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선퇴 추출물의 Sprague-Dawley rat를 이용한 단회 경구 투여 독성시험

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Single Dose Oral Toxicity Study of Cicadidae Periostracum Extracts in Sprague-Dawley Rats

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ABSTRACT

Objective : Cicadae Periostracum (CP), which is the discarded shell of the *Cryptotympana atrata* (Fabricius, 1775), is a recognized component of oriental medicine for treatment sore throat, itching, shock, sedation, edema. However, the safety and toxicity of CP have not yet been established. It has been reported that symptoms of addiction or side effects may occur in patients who take high doses of CP or who are hypersensitive to it. Therefore, we investigated the acute toxicity of an CP extracts in Sprague-Dawley (SD) rats.

Methods : To study acute toxicity, five SD rats of each sex per group were treated with CP extracts at single doses of 0, 500, 1000, or 2000 mg/kg administrated by oral gavage, and body weight, clinical signs, and mortality were observed after dosing. At the end of 14-day observation period, all animals were sacrificed and complete hematological and macroscopic examinations were performed.

Results : There were no dead animal and test article-related effects on body weight change or the gross finding. No toxicologically significant results were observed between control and treated groups in hematology. Although salivation related to stress at the highest dose was observed in clinical signs immediately after administration, it is considered to have no toxicological significance.

Conclusion : As the results, we did not find any adverse effect at the dose levels of 500, 1000, or 2000 mg/kg in rats. The minimal lethal dose was considered to be over 2000 mg/kg body weight in rats.

Key words : *Traditional Medicine, Cicadae Periostracum, Acute toxicity, Oral administration, SD Rats*

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Ⅰ. Introduction

Utilizing diverse natural resources such as plants, animals, and mineral components is a widespread tradition in traditional medicine. Medicines made from animals and derivatives from their organs have been used in various culture since ancient times [1-3]. Not only plant materials are commonly utilized in traditional medicine, but also animal products are also widely employed [4,5]. Therapeutic utilization of animals and their products has been a common practice in ancient cultures worldwide. It remains prevalent in many modern societies [2,4]. In India, traditional medicinal practices involve the use of at least 109 animal species. In Northeast Brazil, around 250 animal species are utilized medicinally [6]. Similarly, in Africa, certain studies have reported that various animals are believed to possess healing properties for different diseases [1]. Over 1,500 animal species have been recognized for their therapeutic properties in China [7].

Cicadae Periostracum (CP), which is the discarded shell of the *Cryptotympana atrata* (Fabricius, 1775), is a recognized component of traditional Chinese medicine known for its functions such as "dispelling wind and heat," "clearing vision and reducing cloudiness," and "relieving convulsions by dispelling wind" [8,9]. CP has a history of being employed to alleviate symptoms like sore throat, hoarseness, itching, and spasms in China [10,11]. CP is commonly referred to as "Cicadas" or "Sun-Tae" in Korea. Its origins trace back to an ancient Korean medical text, the *Dongui Bogam*, classified within the Chung-bu category [12]. In Korean traditional medicine, CP has been utilized for treating conditions such as epilepsy, shock, smallpox, sedation, edema, and symptoms of night terror [12]. CP has been reported to have various pharmacological effects, such as dermatitis symptoms-alleviating, anticonvulsant, neuronal apoptosis-protecting, and antioxidant effect [13-17].

However, it has been reported that symptoms of addiction or side effects may occur in patients who take high doses of CP or who are hypersensitive to it [18]. This study conducted a single-dose toxicity test using CP, observing mortality rates, clinical symptoms, changes in body weight, hematological examinations, and necropsy results. In this study, we attempted to present scientific evidence on the toxicity effects of CP.

Ⅱ. Materials and Methods

1. Test substances

CP was purchased from Kwong-Mung-dang Company (Ulsan, Korea) and authenticated by Dr. Goya Choi (Herbal Medicine Resources Research Center, Korea Institute of Oriental Medicine, Naju, Korea). A voucher specimen (3-18-0038) was deposited at the Herbal Medicine Resources Research Center, Korea Institute of Oriental Medicine. Briefly, CP was extracted in distilled water for 3 h under reflux (100 ± 2℃). The extract was filtered, evaporated on a rotary vacuum evaporator, and lyophilized (yield, 6.30%). The powder was kept at 4℃ until use. [14].

2. Animals

Seven-week-old male and female SD (Sprague-Dawley) rats were purchased from Orientbio (Seongnam, Korea) and used acclimatization for 6 days. During acclimatization and experimental periods, animals were kept under free access to food pellets (Teklad Certified Irradiated Global 18 % Protein Rodent Diet, Envigo, USA) and tap water. Animals were housed at 22 ± 2℃, relative humidity of 55 ± 10% in a 12 hr light/12 hr dark cycle. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Korea Conformity Laboratories (Incheon, Korea) based on Animal Protection Act of Republic of Korea (Approval No.: IA20-02696)

3. Experimental Group

Animals (20 males and 20 females) were allocated to four experimental group of CP extract receiving 0, 500, 1000 or 2000 mg/kg.

4. Treatment

After starvation for 4 hr, animals were orally administered with CP extract dissolved in water at doses of 500, 1000, or 2000 mg/kg or its vehicle alone. The application volume (10 mg/kg) was calculated according to the body weight on the treatment day. The oral route is the clinically intended route for the test article.

5. Clinical signs and mortality

Gross appearances of animals were observed immediately and after 30 min, 1, 2, 3, 4, 5, and 6 hr of the administration. Thereafter, they were observed once a day for 14 days. Rats were observed once daily for morbidity and mortality for 14 days. Clinical signs

such as posture, gait, fur, skin, eye/pupil, mucus, respiration, response to handling, convulsions, stereotypy and bizarre movements were observed.

6. Body Weight

Individual body weights of animals were measured shortly before test article administration and on 1, 3, 7, and 14 day after treatment thereafter.

7. Macroscopic examination

On day 14 after treatment, all surviving rats were weighted and then sacrificed by cutting the abdominal aorta with posterior vena cava under isoflurane anesthesia for macroscopic observation. These animals were subjected to gross necropsies such as inspection of the outer body surface, cranial, abdominal and thoracic cavity and their contents.

8. Hematological analysis

On the 14^{th} day, animals were weighed, anaesthetized, and euthanized for collection of blood samples. Blood samples were examined for hematology analysis. Hematological parameters were determined using ADVIA 2120i hematology analyzer (Siemens Ireland, Dublin, Ireland). Hematological analysis included measurement of White Blood Cell (WBC) count, differential WBC count, red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT).

9. Statistical analyses

Weight changes and hematological results of the control group and experimental groups were analyzed for significance by one-way analysis of variance (ANOVA) using SPSS software (SPSS Inc., Chicago, IL, USA). P-value < 0.05 was considered to be significant. Results are expressed as mean ± standard deviation (SD).

Ⅲ. Results

1. Mortality and Clinical findings

Mortality and abnormalities in gross appearance of the animals were not observed during the experimental period. During clinical observation, fur, skin, eyes, mucous, membrane, gait, posture and respiration appeared normal. Lacrimation, clonic or tonic movement, piloerection, diarrhea, stereotype and bizarre behaviors were not observed. Salivation was transiently observed at 30 minutes post treatment (Table 1).

Table 1. Mortalities and clinical signs of rats

SEX : MALE		GROUP(mg/kg)			
		G1(0)	G2(500)	G3(1,000)	G4(2,000)
MORTALITIES	No. of dead animals	0 / 5 #	0 / 5	0 / 5	0 / 5
	%	0	0	0	0
CLINICAL SIGNS	No abnormalities detected	5 / 5	5 / 5	5 / 5	4 / 5
	Salivation	0 / 5	0 / 5	0 / 5	1 / 5
SEX : FEMALE		**GROUP(mg/kg)**			
		G1(0)	**G2(500)**	**G3(1,000)**	**G4(2,000)**
MORTALITIES	No. of dead animals	0 / 5	0 / 5	0 / 5	0 / 5
	%	0	0	0	0
CLINICAL SIGNS	No abnormalities detected	5 / 5	5 / 5	5 / 5	5 / 5
	Salivation	0 / 5	0 / 5	0 / 5	1 / 5

: Number of animals with the signs / Number of animals examined

2. Body weights

Body weight gain of males and females in experimental groups (CP 500, 1000, and 2000 mg/kg groups) were similar to those of corresponding control groups, showing no significant ($p>0.05$) differences (Fig. 1).

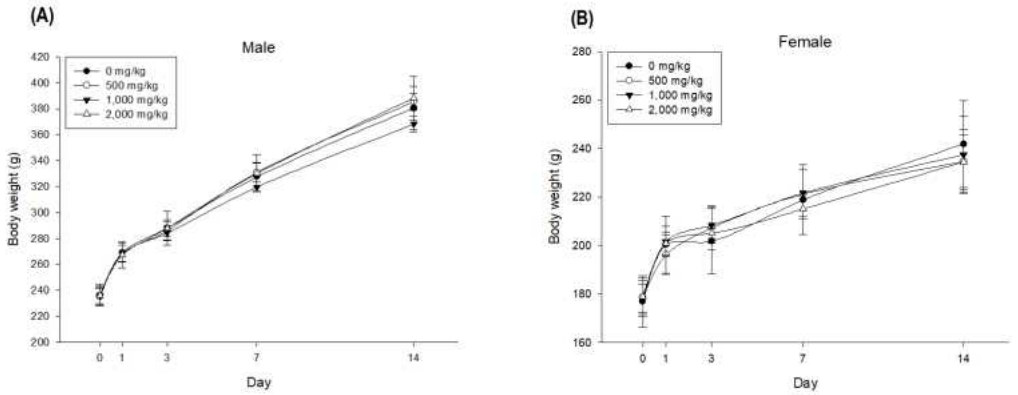

Fig. 1. Change on Body weights
(A) Male or (B) female rats were fed with CP extract (500, 1000, or 2000 mg/kg) and body weight changes were monitored for 14 days. Data represent the mean ± S.D.

3. Macroscopic examination

No abnormal findings were observed in macroscopic observation on day 14 (Table 2).

Table 2. Gross findings of rats

SEX : MALE					SEX : FEMALE				
ORGANS	SIGNS				ORGANS	SIGNS			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)		G1(0)	G2(500)	G3(1,000)	G4(2,000)
All organs	No gross finding detected	5 / 5 #	5 / 5	5 / 5	All organs	No gross finding detected	5 / 5	5 / 5	5 / 5

#: Number of animals with the signs / Number of animals examined

4. Hematological analysis

Hematological values are reported in Table 3 and 4. Relative levels of neutrophils and monocytes were significantly increased in female rats treated with 2000 mg/kg. However, there was no significant hematological change in male rats treated with CP extracts at any concentration compared with vehicle-treated controls.

Table 3. Hematological values of male rats

TEST ITEM	GROUP(㎎/㎏)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
WBC[1](K/μ L)	10.75 ± 1.78 (5)	12.03 ± 1.51 (5)	9.07 ± 1.89 (5)	11.56 ± 1.80 (5)
NE[2](K/μ L)	1.42 ± 0.30 (5)	1.24 ± 0.43 (5)	1.23 ± 0.33 (5)	1.68 ± 0.42 (5)
EO[3](K/μ L)	0.10 ± 0.02 (5)	0.09 ± 0.03 (5)	0.06* ± 0.01 (5)	0.05* ± 0.03 (5)
BA[4](K/μ L)	0.00 ± 0.01 (5)	0.01 ± 0.01 (5)	0.01 ± 0.01 (5)	0.00 ± 0.01 (5)
LY[5](K/μ L)	8.86 ± 1.55 (5)	10.22 ± 1.43 (5)	7.45 ± 1.68 (5)	9.46 ± 1.38 (5)
MO[6](K/μ L)	0.25 ± 0.06 (5)	0.35 ± 0.12 (5)	0.25 ± 0.08 (5)	0.27 ± 0.10 (5)
LUC[7](K/μ L)	0.13 ± 0.06 (5)	0.12 ± 0.02 (5)	0.07 ± 0.03 (5)	0.09 ± 0.03 (5)
NEP[8](%)	13.2 ± 1.8 (5)	10.3 ± 3.3 (5)	13.7 ± 3.1 (5)	14.4 ± 2.1 (5)

TEST ITEM	GROUP(mg/kg)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
EOP[9](%)	0.9 ± 0.3 (5)	0.7 ± 0.2 (5)	0.7 ± 0.1 (5)	0.4* ± 0.2 (5)
BAP[10](%)	0.1 ± 0.1 (5)	0.1 ± 0.1 (5)	0.1 ± 0.1 (5)	0.1 ± 0.1 (5)
LYP[11](%)	82.3 ± 2.5 (5)	84.9 ± 4.1 (5)	81.9 ± 4.1 (5)	82.0 ± 2.8 (5)
MOP[12((%)	2.4 ± 0.6 (5)	2.9 ± 0.7 (5)	2.9 ± 1.1 (5)	2.3 ± 0.8 (5)
LUP[13](%)	1.2 ± 0.6 (5)	1.1 ± 0.3 (5)	0.8 ± 0.4 (5)	0.8 ± 0.2 (5)
RBC[14](M/μL)	7.39 ± 0.27 (5)	7.03 ± 0.30 (5)	7.17 ± 0.21 (5)	7.03 ± 0.26 (5)
Hb[15](g/dℓ)	14.1 ± 0.3 (5)	13.9 ± 0.6 (5)	14.1 ± 0.6 (5)	13.6 ± 0.3 (5)
RDW[16](%)	11.7 ± 0.4 (5)	11.9 ± 0.3 (5)	11.6 ± 0.4 (5)	12.0 ± 0.1 (5)
HCT[17](%)	44.0 ± 1.0 (5)	43.2 ± 1.8 (5)	43.6 ± 1.5 (5)	42.5 ± 0.9 (5)
MCV[18](fL)	59.5 ± 1.4 (5)	61.4 ± 0.6 (5)	60.8 ± 1.6 (5)	60.4 ± 1.0 (5)
MCH[19](pg)	19.2 ± 0.4 (5)	19.8 ± 0.2 (5)	19.6 ± 0.5 (5)	19.4 ± 0.3 (5)
MCHC[20](g/dℓ)	32.2 ± 0.3 (5)	32.3 ± 0.3 (5)	32.2 ± 0.4 (5)	32.1 ± 0.3 (5)
PLT[21](K/μL)	1188 ± 82 (5)	1074 ± 168 (5)	902* ± 95 (5)	1084 ± 93 (5)
MPV[22](fL)	6.1 ± 0.2 (5)	5.8** ± 0.1 (5)	5.5** ± 0.1 (5)	5.7** ± 0.2 (5)

Data represent the mean ± S.D (Number of animals). 1: White blood cell, 2: Neutrophil, 3: Eosinophil, 4: Basophil, 5: Lymphocyte, 6: Monocyte, 7: Large unstained cell, 8: Percent of neutrophil, 9: Percent of eosinophil, 10: Percent of basophil, 11: Percent of lymphocyte, 12: Percent of monocyte, 13: Percent of large unstained cell, 14: Red blood cell, 15: Hemoglobin, 16: Red cell distribution width, 17: Hematocrit, 18: Mean corpuscular volume, 19: Mean corpuscular hemoglobin, 20: Mean corpuscular hemoglobin concentration, 21: Platelet, 22: Mean platelet volume. *: Significant difference compared with the control group values, $p<0.05$. **: Significant difference compared with the control group values, $p<0.01$

Table 4. Hematological values of female rats

TEST ITEM	GROUP(mg/kg)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
WBC[1](K/μL)	8.93 ± 1.88 (5)	9.47 ± 2.11 (5)	9.42 ± 1.27 (5)	6.82 ± 1.84 (5)
NE[2](K/μL)	0.82 ± 0.26 (5)	0.78 ± 0.36 (5)	1.01 ± 0.34 (5)	1.13 ± 0.69 (5)
EO[3](K/μL)	0.09 ± 0.01 (5)	0.08 ± 0.01 (5)	0.11 ± 0.05 (5)	0.09 ± 0.02 (5)
BA[4](K/μL)	0.00 ± 0.01 (5)	0.01 ± 0.00 (5)	0.00 ± 0.00 (5)	0.00 ± 0.00 (5)
LY[5](K/μL)	7.79 ± 1.80 (5)	8.27 ± 1.70 (5)	8.05 ± 1.56 (5)	5.29* ± 1.39 (5)
MO[6](K/μL)	0.17 ± 0.04 (5)	0.23 ± 0.08 (5)	0.18 ± 0.06 (5)	0.23 ± 0.08 (5)
LUC[7](K/μL)	0.07 ± 0.04 (5)	0.11 ± 0.02 (5)	0.07 ± 0.03 (5)	0.07 ± 0.03 (5)
NEP[8](%)	9.3 ± 2.9 (5)	8.0 ± 2.1 (5)	11.2 ± 4.9 (5)	16.3* ± 6.7 (5)
EOP[9](%)	1.0 ± 0.3 (5)	0.9 ± 0.1 (5)	1.2 ± 0.4 (5)	1.5 ± 0.4 (5)
BAP[10](%)	0.1 ± 0.1 (5)	0.1 ± 0.1 (5)	0.0 ± 0.1 (5)	0.0 ± 0.1 (5)
LYP[11](%)	86.9 ± 2.9 (5)	87.5 ± 2.1 (5)	84.9 ± 5.4 (5)	77.7* ± 5.9 (5)
MOP[12((%)	1.9 ± 0.4 (5)	2.3 ± 0.4 (5)	1.9 ± 0.7 (5)	3.3** ± 0.6 (5)
LUP[13](%)	0.8 ± 0.3 (5)	1.3 ± 0.4 (5)	0.7 ± 0.3 (5)	1.1 ± 0.4 (5)
RBC[14](M/μL)	7.29 ± 0.25 (5)	7.18 ± 0.22 (5)	7.01 ± 0.37 (5)	7.04 ± 0.27 (5)
Hb[15](g/dℓ)	13.5 ± 0.5 (5)	13.9 ± 0.3 (5)	13.4 ± 0.6 (5)	13.3 ± 0.5 (5)
RDW[16](%)	10.8 ± 0.5 (5)	10.9 ± 0.7 (5)	10.8 ± 0.4 (5)	11.0 ± 0.6 (5)
HCT[17](%)	40.6 ± 1.6 (5)	41.4 ± 0.9 (5)	40.0 ± 1.9 (5)	40.3 ± 1.5 (5)
MCV[18](fL)	55.6 ± 0.5 (5)	57.7* ± 1.0 (5)	57.2 ± 1.1 (5)	57.2 ± 2.9 (5)

TEST ITEM	GROUP(mg/kg)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
MCH[19](pg)	18.5 ± 0.2 (5)	19.3** ± 0.3 (5)	19.1 ± 0.7 (5)	19.0 ± 0.9 (5)
MCHC[20](g/dℓ)	33.3 ± 0.2 (5)	33.5 ± 0.3 (5)	33.4 ± 0.9 (5)	33.1 ± 0.4 (5)
PLT[21](K/μL)	1005 ± 143 (5)	975 ± 61 (5)	1081 ± 91 (5)	1057 ± 78 (5)
MPV[22](fL)	5.8 ± 0.4 (5)	5.9 ± 0.2 (5)	6.6** ± 0.3 (5)	6.8** ± 0.3 (5)

Data represent the mean ± S.D (Number of animals). 1: White blood cell, 2: Neutrophil, 3: Eosinophil, 4: Basophil, 5: Lymphocyte, 6: Monocyte, 7: Large unstained cell, 8: Percent of neutrophil, 9: Percent of eosinophil, 10: Percent of basophil, 11: Percent of lymphocyte, 12: Percent of monocyte, 13: Percent of large unstained cell, 14: Red blood cell, 15: Hemoglobin, 16: Red cell distribution width, 17: Hematocrit, 18: Mean corpuscular volume, 19: Mean corpuscular hemoglobin, 20: Mean corpuscular hemoglobin concentration, 21: Platelet, 22: Mean platelet volume. *: Significant difference compared with the control group values, $p<0.05$. **: Significant difference compared with the control group values, $p<0.01$

Ⅳ. Discussion

CP is an herbal medicine widely used in oriental medicine to relieve rashes and itching, improve vision, relieve spasms, and soothe the throat. However, it has been reported that CP can cause toxicity and side effects in patients who take too much of it or have a hypersensitive constitution. Thus, it is considered necessary to look into its toxicity [18]. CP has been reported to decrease activity in mice and rabbits. When administered at 1.12 g/kg for 7 days, it can cause changes in kidney-related serum biochemistry in rabbits [18].

The test article CP extracts at a dose of 0, 500, 1000, or 2000 mg/kg was orally administrated to male and female SD rats to evaluate its acute toxicity. Clinical signs, mortality, body weight changes, and macroscopic examination were observed for 14 days following a single treatment. No significant changes were observed in this study. However, salivation in clinical signs was observed in a male rat treated with the highest doses of CP extracts. Salivation in rats has been reported to be dose-related [19]. Test substances that are quite unpleasant or have immediate side effects may cause stress during administration. Clinical signs such as salivation may indicate that the animal is experiencing stress [20]. However, it was not accompanied by other pathological changes such as body weight or macroscopic examination. Clinical signs observed transiently after administration were not observed from one hour after treatment. Therefore, it was considered to have no toxicological significance.

In terms of hematology, increase of relative levels of neutrophils and monocytes were observed in female rats after administration of the highest doses of CP extracts. However, these hematological changes were within the normal historical range [21,22]. They were detected only in female rats, suggesting that they were not related to test article toxicity.

In this study, we did not find any toxicological significant clinical changes. Body weight changes and macroscopic examinations were not significant compared to the control group. Thus, LD_{50} of CP extracts was considered to be greater than 2000 mg/kg. Overall, results showed no toxicity significance. However, a single oral dose acute toxicity test is not sufficient to determine toxicity to CP extracts. Further investigation should include 4- and 13-week repeated oral dose toxicity studies and genotoxicity studies. This study observed toxicity after a single dose of CP extracts. It could be used as a basis for the safety of CP.

Ⅴ. Conclusions

In a single oral toxicity study with CP extracts in SD rats, no mortality was observed following administration of the test article. No toxicological changes were observed in clinical findings, body weight changes, hematological analysis, or macroscopic examination attributable to CP extracts. Therefore, the LD_{50} of CP extracts is considered to exceed 2000 mg/kg.

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