Regular Article

Ninety-Day Subchronic Oral Toxicity Study of *Senecio scandens* Extract in Rats

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The present study assessed the safety/toxicity of *Senecio scandens*, a well-known Chinese herb that is used as an anti-inflammatory, antibiosis, and antipyretic drug. A 90-d subchronic oral toxicity study of *S. scandens* was performed in Wistar rats. The extract of *S. scandens* was administered orally to male and female rats at a single dose of 225, 450, and 900 mg/kg/d. There was no obvious toxicity. Certain changes in hematology and coagulation parameters (red cell distribution width (RDW), platelet count (PLT), monocyte percentage (Mo%), activated partial thromboplastin time (APTT), prothrombin time (PT)) were observed in some administration groups. In regards to the blood biochemical parameters, the levels of creatinine (CRN), potassium, and chloride were increased in a number of the treated rats. There were no significant changes in other hematology, coagulation, or biochemical parameters in rats orally administered *S. scandens*. *S. scandens* has a slight effect on rat coagulation and metabolism systems. The herb was safe at all doses tested, but caution should be taken when administering *S. scandens* at higher doses.

Key words *Senecio scandens*; subchronic toxicity; rat; safety

Plants of the genus *Senecio* are found in different parts of the world.[1–3] Belonging to the tribe *Senecioneae*, the genus *Senecio* is the largest and most complex genus in the *Asteraceae* (Compositae) family and includes more than 1500 species with a worldwide distribution.[4–6] These plants contain toxic pyrrolizidine alkaloids (PAs) and are frequently ingested accidentally because of food grain contamination or intentionally in the form of herbal medicines and, if consumed over an extended period of time, can induce irreversible liver damage.[7–10] It is estimated that over 2000 deaths reported in numerous countries, such as Afghanistan, China, Ethiopia, Iraq, South Africa, and Uzbekistan, have been caused by PAs.[10,11] Many *Senecio* spp. throughout the world have been shown to contain hepatotoxic PAs,[12–14] and the World Health Organization (WHO) has been pronounced some species as forbidden for use.[15]

Most species of *Senecio scandens* distributed in China also contain toxic PAs.[16,17] *Senecio scandens* has been used in Chinese traditional and folk medicine for its antipyretic, antibacterial, anti-inflammatory and liver- and eye-protective properties for more than a thousand years. Some compounds isolated from the genus *Senecio* and some crude extracts are known to possess significant antimicrobial activities, including antibacterial, antifungal, and antitubercular activities.[18–21] *Senecio scandens* Buch.-Ham. locally known as "Qianliguang," is one of the most popular species used as a Chinese medicinal herb. Herbal proprietary products of *Senecio scandens* are registered as over-the-counter remedies in China and are exported to Western countries. The main active ingredients of *Senecio scandens* are the PAs and eremophilane sesquiterpenes. PAs of *Senecio scandens* have been reported to contain adonifoline, monocrotaline, senkirkine, dehydrosenkirkine, senecionine, neoplatyphylline and seneciphylline *etc.*[22,23] Belonging to retronecine esters-type PAs (RET-PAs), adonifoline is one of the main hepatic PAs. It was identified as a specific marker for *Senecio scandens*, and subsequent hepatotoxicity studies showed that *Senecio scandens* exhibited the potential for low toxicity.[22] Orally administrated adonifoline could be absorbed fast with lower bioavailability and quickly metabolized to PA *N*-oxides and hydroxylation products of PAs or their *N*-oxides.[24] PAs toxicity of *Senecio scandens* ranking criteria is not well defined in the Chinese Pharmacopoeia. The potential toxicity of *Senecio scandens* has not been fully recognized to date. Despite the many pharmacological effects of *Senecio scandens* and its products the safety of their use has raised general concerns because of the risk of the presence of hepatotoxic PAs.[17,25–28]

As part of a project to provide safety data required for the use of *Senecio scandens* as drugs, the present study was conducted to evaluate the toxicity of the *Senecio scandens* extract (SCE) qualitatively and quantitatively after 90 d of repeated oral administration in Wistar rats and to determine the target organs of SCE.

MATERIALS AND METHODS

Preparation of *Senecio scandens* Extract The aerial portions of *Senecio scandens* collected from Henan province, China, were identified as *Senecio scandens* Buch.-Ham. ex D. Don by Prof. Xulin Guo, School of Pharmaceutics Sciences, Peking University. The dried *Senecio scandens* was ground into powder. Then, the powder (100 kg) was extracted by decoction using hot water (twice, once for 2 h and once for 1 h). The filtrate was concentrated and then lyophilized using a freeze-drying system. The total acquired SCE was 14.9 kg (yield 14.9%). SCE was prepared for gavage by dissolving the

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extract with sterile water to obtain the concentrations necessary for the doses for each experimental group in the repeated dose toxicity studies.

Analysis of the Extracts The contents of alkaloids in SCE are shown in Table 1. The content of total alkaloids was measured by ultraviolet spectrophotometer. Adopting monocrotaline as reference, the absorbance was determined by UV spectrophotometer in 216 nm wavelength. The equation obtained for the calibration curve of monocrotaline in the range of 0.0200–0.1970 μg/mL was $y=0.03759+5.3757x$ ($r=0.9992$). The experiments were conducted in quintuplicate. The total RET-PAs content was performed using a pre-column derivatization HPLC method described by Zhang *et al*.[29] The determination of adonifoline (Fig. 1), the main hepatotoxic PAs in *Senecio scandens* Buch.-Ham. ex D. Don, was performed using HPLC-MS^2 as described by Zhang *et al*.[30]

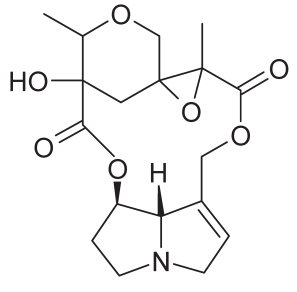
Experimental Animals and Animal Husbandry The experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals issued by the National Institutes of Health, and the procedures were approved by the Institutional Animal Care and Use Committee of the China Academy of Chinese Medical Sciences. Young adult Wistar rats (5 weeks old) of both sexes were purchased from the animal department of China Medical University (CMU) and acclimated for 1 week prior to commencing the studies. The animals were housed in groups of five in stainless steel wire mesh cages and were provided sterilized tap water and commercial rodent chow (2.0 Mrad γ-ray sterilized EP pellet, Beijing Keao Xieli Feed Co., Ltd.). The animals were housed in a room maintained at a temperature of 23±3°C and a relative humidity of 55±5% with artificial lighting from 08:00 to 20:00 and with 10–20 air changes per hour. Only healthy animals were used in the studies.

Administration SCE was administered to rats by oral gavage. Administration in the repeated oral dose toxicity study was performed repeatedly once per day without fasting. The dosing volume was set to 10 mL/kg, based on the most recent body weights of the rats.

Table 1. Contents of Alkaloids in SCE

Content	(%)
Total alkaloids	3.57[a]
RET-PAs	0.0966[b]
Adonifoline	0.0069[c]

a) Analyzed by ultraviolet spectrophotometer. *b*) Analyzed by HPLC with pre-column derivatization. *c*) Analyzed by HPLC-MS^2.



Adonifoline

Fig. 1. Chemical Structure of a Key Compound in SCE, China

Experimental Design After a one week acclimatization period, forty healthy animals of each gender at 6 weeks of age were included in the experiments. A dose of 900 mg/kg/d (approximate 1/8 LD_{50} of mice, 1/16 LD_{50} of rats) was set as the high dose based on the results of our previous acute oral toxicity study.[31] The other doses were 450 and 225 mg/kg/d and were set using a common ratio of 2. The vehicle control group was given sterile water by gavage. Each group consisted of 10 male and 10 female rats and all rats were sacrificed after 90 d of treatment. The following items were examined during the experimental period: mortality, clinical signs, body weight, food consumption, urinalysis, hematology, coagulation, serum biochemistry, necropsy findings, organ weight and histopathology.

Mortality and Clinical Signs

All animals were observed thoroughly for the onset of any immediate signs of toxicity and throughout the observation period to record any delayed acute effects and mortality. All animals were observed once a day for 90 d after drug administration.

Body Weight (BW) and Food Consumption

The individual animal BWs were recorded at receipt, on the day of initiation of treatment and weekly thereafter (±2 d) during the course of study. Fasting BWs were recorded at terminal euthanasia. The amount of food was measured before it was supplied to each cage, and food remaining was measured weekly thereafter (±2 d) to calculate the daily food consumption (g/rat/d).

Urinalysis

After 90 d of treatment, urine was collected in metabolic cages for 3 h and was used for urinalysis. Additionally, the total urine volume was measured from urine samples collected for 24 h. Urinalysis was conducted to determine glucose, bilirubin, ketone body, specific gravity, occult blood, pH, protein, urobilinogen, nitrite and leukocyte levels using the Bayer multistix 10SG and urine analyzer (Bayer 50, Germany).

Hematology and Coagulation

Animals were fasted overnight, and following pentobarbital sodium anesthesia, blood samples for hematology and clinical chemistry were collected from the abdominal aorta using a syringe and a 24-gauge needle. Hematology parameters in ethylenediaminetetraacetic acid (EDTA) treated blood, including total white blood cell (WBC) count, differential leukocyte percentage, total red blood cell (RBC) count, hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RDW, PLT, mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), lymphocytes (LYM), missouri (MO), granulocyte (GR), lymphocyte percentage (LYM%), MO% and granulocyte percentage (GR%), were measured using a hematological autoanalyzer (Nihon Kohden Celltac MEK-6318 K, Japan). PT and APTT were measured in sodium citrate-treated blood using an automated hematocoagulation analyzer (Coagrex-100S, Japan).

Biochemistry

Blood collected for serum biochemistry was untreated and was centrifuged at 3000 rpm for 10 min. The serum obtained was examined for the following parameters using a biochemistry autoanalyzer (Hycel Lisa 300, France): aspartate ami-

notransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBIL), alkaline phosphatase (ALP), total protein (T.P.), albumin (ALB), CRN, blood urea nitrogen (BUN), total cholesterol (CHOL), triglycerides (TG), r-glutamyl transferase (r-GT), glucose (GLU) and creatine kinase (CK-NAC). Serum electrolytes, such as chloride, sodium and potassium, were measured using an ion autoanalyzer (Bayer 644 Na/K/Cl analyzer, U.S.A.).

Necropsy Findings

Complete gross postmortem examinations were performed on all terminated animals. All organs were fixed and stored individually for histopathological examination.

Organ Weights

The absolute and relative (organ-to-BW ratios) weights of the following organs were measured in all survivors following sacrifice: heart, liver, spleen, lungs, thymus, kidneys, brain, stomach, adrenal glands, uterus, ovaries, testis, epididymis and prostate.

Histopathology

The following tissues were obtained from all animals: heart, liver, spleen, lungs, thymus, kidneys, brain, stomach, adrenal glands, uterus, ovaries, testis, epididymis and prostate. Tissues were fixed with 10% neutral buffered formalin solution and sectioned to 3–5 μm. The sections were stained with hematoxylin and eosin for microscopic examination.

Statistical Analysis The following parameters were analyzed using SPSS /13.0 software: body weight, food consumption, organ weight, urinalysis parameters, hematology parameters and serum biochemistry parameters. Means and standard deviations were calculated, and the homogeneity of the variance was analyzed. Differences between the control group and each test group were evaluated using Dunnett's test or Steel's test. A $p<0.05$ was considered significant.

RESULTS

Mortality and Clinical Signs Neither treatment-related mortality nor obvious clinical signs, including hair loss, scabbing, soft or mucoid feces, decreased defecation or feces smaller than normal, wet yellow material in the urogenital area or vocalization upon handling, were found in any of the treatment groups throughout the experimental period.

Body Weight and Food Consumption Figure 2 shows the mean body weights of the female and male rats. There were no differences in body weights between the treatment groups and the control group for either gender throughout the experimental period. There were no treatment-related changes in body weight in the rats.

As shown in Fig. 3, no significant changes were observed in food consumption during the experimental period. There were no significant differences in food consumption for the female and male rats in the treatment groups compared to those in the control group.

Urinalysis No significant differences were observed in the qualitative analysis of urine parameters, including pH, specific gravity and glucose, protein, bilirubin, urobilinogen, ketone body, occult blood, nitrite and leukocyte levels in the urine of either gender of rats collected for 90 d (Table 2).

Hematology and Coagulation However, compared to the control group some statistically significant changes were noted, as described below. RDW was increased in female rats of all groups ($p<0.05$). PCT was decreased in female rats of all groups ($p<0.05$ or $p<0.01$). MO% was increased in male and female rats in the 900 mg/kg/d SCE group ($p<0.05$). Although PLT was decreased in all SCE groups, it was decreased slightly in male rats and more significantly in female rats ($p<0.05$). PT was prolonged in male and female rats in the 450 and 900 mg/kg/d SCE groups ($p<0.05$). APTT was prolonged in male and female rats in all SCE groups ($p<0.05$). There were no significant changes in the other hematological parameters in the rats orally administered SCE (225, 450 and 900 mg/kg/d) for 90 d (Table 3).

Biochemistry The results of the serum biochemical analysis of rats orally administered SCE for 90 d are shown in Table 4. Some significant changes were observed and are described below. CRN was increased in male rats in all SCE

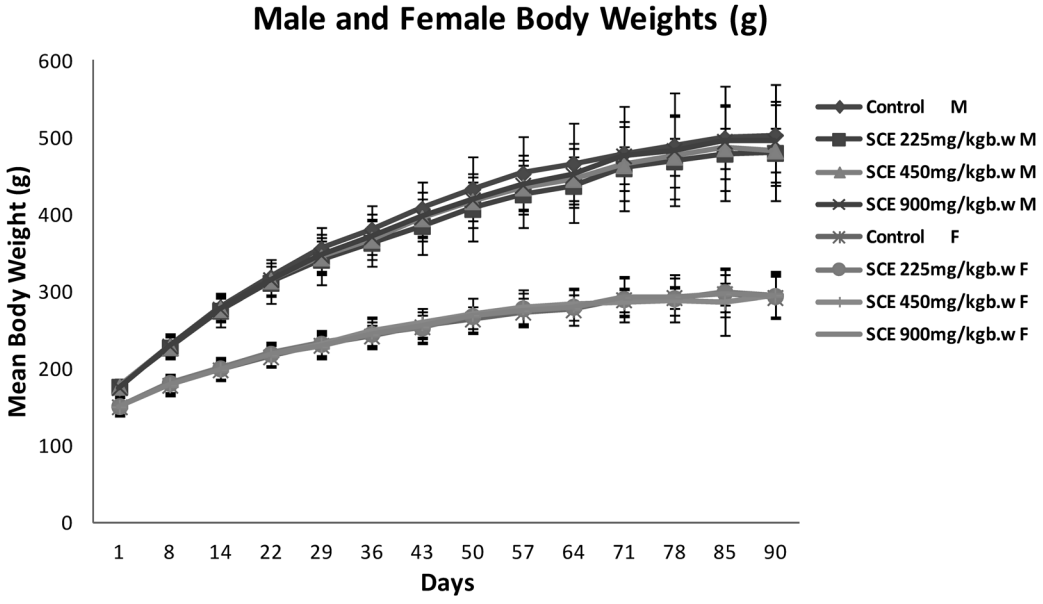


Fig. 2. Effect of SCE on Body Weights in Male (M) and Female (F) Rats

Mean body weights for male and female rats during a 90-d oral (gavage) toxicity study. The values are presented as mean±S.D. (N=10/sex).

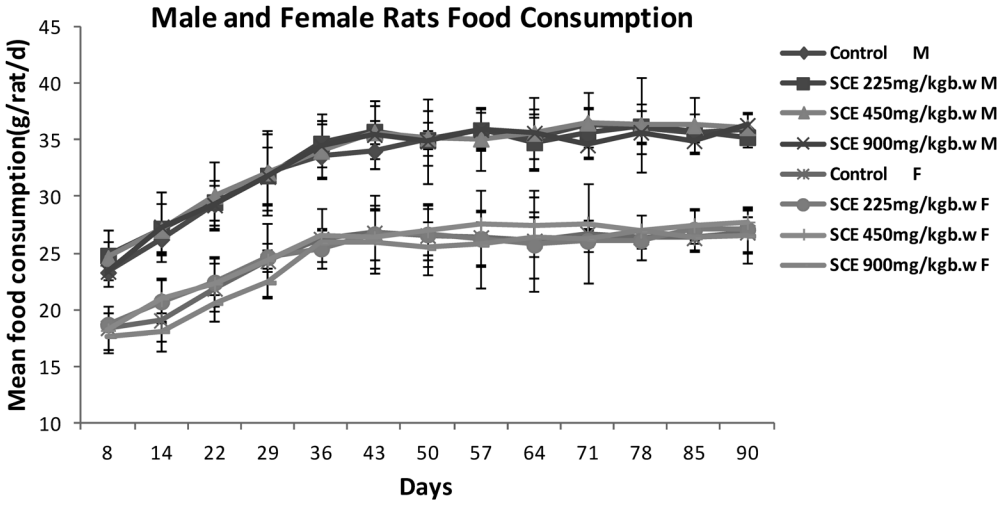


Fig. 3. Effect of SCE on Food Consumption in Male (M) and Female (F) Rats during a 90-d Oral (Gavage) Toxicity Study
The values are presented as mean±S.D. (N=10/sex).

Table 2. Urinalysis of Male and Female Rats Treated Orally with SCE for 90 d

Parameters	Results	Dose (mg/kg/d)							
		Male				Female			
		0	225	450	900	0	225	450	900
Urine volume (mL/24 h)		38.0±5.8	37.67±4.5	38.5±1.2	39.3±9.7	26.17±2.0	26.5±3.5	28.7±9.9	27.3±4.4
Glucose	0	10	10	10	10	8	9	8	10
	Trace (5 mg/dL)	0	0	0	0	2	1	2	0
Bilirubin	Negative	10	10	10	10	10	10	10	10
Ketone body (mg/dL)	Negative	10	10	10	9	10	10	10	10
	5 (Trace)	0	0	0	1	0	0	0	0
Specific gravity	≤1.005	8	10	8	10	6	7	10	7
	1.01000	2	0	2	0	0	3	0	2
	1.01500	0	0	0	0	4	0	0	1
pH	6.5	0	0	0	0	0	0	4	3
	7.0	0	4	0	4	6	6	0	3
	7.5	4	0	3	4	2	2	4	0
	8.0	6	6	5	1	2	2	0	2
	8.5	0	0	2	1	0	0	2	2
Protein (mg/mL)	Negative	0	0	2	3	1	1	0	0
	Trace	8	7	6	5	7	3	5	6
	30	2	3	2	2	2	6	5	4
Urobilinogen (mg/dL)	Normal (1)	10	10	10	10	10	10	10	10
Nitrite	Negative	10	10	10	10	10	10	10	10
Occult blood	Negative	10	10	9	9	8	8	10	8
	Trace	0	0	1	1	0	1	0	2
	Small	0	0	0	0	2	1	0	0
Leukocyte	Negative	10	10	10	10	10	10	10	9
	Trace (25 leu/μL)	0	0	0	0	0	0	0	1

n=10 per sex in each group.

groups (compared with control, $p<0.05$). The level of potassium was increased in female rats in the 450 and 900 mg/kg/d SCE groups and in male rats in all SCE groups (compared with control, $p<0.05$). The level of chloride in the serum of all rats in all SCE groups was increased (compared with control, $p<0.01$). There were no significant differences in the other biochemical parameters measured between the rats orally administered SCE (225, 450 and 900 mg/kg/d) for 90 d and the control rats.

Necropsy Findings and Organ Weights There were no grossly visible findings or lesions in any group. SCE did not cause any significant changes in body or organ weights, as shown in Table 5.

Histopathology Gross pathological examination revealed no detectable abnormalities in the selected organs, including the liver and kidney, as shown in Fig. 4. In addition, no exposure-related histopathological changes were observed in any of the organs examined.

Table 3. Hematology Data of Rats Orally Administered SCE for 90 d

Parameter	Dose (mg/kg/d)			
	0	225	450	900
Male				
WBC (10^9/L)	7.82±1.26	7.20±1.50	7.68±1.80	7.22±1.33
RBC (10^{12}/L)	7.99±0.30	7.96±0.63	7.77±0.99	8.15±0.66
HGB (g/dL)	15.2±0.84	15.0±1.26	14.5±1.51	15.0±1.26
HCT (%)	40.2±2.44	39.2±3.02	37.8±4.62	39.5±2.89
MCV (fL)	50.3±2.29	49.3±1.02	58.7±0.89	48.5±1.17
MCH (pg)	18.9±1.68	18.5±0.66	18.4±0.81	18.2±0.51
MCHC (g/L)	36.8±1.50	37.8±1.72	38.0±1.55	37.7±0.82
RDW (%)	13.2±0.96	12.9±0.67	13.3±0.83	13.5±0.70
PLT (10^9/L)	390.8±59.7	364.2±67.7	344.5±57.7	377.5±78.1
MPV (fL)	8.69±0.45	8.48±0.38	8.57±0.36	8.35±0.20
PDW (10GSD)	13.5±0.62	12.9±0.43	13.4±0.28	13.2±0.33
PCT (mL/L)	0.33±0.09	0.31±0.07	0.31±0.05	0.31±0.07
GR (10^9/L)	2.06±2.08	1.15±0.88	1.16±0.58	1.57±0.71
LYM (10^9/L)	9.4±3.51	9.48±3.30	8.02±2.34	9.93±2.71
MO (10^9/L)	1.03±0.61	0.88±0.12	1.12±0.30	1.12±0.38
GR% (%)	12.0±9.79	11.6±2.02	11.7±5.79	12.3±5.49
LYM% (%)	80.6±12.4	80.5±12.0	77.5±7.03	77.9±8.01
MO% (%)	7.30±2.69	7.96±2.05	9.80±2.89	10.8±1.47*
PT (s)	13.7±0.29	14.3±0.55	14.7±0.69*	14.5±0.51*
APTT (s)	22.0±1.12	25.2±1.40*	25.9±2.80*	25.4±1.83*
Female				
WBC (10^9/L)	6.16±1.75	6.65±1.71	6.98±0.63	6.30±2.36
RBC (10^{12}/L)	7.56±0.41	7.04±0.65	6.92±0.67	7.42±1.32
HGB (g/dL)	14.67±1.21	14.0±1.41	13.6±0.55	14.0±2.89
HCT (%)	38.5±1.92	36.3±3.47	36.5±1.31	37.6±6.92
MCV (fL)	50.8±1.79	51.5±1.16	50.4±1.05	50.6±1.48
MCH (pg)	19.2±1.30	19.9±1.16	18.8±0.40	18.8±0.78
MCHC (g/L)	38.0±2.19	38.7±1.75	37.3±0.76	37.1±1.46
RDW (%)	12.9±0.51	12.2±0.49*	12.0±0.56*	12.2±0.51*
PLT (10^9/L)	387.0±13.7	329.2±61.5*	327.8±18.8*	318.8±47.7*
MPV (fL)	8.68±0.53	8.47±0.32	8.17±0.35	8.31±0.30
PDW (10GSD)	12.9±0.19	13.1±0.41	13.3±0.43	13.0±0.41
PCT (mL/L)	0.34±0.02	0.27±0.05*	0.24±0.02**	0.28±0.06*
GR (10^9/L)	0.72±0.83	0.48±0.23	0.71±0.36	0.44±0.35
LYM (10^9/L)	5.98±1.55	5.58±1.60	5.61±0.79	6.07±2.87
MO (10^9/L)	0.68±0.22	0.58±0.21	0.66±0.17	0.79±0.29
GR% (%)	8.34±1.14	7.80±1.74	10.6±2.48	6.19±1.87
LYM% (%)	84.4±11.5	83.6±5.58	80.0±7.19	82.9±4.19
MO% (%)	7.24±2.34	8.65±1.30	9.39±2.07	10.9±1.97*
PT (s)	13.7±0.37	14.2±0.57	14.3±0.51*	14.3±0.42*
APTT (s)	22.3±1.18	24.3±0.79*	24.9±1.25*	24.8±1.10*

Values are mean±S.D. for 10 rats per sex in each group. * Significantly different from controls with $p<0.05$. ** Significantly different from controls with $p<0.01$.

DISCUSSION

Poisonings from the intentional use of plants containing PAs as foods or herbs have occurred throughout the world and have been formally reported in a number of articles, since 1920.[7] *Senecio* is a genus that is distributed worldwide. Plants in this genus contain PAs, and many species have been used for medicinal treatment for more than a thousand years.[26,32] Therefore, individuals who consume herbal remedies or herbal teas made from *Senecio* plants are at risk of developing irreversible acute and/or chronic toxicities,[33,34] including acute liver failure, cirrhosis, pneumonitis, pulmonary hypertension and heart failure.[35] Poisoning cases are widely distributed in all ages, but are more common in children.[9] Today, several countries have restricted the use of *Senecio*.

Senecio vulgaris L. and *scandens* Buch.-Ham. are two of the main medicinal herbs used in Europe and China that contain PAs. Because *Senecio vulgaris* L. contains high contents of several toxic PAs, its use as a herbal tea has caused several deaths, and has been restricted for medical use only.[9,36,37] More than 160 species of *Senecio* spp. are distributed throughout China, including 17 species that are used as Chinese herbal medicines. *Senecio scandens* Buch.-Ham., one of the main medicinal herbs, has been used for a variety of ailments, such as bacterial diarrhea, enteritis, conjunctivitis and respiratory tract infections, for more than a thousand years. At pres-

Table 4. Biochemistry Data of Rats Orally Administered SCE for 90 d

Parameter	Dosage (mg/kg/d)			
	0	225	450	900
Male				
ALT (U/L)	27.8±3.83	27.3±2.75	29.5±3.42	30.0±5.15
AST (U/L)	114.0±10.0	103.5±17.3	117.0±17.1	122.5±17.7
TBIL (μmol/L)	4.52±1.11	4.55±1.13	3.56±1.59	3.27±1.49
ALP (U/L)	69.0±23.6	63.3±13.3	69.5±18.4	66.7±25.5
r-GT (U/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CRN (μmol/L)	47.1±4.31	66.5±17.1*	62.2±11.4*	59.7±5.89*
BUN (mmol/L)	6.92±1.31	7.75±1.40	8.23±1.48	8.65±1.43
CK-NAC (U/L)	881±183	719.4±181.4	999.0±233.3	1048.8±128.7
TG (mmol/L)	0.46±0.19	0.61±0.27	0.50±0.24	0.65±0.31
CHOL (mmol/L)	1.79±0.27	1.84±0.40	1.80±0.32	2.03±0.32
T.P. (g/L)	77.8±6.57	80.0±3.47	78.4±6.67	82.7±8.45
ALB (g/L)	30.6±1.45	32.1±2.02	31.8±1.59	32.3±1.67
GlU (mmol/L)	5.54±0.93	5.57±0.63	5.79±1.15	5.60±0.70
Sodium (mmol/L)	300±41.7	306.4±24.9	320.6±19.0	285.3±14.4
Potassium (mmol/L)	5.20±0.63	5.69±0.36	6.42±0.60**	6.16±0.95**
Chloride (mmol/L)	32.9±4.52	42.4±3.91**	46.1±4.80**	87.60±15.96**
Female				
ALT (U/L)	20.7±5.72	19.3±2.94	19.9±3.18	17.28±4.46
AST (U/L)	119.3±8.73	110.0±7.79	120.3±12.2	120.0±18.2
TBIL (μmol/L)	4.69±0.93	3.98±0.84	3.96±1.03	3.72±1.34
ALP (U/L)	44.67±27.20	34.67±16.8	37.85±21.5	34.00±6.90
r-GT (U/L)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
CRN (μmol/L)	47.75±8.38	55.4±14.97	57.7±13.76	54.2±8.58
BUN (mmol/L)	6.31±1.70	7.10±0.86	6.52±1.31	6.18±1.43
CK-NAC (U/L)	888.8±110.3	885.7±299.1	1156.7±310.7	1092.7±212.9
TG (mmol/L)	0.41±0.12	0.41±0.14	0.37±0.08	0.37±0.12
CHOL (mmol/L)	1.67±0.58	1.70±0.30	1.53±0.40	1.42±0.43
T.P. (g/L)	84.8±7.55	82.3±5.66	80.0±7.26	77.4±6.50
ALB (g/L)	33.63±1.65	33.08±1.32	33.34±2.02	33.61±2.46
GlU (mmol/L)	4.98±1.18	4.83±0.32	4.87±0.69	4.17±0.63
Sodium (mmol/L)	269.4±34.0	285.5±31.6	286.2±10.8	298.5±24.7
Potassium (mmol/L)	4.46±0.25	5.03±0.30**	5.27±0.52**	5.70±0.89**
Chloride (mmol/L)	38.11±2.53	44.53±2.66**	43.87±2.34**	45.6±5.82**

Values are mean±S.D. for 10 rats per sex in each group. * Significantly different from controls with $p<0.05$. ** Significantly different from controls with $p<0.01$.

ent, *Senecio scandens* Buch.-Ham. is officially recorded in the Chinese Pharmacopeia and is used alone or as an ingredient in more than 100 preparations, mainly for anti-inflammatory purposes. There have been few reports about the toxicity of *Senecio* spp., and only several species of *Senecio* have caused liver damage in livestocks in China. The source and species that caused the reported clinical deaths are unclear, but several PAs have been found in *Senecio scandens*.[15,17,26]

Our previous studies showed that the toxicity of *Senecio scandens* varies with its production area in China. *Senecio scandens* plants obtained from the Guangxi and Hubei provinces have no marked toxicity, while there is some degree of toxicity in *Senecio scandens* plants obtained from the Jiangsu, Zhejiang, Sichuan, and Henan provinces. The *Senecio scandens* plants obtained from Henan are the most toxic, the LD_{50} for Henan SCE in mice and rats are 6.85 g/kg[29] and 14.66 g/kg (data not shown) respectively. The plant's toxicity is related to species variation, and the tolerance in animals.

The present study evaluated the potential toxicity of *Senecio scandens* obtained from Henan province after 90-d repeated oral administration in Wistar rats; gross and target organ toxicities were determined. The doses used were 225, 450, and 900 mg/kg/d, which approximately equaled to the 1/32, 1/16, 1/8 of the LD_{50} in mice, and the 1/64, 1/32, 1/16 of the LD_{50} in rats, respectively. In clinical practice, the conventional dose of the herb *Senecio scandens* is 15–30 g/70 kg body weight, which is equal to 0.03–0.06 g/kg SCE in our experiments. Thus, the highest dose of *Senecio scandens* (900 mg/kg) given to the Wistar rats in this experiment was 15–30 times higher than the conventional clinical dose. The results of this 90-d repeated oral dose toxicity study indicated that repeated oral administration of SCE at dosages of 225, 450 and 900 mg/kg/d did not result in death. No treatment-related effects on body weight, food consumption, urinalysis, histopathology or morphology were observed.

Some effects on hematological and serum biochemical parameters were observed in the treatment groups. The significant decreases in PLT and PCT in female rats were considered to be due to differences in gender. The significant increases in RDW, APTT and PT in the SCE groups were considered to be a treatment-related effect, as they were observed throughout the entire treatment period. It is well-known that increased

Table 5. Absolute and Relative Organ Weights of Male and Female Rats Treated Orally with SCE for 90 d

Items	Dosage (mg/kg/d)			
	0	225	450	900
Male				
Fasted body weight (g) [a)]	470.0±16.49	461.4±21.79	461.2±23.32	470.2±63.70
Heart (g)	1.331±0.125	1.279±0.066	1.314±0.138	1.365±0.182
% of body weight	0.283±0.026	0.278±0.018	0.284±0.017	0.291±0.023
Liver (g)	10.710±1.493	10.374±0.453	11.182±0.869	11.861±1.463
% of body weight	2.279±0.313	2.252±0.143	2.423±0.104	2.524±0.172
Spleen (g)	0.921±0.133	0.865±0.070	0.833±0.109	0.890±0.114
% of body weight	0.196±0.027	0.187±0.020	0.181±0.023	0.191±0.021
Lung (g)	1.771±0.227	1.663±0.133	1.770±0.081	1.890±0.114
% of body weight	0.377±0.045	0.360±0.045	0.384±0.021	0.398±0.061
Kidney (g)	2.665±0.420	2.653±0.146	2.679±0.201	2.728±0.225
% of body weight	0.564±0.082	0.575±0.019	0.581±0.030	0.601±0.043
Brain (g)	2.104±0.103	1.917±0.189	2.072±0.113	2.002±0.117
% of body weight	0.448±0.020	0.417±0.052	0.450±0.027	0.432±0.066
Stomach	1.816±0.424	1.942±0.209	1.854±0.488	1.968±0.270
% of body weight	0.386±0.058	0.422±0.052	0.394±0.013	0.422±0.024
Adrenal gland (g)	0.094±0.009	0.083±0.013	0.092±0.027	0.088±0.023
% of body weight	0.020±0.002	0.018±0.003	0.019±0.005	0.019±0.002
Thymus (g)	0.380±0.119	0.409±0.128	0.392±0.073	0.385±0.114
% of body weight	0.081±0.026	0.080±0.027	0.085±0.017	0.084±0.029
Testis (g)	3.419±0.299	3.367±0.310	3.353±0.170	3.373±0.326
% of body weight	0.728±0.059	0.733±0.094	0.728±0.050	0.718±0.106
Epididymis (g)	1.473±0.117	1.567±0.142	1.480±0.151	1.523±0.132
% of body weight	0.313±0.018	0.340±0.034	0.321±0.036	0.324±0.034
Prostate gland (g)	0.502±0.158	0.533±0.183	0.518±0.069	0.470±0.164
% of body weight	0.103±0.032	0.116±0.042	0.112±0.014	0.105±0.053
Female				
Fasted body weight (g) [a)]	286.0±9.187	287.6±26.16	277.4±10.43	279.20±11.30
Heart (g)	0.944±0.072	0.938±0.045	0.938±0.059	0.944±0.055
% of body weight	0.331±0.032	0.327±0.020	0.338±0.016	0.338±0.011
Liver (g)	7.458±0.882	7.192±0.948	7.243±0.415	7.483±0.918
% of body weight	2.613±0.354	2.495±0.153	2.611±0.063	2.674±0.220
Spleen (g)	0.668±0.055	0.641±0.096	0.639±0.061	0.622±0.191
% of body weight	0.234±0.024	0.222±0.016	0.230±0.016	0.221±0.064
Lung (g)	1.391±0.104	1.365±0.212	1.412±0.261	1.407±0.116
% of body weight	0.486±0.030	0.474±0.052	0.508±0.080	0.505±0.005
Kidney (g)	1.858±0.156	1.814±0.176	1.874±0.130	1.905±0.166
% of body weight	0.651±0.069	0.632±0.056	0.676±0.005	0.681±0.039
Brain (g)	1.873±0.140	1.960±0.153	2.008±0.071	1.984±0.089
% of body weight	0.654±0.032	0.684±0.050	0.724±0.015	0.711±0.035
Stomach (g)	1.649±0.142	1.707±0.208	1.642±0.128	1.666±0.053
% of body weight	0.578±0.066	0.593±0.030	0.592±0.036	0.597±0.024
Adrenal gland (g)	0.105±0.013	0.106±0.016	0.099±0.011	0.103±0.032
% of body weight	0.037±0.004	0.037±0.007	0.036±0.004	0.037±0.012
Thymus (g)	0.373±0.067	0.329±0.083	0.327±0.060	0.337±0.092
% of body weight	0.131±0.027	0.113±0.022	0.118±0.024	0.121±0.034
Uterus (g)	0.732±0.152	0.631±0.189	0.644±0.184	0.571±0.059
% of body weight	0.255±0.048	0.217±0.052	0.232±0.065	0.205±0.020
Ovary (g)	0.183±0.041	0.176±0.042	0.163±0.023	0.166±0.033
% of body weight	0.064±0.016	0.061±0.013	0.058±0.006	0.059±0.011

a) Body weight was measured immediately before necropsy after an overnight fast. Values are mean±S.D. for 10 rats per sex in each group.

RDW is an indicator of anemia and that decreased PLT can cause excessive bleeding. The prolongation of APTT and PT are associated with the risks of thrombosis and coagulation disorders. However, it should be mentioned that the overall clotting assays, such as the PT and APTT in our experiment, do not accurately mirror *in vivo* coagulation. Nevertheless, these assays are a readily available means for swiftly assessing the level of one or more clotting factors. SCE may be an inducer of coagulation disorders in rats, with some intrinsic factors of coagulation.

The slight increase in MO% in the female rats in the 900 mg/kg/d group was not regarded as an adverse effect of

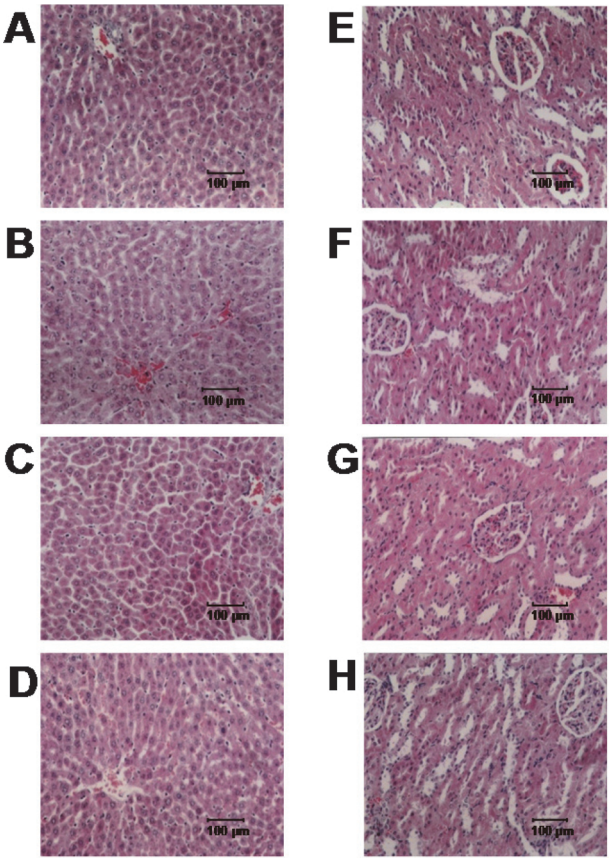


Fig. 4. Liver and Kidney Sections of Rats in 90-d Subchronic Oral Toxicity Test Showing No Gross Pathological Changes (H&E Stain, ×200)

A–H: Representative HE-stained histological sections of female rats (19 weeks old) in each experimental group; (A and E) liver and kidney sections of control rat; (B and F) liver and kidney sections of rat administered with 225 mg/kg/d dose of SCE; (C and G) liver and kidney sections of rat administered with 450 mg/kg/d dose of SCE; (D and H) liver and kidney sections of rat administered with 900 mg/kg/d dose of SCE.

the extract. The slight increase in CRN in female rats was not statistically significant, but the increase in CRN in male rats was considered to be related to SCE treatment. CRN, potassium and chloride increases in the treatment groups may indicate a metabolic disease or disorder, although in this study, there were no abnormal findings in the metabolic organs by histopathology analysis.

In this study, there were no obvious differences in the histopathological results in the heart, spleen, lungs, thymus, kidneys, brain, stomach, adrenal glands, uterus, ovaries, testis, epididymis or prostate of the treated rats compared to the control rats. PAs have been shown to induce apoptosis in rat hepatocytes,[38] and apoptosis of hepatocytes is the toxic characteristic in the liver of veno-occlusive disease (VOD), which has the histologic features of massive congestion, thickening of venous vessel walls, perivenular necrosis, fibrosis and signs of inflammation, *etc*.[8,39] The principal histopathological findings observed in the treatment groups were no organic pathologic changes in the liver and only a slight effect on liver functions, as indicated by changes in some hematological and serum biochemical parameters. However, the liver histopathological analysis indicated no hepatic toxicities at the dosages tested.

CONCLUSION

Overall, the results of this study clearly indicate that SCE did not produce major organ or general systemic toxicity when fed to male and female rats. The overall health, body weight gain, food consumption and clinical pathology parameters were similar between the rats fed SCE and the control rats. Although the liver was not identified as the target organ of SCE containing hepatotoxic PAs (mainly adonifoline), some parameters in metabolism were changed. The hepatotoxic effects of *Senecio scandens* have not always been made aware to the public, and this needs to be corrected.

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Conflict of Interest The authors declare no conflict of interest.

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