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Sub-Chronic Oral Toxicity Study of Deer Velvet Antler Extracts in ICR Mice

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ABSTRACT - This study evaluated the sub-chronic oral toxicity of freeze-dried deer velvet antler (DVA) extract in ICR mice over a 90-day period. The extract was administered orally to male and female mice at doses of 1,000, 2,000, and 5,000 mg/kg body weight daily; the control group (0 mg/kg body weight) received vehicle only. No clinical signs of toxicity or mortality were observed. Body weight gain, food consumption, and general behavior remained normal across all groups. Hematological analysis revealed no dose-dependent alterations in hemoglobin levels, platelet characteristics, or red or white blood cell counts. Males administered 2,000 mg/kg DVA exhibited a slight increase in reticulocyte count, whereas females exhibited reduced count at 5,000 mg/kg; however, neither finding was dose-responsive or toxicologically significant. Serum biochemistry including liver and kidney function markers (ALT, AST, γ-GT, BUN, and creatinine) remained within normal range, and no evidence of organ damage was observed. Notably, male mice exhibited a significant reduction in serum triglycerides compared with those in the control group, suggesting potential lipid-modulating effects of DVA; however, this finding requires further investigation. Based on these findings, the no-observed-adverse-effect level (NOAEL) of DVA extract was determined to be greater than 5,000 mg/kg/day in both sexes. These results support the safety profile of DVA extract at the tested doses, and its potential metabolic benefits.

Key words: Deer antler, Sub-chronic, Oral toxicity, NOAEL

Deer velvet antler (DVA) is a unique frontal extension of the deer skull that develops postnatally from permanent bony structures known as pedicles[1)]. In traditional Korean medicine, as well as in other Asian countries, deer antlers are highly valued due to their reported benefits in treating a variety of conditions, including enhancement of forming blood cells, improving liver function, and restoring vitality[2–7)]. But limited safety data have hindered its broader therapeutic application. Our previous research aimed to establish a scientific basis for its safe use by investigating its nutritional content, presence of bioactive compounds, and potential toxicological effects *in vitro* and *in vivo*. Therefore, we evaluated the biochemical composition, cytotoxicity, and acute oral toxicity of three types of deer velvet antler (DVA) extracts—raw, dried, and freeze-dried samples[8)].

According to the results from previous studies, deer velvet antler extracts contain diverse bioactive compounds and possess no significant acute toxicity in animal models at standard doses. Also, the findings suggested that the identified marker compounds can be utilized for standardizing and enhancing the therapeutic reliability of commercial DVA products. Especially, the study has identified that deer antler velvet contains water-soluble bioactive compounds including sialic acid, uronic acid, etc[8)]. Though it possesses various medicinal values, improper use of deer antler velvet has a potential for side effects, including fever, epistaxis, headache, palpitations, diarrhea, indigestion, vomiting, and nausea. Therefore, it is advisable to use deer antler medication with caution[9)]. With this backdrop, this study aimed to investigate any adverse effects associated with prolonged oral administration for 90 days, and to establish a no-observed-adverse-effect level (NOAEL) for the deer antler velvet (*Cervus canadensis* Erxleben) extract.

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Materials and Methods

Preparation of deer velvet antler extract

From the Korea Rabbit and Deer Agricultural Cooperative Federation (Seoul, Korea), raw deer velvet antlers were obtained. The tissues from the antlers were stored at 4ºC until further processing. To improve the extraction process, impurities from raw deer velvet antlers were removed. Initially, the velvet antlers were cut into small pieces. Then, each 4 kg of velvet antlers was suspended in 12 L of distilled water and thoroughly mixed to eliminate impurities; this process was repeated five times. Subsequently, the antlers were subjected to extraction; an electric extractor (Gyeongseo ENP, Incheon, Korea) was used at 95ºC for 12 hours with distilled water, and filtered using a 120-mesh filter to remove residues. After being concentrated under lower pressure, the sample was freeze-dried and kept at 4ºC for use in subsequent *in vivo* experiments. The extraction protocols were approved by the Animal Ethics Committee, with approval numbers IAC2022-1319 for the raw extract, IAC2022-1320 for the dried extract, and IAC2022-1325 for the freeze-dried extract, in accordance with the Animal Protection Act no. 16977[8].

Test substance and dose selection

The test substance, a freeze-dried powder of deer antler concentrate, was reconstituted in sterile distilled water, which served as the vehicle, with dosing solutions freshly prepared each day prior to administration. The dose levels were selected based on a comprehensive approach combining preliminary toxicity data, traditional use patterns, and regulatory guidelines. A preliminary acute toxicity study was conducted using single oral doses up to 5,000 mg/kg, with no mortality or clinical signs of toxicity observed at any tested dose, establishing 5,000 mg/kg as a safe upper limit for the subchronic study. While conventional toxicology studies often employ 3- or 10-fold dose spacing[10], doses of 1,000, 2,000, and 5,000 mg/kg (representing 2-fold and 2.5-fold intervals) were selected because the preliminary study indicated an exceptionally high safety margin for deer velvet antler extract, intermediate dose levels were needed to better characterize potential dose-response relationships, the highest dose (5,000 mg/kg) represented the maximum feasible dose considering gavage volume limitations (20 mL/kg) and extract solubility constraints, and the lowest dose (1,000 mg/kg) represents approximately 100-fold the estimated human equivalent dose based on traditional usage patterns[11]. Dose selection followed OECD Test Guideline 408 principles, using the highest dose that produces minimal toxicity while allowing adequate safety margin assessment[12]. Based on the results of a preliminary 14-day single-dose toxicity study, the dose levels were set at 0 mg/kg, 1,000 mg/kg, 2,000 mg/kg, and 5,000 mg/kg, with the dosing volume standardized at 20 mL/kg and the actual dose administered to each animal calculated based on its individual body weight measured immediately before dosing.

Experimental animals and housing conditions

Five-week-old ICR mice were obtained from J-Bio Co., Ltd. (Gwangju, Korea). After a one-week acclimatization period, only healthy animals showing no abnormal signs were selected for the study. Without any selection method, mice were randomly assigned to experimental groups with 10 males and 10 females per group (n=10/sex/group). Individual animals were kept in cages under controlled environmental conditions, including a 12-hour light/dark cycle, temperature of 22 ± 3ºC, relative humidity of 50 ± 20%, and light intensity maintained between 150 and 300 lux. A sterilized AIN-76A purified diet was provided *ad libitum*, and autoclaved distilled water was freely available throughout the study. The experimental protocols used in this study were approved by the Animal Ethics Committee of Chonnam National University (No. CNU IACUC-YS-2020-9). The composition of the experimental groups receiving deer antler extract and their respective dosing regimens are described in Table 1.

Table 1. Experimental groups and dosing information

Group	Sex	Number of Animals	Dose (mg/kg B.W)	Dose amount (mL/kg B.W)	Route of administration
G1	Male Female	10	0	20	Oral administration
G2	Male Female	10	1,000	20	Oral administration
G3	Male Female	10	2,000	20	Oral administration
G4	Male Female	10	5,000	20	Oral administration

General clinical signs

From the day of oral administration, animals were monitored hourly from 1 to 8 hours post-dosing for the first 7 days. Thereafter, observations were conducted once daily until the end of the study. All animals were assessed for general clinical signs, symptoms of toxicity, and mortality[13].

Body weight, feed, and water intake measurements

Before starting dosing, the body weights of every animal were noted, and they were then measured once a week for the duration of the treatment. A final body weight measurement was taken on the day of necropsy. Feed and water were provided *ad libitum* to ensure unrestricted access. Feed intake was calculated weekly based on the difference between the amount supplied and the residual amount remaining[13].

Necropsy and organ weights

No mortality was observed during the 90-day oral administration period. At the end of the study, animals were euthanized by exsanguination under isoflurane anesthesia (Hana Pharm, Seoul, Korea), followed by a gross necropsy. No specific toxicological lesions were observed upon macroscopic examination; therefore, histopathological analysis was not conducted[14].

Hematological analysis

Following the completion of the trial, animals were allowed free access to water and fasted the previous day. After inducing anesthesia with inhaled anesthetics, a surgical procedure was performed to collect blood. The blood was transferred into collection tubes containing EDTA, an anticoagulant. Hematological parameters were then analyzed using a Mindray 5100 hematology analyzer. The following parameters were assessed: red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW-CV, RDW-SD), mean corpuscular hemoglobin concentration (MCHC), platelet distribution width (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW), and reticulocyte count[13].

Blood biochemical analysis

Blood samples were centrifuged at 1,100 g for 15 minutes to separate the serum, which was then analyzed using a BECKMAN AU-480 biochemical analyzer. The following biochemical parameters were measured: triglycerides (TG), high-density lipoprotein (HDL), Low-Density Lipoprotein (LDL), gamma-glutamyltransferase (γ-GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)[13].

Statistical analysis

The mean ± standard deviation (mean ± SD) is used to display all data. IBM SPSS Statistics version 20 (SPSS Inc., Chicago, IL, USA) was used to conduct the statistical analysis. The data was analyzed using one-way analysis of variance (ANOVA), and the statistical significance between sample groups ($P<0.05$) was evaluated using Tukey's multiple range test.

Results and Discussion

General symptoms, body weight, food intake and mortality

Throughout the 90-day study period, normal body weight gain was observed in both male and female animals across all groups. No significant changes in food intake were observed in any group following the administration of the

Table 2. Effects of antler's freeze-dried extract of body weight and food consumption of ICR mouse, and mortality with clinical signs.

Sex	Group	Dose (mg/kg B.W.)	N	Initial body weight (g)	Final body weight (g)	Body weight gain (g/day)	Food intake (g/day)	Mortality (%)	Clinical signs
Male	G1	0	10	24.10±2.92[a]	41.80±4.69[a]	17.70±6.82[a]	27.97±1.35[a]	0% (0/10)	Normal
	G2	1000	10	24.10±2.47[a]	42.70±2.83[a]	18.60±4.44[a]	30.45±1.41[a]	0% (0/10)	Normal
	G3	2000	10	24.00±3.02[a]	41.50±5.89[a]	17.50±6.20[a]	28.51±1.00[a]	0% (0/10)	Normal
	G4	5000	10	24.00±2.83[a]	39.60±4.33[a]	15.60±5.30[a]	27.92±1.36[a]	0% (0/10)	Normal
Female	G1	0	10	22.70±0.82[a]	30.20±2.62[a]	7.5±3.39[a]	25.11±1.51[a]	0% (0/10)	Normal
	G2	1000	10	23.00±0.00[a]	26.80±3.46[a]	3.8±3.66[a]	25.09±1.54[a]	0% (0/10)	Normal
	G3	2000	10	22.70±2.16[a]	27.11±1.62[a]	4.44±2.90[a]	24.43±2.66[a]	0% (0/10)	Normal
	G4	5000	10	22.70±1.57[a]	27.11±1.96[a]	4.44±1.25[a]	23.77±3.22[a]	0% (0/10)	Normal

B.W: Body weight; N: Number of animals; Mortality: The number of deaths/number of animals tested.

freeze-dried deer antler extract (Table 2). When compared to control groups, there were no statistically significant variations in food intake or body weight growth. Both male and female mice showed normal growth patterns and consistent dietary intake across all treatment groups. Based on the observation the study suggest that the extract did not adversely affect metabolic health or appetite.

During the observation period, no mortality related to the test substance could be observed in any of the groups, including the control group. No abnormal symptoms attributable to the extract were detected in the low (1,000 mg/kg/day), medium (2,000 mg/kg/day), or high (5,000 mg/kg/day) dose groups for both male and female animals. Based on these findings, the NOAEL of the deer antler extract in mice is estimated to be above 5,000 mg/kg body weight (B.W.) for both sexes (Table 2). There is other evidence from animal studies indicating that deer antler extract has a high safety margin, with no significant toxicity observed at doses up to 5,000 mg/kg in mice and 2,000 mg/kg in rats, which result is similar to this study[15,16].

Autopsy findings and organ weights

The effects of oral administration of freeze-dried deer antler extract on organ weights were assessed. When all individuals' organs were autopsied, no notable alterations or unusual discoveries pertaining to the extract's treatment were observed. In male mice, we observed statistically significant changes ($P<0.05$) in both heart and liver weights. Heart weight showed a significant decrease in the highest dose group (G4: 0.49 g/100 g BW) compared to control group (G1: 0.59 g/100 g BW), while liver weight were significantly reduced in deer antler extract treated groups (G2-G4: 3.51-3.58 g/100 g BW) compared to control group (G1: 4.08 g/100 g BW) (Table 3).

Importantly, these alterations did not exhibit a dose-dependent pattern and were no clinical signs of organ dysfunction. Moreover, no other significant findings related to the deer antler extract were observed in the autopsy. In contrast, female mice showed no significant organ weight changes were observed in any treatment groups, suggesting sex-specific responses to the deer antler extract. This difference could be attributed to differential metabolic processing of bioactive compounds in deer antler extract, potentially mediated by sex hormones affecting liver enzyme expression and cardiac metabolism[17,18]. Therefore, these findings are not considered to be of toxicological concern in both male and female mice.

Hematological examination

Following 90 days oral administration of the freeze-dried powder of velvet antler extract, hematological parameters were evaluated against established normal reference ranges for ICR mice to assess clinical significance (Table 4). White blood cell (WBC) counts were consistently within the normal range of 1.06-56.0×10^3 cells/μL in all experimental groups (0.9-3.1×10^3 cells/μL), including control animals (1.8×10^3 cells/μL). In male mice, WBC counts showed variable responses with G2 showing elevation (2.5×10^3 cells/μL), while G3 showed suppression (0.9×10^3 cells/μL) compared to control (G1: 1.8×10^3 cells/μL). The results in female mice demonstrated more consistent WBC suppression across all treatment groups (G2-G4: 1.2-1.9 x 10^3 cells/μL) compared to control (3.1×10^3 cells/μL). However, these alterations exhibited no dose-dependency, and all values remained within the normal physiological ranges, with no associated clinical signs or mortality observed in ICR mice (normal range: 1.06-56.0×10^3 cells/μL)[19,20]. The absence of clinical signs or mortality suggests these variations represents normal biological fluctuation rather than immunotoxicity. Moreover, similar sex-specific trends were seen in lymphocyte counts, with females consistently showing decreases in velvet antler extract treated groups and males displaying varied responses. In both sexes, neutrophil, monocyte, eosinophil, and basophil counts did not change

Table 3. Effects of antler's freeze-dried extract of organ weight of ICR mouse (unit: g/100g BW)

Sex	Group (mg/day)	Heart	Kidney		Liver
			left	right	
Male	G1	0.59±0.06^{a}	0.77±0.09^{a}	0.87±0.09^{a}	4.08±0.044^{a}
	G2	0.53±0.04^{ab}	0.73±0.07^{a}	0.81±0.06^{a}	3.58±0.34^{b}
	G3	0.54±0.11^{ab}	0.67±0.15^{a}	0.80±0.17^{a}	3.51±0.31^{b}
	G4	0.49±0.07^{b}	0.68±0.11^{a}	0.78±0.12^{a}	3.53±0.31^{b}
Female	G1	0.47±0.07^{a}	0.61±0.10^{a}	0.60±0.08^{a}	4.21±0.37^{ab}
	G2	0.50±0.14^{a}	0.64±0.13^{a}	0.64±0.09^{a}	3.36±1.17^{ab}
	G3	0.51±0.08^{a}	0.71±0.06^{a}	0.71±0.07^{a}	4.43±0.59^{a}
	G4	0.54±0.05^{a}	0.68±0.06^{a}	0.68±0.06^{a}	4.29±0.49^{ab}

Table 4. Leukocyte values in the male and female ICR mouse treated orally with antler's freeze-dried extract for 90 days (units: 10^3 cells/μL).

Sex	Group	WBC	Neutrophil	Lymopocyte	Monocyte	Eosinophil	Basophil
Normal range		1.06-56.0[19,20]	0.03-3.1[19,20]	0.12-23.4[19]	0.04-5.08[19]	0.01-2.0[19,22]	0.0-2.33[19,22]
Male	G1	1.8±0.2^{ab}	0.4±0.1^{ab}	1.1±0.2^{ab}	0.2±0.1^{a}	0.1±0.03^{a}	0.1±0.1^{a}
	G2	2.5±0.6^{a}	0.5±0.1^{a}	1.8±0.5^{a}	0.1±0.1^{a}	0.1±0.01^{a}	0.0±0.0^{b}
	G3	0.9±0.4^{b}	0.2±0.1^{b}	0.7±0.3^{b}	0.1±0.01^{a}	0.1±0.00^{a}	0.0±0.0^{b}
	G4	1.8±1.2^{b}	0.8±0.64^{ab}	0.8±0.4^{b}	0.1±0.1^{a}	0.1±0.02^{a}	0.0±0.01^{ab}
Female	G1	3.1±0.7^{a}	0.7±0.2^{a}	2.0±0.5^{a}	0.2±0.1^{a}	0.2±0.07^{a}	0.1±0.0^{a}
	G2	1.4±0.3^{ab}	0.4±0.1^{a}	0.7±0.1^{b}	0.1±0.1	0.3±0.16^{a}	0.1±0.01^{a}
	G3	1.9±0.5^{ab}	0.5±0.2^{a}	1.0±0.2^{b}	0.1±0.1^{a}	0.3±0.18^{a}	0.0±0.0^{a}
	G4	1.2±0.2^{b}	0.2±0.02^{a}	0.8±0.2^{b}	0.1±0.0^{a}	0.2±0.10^{a}	0.1±0.01^{a}

Table 5. Erythrocyte and thrombocyte values in the male and female ICR mouse treated orally with antler's freeze-dried extract for 90 days

Sex	Group	RBC (10^6 cells/μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (10^3 cells/μL)	MPV (fL)	Reticulocyte (%)
Normal range		6.1-12.2[20-22]	10.8-19.2[22,23]	33.5-62[22,24]	42.7-56[20,23]	11.7-17.6[20,22]	27-37.6[19,20]	15.9-23[22,25]	620-2159[22-24]	4.3-6.2[22]	3.0-5.8[19,20]
Male	G1	8.12 ±1.47^{a}	13.20±2.96^{a}	38.54±6.81^{a}	47.52±1.13^{a}	16.20±1.06^{a}	34.12±2.05^{a}	14.02±1.05^{a}	748.60±597.07^{a}	6.34±0.18^{a}	2.93±0.30^{b}
	G2	8.88 ±0.53^{a}	14.18±0.56^{a}	40.00±1.43^{a}	45.10±1.48^{a}	15.98±0.55^{a}	35.46±0.18^{a}	14.68±1.46^{a}	1302.8±297.52^{a}	6.64±1.06^{a}	3.23±0.71^{ab}
	G3	7.74±2.78^{a}	12.62±4.54^{a}	35.24±12.68^{a}	45.60±2.02^{a}	16.34±0.68^{a}	35.86±0.11^{a}	14.18±0.62^{a}	1173.80±474.33^{a}	6.42±0.30^{a}	3.55±0.19^{a}
	G4	8.36±0.56^{a}	13.58±0.94^{a}	37.82±2.47^{a}	45.22±1.05^{a}	16.22±0.50^{a}	35.88±1.09^{a}	14.00±0.82^{a}	1401.2±352.92^{a}	6.38±0.43^{a}	3.18±0.20^{ab}
Female	G1	8.38±0.64^{a}	14.24±0.78^{a}	39.18±2.07^{a}	46.82±1.52^{a}	17.00±0.50^{a}	36.34±0.38^{a}	13.78±0.61^{a}	1037.2±307.47^{a}	6.28±0.37^{a}	3.74±0.37^{a}
	G2	8.59±0.66^{a}	14.22±0.72^{a}	38.92±2.28^{a}	45.40±2.49^{a}	16.60±0.99^{a}	36.56±0.65^{a}	14.18±1.00^{a}	1083±469.99^{a}	6.52±0.39^{a}	3.16±0.75^{ab}
	G3	8.16±0.77^{a}	13.75±1.13^{a}	38.23±2.69^{a}	46.90±1.21^{a}	16.85±0.33^{a}	35.95±0.76^{a}	14.68±1.65^{a}	1170.7±5278.21^{a}	6.43±0.78^{a}	3.63±0.46^{ab}
	G4	8.30±0.63a	14.28±0.69^{a}	39.05±2.14^{a}	47.15±1.42^{a}	17.25±0.66^{a}	36.55±0.44^{a}	17.80±9.21^{a}	1259.5±77.52^{a}	5.88±0.29^{a}	2.6±1.14^{b}

fL: Femtoliters; pg: Picograms.

in any of the treatment groups, preserving normal differential percentages in relation to total WBC counts.

Changes in erythrocyte parameters due to velvet antler extract treatment were observed in both male and female groups (Table 5). Erythrocyte parameters demonstrated clinically acceptable profiles when compared to reference ranges. Red blood cell counts (7.74-8.88×10^6 cells/μL in males, 8.16-8.59×10^6 cells/μL in females), haemoglobin levels (12.62-14.28 g/dL), and haematocrit values (35.24-40.00% in males, 38.23-39.18% in females) all stayed within established normal ranges (RBC: 6.1-12.2×10^6 cells/μL; HGB: 10.8-19.2 g/dL; HCT: 33.5-62.0 %)[20-24], confirming the absence of treatment-related anaemia or polycythaemia. Reticulocyte counts showed significant differences in both sexes, with the most notable change in female G4 group (2.6%) compared to controls (3.74%). While, the progressive increases were observed in male mice from G1 (2.93%) to G3 (3.55%). However, all reticulocyte values remained within the normal reference range (3.0-5.8%)[19,20], indicating these variations likely represent adaptive responses to the extract rather than adverse effects. Platelet parameters, including platelet count (748.60-1401.2×10^3 cells/μL) and mean platelet volume (5.88-6.64 fL), remained within normal ranges (PLT: 620-2159×10^3 cells/μL; MPV: 4.3-6.2 fL)[22-24] across all treatment groups, indicating normal hemostatic function. Overall, the safety profile of the deer velvet antler extract is supported by the clinical interpretation based on normal reference ranges, which shows that essential haematological parameters remained within physiologically acceptable limits, even though statistical differences were noted between some treatment groups.

Serum biochemical analysis

To evaluate the potential toxicity of freeze-dried antler powder, serum biochemical parameters were analyzed in each experimental group (Table 6). Key markers assessed, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) for liver function. The resulting values of AST, ALT, and gamma-glutamyl transferase (γ-GT) exposed that no dose-dependent elevations occurred in

Table 6. Serum biochemical values in the male and female ICR mouse treated orally with antler's freeze-dried extract for 90 days

Sex	Group	AST (U/L)	ALT (U/L)	γ –GT (U/L)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Normal range		83-405[22,25]	20-129[22,25]	0-8[22,25]	67-120[22,25]	70-126[29,30]	11.7-30[29,30]
Male	G1	325.8±183.1[a]	45.0±16.9[a]	-5.6±2.3[b]	199.0±41.3[a]	197.8±25.7[a]	29.6±13.1[a]
	G2	163.2±66.6[a]	37.0±7.7[a]	-3.2±0.5[ab]	115.6±27[b]	225.8±20.5[a]	39.0±10.2[a]
	G3	121.2±44.9[a]	34.6±12.7[a]	-2.4±0.6[a]	83.4±25.1[b]	220.6±47.8[a]	38.2±24.9[a]
	G4	242.0±202.6[a]	41.2±11[a]	-2.2±1.3[a]	103.2±26.6[b]	193.0±27.6[a]	25.4±7.4[a]
Female	G1	486.8±220.4[a]	49.6±22.6[a]	-4.2±0.8[a]	72.2±20.1[a]	120.4±38.3[a]	23.2±11.1[a]
	G2	682.2±260.5[a]	132.4±98.9[a]	-3.6±1.1[a]	57.2±23.7[a]	109.0±35.6[a]	18.4±6.8[a]
	G3	705.2±385[a]	117.4±104.3[a]	-4.2±1.3[a]	74.2±25.1[a]	126.4±20.7[a]	20.4±6.4[a]
	G4	422.0±233.4[a]	47.4±24.9[a]	-2.8±1.6[a]	57.4±9.2[a]	111.6±37.9[a]	18.0±9.1[a]

either male or female mice, indicating absence of hepatotoxicity. Moreover, AST levels remained within normal ranges (83-405 U/L)[22,25] across all groups, with male mice showing values of 121.2-325.8 U/L and female mice showing 422.0-705.2 U/L. ALT levels also remained within normal ranges (20-129 U/L)[22,25] for all groups (males: 34.6-45.0 U/L; females: 47.4-132.4 U/L). However, liver enzyme patterns exibited notable sex-specific variations. While male mice showed relatively stable and stayed within the normal ranges of AST (83-405 U/L) and ALT (20-129 U/L) levels across all groups, female mice demonstrated considerable numerical variations in AST levels (422-705 U/L) and ALT (G2: 132 U/L) levels, though without dose-dependency. These elevated enzyme levels in females, while statistically non-significant due to high inter-individual variation, may be attributed to physiological stress responses or fasting-induced metabolic changes rather than hepatotoxic effects[26,27]. The lack of correlation between dose and liver enzyme levels, combined with low γ-GT values, confirms the absence of hepatotoxicity. Furthermore, γ-GT remained unchanged across all groups, supporting the absence of cholestatic or biliary injury[28].

Serum triglyceride (TG) levels were significantly decreased in all treatment male groups (G2: 115.6, G3: 83.4, G4: 103.2 mg/dL) compared to the control group (G1: 199 mg/dL), representing a 42-58% reduction in triglyceride levels. This suggests a potential lipid-lowering effect of the antler extract. However, the toxicological significance of this finding in healthy animals remains unclear, as this study was designed to assess safety rather than therapeutic efficacy. Investigating the mechanism behind this sex-specific response in suitable illness models is necessary to ascertain its clinical significance. In female mice's TG levels remained constant between 57.2 and 74.2 mg/dL in all treatment groups, indicating no significant changes across any treatment groups. These results suggesting some sex-specific metabolic responses potentially mediated by differences in sex-enzymes balance may affect lipid metabolism, and particularly regarding liver enzyme variability in females. Such differences may be consistent with sex hormones, which can influence liver enzyme expression and lipid metabolism[17]. These observational differentiations reflect inherent physiological distinctions rather than deer antler extract-induced toxicity. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels remained relatively stable across all groups in both sexes, with no dose-dependent patterns observed. Consequently, the range of HDL levels was 193.0-225.8 mg/dL for males and 109.0-126.4 mg/dL for females, whereas the range of LDL levels was 25.4-39.0 mg/dL for males and 18.0-23.2 to females.

In summary, the present study indicates that freeze-dried deer velvet antler extract is well acceptable in ICR mice following 90 days oral administration even at high doses up to 5,000 mg/kg per day. No deaths or clinical signs of toxicity were observed in any treatment group. Generally, animals showed normal body weight gain, food intake, and behavior, suggesting that the deer velvet antler extract did not negatively impact gross health or metabolism.

The results of blood analysis and serum biochemistry further supported the safety of the deer velvet extract. Comprehensive haematological evaluation revealed that all essential parameters remained within normal reference ranges for ICR mice. While minor variations in white blood cell and reticulocyte counts were observed between treatment groups, these changes lacked dose-dependency and clinical significance, likely representing normal biological variation rather than treatment-related effects. The absence of treatment-related haematological toxicity is confirmed by the maintenance of erythrocyte parameters within normal ranges and normal reticulocyte activity. Despite minor statistical differences between treatment

groups, clinically significant haematological measures stayed within physiologically normal limits, which supports our conclusion that the extract retains an appropriate safety profile.

Liver and kidney function markers, including ALT, AST, and γ-GT remained within normal ranges and showed no dose-related changes. Although there were some variations in hematological parameters and organ weights, these findings were not dose-dependent and were not associated with any signs of organ damage, ensuring the safety of deer velvet antler extract from potential toxicity. Male mice treated with the extract showed significantly lower triglyceride levels compared to control. However, since this was a toxicological study conducted on healthy animals, it is impossible to assess the therapeutic importance of this finding without doing suitable efficacy investigations in relevant disease models.

Overall, based on the comprehensive evaluation of clinical observations, hematological parameters, and serum biochemistry, the freeze-dried deer velvet antler extract was found to be safe at all tested doses, with a NOAEL greater than 5,000 mg/kg/day in both male and female mice. Along with the lack of clinical symptoms or mortality and the absence of dose-dependent toxicity patterns across all assessed parameters confirm the safety profile of antler extract at the tested doses. These results strongly support the safety of deer velvet antler extract for oral use within the dose range studied. While statistically significant changes in triglyceride levels were observed in male mice, the toxicological and clinical significance of this finding in healthy animals cannot be determined from this safety study. These results suggest the safety of deer velvet antler extract for oral use within the tested dose range and its potential not only as a traditional health supplement but also as a candidate for further development in metabolic health applications.

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국문요약

본 연구는 동결건조 녹용(DVA) 추출물의 반복 경구 투여에 따른 아급성 독성을 평가하기 위해 ICR 생쥐를 대상으로 90일간 독성시험을 실시하였다. 실험군은 수컷 및 암컷 생쥐에게 각각 체중당 1,000, 2,000, 5,000 mg/kg의 용량으로 DVA 추출물을 매일 경구 투여하였으며, 대조군은 동일한 조건에서 용매만을 투여하였다. 실험 기간 동안 모든 군에서 임상적 독성 징후나 사망 사례는 관찰되지 않았으며, 체중 증가, 사료 섭취량, 일반적인 행동에서도 유의한 이상 소견은 나타나지 않았다. 혈액학적 분석 결과, 적혈구, 백혈구, 혈소판 수 및 혈색소 수치 등에서 용량 의존적인 변화는 확인되지 않았으며, 2,000 mg/kg 용량군 수컷에서 망상적혈구 수치의 소폭 증가, 5,000 mg/kg 용량군 암컷에서의 감소가 관찰되었으나, 이는 독성학적으로 유의적인 차이를 보이지 않았다. 또한, 혈청 생화학 분석을 통해 간 및 신장 기능 지표(ALT, AST, γ-GT, BUN, creatinine)는 모든 군에서 정상 범위를 유지하였으며, 장기 손상 소견은 관찰되지 않았다. 한편, 수컷 생쥐에서 혈청 중성지방 수치가 유의하게 감소하여, DVA 추출물의 지질 대사 조절 가능성을 확인하였다. 이상의 결과를 종합할 때, 본 녹용추출물의 체내 무독성량 NOAEL은 수컷 및 암컷 ICR 생쥐 모두에서 5,000 mg/kg/day 이상으로 판단되며, DVA 추출물은 경구 투여 시 안전성이 확보된 소재로 사료된다.

Conflict of interests

The authors declare no potential conflict of interest.

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