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Original Article

A subchronic toxicity study of ethanol root extract of baked *Aconitum flavum* in rats

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ABSTRACT

The genus *Aconitum* has strong toxicity, but the acute toxicity of baked *Aconitum flavum* Hand.-Mazz., Ranunculaceae, was reduced significantly on the premise of keeping anti-inflammatory and anti-nociceptive activities. However, the risk associated with long-term use is unknown. In a sub-chronic toxicity study, rats were orally administered *A. flavum* at doses of 0.76–3.03 g/kg for 90 days and further recovered for 14 days. Our results showed that oral treatment with *A. flavum* for 90 days caused significant changes in some hematological indicators at doses of 3.03 and 1.52 g/kg, such as red blood cell, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. These results indicated that the *A. flavum* affects the structure and function of red blood cell. Furthermore, significant changes were observed in the white blood cell at dose of 3.03 g/kg in male rats, which confirmed tissue damage or toxicity. The liver function tests exhibited non-significant alterations in aspertate aminotransferase, alanine aminotransferase and avenin-like storage proteinsgene. But other parameters, such as total protein and albumin were obviously decreased at all doses. *A. flavum* also caused a significant decrease in glucose, cholesterol and triacylglyceride at all doses. For kidney function, there were significant elevations in urea and creatinine at doses of 3.03 and 1.52 g/kg. The levels of certain electrolytes (Na^+, K^+ and Cl^-) were significantly different after 90 days of treatment with *A. flavum* (3.03 and 1.52 g/kg). Organs were observed by light microscopy after hematoxylin-eosin staining. Hemosiderin depositions in the spleen were observed in the *A. flavum* group. These data demonstrated that the subtoxicity of *A. flavum* was reduced considerably by baked, but the subchronic toxicity effects on the liver, kidney and spleen should not be ignored.

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Introduction

The genus *Aconitum* consists of over 300 species distributed in Asia, Europe and North America. Most of them grow in high altitudes in the northern hemisphere. There are 200 species in China, such as *Aconitum kusnezoffii* Reichb., *A. carmichaelithe* Debx. and *A. flavum* Hand.-Mazz. (Xiao, 2006). In traditional Chinese medicine, the genus *Aconitum* has similar pharmacological actions and has been commonly used for the treatment of various diseases, such as rheumatic fever, painful joints, gastroenteritis, edema, bronchial asthma and various tumors (Singhuber et al., 2009; Qin et al., 2012). However, with increasing popularity, *Aconitum* poisoning can occur in any part of the world because of the highly toxic and narrow margin of safety between therapeutic and toxic doses of these herbs. Many studies have demonstrated that the high toxicity of *Aconitum* herbs is primarily attributed to diester diterpene alkaloids (DDA) (Chan, 2009, 2012; Singhuber et al., 2009). Aconitine, mesaconitine, hypaconitine and other DDA are potent cardiotoxins and neurotoxins found in all parts of the *Aconitum* species, especially in the tubers and roots. Patients with *Aconitum* poisoning often present with a combination of cardiovascular, neurological, gastrointestinal and other signs and symptoms (Chan, 2012; Qin et al., 2012). Although patients face a huge risk, the enthusiasm for the use of *Aconitum* has not diminished. Thus, it has become a popular research topic to determine how to reduce the toxicity and retain the therapeutic effect of the genus *Aconitum*.

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"Paozhi", a traditional Chinese medicine (TCM) herbal processing methods might exert much influence on the conversion or transformation of plant contents and especially secondary plant metabolites to change the toxicity or effect of herbs. To meet the safety of clinical use, the genus *Aconitum* are applied after careful processing (usually soaking, steaming, boiling, baking and processing with Chebulae Fructus decoction) in TCM (Wu et al., 1990; Kim et al., 2012), which could greatly reduce the toxicity of the drug by decomposing the DDA to the less toxic monoester diterpene alkaloids (MDA). The content of DDA becomes relatively lower after the application of these traditional processing techniques, which means that the toxicity of the drug drops remarkably (Singhuber et al., 2009).

Aconitum flavum Hand.-Mazz., Ranunculaceae, belongs to the genus *Aconitum* and is widely used in western China (Shanxi, Ningxia, Gansu, Inner Mongolia, Qinghai and Tibet). It has been used for the treatment of rheumatism, traumatic injury and toothache in folk and clinical medicine (Wang et al., 2008). Water decoction and powders of *A. flavum* were used in traditional medicine for internal and external use.

This herb is allowed to be sold on the herbal commercial market by the government, although it has notable toxicity in oral application. Like other herbs of the genus *Aconitum*, the main chemicals of *A. flavum* are diester diterpene alkaloids. Thus, *A. flavum* has strong toxicity. Our laboratory used a variety of traditional and modern processing methods to process *A. flavum* and studied the oral acute toxicity, anti-inflammatory and anti-nociceptive activity of differently processed products. The research showed that the ethanol extract of baked *A. flavum* reduced its acute toxicity (LD_{50} 22 g/kg) on the premise of maintaining anti-inflammatory and anti-nociceptive activities compared to the toxicity of *A. flavum* (LD_{50} 1.02 g/kg) in mice (Fu et al., 2013). This study laid a solid foundation for the safety of *A. flavum* applications, but the evidence of risk from long-term use is unknown. In order to ensure the clinical safety of baked *A. flavum* (BA), a study of its long-term toxicity is necessary.

Materials and methods

Preparation of ethanol root extract of baked Aconitum flavum

The roots of *Aconitum flavum* Hand.-Mazz., Ranunculaceae, were purchased from an Herbal Medicinal Materials Company of the Mingde Pharmaceutical Co., Ltd. (Ningxia, China). The plant grows in the Liupan mountains, Longde, Guyuan, and Ningxia, in China. The roots were harvested from August to September. The plant samples were authenticated by Lin Dong in the Pharmacognosy Department, College of Pharmacy, Ningxia Medical University, and a voucher specimen was deposited in the same unit (Herbarium number: 20130924). The herb was soaked in water for 6 h, then sliced into smaller pieces and further baked at 105 °C for 3 h in a vacuum oven.

Baked *A. flavum* (named BA) (3 kg) was extracted three times with 70% ethanol (mass ratio of solid to liquid was 1:10). The extract was then combined and evaporated to dryness under reduced pressure, then dried in a vacuum oven at 60 °C, which yielded 616.32 g of residue.

Experimental animals

Sprague-Dawley rats (100–130 g) were obtained from the Experimental Animal Center of Ningxia Medical University (Ningxia, China). The rats were maintained in standardlaboratory cages, in moderate humidity (50% ± 5%), at constant temperature (22 ± 1 °C) in a 12-h light-dark cycle room. All animals had free access to food and water during the experimental period. The experimental protocol was approved by the Ethics Committee of Ningxia Medical University (Ningxia, China, Ethics approval: 2013-146).

Subchronic oral toxicity study

A subchronic toxicity test was performed following the reported technique (Zhang and Du, 2012). Rats of both sexes were randomly divided into four groups: control groups and three treatment groups (n = 40; 20 males and 20 females). The LD_{50} of rats for a period of 14 days was 18.2 g/kg (this value was calculated by the Bliss method), meaning that the doses of 3.03, 1.52 and 0.76 g/kg were selected in this subchronic toxicity study. The BA extract was suspended in water with 0.5% (w/v) sodium carboxyl methyl cellulose (Na-CMC) and administered orally on a daily basis for 90 days, while the control group received only 0.5% sodium carboxyl methyl cellulose (Na-CMC). Twenty rats (ten males and ten females) of each group were sacrificed at the end of the 90 days. The remaining rats in each group were continuously observed in the next 14 days and sacrificed at the end. The extract was freshly prepared with vehicle on a daily basis.

The rats were weighed and visually observed for mortality, behavioral patterns (salivation, coat, lethargy, and sleep), changes in physical appearance, injury, pain and signs of illness were conducted once daily during that period by one researcher. At the end of the experiment, blood samples were collected from all animals via abdominal aorta puncture for biochemical and hematological analyses. The organs were excised, weighed, and examined macroscopically. The relative organ weight was calculated. Principal vital organs (liver, kidney, lung, heart and spleen) were preserved in a fixation medium of 10% buffered formalin for histopathological study.

Clinical biochemistry analysis

The blood samples were centrifuged for 15 min to collect the serum. Serum was analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), total protein (TP), triacylglycerol (TG), glucose (GLU), creatinine (CREA), urea, cholesterol (CHO), sodium (Na^+), potassium (K^+), chloride (Cl^-).

Hematology analysis

The blood samples that were collected complete blood count using the automatic hematology system to evaluate red blood cell (RBC), white blood cell (WBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT), granulocyte (Gran), lymph, monocyte (MONO).

Histopathological study

After sacrificing the rats, parts of the liver, kidney, lung, heart, and spleen tissues were collected for histological studies. The tissues were washed in normal saline and fixed immediately in 10% formalin for a period of at least 24 h, dehydrated with alcohol, embedded in paraffin, cut into 4–5 μm thick sections, and stained with hematoxylin-eosin dye for photomicroscopic observation. The microscopic features of the organs of male and female rats were compared with the control group.

Statistical analysis

The means and standard deviations were calculated for measurement data in each group, which included body weight, clinical pathological data, and relative organ weights. The homogeneity of variance was performed; if the variances were homogeneous, the single factor analysis of variance was performed for inter-group comparison. When the ANOVA showed significant differences, a Dunnett t test was performed. When the variances were not homogeneous, the Dunnett's T3 test was performed for inter-group comparisons. Statistical significance was considered at $p < 0.05$.

Results

Clinical signs, necropsy findings and body weight

The animals from all treatment groups did not appear to have any obvious symptoms of toxicity in rats of both sexes. No deaths or serious clinical signs were found in all groups throughout the experimental period. Physical observation of the treated rats indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, diarrhea, tremors, salivation, coma and behavioral changes. No abnormal gross findings were observed in the necropsies of any of the rats. Compared with the control group, all treatment groups had nonsignificant lower weight gain from all weeks (data not show).

Relative organ weights

The relative organ weights of rats during the treatment period and recovery period are shown in Tables 1 and 2. The relative organ weight of each organ recorded at necropsy in the treatment groups did not show a significant difference compared to the control.

Hematology analysis

The effect of BA extract on hematological parameters for 90 days (treatment period) is shown in Table 3. Compared to the control group, MCV and MCH significantly increased in both genders at dose of 3.03 g/kg, while MCHC value significantly increased at doses of 1.52 and 3.03 g/kg. There were significant increases in HGB and RBC indices in male animals at 1.52 and 3.03 g/kg of BA groups. All other measured hematological parameters were not significantly different from the control except for WBC in respect to a significant increase in male rats at dose of 3.03 g/kg compared to the control value.

At the end of the recovery period, significant increases were observed in the levels of MCHC and WBC (at a dose of 3.03 g/kg) in male animals compared to control values. MCV only significantly increased at a dose of 3.03 g/kg in female rats. Additionally, no significant alterations in other indicators were observed (Table 4).

Table 1
Relative organ weights of rats after 90 days of treatment with BA.

Organs	Control	BA extracts (g/kg)		
		3.03	1.52	0.76
Male				
Heart	0.30 ± 0.04	0.30 ± 0.05	0.28 ± 0.04	0.28 ± 0.02
Liver	2.79 ± 0.41	2.59 ± 0.30	2.58 ± 0.34	2.50 ± 0.10
Spleen	0.18 ± 0.02	0.18 ± 0.03	0.16 ± 0.03	0.17 ± 0.02
Lungs	0.36 ± 0.05	0.34 ± 0.03	0.36 ± 0.04	0.32 ± 0.04
Kidneys	0.65 ± 0.06	0.62 ± 0.04	0.63 ± 0.06	0.63 ± 0.05
Brain	0.56 ± 0.05	0.54 ± 0.05	0.51 ± 0.06	0.53 ± 0.04
Thymus	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.03	0.08 ± 0.01
Paranephros	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Testis	0.81 ± 0.07	0.78 ± 0.08	0.80 ± 0.09	0.78 ± 0.09
Epididymis	0.28 ± 0.05	0.28 ± 0.04	0.26 ± 0.04	0.27 ± 0.03
Female				
Heart	0.28 ± 0.03	0.25 ± 0.05	0.28 ± 0.03	0.29 ± 0.04
Liver	2.74 ± 0.15	2.60 ± 0.39	2.67 ± 0.25	2.68 ± 0.19
Spleen	0.17 ± 0.02	0.14 ± 0.03	0.16 ± 0.02	0.17 ± 0.03
Lungs	0.43 ± 0.04	0.38 ± 0.13	0.40 ± 0.03	0.41 ± 0.05
Kidneys	0.60 ± 0.06	0.55 ± 0.14	0.60 ± 0.12	0.60 ± 0.07
Brain	0.72 ± 0.06	0.71 ± 0.04	0.69 ± 0.05	0.61 ± 0.11
Thymus	0.11 ± 0.01	0.11 ± 0.02	0.10 ± 0.01	0.11 ± 0.01
Paranephros	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
Uterus	0.21 ± 0.04	0.17 ± 0.06	0.20 ± 0.06	0.21 ± 0.07
Ovary	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01

Relative organ weight was calculated as (organ weight/body weight) × 100%.
The values are presented as means ± standard deviation of mean (10 rats/sex/group).

Table 2
Relative organ weights of rats after 14 days of recovery.

Organs	Control	BA extracts (g/kg)		
		3.03	1.52	0.76
Male				
Heart	0.30 ± 0.02	0.29 ± 0.03	0.29 ± 0.03	0.29 ± 0.03
Liver	2.76 ± 0.33	2.63 ± 028	2.65 ± 0.22	2.68 ± 0.26
Spleen	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02
Lungs	0.35 ± 0.04	0.35 ± 0.04	0.36 ± 0.03	0.35 ± 0.05
Kidneys	0.65 ± 0.05	0.64 ± 0.05	0.65 ± 0.05	0.64 ± 0.05
Brain	0.55 ± 0.04	0.51 ± 0.05	0.51 ± 0.04	0.53 ± 0.03
Thymus	0.08 ± 0.01	0.07 ± 002	0.07 ± 0.02	0.08 ± 0.01
Paranephros	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Testis	0.79 ± 0.07	0.78 ± 0.07	0.79 ± 0.04	0.79 ± 0.05
Epididymis	0.28 ± 0.05	0.28 ± 0.02	0.28 ± 0.04	0.28 ± 004
Female				
Heart	0.29 ± 0.03	0.29 ± 0.02	0.29 ± 0.02	0.30 ± 0.02
Liver	2.71 ± 0.24	2.76 ± 0.28	2.77 ± 0.24	2.71 ± 0.18
Spleen	0.18 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.17 ± 0.02
Lungs	0.41 ± 0.05	0.40 ± 0.04	0.41 ± 0.03	0.40 ± 0.03
Kidneys	0.59 ± 0.05	0.59 ± 0.12	0.61 ± 0.08	0.59 ± 0.06
Brain	0.69 ± 0.06	0.69 ± 0.05	0.69 ± 0.04	0.69 ± 0.06
Thymus	0.10 ± 0.00	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Paranephros	0.20 ± 0.02	0.19 ± 0.07	0.19 ± 0.05	0.19 ± 0.05
Uterus	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Ovary	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.01

Relative organ weight was calculated as (organ weight/body weight) × 100%.
The values are presented as means ± standard deviation of mean (10 rats/sex/group).

Clinical biochemistry analysis

Table 5 shows the results of biochemical parameters at the end of treatment periods. Compared to the control group, the levels of serum Na^+, Cl^-, TP, GLU and TG significantly decreased but UREA significantly increased in the treatment groups at doses of 3.03 and 1.52 g/kg in female rats. ALB significantly decreased at a dose of 3.03 g/kg in female rats. In addition, a statistically significant reduction in K^+ was noted in all BA groups in female rats. For male rats, Na^+ at all doses, Cl^- at 3.03 and 1.52 g/kg, and K^+ at dose of 3.03 g/kg were remarkably decreased compared with control. Significant decreases of GLU at doses of 3.03 and 1.52 g/kg, and TP at dose of 3.03 g/kg were observed, whereas the UREA at dose of 3.03 g/kg was significantly increased compared to control values.

As shown in Table 6, withdrawal of repeated daily oral treatments of BA extract for 14 days caused reductions in the levels of TP, ALB and TG, significantly in male rats at all doses compared to the control groups. While, in female rats at all doses, significant decreases in CHO and ALP were showed. The concentration of TG was significantly decreased in female rats at doses of 3.03 and 1.52 g/kg. Additionally, rats treated with a dose of 3.03 g/kg exhibited significant decreases in GLU in both sexes and remarkable decreases in CHO in male rats at compared to the control values. Significant increases were observed in the serum levels of UREA and CREA, in female rats at a dose of 3.03 g/kg compared to control values.

Table 3
Hematological parameters of rats after 90 days of treatment with BA.

Parameter	Unit	Control	BA extracts (g/kg)		
			3.03	1.52	0.76
Male					
HGB	g/l	160.50 ± 5.10	166.40 ± 6.38[a]	166.80 ± 7.19[a]	164.60 ± 3.27
RBC	10^{12}/l	8.56 ± 0.32	10.08 ± 0.23[a]	8.97 ± 1.18[a]	8.29 ± 0.32
MCV	fL	54.91 ± 2.11	56.08 ± 1.91[a]	55.00 ± 2.06	53.18 ± 2.45
MCH	pg	18.76 ± 0.58	19.89 ± 0.72[a]	19.14 ± 0.86	18.98 ± 0.99
MCHC	g/l	348.20 ± 4.49	356.90 ± 10.72[a]	354.60 ± 4.65[a]	348.50 ± 6.04
WBC	10^9/l	5.87 ± 1.87	8.30 ± 2.47[a]	5.48 ± 1.02	7.53 ± 1.85
Gran	%	24.66 ± 9.79	16.90 ± 5.34	16.78 ± 5.25	19.32 ± 3.12
Lymph	%	75.50 ± 8.70	74.81 ± 9.15	79.33 ± 6.13	75.33 ± 5.51
MONO	%	5.67 ± 2.54	4.76 ± 5.22	4.40 ± 183	4.16 ± 2.97
PLT	10^9/l	834.80 ± 332.71	605.00 ± 308.52	1035.70 ± 195.54	954.00 ± 131.15
Female					
HGB	g/l	162.40 ± 4.50	160.70 ± 7.30	161.70 ± 5.19	159.00 ± 13.59
RBC	10^{12}/l	8.29 ± 0.30	8.96 ± 1.37	8.41 ± 0.36	8.11 ± 0.80
MCV	fL	54.51 ± 0.71	59.07 ± 1.93[a]	55.00 ± 1.80	53.99 ± 1.70
MCH	pg	19.58 ± 0.26	20.29 ± 0.49[a]	19.25 ± 0.38	19.63 ± 0.76
MCHC	g/l	343.80 ± 5.16	363.70 ± 6.62[a]	359.40 ± 1.78[a]	350.00 ± 6.02
WBC	10^9/l	5.80 ± 0.85	6.93 ± 2.04	5.45 ± 0.91	5.99 ± 2.01
Gran	%	15.99 ± 5.13	16.41 ± 3.59	15.55 ± 4.86	14.76 ± 2.65
Lymph	%	78.41 ± 5.32	79.03 ± 16.59	77.49 ± 5.71	75.78 ± 4.24
MONO	%	4.80 ± 2.24	5.39 ± 1.86	4.66 ± 1.88	5.62 ± 3.90
PLT	10^9/l	672.60 ± 234.58	910.40 ± 521.40	889.70 ± 259.18	773.60 ± 318.33

The values are presented as means ± standard deviation of mean (10 rats/sex/group).
[a] $p < 0.05$ compared with the control.

Table 4
Hematological parameters of rats after 14 days of recovery.

Parameter	Unit	Control	BA extracts (g/kg)		
			3.03	1.52	0.76
Male					
HGB	g/l	165.80 ± 11.97	167.30 ± 7.29	182.30 ± 3.74	160.10 ± 15.55
RBC	10^{12}/l	8.76 ± 0.39	7.98 ± 2.49	8.73 ± 0.29	8.59 ± 0.62
MCV	fL	54.81 ± 1.70	52.96 ± 1.59	49.23 ± 0.99	53.13 ± 2.56
MCH	pg	18.82 ± 0.90	18.73 ± 1.86	18.09 ± 0.21	18.61 ± 1.14
MCHC	g/l	343.50 ± 16.17	367.40 ± 8.27[a]	354.70 ± 32.35	350.10 ± 11.25
WBC	10^9/l	6.69 ± 1.11	10.94 ± 0.86[a]	6.56 ± 0.90	7.73 ± 1.73
Gran	%	25.42 ± 6.76	22.31 ± 9.88	17.68 ± 7.25	18.91 ± 8.46
Lymph	%	70.55 ± 1.35	69.05 ± 7.52	72.45 ± 3.46	69.74 ± 9.34
MONO	%	7.18 ± 1.43	6.04 ± 2.94	7.28 ± 2.53	6.51 ± 4.69
PLT	10^9/l	551.20 ± 174.58	690.40 ± 237.47	610.44 ± 100.09	542.00 ± 257.38
Female					
HGB	g/l	156.00 ± 19.56	166.90 ± 7.29	163.40 ± 11.24	159.60 ± 2.41
RBC	10^{12}/l	8.44 ± 0.43	8.85 ± 0.45	8.58 ± 0.56	8.15 ± 0.21
MCV	fL	53.53 ± 0.99	56.09 ± 1.36[a]	54.15 ± 1.82	52.54 ± 2.80
MCH	pg	18.47 ± 2.14	18.89 ± 1.00	19.05 ± 0.51	19.58 ± 0.51
MCHC	g/l	345.00 ± 38.88	359.60 ± 11.24	351.80 ± 4.32	349.40 ± 3.60
WBC	10^9/l	7.79 ± 2.23	5.76 ± 1.08	7.48 ± 0.98	7.61 ± 1.37
Gran	%	18.44 ± 3.85	16.18 ± 4.58	18.32 ± 4.81	14.91 ± 3.38
Lymph	%	71.68 ± 6.25	77.65 ± 5.25	76.28 ± 7.67	81.81 ± 3.00
MONO	%	6.33 ± 3.95	6.04 ± 2.05	5.95 ± 2.80	6.72 ± 2.59
PLT	10^9/l	542.60 ± 228.11	683.20 ± 272.71	526.90 ± 191.35	656.70 ± 302.88

The values are presented as means ± standard deviation of mean (10 rats/sex/group).
[a] $p < 0.05$ compared with the control.

Macropathology and histopathology

In the macroscopic examination, no significant difference was observed in gross pathology. As shown in Fig. 1, in addition to bleeding, there were no remarkable histological changes observed in the heart, liver, lungs and kidneys. However, plenty of macrophages phagocytosed amounts of hemosiderin granules in the spleen was observed compared to the organs of the control group. Hemosiderin accumulation was showed by arrows in spleen of Figs. 1 and 2. These results were not significantly changed in the reversibility study (Fig. 2).

Discussion

Subchronic studies assess the undesirable effects of continuous or repeated exposure of plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents. They provide information on target organ toxicity and are designed to identify adverse effect levels (Yuet Ping et al., 2013). In this study, the subchronic toxicity of a BA extract was evaluated in rats at doses of 0.76–3.03 g/kg/day for 90 days. Consideration should be given to an additional satellite group of reversibility, persistence, or delayed occurrence of systemic toxic effects and recovery from toxic effects

Table 5
Biochemical parameters of rats after 90 days of treatment with BA.

Parameter	Unit	Control	BA extracts (g/kg)		
			3.03	1.52	0.76
Male					
Na	mmol/l	176.80 ± 10.60	140.10 ± 2.56[a]	144.70 ± 2.83[a]	164.50 ± 14.80[a]
K	mmol/l	8.12 ± 1.00	6.98 ± 0.45[a]	7.77 ± 0.33	7.92 ± 0.54
Cl	mmol/l	137.10 ± 9.43	109.20 ± 1.32[a]	109.40 ± 2.88[a]	138.60 ± 12.26
UREA	mmol/l	7.68 ± 1.08	10.31 ± 0.91[a]	7.01 ± 1.54	7.13 ± 0.83
CREA	μmol/l	46.71 ± 13.89	44.66 ± 3.91	47.70 ± 3.79	49.49 ± 3.68
TP	g/l	63.72 ± 3.80	58.41 ± 1.96[a]	64.51 ± 3.04	64.61 ± 3.63
ALB	g/l	33.98 ± 2.17	33.17 ± 5.26	35.05 ± 2.13	34.86 ± 1.17
ALP	U/l	224.50 ± 3.80	221.60 ± 59.88	208.60 ± 28.07	196.80 ± 32.44
AST	U/l	158.24 ± 24.65	155.06 ± 24.42	146.64 ± 17.38	137.05 ± 28.86
ALT	U/l	58.40 ± 5.83	53.21 ± 7.30	56.71 ± 8.36	58.67 ± 8.00
GLU	mmol/l	5.78 ± 0.78	4.47 ± 0.56[a]	4.74 ± 0.66[a]	5.51 ± 0.50
CHO	mmol/l	1.55 ± 0.24	1.58 ± 0.23	1.65 ± 0.20	1.66 ± 0.22
TG	mmol/l	0.54 ± 0.15	0.59 ± 0.0.08	0.52 ± 0.16	0.56 ± 0.11
Female					
Na	mmol/l	172.20 ± 12.78	140.60 ± 5.60[a]	143.70 ± 1.83[a]	174.60 ± 13.78
K	mmol/l	8.11 ± 0.45	6.48 ± 1.20[a]	7.42 ± 0.38[a]	7.42 ± 0.63[a]
Cl	mmol/l	142.90 ± 7.25	105.90 ± 5.53[a]	111.30 ± 2.06[a]	138.00 ± 12.84
UREA	mmol/l	7.61 ± 1.48	10.28 ± 1.40[a]	10.42 ± 1.91[a]	8.60 ± 2.06
CREA	μmol/l	45.13 ± 6.12	45.05 ± 5.78	48.91 ± 4.75	45.96 ± 11.08
TP	g/l	75.36 ± 6.22	64.75 ± 5.94[a]	69.02 ± 4.65[a]	73.07 ± 4.05
ALB	g/l	39.77 ± 3.11	35.50 ± 4.52[a]	40.39 ± 2.16	37.48 ± 2.89
ALP	U/l	195.60 ± 31.74	183.20 ± 51.35	179.60 ± 21.73	181.90 ± 19.04
AST	U/l	157.36 ± 59.75	147.09 ± 39.30	171.40 ± 39.58	139.63 ± 38.42
ALT	U/l	57.28 ± 10.41	58.47 ± 9.23	53.03 ± 21.62	61.34 ± 37.67
GLU	mmol/l	6.45 ± 0.47	3.90 ± 1.23[a]	4.88 ± 0.64[a]	5.88 ± 0.64
CHO	mmol/l	2.28 ± 0.47	1.62 ± 0.42	2.14 ± 0.47	2.28 ± 0.65
TG	mmol/l	1.46 ± 0.29	0.74 ± 0.29[a]	0.92 ± 0.23[a]	1.56 ± 0.43

The values are presented as means ± standard deviation of mean (10 rats/sex/group).
[a] $p < 0.05$ compared with the control.

Table 6
Biochemical parameters of rats after 14 days of recovery.

Parameter	Unit	Control	BA extracts (g/kg)		
			3.03	1.52	0.76
Male					
Na	mmol/l	141.00 ± 1.05	142.50 ± 1.51	142.20 ± 2.25	142.30 ± 1.49
K	mmol/l	6.71 ± 0.32	7.15 ± 0.34	6.98 ± 0.64	6.96 ± 0.38
Cl	mmol/l	105.80 ± 1.69	106.70 ± 1.49	107.20 ± 0.79	106.60 ± 1.78
UREA	mmol/l	8.08 ± 1.15	7.18 ± 1.14	7.73 ± 0.72	7.10 ± 0.49
CREA	μmol/l	44.39 ± 1.31	47.28 ± 16.22	46.44 ± 4.40	44.16 ± 9.03
TP	g/l	75.52 ± 4.25	70.98 ± 1.70[a]	70.11 ± 4.54[a]	69.68 ± 1.71[a]
ALB	g/l	45.57 ± 2.04	41.65 ± 1.36[a]	42.48 ± 2.17[a]	41.42 ± 0.81[a]
ALP	U/l	211.30 ± 14.61	156.50 ± 55.54	183.20 ± 26.87	261.10 ± 48.56
AST	U/l	175.51 ± 18.20	157.47 ± 25.83	180.22 ± 22.71	195.78 ± 77.08
ALT	U/l	96.93 ± 8.98	92.51 ± 8.36	96.51 ± 10.73	97.78 ± 13.66
GLU	mmol/l	7.76 ± 0.57	7.42 ± 2.72	8.18 ± 0.74	8.19 ± 1.30
CHO	mmol/l	2.71 ± 0.68	1.77 ± 0.67[a]	2.00 ± 0.48	2.48 ± 0.45
TG	mmol/l	1.02 ± 0.17	0.53 ± 0.19[a]	0.81 ± 0.13[a]	0.79 ± 0.15[a]
Female					
Na	mmol/l	143.40 ± 2.07	142.20 ± 1.48	142.70 ± 1.70	142.10 ± 2.02
K	mmol/l	6.56 ± 0.16	7.03 ± 0.74	6.19 ± 0.56	6.96 ± 0.46
Cl	mmol/l	106.30 ± 1.77	107.30 ± 2.75	108.00 ± 1.32	106.90 ± 2.42
UREA	mmol/l	6.83 ± 0.56	9.29 ± 0.53[a]	7.79 ± 1.72	7.86 ± 1.46
CREA	μmol/l	39.92 ± 1.07	57.14 ± 9.07[a]	46.57 ± 8.77	45.04 ± 18.87
TP	g/l	74.83 ± 2.81	74.94 ± 3.52	77.65 ± 2.45	76.94 ± 4.59
ALB	g/l	45.10 ± 2.65	45.44 ± 0.98	46.55 ± 1.22	46.23 ± 1.99
ALP	U/l	230.70 ± 10.13	78.40 ± 19.88[a]	168.20 ± 56.23[a]	186.70 ± 26.97[a]
AST	U/l	157.93 ± 25.66	172.66 ± 52.78	192.96 ± 35.17	155.92 ± 22.19
ALT	U/l	77.03 ± 4.74	68.37 ± 12.70	88.60 ± 17.11	88.98 ± 18.75
GLU	mmol/l	8.46 ± 0.63	6.14 ± 0.80[a]	8.44 ± 0.77	8.05 ± 1.34
CHO	mmol/l	2.61 ± 0.14	2.06 ± 0.10[a]	2.27 ± 0.15[a]	2.19 ± 0.28[a]
TG	mmol/l	1.46 ± 0.29	0.74 ± 0.29[a]	0.92 ± 0.23[a]	1.56 ± 0.43

The values are presented as means ± standard deviation of mean (10 rats/sex/group).
[a] $p < 0.05$ compared with the control.

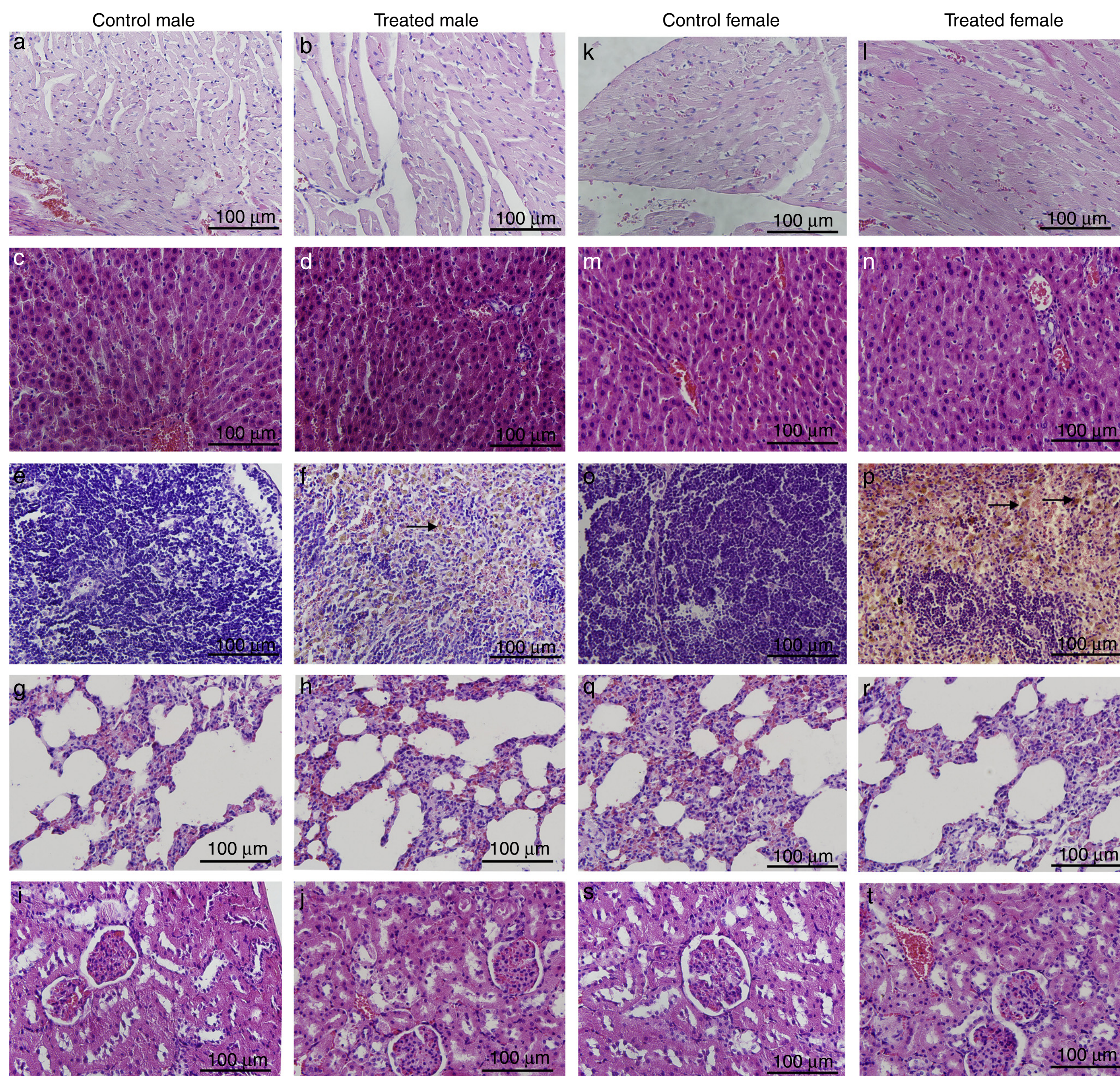

Fig. 1. Effects of 3.03 g/kg of BA extract on the microstructures of various rat organ after 90 days (HE 400×). (a), (b), (k) and (l): heart; (c), (d), (m) and (n): liver; (e), (f), (o) and (p): spleen; (g), (h), (q) and (r): lung; (i), (j), (s) and (t): kidney.

for at least 14 days after treatment (Yuet Ping et al., 2013). The satellite group in this study was orally administered a BA extract at a daily dose of 0.76–3.03 g/kg/day for 90 days with no further treatment for the following 14 days before the termination of the study.

In this study, no significant changes in the weights of the heart, spleen, lungs and kidneys were observed, suggesting that administration of BA extracts at subchronic oral doses had no effect on normal growth. The usefulness of weighing organs in toxicity studies includes their sensitivity to toxicity, enzyme induction, physiologic perturbations, and acute injury. It is frequently a target organ of toxicity and correlates well with histopathological changes (Michael et al., 2007). The relative organ weights have been observed in toxicity studies to be a relatively sensitive indicator for particular organs and therefore define toxicity as significant changes observed in those particular organs (Kluwe, 1981). The results of this study revealed that the essential organs, such as heart, liver, spleen, kidneys, and lungs, were not adversely affected and showed no clinical signs of toxicity throughout the treatment. Because there was no reduction in relative organ weights of the treated animals at any of the tested doses, we concluded that the BA extract has no obvious toxicity in the analyzed organs.

Evaluation of hematological parameters can be used to determine the extent of the deleterious effect of extracts on an animal's blood. This approach can also be used to explain blood relevant functions of a plant extract or its products (Yakubu et al., 2007). A hemogram was conducted for all BA extract treated and control groups and the results show significant increases in Hb, RBC, MCV, MCH and MCHC at any dose. The significant effect of the extract indicates that the BA extract affects the erythropoiesis, morphology, or osmotic fragility of the RBC. Leukocytes are the first line of cellular defense that responds to infectious agents, tissue

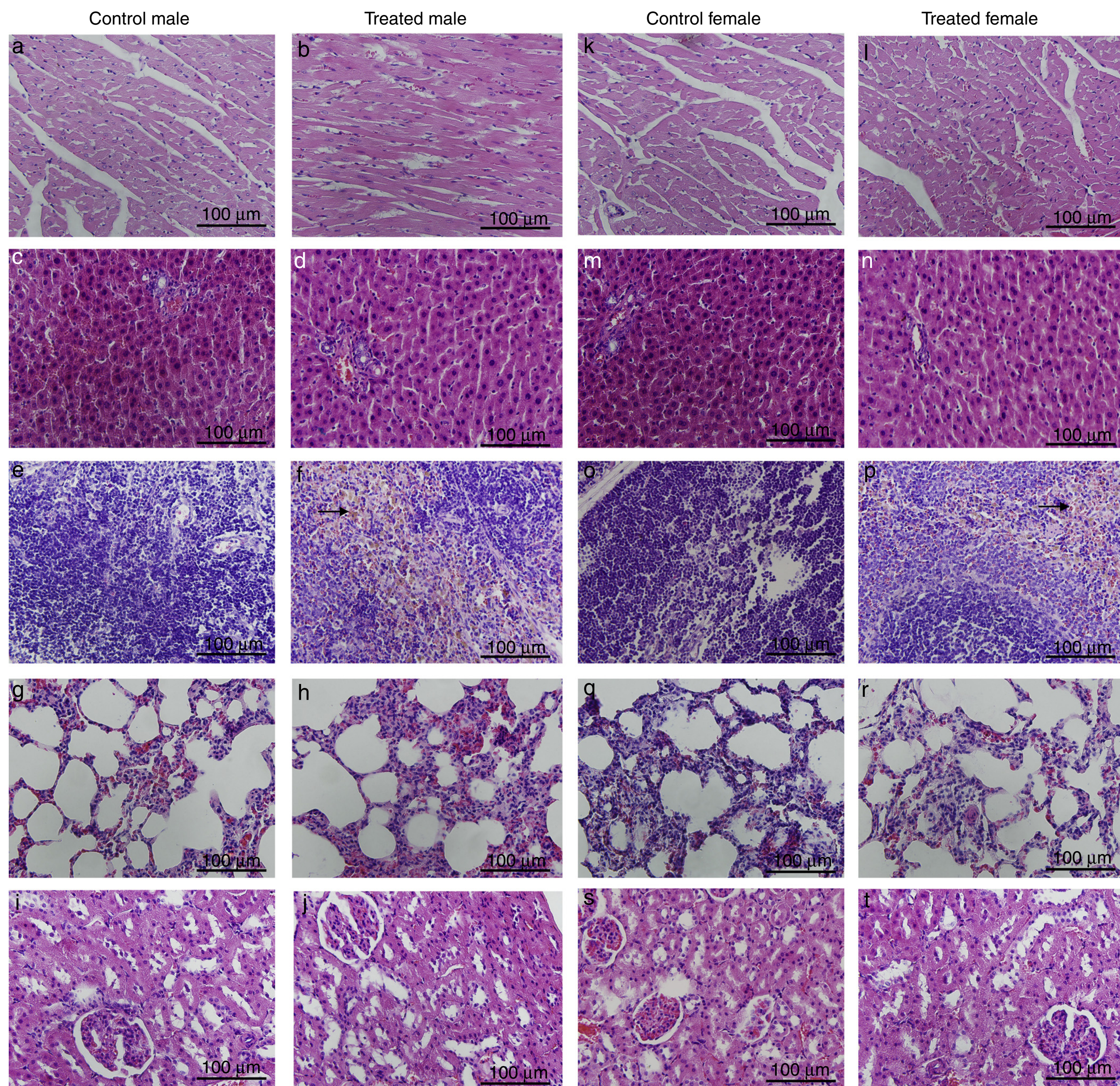

Fig. 2. Effects of 3.03 g/kg of BA extract on the microstructures of various rat organ after 14 days of recovery (HE 400×). (a), (b), (k) and (l): heart; (c), (d), (m) and (n): liver; (e), (f), (o) and (p): spleen; (g), (h), (q) and (r): lung; (i), (j), (s) and (t): kidney.

injury, toxic effect or inflammatory processes. Furthermore, significant change was observed in the WBC in the BA group at a dose of 3.03 g/kg, which confirmed tissue damage or toxicity.

The serum hematology and clinical biochemistry analyses were performed to evaluate the possible alterations in hepatic and renal functions influenced by the extracts. Liver and kidney function analysis is very important in the toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of an organism (Yuet Ping et al., 2013). Liver is the major organ involved in drug metabolism and elimination. High levels of ALT, AST, and ALP are reported in liver diseases or hepatotoxicity (Brautbar and Williams, 2002). Production of TP is another typical function of liver. A decrease in TP and ALB is a sign of the reduced synthetic function of the liver or might be due to impaired hepatocellular function (Yuet Ping et al., 2013). There were no significant increases in serum ALT, AST and ALP values in either male or female rats at all doses, but other hepatic function parameters, such as TP and ALB, were significantly decreased at all of the doses tested. The changes of TP and ALB were irreversible with the extract withdrawal for 14 days in male rats at all doses. Thus, the significant changes in serum concentrations of TP and ALB confirmed that the BA extract damaged the hepatocellular or secretory functions of the liver. At the same time, the toxic effects in liver appeared delayed in male rats at all doses. Liver as an important organ in the synthesis of CHO and TG, liver injury may hinder the normal synthesis of CHO and TG. In our results, BA caused a significant decrease in CHO and TG. These results indicate that BA affects the live function and lipid metabolism imbalance.

The function of kidney has important influence on drug metabolism. Many medicines can cause kidney injury (Gobe et al.,

2015). Renal dysfunction can be assessed by concurrent measurements of electrolytes, urea and creatinine (Akindele et al., 2014). Changes in Na^+, K^+ and Cl^- levels in some BA extract groups showed significant differences indicating an abnormal renal function. This damage was further confirmed by significant increases in urea and creatinine levels in all groups.

Although clinical biochemistry analysis showed liver and kidney damage, none of these organs from the BA extract treated rats showed any significant alteration in cell structure or any unfavorable effects compared to the control group when viewed under the light microscope. Hemosiderin depositions were observed in the spleens after BA extract treatment. An increase of hemoglobin indicated that the BA extract damaged the spleen function.

In general, the subchronic toxicity of baked *A. flavum* was reduced enormously by the method described in "Preparation of ethanol root extract of baked *Aconitum flavum*" section. In our research, no deaths or serious clinical signs were found. At the same time, the morphological and histopathological changes were not observed in the main organs except for spleen. The changes in hematological parameters and clinical biochemistry analyses showed that the damage to metabolic organs (liver and kidneys) was slight. It is worth noting that the damage to the spleen may be related to an inhibitory effect on immune function. Thus, regarding damage to the liver, kidney and spleen functions, caution should be exercised when using the BA extract for long term therapy. However, these toxicities may not be overly serious because BA toxicity is significantly less than *A. flavum*. The ancient Chinese said that toxicity is the profile of a drug; therefore, we believe that BA will improve the patient's life if BA is applied reasonably.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Authors' contributions

YZ, EG and XZ contributed in collecting plant samples and conducting the laboratory work. XZ and BW analyzed the data. LY, LZ and JH read the manuscript critically. XF designed the study, supervised the laboratory work and contributed to the critical reading of the manuscript. All the authors have read the final manuscript and approved its submission.

Conflicts of interest

The authors declare no conflicts of interest.

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