



Published in final edited form as:

Zhong Xi Yi Jie He Xue Bao. 2010 May ; 8(5): 438–447.

Preclinical safety evaluation of the aqueous acetone extract of Chinese herbal formula Modified Huo Luo Xiao Ling Dan

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Abstract

Objective—To investigate the safety of oral administration of Modified Huo Luo Xiao Ling Dan (HLXLD), a compound traditional Chinese herbal medicine.

Methods—The toxicological information of HLXLD and its individual constituent herbs was searched in *cintcm* or *TCMLars* (www.cintcm.com), PubMed (MEDLINE), Chinese Herbal Medicine (1999) and WHO Monographs on Selected Medicinal Plants (Vol. I—III). Single-dose acute toxicity was assessed by using the highest possible dosage. Motor function test was used to determine whether the herbal formula might cause motor impairment. Nine-day HLXLD repeat-dose sub-chronic toxicity/adverse effects, and 42-day chronic toxicity/adverse effects in rats were also assessed.

Results—The literature searches showed that HLXLD and its eleven ingredient herbs had no side/adverse effects listed in the traditional Chinese medicine literature. Under the dosages proposed in the formula, the HLXLD formula had no side/adverse effects according to MEDLINE, Chinese Herbal Medicine and WHO Monographs on Selected Medicinal Plants. The studies in rats showed: (1) in single-dose acute toxicity assessment, the maximal feasible single oral dose, 9.20 g/kg HLXLD, showed no significant effect on clinical signs, or body weight and mortality over a 14-day period in rats; (2) during motor function test, nine-day repeat-dose of daily HLXLD treatment at 4.60 g/kg did not cause motor impairment; (3) in nine-day HLXLD repeat-dose sub-chronic toxicity/adverse effects assessment, there were no noticeable abnormal behavioral changes or obvious adverse reactions and signs in complete Freund's adjuvant inflamed rats (highest observed dosage: 4.60 g/kg), and no noticeable adverse effects were observed during, or 14 days after nine-day treatment at 4.60 g/kg in non-inflamed rats; (4) during 42-day chronic toxicity/adverse effects assessments, no noticeable abnormal behavioral changes, no obvious adverse reactions and signs were observed in normal rats administered with HLXLD at a dose of 2.30 g/kg and the values of serum biochemistry and histopathology were in normal range.

Conclusion—Both existing information and animal data support that Modified HLXLD is a safe herbal product for clinical application.

Keywords

Chinese herb formula; Modified Huo Luo Xiao Ling Dan; Safety; Literature investigation; Acute toxicity tests; Motor function; Sub-acute toxicity; Chronic toxicity tests; Rats

Chinese herbal medicine, mainly in herbal formulations, is a major modality of traditional Chinese medicine (TCM) and has been used for thousands of years in China and other Asian countries to treat a variety of inflammatory diseases [1]. In recent years, the medicinal use of herbs, including Chinese herbs, has received increasing public interest in Western countries [2]. In 2002 alone, there was 19% of American adults using herbs for their health issues [3]. Although Chinese herbs have been used widely, their safety evaluation is not well conducted, especially in herbal formulations.

Herbal formulations, typically of 10 or more herbs, are commonly prescribed by Chinese herbalists, and numerous such formulas are well documented in ancient and modern Chinese literature [4–6]. Although the use of individual Chinese herbs also is prolifically documented [7–9], it is believed that a combination of herbs of various functions can augment the effectiveness of a treatment. According to the theory of Chinese herbalism, the interactions among the herbs may produce synergistic effects and neutralize potential toxicity or side effects of the individual constituents [1,10–12]. The Chinese formula Huo Luo Xiao Ling Dan (HLXLD) (fantastically effective pill to invigorate the collaterals), actually a group of formulations with the core herbs *Boswellia carterii* Birdw. (Ruxiang), *Commiphora myrrha* Engl. (Moyao), *Angelica sinensis* (Oliv.) Diels (Danggui) and *Salvia miltiorrhiza* Bge. (Danshen), is an effective and popular formula used in current clinical practice in treating various inflammation or pain disorders, and has been modified to suit the various clinical conditions or patient's constitution [13–22]. The main actions of this formula and its modifications are promotion of circulation, alleviation of pain and reduction of swelling [6,10]. In our previous studies, one of HLXLD formulas containing eleven herbs (Table 1) was used in arthritis and other inflammatory disorders, and the whole formula was proved having significant effects against inflammatory hyperalgesia and edema [23,24], and arthritis [25,26].

In the present study, we evaluated this formula by both literature review and experiments in rats. Specifically, the aims of the present study were to review the safety data of HLXLD related in literature, evaluate its acute toxicity, assess the effect on motor function, evaluate potential adverse effects and toxicity in inflammatory rats, arthritis rats and naive rats under repeat-dose treatments, and investigate its chronic toxicity in naive rats.

1 Materials and methods

1.1 Literature searches

The toxicological information from web sites, Chinese Herbal Medicine (1999) [9] and WHO Monographs on Selected Medicinal Plants (Vol. I — III) was searched [27]. The web sites included Chinese medicinal literature database known as cinctcm or TCMIars (www.cinctcm.com) and PubMed (MEDLINE). Three searches were conducted in the Chinese Medicinal Literature Database (cinctcm) and one search in PubMed for evidence of clinical use of HLXLD and side effects of the whole HLXLD formula or any of its individual constituent herbs. Search methods were as follows: (1) TCMIars was searched for Huo Luo Xiao Ling Dan and for each of the 11 constituent herbs in current HLXLD formulation; (2) The monographs in TCMIars (Chinese Materia Medica and Chinese

Formulary) of the 11 individual herbs in current formulation of HLXLD were searched; (3) One search was conducted in TCMLars for the keyword: “side effect” or “adverse effect”; (4) One PubMed search was conducted for side effects of HLXLD and each of the 11 constituent herbs in current formula of HLXLD. In Chinese Herbal Medicine (1999) [9] and WHO Monographs on Selected Medicinal Plants (Vol. I—III) [27], the toxicity and side effects of HLXLD related with 11 herbs were searched.

1.2 Animal assessments

1.2.1 Animal preparation—Sprague-Dawley rats weighing from 250 to 270 g (Harlan, Indianapolis, IN, USA) were purchased 1 week before the experiment and habituated to the living and testing environments. The rats were kept under controlled environmental conditions with temperature of (22±0.5) °C, relative humidity of 40% to 60%, 7 am to 7 pm alternate light- dark cycles, food and water ad libitum. The animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland School of Medicine, Baltimore. Ethical guidelines for the treatment of animals of the International Association for the Study of Pain were followed [28]. In most parts of current study, toxicity assessments were conducted in normal rats [29–31]. In observation conducted in rats with inflammation and hyperalgesia, the inflammation and hyperalgesia were induced by injecting complete Freund's adjuvant (CFA) (suspended in an 1:1 oil/saline emulsion, 0.08 mL, 0.04 mg heat-killed Mycobacterium tuberculosis) into the plantar surface of left hind paw of the rat subcutaneously with a 25-gauge hypodermic needle [32], one hour after HLXLD or vehicle administration in the second day. CFA-inflamed rats showed normal grooming behavior and levels of activity, and the effect of hyperalgesia on their normal behavior seemed minimal.

1.2.2 HLXLD extract preparation—Herbs used in the HLXLD were purchased from the Tongrentang Pharmaceutical (Beijing, China) and identified at the Institute of Materia Medica, Chinese Academy of Medical Sciences in Beijing, using the latest Pharmacopoeia of the People's Republic of China (2000) [13]. They were ground into powder (through 80-mesh screen), and both non-polar and polar components were completely extracted with 70% aqueous acetone at room temperature. Evaporation temperatures were kept below 55 °C to minimize the possible breakdown of thermo-labile compounds that may be present in extracts. This initial crude extract was concentrated under reduced pressure, and the dried residue was coded, weighed, and divided into two portions: one for bioassay studies, the other to be deep-frozen for future reference. The weight of the final HLXLD extract was about 25% that of the raw herbs. The quality of the extract was monitored by high-performance liquid chromatography (HPLC) to assure lot-to-lot consistency. Each individual herb was traced, and its major representative peaks were identified [26].

1.2.3 Toxicity and adverse effects assessments—Assessment encompassed the following tests or observations: (1) Single-dose acute toxicity was assessed by using the highest possible dosage; (2) Motor function test to determine whether the herbal formula might cause motor impairment; (3) Nine-day repeat-dose sub-chronic toxicity or adverse effect assessment; (4) Forty-two-day repeat- dose chronic toxicity or adverse effect assessment. The studies were carried out in compliance with the guidelines and guidance for developing new herbal medicines or botanic drugs in USA and China [29–31]. Herb extraction or vehicle administration was performed gently and swiftly, and no analgesics were required for the procedure. All assessments and observations were performed by investigators who were blinded to treatment group assignments.

1.2.3.1 Single-dose acute toxicity assessment: Twenty rats, half male and half female, were treated with a single dose of HLXLD at 9.20 g/kg, which is the maximal feasible dosage in

rats — the maximal amount that can be given intragastrically (i.g.) considering the volume of the rat's stomach [30,33]— that can be dissolved in a 4 mL vehicle, this being the maximal volume of sesame oil that did not cause diarrhea in the rats. Mortality, clinical signs, body weight and behavioral activities were monitored for 14 days after herbal administration. After the observation period, all rats were euthanized and gross necropsy was performed as described in literature [34–36].

1.2.3.2 Motor function test: Rats given HLXLD extract treatments of 4.60 g/kg or vehicle (n=4 per group, i.g.) were tested for motor function, by a method described by Wei et al [37]. HLXLD extract was dissolved in a 2 mL mixture of distilled water and sesame oil (2:1), and administered once a day (i.g.) by using a 5 mL syringe with a 4 cm long gavage needle through the mouth to the stomach. Control rats received 2 mL vehicle only. Each rat received one dose of HLXLD or vehicle daily for subsequent 9 days [24], and the motor function was tested at the same time points as the paw withdrawal latency were tested in our previous HLXLD anti-inflammatory studies [24], i.e., in the first day, immediate before and one hour after HLXLD administration (one day before CFA injection); the second day, three and six hours after CFA injection (the day CFA injected, i.e., 0 d); the third, the fifth, the seventh and the ninth day (i.e., 1, 3, 5 and 7 d, post-CFA injection) immediate before and one hour after HLXLD administration. An accelerating rotarod treadmill (IITC Model 720A Woodland Mills, CA) accelerated to full speed within 30 seconds. The timer was set to zero when the rat was in position and started when the rod began to rotate. When the rat fell off the rod onto a platform, a switch was triggered and the timer stopped. The time of the fall was recorded, and a 60-second cutoff was used. Prior to the formal test, three one-hour training sessions were given to acclimate the animals to the rotarod apparatus. Rats were tested three times at 10-minute intervals, at 2.3 r/min, then 15.0 r/min, and finally at 45.0 r/min.

1.2.3.3 Nine-day repeat-dose sub-chronic toxicity and adverse effects assessment: Standard pharmacological categories of toxic or adverse behavioral reactions were adopted in our experiment according to the method [33,35] during the 9 days of herbal treatments, which was the same as in our previous HLXLD anti-inflammation study [24], both CFA-inflamed rats and normal rats used in the motor function test were closely monitored for unusual behavioral changes, loss of appetite, diarrhea, weight loss, vomiting, fur discoloration, sedation, irritation, and convulsion. To detect adverse effects that may occur some time after the last administration of a drug [38,39], the rats used in the motor function test were similarly observed for another 14 days after the last HLXLD administration. In the observations conducted in inflamed rats, rats were randomly divided into four HLXLD treatment groups (0.575, 1.15, 2.30 and 4.60 g/kg per day, extract weight) and one control group (n=8 per group). Each dose was dissolved in a 2 mL mixture of distilled water and sesame oil (2:1) and administered once a day (i.g.) by using a 5 mL syringe with a 4 cm long gavage needle through the mouth to the stomach. Control rats received 2 mL vehicle only. Each animal received one dose of HLXLD or vehicle daily for 9 days: the first, one day before the CFA injection; the second, one hour before; and then, once on each of the 7 subsequent days after CFA injection. After the observation period, all rats in the HLXLD-treated and control groups and motor function groups were euthanized, and gross necropsy was performed.

1.2.3.4 Forty-two-day chronic toxicity and adverse effects assessment: Thirty normal rats were randomly divided into HLXLD group (20 rats, half male and half female) and vehicle control (10 rats, half male and half female). The treatments, either HLXLD (2.3 g/kg per day) or vehicle (same amount of distilled water and sesame oil, 2:1, as that in HLXLD-treated group), were given (i.g.) for consecutive 42 days which is the same as that in our

proposed clinical trial in future. The same items above, i.e. behavior changes, clinical signs were monitored during this period. The blood samples were collected by cutting-tail method, before using HLXLD or vehicle at baseline and at the end of experiment under the anesthesia (sodium pentobarbital, 80 mg/kg, intraperitoneal injection). After the observation period, all rats in the HLXLD-treated and control groups were euthanized, gross necropsy and histopathological studies were performed.

1.2.3.5 Blood biochemistry test: The blood biochemistry tests were conducted in another independent laboratory, the research animal diagnostic laboratory in University of Missouri, for detecting if HLXLD affects the function of liver, kidney and heart. The sera alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, and blood urea nitrogen (BUN), creatinine (Cr), lactate dehydrogenase (LDH), creatine kinase (CK), total protein, albumin and globulin contents were tested by using commercial kits.

1.2.3.6 Gross necropsy and histopathology: Each rat was subjected to a full necropsy including examination of the external surface of the body, all orifices and the thoracic, abdominal and cranial cavities and their contents. The organs of brain, lungs, heart, liver, kidneys, spleen, stomach, adrenals, testes and ovaries etc. were checked immediately after the dissection. And the tissues of liver, kidneys, heart and spleen from each rat in chronic toxicity study were preserved in 10% neutral buffered formalin for histopathological examination. Gross lesion was also examined, if noted in any test group. The preserved tissues were buried in paraffin, and 5 μ m-thick sections were cut and were stained with the standard haematoxylin and eosin (HE) method. The histopathological observations were conducted under a light microscope (Nikon E600; magnification, $\times 20$). The investigator responsible for the histopathological observations was blinded to the treatment assignment of each animal.

1.3 Statistical analysis

The number of animals used for each specific test was estimated via a power analysis using either our own preliminary data or published results employing similar experimental procedures and physiologic parameters [40]. The results were presented as $\bar{X} \pm s$. In motor function test, one-way ANOVA was used; all post hoc comparisons were conducted by using the Dunnett test. In blood biochemistry tests, student-t test was used to reveal significant differences between groups. $P < 0.05$ was considered significant in all cases.

2 Results

2.1 Literature searches

In database of TCMLars, there were 151 papers published in Chinese between 1998 and 2003 on HLXLD. No descriptions of side effects or adverse reactions were found for any whole formula or for any individual constituent herbs in the formula. Almost all papers were related to modified HLXLD, because the cultural norms of treatment involved individualized decoctions rather than standardized products. The main diseases or disorders that were treated by the formula were various forms of arthritis, sciatic nerve pain, post-traumatic pain syndrome, ischemic heart disease, stroke, and some conditions in obstetrics and gynecology. There were several laboratory experiments testing HLXLD. However, no experiments focused on the safety aspects of it. There were several similar formulations as current HLXLD were searched in these studies. The monographs in TCMLars (Chinese Materia Medica and Chinese Formulary) of the 11 individual herbs in the current formulation of HLXLD were searched, which showed no side effects were listed.

The search was conducted in TCMLars for the keyword “side effect” or “adverse effect”. There were 66 papers reporting either side effects of any herbs, acupuncture, massage or qigong, or the correction of side effects of chemotherapy or hormone therapy by the use of TCM therapy. No side effect or adverse effect was related to current HLXLD or its similarities, or any of its 11 constituent herbs in the formulation proposed for study.

The PubMed (MEDLINE) search was conducted for side effects of HLXLD and for each of the 11 constituent herbs in the formula of HLXLD proposed for study. There was no literature about side or adverse effects related to HLXLD. However, three of the constituent herbs had concerns registered: (a) Gancao (Glycyrrhiza uralensis Fisch.) may cause hypertension or water-sodium retention mainly in hypertension patients, when at a dose over 50 g per day [41]. The formula proposed for study has a dosage of 3 g per day. (b) Danggui (Angelica sinensis (Oliv.) Diels.) may increase photosensitivity, so individuals taking the herb should be cautioned that too much exposure to sunlight may result in a rash. Also, it has been reported to potentiate the effects of warfarin and should be kept to doses under 5 to 30 g per day. It should not be used during pregnancy [42,43]. (c) Yanhusuo (Corydalis ternate W.T. Wang) has been found to potentially influence motor function in rats, when used by intraperitoneal injection as opposed to the oral route of delivery proposed in current study [37].

The search in Chinese Herbal Medicine (1999) [9] and WHO Monographs on Selected Medicinal Plants (Vol. I — III) [27] showed there were no adverse effects when the daily dose of each 11 constituent herbs was at the level of current HLXLD. The acute toxicity data of these 11 herbs listed in these two kinds of literature are shown in Table 1.

In summary, HLXLD and its 11 ingredient herbs have no side or adverse effects listed in the Chinese TCM literature. Under the dosages proposed in current formula, the HLXLD formula has no side or adverse effects according to literature found in MEDLINE, Chinese Herbal Medicine (1999) [9] and WHO Monographs on Selected Medicinal Plants (Vol. I — III) [27].

2.2 Toxicity and adverse effects of HLXLD

2.2.1 Single-dose acute toxicity assessment—The maximal feasible single oral (i.g.) dose, 9.20 g/kg HLXLD, showed no effect on animal clinical signs, or body weight in any of the 20 animals observed over a 14-day period, and no rat died during the experiment. Gross necropsy revealed no damage to organs or tissues, and no stomach ulcer or bleeding foci were found.

2.2.2 Effect of HLXLD on motor function—At the baseline, there was no statistical difference between the two groups at three different speeds. During the 9 days of daily HLXLD treatment at dose of 4.60 g/kg, speed of 2.3 r/min, no significant changes were observed after treatment as compared with baseline or control. The durations of rats staying at the rotarod treadmill were all over 60 seconds, and the durations at the speeds of 15.0 and 45.0 r/min are shown in Table 2.

2.2.3 Nine-day repeat-dose sub-chronic toxicity or adverse effect assessment—During the 9 days of daily HLXLD treatment, there were no noticeable abnormal behavioral changes or obvious adverse reactions and signs. No sedation, fur discoloration, diarrhea or weight loss was observed in either vehicle control or HLXLD-treated (0.575 to 4.60 g/kg) groups in inflamed rats, and normal rats (4.60 g/kg), nor were any obvious late effects seen during 2 weeks after nine daily repeat-dose HLXLD oral administration at 4.60 g/kg. No rat died during the assessment period. Gross necropsy showed no damage to organs or tissues, and no stomach ulcer or bleeding foci were found.

2.2.4 Forty-two-day chronic toxicity or adverse effect assessment—During the 42 days of repeat-dose HLXLD (2.30 g/kg) treatment in normal rats, no noticeable abnormal behavioral changes, no obvious adverse reactions and signs, such as sedation, fur discoloration, diarrhea and weight loss, were observed in either vehicle control or HLXLD-treated groups. One rat in the HLXLD-treated group died in the 6th day. Gross necropsy showed that the esophagus was injured, caused by a feeding accident. In other rats, gross necropsy showed no damage to organs or tissues, and no stomach ulcer or bleeding foci were found.

2.2.5 Blood biochemistry test—No abnormality was found in serum biochemistry assays, all data of ALT, AST, BUN, Cr, LDH, CK, total protein, albumin and globulin were in rats' normal ranges [44], and there were no significant difference between HLXLD (2.30 g/kg per day) group and vehicle group in baseline and end of experiment (Table 3 and 4), in 42-day chronic toxicity or adverse effects assessments. Comparing the data at the end of experiment with its baseline, there were some increases in ALT and AST activities, and total protein, albumin, globulin and creatinine contents, and decrease in LDH activities both in HLXLD and vehicle groups, which may be caused by spontaneous changes in Sprague-Dawley rats at this age.

2.2.6 Microscopic examination—There were no microscopic findings that could be attributed to the administration of the test HLXLD extract. Mild cloudy swelling in the hepatic cells was found both in the HLXLD-treated (4/20 rats) or vehicle-treated rats (3/10 rats).

3 Discussion

The present study demonstrated that in neither Chinese medicinal literature nor Western medical literature there were reports related to the toxicity or adverse effects of Chinese herbal formulation of HLXLD; and at the dose level of the HLXLD, there were no reports related to the toxicity and adverse effects of its 11 constituent herbs, although HLXLD and its modification have been used extensively.

The animal study demonstrated that the single oral administration of HLXLD reached 9.2 g/kg, and this maximal feasible dose in rats did not cause any adverse effect during 14-day observation. This indicated HLXLD had a very high safety range in single oral administration, which was far higher than its constituent, *Boswellia carterii* (Ruxiang), maximal feasible dose of which was 2.5 g/kg [23]. The motor function test showed HLXLD had no effect in causing motor impairment. This indicated that HLXLD had no significant negative effect on motor system and that the paw withdrawal latency increase observed in the previous study [24] was due to pharmacological anti-hyperalgesic effects. During nine-day repeat-dose administration both in inflamed rats and normal rats, HLXLD had no observable adverse effects during or after the treatments, even at the highest dosage of 4.60 g/kg per day with the *Boswellia carterii* at 0.90 g/kg which would cause some adverse effects [23,24]. *Boswellia carterii* at 1.80 g/kg per day is very toxic [23], and current study indicated safe range of HLXLD is much higher than its constituent *Boswellia carterii*. During 42-day repeat-dose treatment, HLXLD had no observable toxicity or adverse effects, either in clinical signs, behavior changes, blood biochemistry, or histopathology. It indicated HLXLD was safe in rats when orally used at 2.30 g/kg per day for 42 consecutive days.

Herbal products generally have been used in the types of capsule or pills in America and other Western countries, instead of “herbal tea”, due to the culture tradition. Considering the application and possibility in daily life, the maximal feasible dosage of capsule or pills in real clinical practice should be 1/5 to 1/4 of the dosage in decoction, i.e. 5 g extract or 14

capsules (number 0 capsule). Therefore, the dosage 2.30 g/kg per day is equivalent to about 5 times of maximal feasible dosage of capsule or pills, which could be used in patients. Our data is over-qualified to the requirements of U.S. Food Drug Administration (FDA), which states using a maximum feasible dose and being tested by using the same route of administration as proposed for clinical use, and the duration at least equal to that of the clinical trial [29].

It should be noted that the primary objective of long-term, repeat-dose toxicity studies in animals is to identify the organs and (or) systems that are the targets of the herbal product's toxicity and the threshold dosages of producing toxic effects. The studies provided valuable information for designing long-term clinical studies at safe doses, with appropriate monitoring for predicted adverse reactions. Existing literature on the animal toxicity of herbal product is often limited to single-dose acute studies. These studies would be inadequate to support long-term use. Most of toxicological studies were in individual herb or herbal ingredient, such as purified part, or single chemical [27,31] and, lots of these studies used other than normal clinical route of administration, such as inter-peritoneal route. The information obtained from these studies may not be tightly related to real Chinese herbological practice, especially in herbal formulations. Present study integrated literature review both in formula and individual herb, and animal studies of formula both in normal and disease model animal, which may provide more valuable information in formula toxicity or adverse effect evaluation for clinical application.

It is worth noting that the extract of HLXLD 9.20 g/kg per day in rats is 10 times, 4.6 g/kg is 5 times and 2.3 g/kg is 2.5 times as that used in human daily clinical practice, considering the extract rate in a hot water extract or decoction. Our data provided global information, from literature to animal study, for further HLXLD clinical safety evaluation. However, further investigation on reproductive and developmental toxicity, carcinogenicity, genotoxicity of this formula is needed.

Part of above safety evaluations were briefly reported in former HLXLD efficacy studies [24,26]. The protocol of this preparation and safety evaluations were used to obtain FDA approval of this modified HLXLD as an investigational new drug for a phase I clinical trial, and a phase II clinical trial is being investigated.

Acknowledgments

We thank Dr. Lyn Lowry for her editorial support. This study was supported by NIH grant P50-AT00084

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Table 1

The components of HLXLD and acute toxicity data in literature of its constituents

No.	Chinese herb name in pinyin [Botanic name]	Plant part	Human dose (g) [*]	Extract proportion	Oral administration acute toxicity ^{**}
(1)	Ruxiang [<i>Boswellia carterii</i> Birdw.]	Gum-resin	15	17.8%	N/A
(2)	Qianghuo [<i>Notopterygium incisum</i> Ting ex H. T. Chang.]	Rhizome and root	12	9.5%	MFD: Water exact 12 g/kg
(3)	Danggui [<i>Angelica sinensis</i> (Oliv.) Diels.]	Root	12	11.6%	N/A
(4)	Baishao [<i>Paeonia lactiflora</i> Pall.]	Root, rootlets and bark	12	10.0%	MFD: 6 g/kg (methanol extract)
(5)	Gancao [<i>Glycyrrhiza uralensis</i> Fisch.]	Root	12	9.7%	N/A ^{***}
(6)	Yanhusuo [<i>Corydalis yanhusuo</i> W.T.Wang]	Root	12	1.8%	LD50: 100 g/kg
(7)	Danshen [<i>Salvia miltiorrhiza</i> Bge.]	Root	12	1.8%	N/A ^{***}
(8)	Chuanxiong [<i>Ligusticum chuanxiong</i> S.H. Qiu.]	Root	12	9.2%	MFD: 6 g/kg (methanol extract)
(9)	Qinjiao [<i>Gentiana macrophylla</i> Pall.]	Root	12	10.0%	LD50: 18.96 g/kg (water extract); 17.36 g/kg (ethanol extract)
(10)	Guizhi [<i>Cinnamomum cassia</i> Presl.]	Twig	15	4.1%	MFD: 50 g/kg (water extract)
(11)	Duhuo [<i>Angelica pubescens</i> Maxim.]	Root	12	14.4%	N/A
Total	11 herbs		138	100%	N/A

N/A: Not available; MFD: Maximal feasible dose; LD50: Half lethal dose;

^{*} This daily dose is for raw herb and for using in a formula;^{**} Data were in rats or mice, from latest Chinese Herbal Medicine (1999);^{***} Literature has a special note that oral administration of this herb is safe.

Table 2Motor function test with rotarod treadmill in two groups ($\bar{x}\pm s$, s)

Group	n	Time of fall off					
		-1 d I	-1 d II	3 h of 0 d	6 h of 0 d	1 d I	1 d II
Control							
15 r/min	4	57.89±2.2	58.33±0.33	54.33±6.89	58.33±3.33	58.75±1.73	59.92±0.17
45 r/min	4	44.11±14.11	50.17±9.13	51.50±7.38	52.25±7.34	48.17±9.25	47.67±12.76
HLXLD							
15 r/min	4	60.00±0.00	59.91±0.16	60.00±0.00	58.58±2.83	58.58±2.83	59.92±1.67
45 r/min	4	56.13±4.95	54.50±6.56	53.42±7.6	53.83±7.20	52.83±11.25	52.50±8.69

Group	n	Time of fall off					
		3 d I	3 d II	5 d I	5 d II	7 d I	7 d II
Control							
15 r/min	4	59.92±0.17	59.33±1.33	56.08±4.57	56.58±3.95	60.00±0.00	56.67±5.23
45 r/min	4	49.75±7.59	51.58±10.21	46.83±10.54	46.92±9.17	50.75±7.05	48.08±13.79
HLXLD							
15 r/min	4	59.08±1.83	57.50±3.79	56.17±4.46	58.33±3.33	59.00±2.00	58.33±3.33
45 r/min	4	52.25±10.27	48.67±9.36	50.42±11.07	51.83±9.46	46.33±12.13	50.75±7.77

Notes: -1 d, the time point 1 day before CFA injection used in a inflammation rat model; 3 h of 0 d, 3 hours after HLXLD or vehicle administration; 6 h of 0 d, 6 hours after HLXLD or vehicle administration; 1 d, 3 d, 5 d and 7 d: the time points 1, 3, 5 or 7 days after CFA injection used in a inflammation rat model; I, immediate before HLXLD or vehicle administration; II, 1 hour after HLXLD or vehicle administration. There were no significant changes in motor function after HLXLD administration for 9 consecutive days at rotarod treadmill speed of 15 r/min ($F=0.99$, $P=0.49$) or 45 r/min ($F=0.30$, $P=0.99$).

Table 3

Serum ALT and AST activities and contents of total protein, albumin and globulin in HLXLD- and vehicle-treated rats ($\bar{x}\pm s$)

Group	n	ALT (IU/L)	AST (IU/L)	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
HLXLD						
Baseline	20	44.45±1.23	114.20±2.95	6.32±0.06	3.63±0.03	2.69±0.04
End	20	69.44±2.99**	153.06±8.43**	8.02±0.22**	4.50±0.12**	3.51±0.11**
Control						
Baseline	10	45.70±2.28	115.50±5.76	6.43±0.13	3.70±0.06	2.73±0.08
End	10	65.20±5.21**	146.70±11.62	7.79±0.20**	4.47±0.10**	3.32±0.10**

** $P<0.01$, vs baseline.

Table 4Serum LDH and CK activities and BUN and creatinine contents in HLXLD- and vehicle-treated rats ($\bar{x}\pm s$)

Group	<i>n</i>	LDH (IU/L)	CK (IU/L)	BUN (mg/dL)	Creatinine (mg/dL)
HLXLD					
Baseline	20	1 619.20±92.88	931.05±99.26	0.47±0.04	19.15±1.84
End	20	937.05±119.33 **	1 094.68±290.13	0.51±0.02	25.31±0.67 **
Control					
Baseline	10	1 693.10±167.63	883.40±85.69	0.59±0.09	22.5±3.15
End	10	926.30±107.45 **	630.90±143.90	0.52±0.02	24.9±1.39

** $P < 0.01$, vs baseline.