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# Sub-chronic safety evaluation of the ethanol extract of *Aralia elata* leaves in Beagle dogs

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ABSTRACT

*Aralia elata* Seem. (*A. elata*) is a traditional Chinese medicine to treat some diseases. This investigation aims to evaluate the pharmaceutical safety of the ethanol extract of *A. elata* leaves, namely ethanol leaves extract (ELE), in Beagle dogs. In sub-chronic oral toxicity study, dogs were treated with the ELE at doses of 50, 100 and 200 mg/kg for 12 weeks and followed by 4 weeks recovery period. During experimental period, clinical signs, mortality, body temperature, food consumption and body weight were recorded. Analysis of electrocardiogram, urinalysis, ophthalmoscopy, hematology, serum biochemistry, organ weights and histopathology were performed. The results showed that both food consumption and body weight significantly decreased in high-dose group. Treatment-related side effects and mortality were observed in high-dose female dogs. Some parameters showed significant alterations in electrocardiogram, urinalysis, serum biochemistry and relative organ weights. These alterations were not related to dose or consistent across gender, which were ascribed to incidental and biological variability. The findings in this study indicated that the no-observed adverse effect level (NOAEL) of the ELE was 100 mg/kg in dogs and provided a vital reference for selecting a safe application dosage for human consumption.


## 1. Introduction

Traditional herbal medicines have been used to treat diseases or promote health for centuries in worldwide (Lee et al., 2012). The World Health Organization reported that almost 80% of the population use traditional herbal medicines, such as plant extracts or their active components, as some part of their primary health care needs (Craig, 1999; Gilani and Attaur, 2005). Despite the presumed safety and growing popularity of herbal medicine products, adverse effects could be a major safety concern for some of those products (Ekor, 2014). Toxicity of herbal medicines could be attributed to inherent toxic properties or interaction with co-administered conventional drug substances (Forte and Raman, 2000; Neergheen-Bhujun, 2013). Therefore, continued preclinical safety evaluations of commonly used herbal medicines are urgently needed.

*Aralia elata* Seem., well-known as “Ci Lao Ya or Longya *Aralia chinensis* L.”, is a traditional medicinal plant belonged to the genus of *Aralia* in Family Araliaceae and distributed in Asia (Wang et al., 2014a). The entire plant of *A. elata* has been traditional used as an

*Abbreviations:* ELE, ethanol leaves extract; CFDA, China Food and Drug Administration; ALD, approximate lethal dose; SG, specific gravity; LEU, leukocyte; NIT, nitrite; BLO, urine occult blood; PRO, protein; GLU, glucose; ASC, ascorbic acid; KET, ketone; UBG, urobilinogen; BIL, bilirubin; WBC, white blood cell; RBC, red blood cell; HCT, hematocrit; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; LYM, lymphocytes; PLT, platelet; MPV, mean platelet volume; MON, monocytes; NEUT, neutrophil granulocytes; PCT, plateletocrit; APTT, activated partial thromboplastin time; PT, prothrombin time; ALB, albumin; ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase; γ-GT, gamma glutamyl transpeptidase; GLB, globulin; A/G, albumin-globulin ration; TP, total protein; TBIL, total bilirubin; CREA, creatinine; UA, uric acid; UREA, urea; CK, creatine kinase; TG, triglyceride; TCHO, total cholesterol; LDH, lactate dehydrogenase; GLU, glucose; Na, sodium; K, potassium; Ca, calcium; CL, chloride; NOAEL, no-observed adverse effect level.

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ingredient in traditional Chinese medicine formula to treat some diseases such as rheumatoid arthritis, gastritis, neurasthenia and hepatitis (Saito et al., 1993; Chung et al., 2005; Gao et al., 1983; Zhang et al., 2012). The saponins in *A. elata* have been considered to be the primary known biological active components (Luo et al., 2015; Wang et al., 2014b). Pharmacological studies in animals demonstrated that ethanol extract of *A. elata* leaves (ELE), mainly contained saponins, possessed liver-protecting, anti-myocardial ischemic and anti-cancer activities (Wang et al., 2009; Lu et al., 2011; Li et al., 2013). However, as a potential new drug to treat these diseases, there is no toxicological evaluation of ethanol extract of *A. elata* leaves in non-rodient animals.

The toxicological evaluation of ethanol extract of *A. elata* leaves (ELE) in rodents has been performed in our previous study (Li et al., 2015). In order to further confirm the pharmaceutical safety of the ELE, the sub-chronic toxicity study in Beagle dogs was performed.

## 2. Materials and methods

### 2.1. Plant material

The leaves of *A. elata* were collected in July 2011 from Heilongjiang province of China. The samples were identified by Prof. Wang Zhenyue of Heilongjiang University of Chinese Medicine and were compared with a previous voucher specimen (No.19900063) deposited in Herbarium of Heilongjiang University of Chinese Medicine, Harbin, China.

### 2.2. Preparation of plant extract

The air-dried leaves (10 kg) were extracted thrice, 2 h each time, with 95% ethanol at twenty times amount of the dries leaves. The combined solutions were evaporated to dryness to obtain the crude extract (2.5 kg). The crude extract was subjected to a macroporous resin column, and eluted by water, 30% and 70% ethanol. The portions of water and 30% ethanol were discarded. The portion of 70% ethanol was collected and evaporated to dryness to obtain the final extract (170 g). The saponins content was more than 50% in the final extract.

### 2.3. HPLC analysis

HPLC analysis was carried out to detect the presence of chemical components in previous study (Li et al., 2015). The fingerprints showed that Congmuyenoside G and Congmuyenoside I were the two main ingredients.

### 2.4. Animals and management

Beagle dogs (weighting 5.6–8.5 kg, 6–12 months age) were purchased from Keyu animal breeding center (Beijing, China) and quarantined for 30 days prior to the experiments. The dogs were maintained individually in stainless-steel cages (90 cm × 90 cm × 90 cm) under standardized laboratory conditions (23.3 ± 1.1 °C, 57.0 ± 4.3% humidity, 12 h light/12 h dark cycle, 8–10 air circulating frequency) and allowed free access to standard sterile diets and filtered water *ad libitum*.

All animal experiments were approved by the Heilongjiang University of Chinese Medicine Animal Ethics Research Board and were performed in accordance with the technical guideline of long term toxicity for traditional Chinese medicine and natural drugs (No. [Z]GPT3-1) (CFDA, 2005).

### 2.5. Study design and dose selection

20 healthy female and 20 healthy male dogs were randomly divided into four groups (5/group/sex): ELE 50, 100, 200 mg/kg/day groups and control group (vehicle group). Dogs in treated groups were orally administrated with ELE once per day. After treatment for 12 weeks, 6 dogs (3 males and 3 females) of each group were sacrificed and the remaining dogs were continuously observed for another 4 weeks and sacrificed after recovery period.

In acute toxicity study, approximate lethal dose (ALD) of ELE in dogs was detected to be more than 2000 mg/kg in oral route. In sub-chronic toxicity study, 0.1 ALD (200 mg/kg) was chosen to be the high dose (Wang et al., 2012).

### 2.6. Clinical observation

During experimental period, mortality and morbidity were observed twice daily. The body weight, temperature and food consumption were recorded weekly. Electrocardiographic and ophthalmologic examination were performed prior to study initiation (pretest period), after treatment period (at the end of 12th week) and after recovery period (at the end of 16th week). Urinalysis was conducted after treatment period and recovery period.

Electrocardiographic parameters including heart rate, PR interval, QT interval and QRS complex were recorded by using electrocardiograph (ECG-6591e, Nihon Kohden, Japan).

Urinalysis parameters including specific gravity (SG), leukocyte (LEU), nitrite (NIT), pH, urine occult blood (BLO), protein (PRO), glucose (GLU), ascorbic acid (ASC), ketone (KET), urobilinogen (UBG) and bilirubin (BIL) were measured by using a semi-automatic urine chemistry analyzer (Scan500, Combi, Germany).

The ophthalmological examination areas include eyelid, sclera, cornea, conjunctiva, iris, pupil, lens, anterior chamber, fundus and vitreous.

### 2.7. Hematological and blood serum biochemical analysis

All dogs were fasted but allowed access to water for more than 12 h prior to collect blood sample. The blood samples were obtained from forelimb vena into the tubes containing EDTA-$K_2$ or trisodium citrate. Hematological and blood serum biochemical analysis were conducted prior to study initiation, after treatment period and after recovery period, respectively.

Hematological parameters including red blood cell (RBC), white blood cell (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), lymphocytes (LYM), platelet (PLT), mean platelet volume (MPV), plateletocrit (PCT), monocytes (MON), neutrophil granulocytes (NEUT) were assessed using hematology analyzer (MEK-7222K, Nihon Kohden, Japan). The coagulation parameters including prothrombin time (PT) and activated partial thromboplastin time (APTT) were analyzed using blood coagulation analyzer (STA-4, STAGO, France).

Serum samples were isolated by centrifugation at 1600 × *g* for 10 min and stored at −20 °C. Serum biochemical parameters including alanine transaminase (ALT), gamma glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), aspartate transaminase (AST), globulin (GLB), albumin (ALB), albumin-globulin ratio (A/G), total bilirubin (TBIL), total protein (TP), creatinine (CREA), uric acid (UA), total cholesterol (TCHO), urea (UREA), creatine kinase (CK), lactate dehydrogenase (LDH), triglyceride (TG) and glucose (GLU) were evaluated using biochemistry analyzer (COULTER-AU480, BECKMAN, America). The ion of sodium (Na), potassium (K), calcium (Ca), and chloride (CL) in serum were analyzed using an electrolyte analyzer (AVL9181, Roche, America).