

Original Research Article

Acute and subacute toxicity study of *Aucklandia lappa* Decne seed oil

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Abstract

Purpose: To investigate the acute and subacute toxicity of *Aucklandia lappa* Decne. seed oil (ALDO) in mice and rats.

Methods: A single dose of 10 g ALDO/kg was administered to Kunming mice in an acute oral toxicity experiment. Their weight and feed consumption were recorded for 14 days to observe whether they had symptoms of poisoning and mortality. Sprague-Dawley (SD) rats were administered 0.89, 1.77 and 3.54 g/kg for 28 days, and symptoms of poisoning and mortality were monitored daily. Body weight, feed consumption, hematology, serum biochemical parameters, relative organ weight, and histopathology of the experimental and control groups were compared.

Results: The acute oral toxicity study revealed that there was no significant difference in the macroscopic results, including mortality, feed consumption and weight growth between the group dosed with 10 g ALDO/kg ($p > 0.05$) and the control group. In the subacute toxicity test, SD rats had a higher weight growth rate and feed utilization after doses of 0.89 g ALDO/kg ($p < 0.01$). However, compared with the control group ($p > 0.05$), there was also no significant difference in biochemical and hematological parameters, relative organ weight, or in macroscopic and histological features of both animal types. The electrolyte concentrations of Na and Cl increased at the doses of 1.77 and 3.54 g/kg ($p < 0.01$).

Conclusion: These results suggest that ALDO is relatively safe when administered orally to rats and provide a theoretical basis for the development of new food resources.

Keywords: *Aucklandia lappa* seed oil, Acute toxicity, Rodents, Subacute toxicity, Histological features, Hematological properties

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INTRODUCTION

Aucklandia lappa Decne. (ALD) is a type of herbal plant belonging to the Asteraceae family, which is generally grown in alpine areas [1] and

is originally from India. It is primarily distributed in Sichuan, Yunnan and Hubei in China, at an altitude of 2300 m. It is harvested in autumn and winter, and its roots have medicinal uses. This traditional Chinese medicine not only has anti-

inflammatory [2] and antitumor effects but can also relieve the asthma symptoms, cough, diarrhea, vomiting, and indigestion [3].

There have many studies on the roots and their two principal active components (costunolide and dehydrocostus lactone), while ALD seeds are primarily used for propagation; thus there is little known about the possible toxicity, safety, and medicinal value of ALD seeds or ALDO. This experiment tested the fatty acid composition of ALDO, and the results showed that the unsaturated fatty acids included linoleic acid (72.9 %), oleic acid (15.8 %), and linolenic acid (0.204 %), comprising 89.10% of the total, and saturated fatty acids included palmitic acid (8.02 %), stearic acid (2.22 %), and peanut acid (0.281 %), with a total proportion of 10.89 %. In this study, mature ALD seeds were mechanically pressed to obtain ALDO. Toxicological assessment of ALDO was conducted through acute and subacute toxicity in Kunming mice and SD rats to test its food safety and provide a basis for further research.

EXPERIMENTAL

Aucklandia lappa Decne. seeds

Aucklandia lappa Decne. seeds were purchased from Muxiang Planting Base, Guanmian Town, Chongqing. The ripe and full seeds were screened and kept in a drying oven at 60 °C for 48 h until there was no weight change. They were subsequently placed in a sealed bag for standby use.

Extraction

The seeds were pressed using a small household oil press (WOP-KP0202) to obtain the initial oil, which was centrifuged at 4000 rpm for 10 min twice. The apparent density of the oil was calculated (0.89 g/mL) and used to define the exact volume that each animal would receive. The oil was stored at 4 °C until further use.

Animals

Healthy Kunming mice and SD rats, weighing 18 – 22 g and 80 – 100 g, respectively, were supplied by Tengxin Biotechnology Co. Ltd (Chongqing, China). They were maintained under standard environmental conditions (23 ± 2 °C; 12:12 h dark/light cycle), water and chow (Xietong Biology, Jiangsu) were available *ad libitum*. All experimental procedures received ethical approval from the institutional ethical committee and were performed according to the guidelines of the Experimental Laboratory Animal

Committee of Chongqing Academy of Traditional Chinese Medicine and were in strict accordance with the principles and guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23).

Acute toxicity study

Kunming mice were allowed to acclimatize to laboratory conditions for one week before starting the experiment. Rats were fasted overnight, but allowed access to water *ad libitum*. Twenty Kunming mice (n = 10/gender) were orally administered ALDO at doses of 10 g/kg. Animals were observed for general behavior, body weight changes, hazardous symptoms, and mortality in the first six hours after the treatment, and were observed for 14 days. Then, the mice were anesthetized with ether for dissection of the heart, liver, kidney, lungs, spleen, testicles, stomach and intestines.

Subacute toxicity study

Eighty males and females SD rats were randomly divided into four groups (n = 20/group) after acclimatization to laboratory conditions for a week. Control group SD rats orally received 4 ml/kg corn oil daily, and experimental group SD rats received ALDO at doses of 0.89, 1.77, or 3.54 g/kg/day for 28 consecutive days. The SD rats were allowed access to water *ad libitum*, their feed intake was monitored daily, and weight growth were recorded every two days. We observed abnormalities during the treatment period. After the treatment, SD rats were fasted overnight, but allowed access to water *ad libitum*.

All SD rats were anesthetized by intraperitoneal injection of pentobarbitone sodium (45 mg/kg), and blood was collected from the abdominal aorta. We used capillary tubes for hematological and biochemical studies, with and without anticoagulants, respectively. After euthanasia by the excess of anesthetic, dissection was performed (n = 10/group), and the heart, liver, spleen, lungs, kidneys, thymus, adrenal glands, testes, and ovaries were removed. We observed if there were any visible lesions on these organs and weighed them individually to obtain absolute and relative weights (g/100 g of body weight). The organs were subsequently placed in 10% neutral buffered formalin for fixation. After fixation, we prepared 4-µm-thick histological sections, and hematoxylin-eosin stained tissue specimens were placed on cover slips [4].

Microscopic examination was performed for all organs including the heart, liver, spleen, lungs,

kidneys, thymus, adrenal glands, testes, and ovaries from the SD rats.

Biochemical, electrolytic and hematological studies

For biochemical analysis, blood without anticoagulants was centrifuged at 3000 rpm for 10 min to obtain serum, total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glutamyl transpeptidase (GGT), total cholesterol (CHOL), triglycerides (TG), glucose (GLU), creatinine (CREA), and urea nitrogen (Urea) were measured using an automatic biochemical analyzer (AU480, Beckman Coulter). The concentrations of sodium (Na), potassium (K) and chlorine (Cl) were measured with an electrolyte analyzer (XD-685, Schindler, Shanghai).

Hematological analysis was performed using an automatic hematological analyzer (XT-2000i, Hizen Meikang, Japan). The parameters were as follows: red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (HGB), hematocrit (HCT), platelet (PLT) count, mean platelet volume (MPV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were determined [5]. Differential leukocyte counting was performed using optical microscopy after staining and counting 100 cells in each case.

Statistical analysis

The results are expressed as mean \pm SEM. Statistical significance of the differences between the test and control groups was analyzed by one-way analysis of variance (ANOVA) using SPSS v 22.0. Statistical significance was set at $p < 0.05$.

RESULTS

Acute toxicity

The results showed that during the 14-day observation period, there were no any symptoms of poisoning or mortality in Kunming mice at a dose of 10 g/kg ALDO. Feed consumption and weight gain were observed in the next 14 days after ALDO administration, and showed no significant difference compared with the control group. After 14 days, the mice from each group were dissected, a comparison was made, and there were no abnormalities in morphology, behavior, skin, breathing, food intake, body

weight, water consumption, as well as postural abnormalities or hair loss.

Subacute toxicity and treatment outcomes

Because of improper operation, 4 rats died during treatment but showed no symptoms before death. There were no notable changes in behavior, activity, posture, gait, or external appearance in other rats over the duration of the study.

Feed utilization and weight growth rate

The weekly feed utilization (Table 1) and weight growth rate (Table 2) were comparable between rats in the different exposure groups during the feeding study.

Excluding the females in the second week, statistically significant differences in feed consumption were observed between the low-dose group (0.89 g/kg) and control groups of both genders, and there were also significant differences between females in the medium-dose and high-dose groups (1.77 and 3.54 g/kg) during the first week ($p < 0.01$) (Table 1).

Weight growth in the low-dose group was significantly higher than that in the control group. In the medium-dose group, the weight growth rate was significantly different in females during the first week ($p < 0.01$) and in males during the first ($p < 0.01$), second ($p < 0.05$), and fourth ($p < 0.01$) weeks when compared with that in the control group. There was no significant difference in weight growth rates between the control group and other groups, as summarized in Table 2.

Serum biochemistry

Statistically significant differences were found in the toxicological response variables, as shown in Table 3. The mean values of ALP, GGT and K in the males treated with low-dose ALDO were significantly higher than those in the control group ($p < 0.05$); this change was not found in the medium-dose and high-dose groups. The mean values of ALT ($p < 0.01$), CHOL ($p < 0.05$) and K ($p < 0.01$) in the males and the mean value of GGT ($p < 0.05$) in the females treated with medium-dose ALDO were significantly higher than those in the control group. Additionally, the mean value of CHOL ($p < 0.05$) in the males and the mean value of GGT ($p < 0.05$) in the females treated with medium-dose ALDO were significantly lower than those in the control group; this change was not found in the low-dose and high-dose groups.

Table 1: Mean feed utilization of repeated dosing of ALDO-treated rats for 4 weeks

Week	Control	ALDO group		
		Low-dose (0.89 g/kg)	Medium-dose (1.77 g/kg)	High-dose (3.54 g/kg)
<i>Female</i>				
1	0.41±0.01	0.57±0.01**	0.58±0.02**	0.35±0.02**
2	0.40±0.01	0.44±0.01	0.40±0.02	0.39±0.02
3	0.25±0.01	0.31±0.01**	0.25±0.01	0.26±0.01
4	0.24±0.01	0.27±0.01*	0.26±0.01	0.25±0.01
<i>Male</i>				
1	0.51±0.01	0.55±0.01*	0.49±0.01	0.48±0.01
2	0.46±0.01	0.55±0.01**	0.48±0.01	0.47±0.01
3	0.33±0.01	0.37±0.01**	0.33±0.01	0.34±0.01
4	0.33±0.01	0.36±0.01**	0.28±0.01	0.30±0.01

* $P < 0.05$; ** $p < 0.01$ significantly different when compared to the control group

Table 2: Weight growth following repeated dosing of ALDO-treated rats (%) over a 4-week period

Week	Control	ALDO group		
		Low-dose (0.89 g/kg)	Medium-dose (1.77 g/kg)	High-dose (3.54 g/kg)
<i>Female</i>				
1	36.14±1.55	64.18±1.41**	72.15±2.72**	31.25±1.74
2	27.40±1.02	36.28±0.96**	28.46±1.15	26.31±1.53
3	14.33±0.72	18.67±0.89**	14.58±0.43	14.24±0.81
4	11.48±0.65	13.82±0.46*	12.63±0.83	12.74±0.71
<i>Male</i>				
1	49.64±0.98	66.22±1.77**	56.80±1.72**	48.15±1.12
2	37.81±0.78	57.53±1.28**	41.21±0.84*	37.52±0.39
3	21.85±0.49	28.94±0.82**	21.94±0.84	21.55±0.75
4	18.62±0.59	21.49±0.90**	15.78±0.84**	16.34±0.71*

* $P < 0.05$; ** $p < 0.01$ significantly different when compared to the control group

The mean value of Urea in the males and the mean value of CREA in the females treated with high-dose ALDO were significantly higher than those in the control group ($p < 0.05$), this change was not found in the low-dose and medium-dose groups. However these differences were only found in one gender and, thus, were not considered to be related to ALDO. Statistically significant differences in the mean AST values were observed in the medium-dose and high-dose groups in both genders in comparison to the control group.

The values of TP ($p < 0.05$, $p < 0.01$), TG ($p < 0.01$) and GLU ($p < 0.01$) in males treated with medium-dose and high-dose ALDO were significantly lower than those in the control group, while the electrolyte concentrations of Na ($p < 0.01$) and Cl ($p < 0.01$) in both genders treated with medium-dose and high-dose ALDO were significantly higher than those in the control group.

Hematological parameters

The hematological parameters of the control and experimental groups are shown in Table 4. The

mean values of MCV and MCH in the females experimental group with low-dose ALDO were significantly higher than those in the control group ($p < 0.05$), while the value of MCHC was much lower than that in the control group ($p < 0.05$); however, these changes were not found in the medium-dose and high-dose groups. The mean value of LYMPH% in the males experimental group with medium-dose ALDO were significantly lower than those in the control group ($p < 0.05$), and this change was not found in the low-dose and high-dose groups. The mean value of MONO% in the females experimental group receiving high doses ALDO was significantly lower than those in the control group ($p < 0.01$), and this change was not found in the low-dose and medium-dose groups. These differences were only found in one gender, meaning that the existence of ALDO was irrelevant.

The values of EO% in the females experimental group receiving low-dose ($p < 0.05$) and medium-dose ($p < 0.01$) ALDO were significantly lower than those in the control group.

Table 3: Serum biochemistry of repeated dosing of ALDO-treated rats for 4 weeks

Parameter	Control	ALDO group		
		Low-dose (0.89 g/kg)	Medium-dose (1.77 g/kg)	High-dose (3.54 g/kg)
Female				
TP (g/L)	56.30±0.68	53.89±0.39**	56.20±0.39	55.11±0.48
ALB (g/L)	35.90±0.59	35.44±0.24	36.00±0.30	36.56±0.24
AST (U/L)	109.56±2.49	120.78±3.79	132.25±3.94**	142.86±5.64**
ALT (U/L)	38.22±2.43	36.56±1.89	38.10±0.98	36.44±1.14
ALP (U/L)	129.57±8.29	122.43±5.30	124.86±5.83	123.25±8.09
GGT (U/L)	1.67±0.09	1.40±0.16	1.32±0.08*	1.47±0.15
CHOL (mmol/L)	1.61±0.07	1.76±0.07	1.63±0.12	1.55±0.09
TG (mmol/L)	0.45±0.02	0.49±0.05	0.39±0.04	0.35±0.02
Urea (mmol/L)	4.75±0.10	4.61±0.22	4.80±0.16	4.81±0.12
CREA (µmol/L)	41.10±0.60	39.67±0.71	42.00±0.73	42.89±0.31*
GLU (mmol/L)	6.71±0.20	5.91±0.23*	6.12±0.16	6.06±0.18*
K (mmol/L)	4.22±0.07	4.12±0.05	4.19±0.06	4.11±0.11
Na (mmol/L)	142.50±0.12	142.78±0.51	145.17±0.21**	144.21±0.34**
Cl (mmol/L)	104.44±0.23	105.11±0.40	107.22±0.16**	106.73±0.26**
Male				
TP (g/L)	58.30±0.37	58.00±0.56	56.70±0.63*	56.00±0.65**
ALB (g/L)	36.10±0.28	36.60±0.34	35.70±0.42	35.75±0.37
AST (U/L)	96.00±4.21	115.44±3.47**	135.80±5.65**	136.50±5.87**
ALT (U/L)	40.00±1.98	42.90±0.96	46.80±2.56**	43.50±1.93
ALP (U/L)	204.50±10.93	233.86±8.28*	204.78±11.65	201.00±8.14
GGT (U/L)	1.62±0.11	1.79±0.07*	1.30±0.11	1.32±0.14
CHOL (mmol/L)	1.55±0.06	1.44±0.06	1.28±0.051*	1.37±0.06
TG (mmol/L)	0.83±0.06	0.72±0.05	0.65±0.05**	0.61±0.07**
Urea (mmol/L)	4.06±0.11	4.40±0.13	4.35±0.10	4.54±0.15*
CREA (µmol/L)	38.50±0.48	37.70±0.05	38.00±0.33	39.13±0.55
GLU (mmol/L)	7.72±0.91	7.00±0.22*	5.91±0.21**	6.11±0.20**
K (mmol/L)	4.30±0.05	4.56±0.07*	4.60±0.09**	4.40±0.13
Na (mmol/L)	142.99±0.28	142.60±0.15	145.33±0.13**	146.15±0.23**
Cl (mmol/L)	103.71±0.16	103.29±0.24	105.16±0.19**	105.45±0.86**

* $P < 0.05$; ** $p < 0.01$ significantly different when compared to the control group

The mean values of WBC, RBC and HGB in the females experimental group with low-dose and high-dose ALDO were significantly lower than those in the control group, and the mean values of WBC, RBC, HGB and HCT in the males experimental group with medium-dose and high-dose ALDO were much lower than those in the control group.

Relative organ weight

The relative organ weights of animals treated with different doses ALDO are shown in Table 5. The relative weights of the spleen ($p < 0.05$) of the females and the relative weights of the thymus ($p < 0.01$) of the males treated with the low-dose ALDO were significantly higher than those in the control group; this change was not found in the medium-dose and high-dose groups. Additionally, the relative weights of the adrenal glands ($p < 0.01$) of the females treated with the medium-dose ALDO was significantly lower than that of the control group, and the relative weights of the hearts ($p < 0.05$) of the males treated with the medium-dose ALDO were significantly higher

than that of the control group, and this change was not found in the low-dose and high-dose groups; the relative weights of the hearts ($p < 0.05$) of the females treated with high-dose ALDO were significantly higher than that in the low-dose and medium-dose groups. However these differences were only found in one gender and, thus, were not considered to be related to ALDO. The relative weights of the lungs of the females treated with medium-dose ($p < 0.01$) and high-dose ($p < 0.05$) ALDO were significantly lower than those of the control group; the relative weights of the kidneys ($p < 0.05$) and testes ($p < 0.05$, $p < 0.01$) of the males treated with the low-dose and medium-dose ALDO were significantly higher than those of the control group.

The relative weights of the lungs of the females treated with medium-dose ($p < 0.01$) and high-dose ($p < 0.05$) ALDO were significantly lower than those of the control group; the relative weights of the kidneys ($p < 0.05$) and testes ($p < 0.05$, $p < 0.01$) of the males treated with the low-dose and medium-dose ALDO were significantly higher than those of the control group.

Table 4: Hematology analysis of repeated dosing of ALDO-treated rats for 4 weeks

Parameter	Control	ALDO group		
		Low-dose (0.89 g/kg)	Medium-dose (1.77 g/kg)	High-dose (3.54 g/kg)
Female				
WBC($10^9/L$)	6.11±0.38	4.54±0.22**	4.85±0.38*	4.18±0.26**
RBC($10^{12}/L$)	6.62±0.17	6.15±0.13**	6.39±0.07	6.08±0.10**
HGB(g/L)	127.30±2.35	121.11±2.19*	124.20±1.46	119.22±1.63**
HCT(%)	36.83±0.68	35.47±0.59	35.98±0.38	34.66±0.48**
PLT($10^9/L$)	1016.25±31.23	1009.00±26.62	1096.25±19.36	1082.43±28.52
MPV(%)	6.85±0.064	6.72±0.08	6.86±0.07	6.87±0.06
MCV(fl)	55.70±0.58	57.74±0.41*	56.31±0.47	57.00±0.56
MCH(pg)	19.25±0.16	19.71±0.11*	19.43±0.15	19.60±0.13
MCHC(g/L)	345.80±1.73	341.44±1.39*	345.10±1.39	344.11±2.12
NEUT(%)	8.81±0.64	6.93±0.71	9.57±0.49	8.56±0.73
LYMPH(%)	89.38±0.77	90.61±1.02	89.17±0.60	90.03±0.73
MONO(%)	1.14±0.14	1.14±0.13	1.02±0.10	0.66±0.08**
EO(%)	0.68±0.05	0.50±0.06*	0.41±0.06**	0.67±0.11
BASO(%)	0±0.00	0±0.00	0±0.00	0±0.00
Male				
WBC($10^9/L$)	6.55±0.38	5.35±0.47*	5.20±0.24**	4.63±0.28**
RBC($10^{12}/L$)	7.20±0.14	6.97±0.04	6.84±0.12*	6.77±0.15*
HGB(g/L)	138.60±1.95	135.60±0.79	133.56±1.62*	129.50±1.79**
HCT(%)	40.51±0.61	39.56±0.25	38.59±0.35**	37.83±0.50**
PLT($10^9/L$)	1019.90±25.12	1078.00±36.34	996.11±34.95	1059.83±36.61
MPV(%)	6.81±0.06	6.82±0.06	6.93±0.04	6.98±0.09
MCV(fl)	56.35±0.39	56.80±0.44	56.47±0.66	55.96±0.83
MCH(pg)	19.29±0.15	19.47±0.14	19.53±0.19	19.13±0.25
MCHC(g/L)	342.40±1.26	342.80±0.92	346.33±1.27	342.38±1.27
NEUT(%)	10.81±0.88	12.24±0.60	11.40±0.39	12.29±1.19
LYMPH(%)	87.56±0.89	86.01±0.61	84.20±1.35*	85.35±1.33
MONO(%)	1.07±0.13	1.19±0.08	1.23±0.11	1.05±0.11
EO(%)	0.37±0.05	0.43±0.06	0.40±0.04	0.53±0.07
BASO(%)	0±0.00	0±0.00	0±0.00	0±0.00

* $P < 0.05$; ** $p < 0.01$ significantly different when compared to the control group

Table 5: Relative organ weight (g/100 g) after repeated dosing of ALDO-treated rats for 4 weeks

Organ	Control	ALDO group		
		Low-dose (0.89 g/kg)	Medium-dose (1.77 g/kg)	High-dose (3.54 g/kg)
Female				
Heart	0.34±0.01	0.35±0.01	0.36±0.01	0.37±0.01*
Liver	3.12±0.06	3.14±0.03	3.03±0.04	2.99±0.08
Spleen	0.25±0.07	0.29±0.01*	0.28±0.01	0.27±0.01
Lungs	0.52±0.01	0.49±0.01	0.48±0.01**	0.48±0.01*
Kidneys	0.77±0.02	0.82±0.03*	0.77±0.01	0.77±0.02
thymus	0.27±0.01	0.30±0.01	0.29±0.01	0.26±0.01
Adrenal glands	0.03±0.00	0.03±0.00	0.02±0.00**	0.03±0.00
Ovaries	0.05±0.00	0.06±0.00	0.05±0.00	0.06±0.00
Male				
Heart	0.32±0.01	0.33±0.01	0.35±0.01*	0.33±0.01
Liver	3.34±0.05	3.41±0.04	3.46±0.16	3.31±0.03
Spleen	0.23±0.01	0.24±0.01	0.25±0.01	0.23±0.01
Lungs	0.44±0.01	0.46±0.01	0.45±0.02	0.40±0.01
Kidneys	0.74±0.01	0.79±0.01*	0.79±0.01*	0.76±0.01
thymus	0.19±0.03	0.24±0.01**	0.20±0.01	0.20±0.01
Adrenal glands	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
Testes	0.82±0.02	0.87±0.03*	0.88±0.02**	0.83±0.02

* $P < 0.05$; ** $p < 0.01$ significantly different when compared to the control group

Histopathological features

After dissection and analysis, there were no obvious abnormalities between the organs of the ALDO-treated and those of control groups, including in the shape, size, color, and texture. Histological assessment showed no signs of toxic effects (Figure 1).

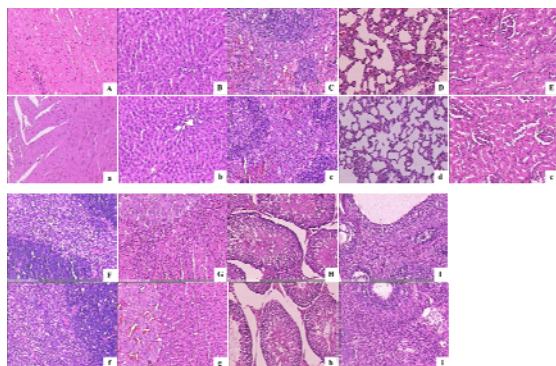


Figure 1: Histopathological assessment of the organs in the control and high-dose ALDO group in the subacute toxicity test. Heart: A (Control, HE 100 \times); a (3.54 g/kg ALDO, HE 100 \times). Liver: B (Control, HE 100 \times); b (3.54 g/kg ALDO, HE 100 \times). Spleen: C (Control, HE 100 \times); c (3.54 g/kg ALDO, HE 100 \times). Lung: D (Control, HE 100 \times); d (3.54 g/kg ALDO, HE 100 \times). Kidney: E (Control, HE 100 \times); e (3.54 g/kg ALDO, HE 100 \times). Thymus: F (Control, HE 100 \times); f (3.54 g/kg ALDO, HE 100 \times). Adrenal gland: G (Control, HE 100 \times); g (3.54 g/kg ALDO, HE 100 \times). Testis: H (Control, HE 100 \times); h (3.54 g/kg ALDO, HE 100 \times). Ovary: I (Control, HE 100 \times); i (3.54 g/kg ALDO, HE 100 \times).

DISCUSSION

The results of the acute toxicity study indicate that oral administration of ALDO by oral doses up to 10 g/kg did not produce any signs of toxicity or death in mice, including that the lowest oral lethal dose of ALDO exceeds 10 g/kg. Therefore, ALDO is not toxic according to the criteria for acute toxic classifications.

In the subacute toxicity test, general behavior and mortality are the primary parameters considered in the evaluation of toxicity [6]. In this study, there were no observable signs of toxicity or mortality during 4 weeks of treatment at all doses used, which is an indication that the extract was well tolerated by the experimental subjects. However, in terms of weight and feed consumption, the feed utilization and weight growth rate in the low-dose group were significantly higher than those in the control group, while there was almost no significant difference in the high-dose group, indicating that

the low-dose ALDO may increase food utilization and increase the weight of rats.

Biochemical measurements are important for investigating toxic effects in tissues [7]. The chemical components in serum primarily originate from the products of tissue cell metabolism and digestive tract decomposition. The components contained in the serum and intratissue fluid are similar, representing the physical and chemical properties of the internal environment of the body and reflecting the state of the body better than whole blood [8]. The increase in serum transaminase enzymes (ALT and AST) is an indicator of hepatocyte damage [9]. Under normal circumstances, these enzymes exist in liver cells, the damaged cells will influence cell permeability and enzymes will penetrate into the blood, which will cause an increase in the concentration of serum enzymes. Other studies have shown that AST and ALT activity increases by 1-fold in the blood which means that 1% of the liver cells are damaged [10].

This study also showed that the concentration of AST was related to dose in both genders, but these values were always within the normal range [11]. Total protein and albumin are indices that reflect metabolism, and triglyceride can reflect lipid metabolism; when the content of triglycerides increases, protein metabolism is enhanced. In this study, the content of TG and TP (in medium-dose and high-dose groups, respectively) in the males and TP (low-dose group) in the females were significantly different, but these values were within the normal range, thus, there was no biological significance [12, 13]. GLU concentrations in each dose group were within the normal physiological range [13].

Electrolytes primarily function to maintain the body fluid osmotic pressure and acid-base balance, maintain the resting potential of red blood cells, participate in the formation of action potential, and metabolism and physiological function. Electrolyte metabolism disorders can cause the whole body organ system and nervous system to abnormally function, and body material metabolism disorders can lead to death [14]. In this study, high-dose ALDO had a greater impact on the electrolyte concentration of Na and Cl, and both electrolytes in males exhibited a dose-response relationship. However, it could be argued that these changes may not be toxicologically significant, as they were not corroborated by the biochemical findings, hematological parameters, or histopathological examination.

Hematological parameters can reflect toxic effects because they are one of the most sensitive targets of toxic chemicals and are important indices for determining the physiological and pathological status in humans and animals [15]. In this study, MONO% in the high-dose female group was significantly lower than the control group, more than the normal range of historical data, but the difference exists in the high-dose female group, so there is no biological significance, which may be associated with experiments design or implementation and without the relationship of ALDO. Indicators other than the control group were all within the normal physiological range [13]. Therefore, ALDO had no significant effect on the hematological parameters in rats.

The relative organ weights reflect the influence of the tested object on the organ, and it is an important index in the toxicity experiments. The increase in the relative organ weights indicates abnormal changes, such as hypertrophy and hyperemia in the organ. The decrease in the relative organ weights indicates abnormal phenomena such as atrophy and degeneration of the organ, but weight change do not indicate the presence of lesions on an organ. This must be determined by through additional histopathological examination [16]. In this study, all organ differences compared to the control group have no biological significance, indicating that the ALDO has no significant effect on the organs of rats.

Histopathological examination of ALDO in rats showed that the liver, heart, spleen, lungs, adrenal glands, thymus, testes, and ovary had no obvious toxic effects.

CONCLUSION

This is the first study to present the acute and subacute toxicological profiles of *Aucklandia lappa* Decne. seed oil. These results suggest that the *Aucklandia lappa* Decne. seed oil is relatively safe when administered orally to rats.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Su contributed to the study design, manuscript preparation, data analysis and management, interpretation of results, and manuscript preparation. Du contributed to the study design, interpretation of results, drafting and preparation of the manuscript. Liang and Ran, Zeng and Li performed experimental tasks. Yang and Zhang contributed to the study design and interpretation of the results. All the authors have read and approved the final draft of the manuscript.

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