.159	0.623±0.136	0.540±0.199
Uterus (g)	—	—	—	0.186±0.038	0.206±0.030	0.202±0.029

\* p<0.05 (compared with control group)

**Table 2** Hematological findings (mean±SD) at 14-days after administration of *Coix lacryma-jobi* L. var. *ma-yuen* Stapf extract

	Male			Female		
	Control (n=7)	500 mg/kg (n=7)	2,000 mg/kg (n=7)	Control (n=7)	500 mg/kg (n=7)	2,000 mg/kg (n=7)
WBC (10 <sup>3</sup> /μl)	7.85±2.00	6.78±1.40	7.42±2.64	6.28±2.39	4.88±1.00	5.63±1.18
RBC (10 <sup>4</sup> /μl)	741.00±52.82	761.50±69.90	732.67±59.55	697.83±35.95	726.83±38.36	739.17±24.18
Hemoglobin content (g/dl)	14.600±0.312	14.55±1.34	14.45±1.15	13.70±0.73	13.82±0.45	14.10±0.44
Hemoglobin value (%)	91.25±2.25	91.00±8.41	90.17±7.31	86.00±4.52	86.50±2.59	88.17±2.86
Hematocrit value (%)	46.89±2.74	47.03±5.29	45.15±3.99	43.32±2.47	42.77±1.60	42.87±1.28
MCV (fl)	63.36±2.05	61.70±2.22	61.65±2.97	62.07±1.86	58.93±3.05	58.00±1.43* <sup>1</sup>
MCH (pg)	19.79±1.34	19.13±0.43	19.73±0.84	19.62±0.37	19.05±0.82	19.08±0.31
MCHC (%)	31.20±1.51	31.00±0.82	32.03±0.87	31.63±0.51	32.30±0.53	32.92±0.57* <sup>2</sup>
Blood platelet (10 <sup>4</sup> /μl)	72.90±9.62	50.87±24.31	54.98±27.57	83.85±9.57	79.32±7.52	84.32±9.03
Neutrophils (%)	59.03±9.38	54.55±1.96	58.18±12.72	56.05±12.83	61.50±5.71	61.63±4.36
Eosinophil (%)	5.93±7.12	5.67±3.81	4.17±2.94	2.28±2.06	1.55±0.52	1.98±0.67
Basophil (%)	0.063±0.092	0.03±0.08	0.00	0.27±0.41	0.00	0.15±0.20
Monocyte (%)	1.790±1.986	3.12±3.54	3.32±4.36	2.35±1.17	1.90±0.83	2.87±1.46
Lymphocyte (%)	33.19±6.12	36.63±2.82	34.33±9.21	39.00±13.01	35.05±5.86	33.37±4.66

WBC: white blood cell count; RBC: red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration

\*<sup>1</sup>, \*<sup>2</sup> p<0.05 (compared with control group)

there were not significant differences in concentration dependence in these results.

### 3.5 Urinalysis

Hidden blood, glucose, and protein levels of test groups were not significantly different than control.

## 4. DISCUSSION

Many foods such as noodles, confections and the like have been developed using Coix seed as a raw material. Normally

when Coix seeds are used in foods, their husks, pellicles and astringent skin are removed by threshing and only the grain portion utilized. In particular, the husk is very hard and cannot be eaten intact, and has thus been thought unsuitable as a food ingredient. The exception is Coix seed tea, which is directly decocted from grains with husks.

Further, “Yokuinin,” described in the Japanese pharmacopoeia and known as a kampo medicine in Japan, is defined as a grain prepared by collecting grains of Coix seed, threshing (husks, pellicles and astringent skin removed) and drying. Yokuinin thus does not contain any husk, pellicle and

**Table 3** Blood biochemical findings (means±SD) at 14-days after administration of *Coix lacryma-jobi* L. var. *ma-yuen* Stapf extract

	Male			Female		
	Control (n=7)	500 mg/kg (n=7)	2,000 mg/kg (n=7)	Control (n=7)	500 mg/kg (n=7)	2,000 mg/kg (n=7)
Total protein (g/dl)	5.69±0.20	5.63±0.34	5.88±0.29	5.73±0.28	5.45±0.29	5.60±0.31
Albumin (g/dl)	2.23±0.07	2.13±0.15	2.25±0.12	2.37±0.13	2.25±0.10	2.28±0.12
AST (IU/l)	110.00±11.16	93.50±11.98* <sup>1</sup>	87.33±5.85* <sup>2</sup>	114.86±33.97	75.67±12.60* <sup>3</sup>	74.33±10.01* <sup>4</sup>
ALT (IU/l)	31.88±3.40	38.00±11.37	35.33±5.79	35.00±4.90	33.50±4.23* <sup>5</sup>	34.00±6.03* <sup>6</sup>
ALP (IU/l)	577.25±93.77	586.00±134.17	661.30±200.42	436.14±107.00	394.17±47.5	338.00±28.31* <sup>7</sup>
γGTP (IU/l)	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Total bilirubin (mg/dl)	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00
Total cholesterol (mg/dl)	64.13±5.67	73.17±11.09	63.50±7.34	71.14±15.91	64.17±5.95	67.33±8.45
Neutral lipid (mg/dl)	133.13±43.94	185.67±102.40	123.83±37.54	62.71±31.37	119.67±54.32	112.17±44.95
HDL-cholesterol (mg/dl)	25.25±2.55	29.17±4.54	24.33±2.58	26.71±5.38	24.33±3.44	25.83±2.71
LDL-cholesterol (mg/dl)	9.00±1.69	8.50±1.22	8.33±1.86	7.14±1.68	6.17±1.17	5.17±1.47
Urea nitrogen (mg/dl)	19.39±2.56	20.25±2.37	18.88±1.48	19.51±3.12	17.90±1.24	17.78±1.96
Creatinine (mg/dl)	0.36±0.05	0.32±0.04	0.31±0.06	0.28±0.02	0.27±0.02	0.27±0.05
Na (mEq/dl)	146.25±1.04	145.00±1.41	146.00±1.79	148.00±5.51	151.50±4.32	150.00±6.63
Cl (mEq/dl)	101.75±1.16	99.50±3.39	102.50±1.87	99.71±4.50	97.50±2.88* <sup>8</sup>	97.67±3.20
K (mEq/dl)	4.28±0.27	5.58±2.32	4.85±0.33	4.93±1.13	4.50±0.86	4.20±1.10
Glucose (blood serum) (mg/l)	159.00±11.12	247.33±179.81	177.83±16.53	161.71±50.34	165.83±34.45	175.83±17.74

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase;

\*<sup>1-8</sup> p<0.05 (compared with control group)

astringent skin. When discussing foods, the term “Coix seed” (Japanese *hato-mugi*) usually refers to the grain after threshing, unless otherwise specified. If Coix seed does contain the husk, pellicle and astringent skin, it is then referred to as Yokuinin with husk, Coix seed with skin, Coix seed with husk, etc.. Hot water extract of Yokuinin is used medically and clinically in Japan, and application for insurance reimbursement for the use of Yokuinin for verruca vulgaris and adolescent verruca has been approved by the Ministry of Health, Labour and Welfare. Recently, some studies have indicated that Coix seed possesses pharmacological effects including anti-cancer<sup>2-4)</sup>, anti-obesity<sup>5,6)</sup>, hypolipemic<sup>7)</sup>, anti-diabetic<sup>8)</sup> and osteoporosis prevention<sup>9)</sup>.

Clinically, Yokuinin extract in the form of a powder or tablet is used in the pharmaceutical preparation. Normally, 6 g of powder/18 tablets contain 2 g of dried water extract. It is known that Yokuinin extract is effective against verruca vulgaris and adolescent verruca plana. Although surgical excision, electrocauterization, cryotherapy and the like are employed against verruca, most cases are actually quite intractable and complete cure is difficult to achieve. Thus, the advent of a new remedy possessing both high safety and efficacy has been much awaited. It is thought that the reason Yokuinin is rarely used outside Japan for treating viral verruca is due to its weak potency.

As a result of intensive studies to improve this potency, Suzuki et al. have succeeded in obtaining a pharmaceutical composition having markedly superior drug efficacy compared to conventional pharmaceutical preparations of Coix seed by using the husks, pellicles and astringent skin of Coix seed (Japan patent No 3590042). Coix seeds are safe in their natural form, and the composition by Suzuki et al may be used safely in both foods and medicaments. Clinical dose is typically 0.03–0.04 g/kg body weight/day, with 2 g/day of product used for an adult. The product is preferably administered between meals, with 1g of product administered in the morning and 1 g in the evening.

Should a pharmaceutical preparation of Coix seed showing improved potency and efficacy over Yokuinin be developed, it is possible that it will find worldwide acceptance.

## 5. CONCLUSION

Oral acute toxicity tests revealed that the lethal dose of hot water extract of whole-grain *Coix lacryma-jobi* L. var. *ma-yuen* Stapf is >2,000 mg/kg in both male and female rats. The composition of CRD extract is derived from natural Coix seed, and this seed has been long used in food preparations, such that we may safely contend that the composition has no (or at least extremely low) toxicity, and thus shows great safety. It would

appear that even the elderly, infants and invalids may safely take this composition long term.

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## 要 旨

### ハトムギ熱水抽出物のラット急性毒性試験

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ハトムギの種皮を除いた種子の部分であるヨクイニン<sup>®</sup>は長年、疣贅に対する伝統的な漢方薬として利用されている。しかしながら、ハトムギの殻、薄皮、渋皮の熱水抽出物についての安全性試験に関する報告は当たらない。そこで、我々はハトムギの殻、薄皮、渋皮、種子のすべての部分を含む熱水抽出物（CRD エキス）を高用量（2,000 mg/kg）摂取した場合の急性毒性試験をラットを用いて行った。エキス摂取2週間後の体重変化、血液検査、臓器重量測定・組織病理検査、尿検査を行った結果、すべての項目において異常は認められなかった。

キーワード： *Coix lacryma-jobi* L. var. *ma-yuen* Stapf, ハトムギ, 急性毒性試験, ラット