

## Animals

Total fifteen rabbits (2.0~2.5Kg, 5 males and 10 females, SCXK (SU) 2010-0002) were acquired from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai). The experimental animals were allowed free access to water and mouse chow, and were housed under controlled environmental conditions (constant temperature, humidity, and 12h dark/light cycle). Rabbits were acclimated to the environment for 7 days before the experiment.

**Tab. 1 Groups**

Groups	Concentration (g/mL)	Dilution ratio	Dose volume
DLS	VB 0.0004, CB 0.002, RG 0.1, AM 0.3		
VB	0.0004		
CB	0.002	1:10	3mL/Kg
RG	0.1		
AM	0.3		

## Experimental protocol

Five groups of total fifteen rabbits received DLS, VB, CB, RG, and AM, the dosages were listed in table 1. The infusion was single administrated, and the congestion, dropsy, hemorrhage and putrescence were visually observed on the injection site after administration. The rabbits were ethically sacrificed after 48h observation, and a piece of vascular tissue on the injection site was removed for histopathological examination.

In this experiment we selected the self-control, the left ear of the rabbit to received the tested drug and the right ear received 0.9% sodium chloride injection as control. Symptoms 0 for no obvious irritation; 1 for erythema; 2 for edema; 3 for ulcers; Severity: + mild; ++ obvious; +++ severe.

## Results

Macroscopic observation showed that, after the single injection to rabbits, the ear vein in DLS, VB and CB showed different degrees of erythema. The single herb group exhibit less irritation symptom than DLS, DLS exhibited the strongest irritation symptom, followed by VB and CB. No irritation symptoms such as erythema, edema, and ulcer were observed in RG group, AM group and the control group.

Pathological examination showed that DLS was also the most intent irritation, followed by VB and CB. The main pathological features were vascular endothelium swelling, vascular edema, bleed and inflammatory cell infiltration. No pathological changes were found in RG group, AM group and the control group.

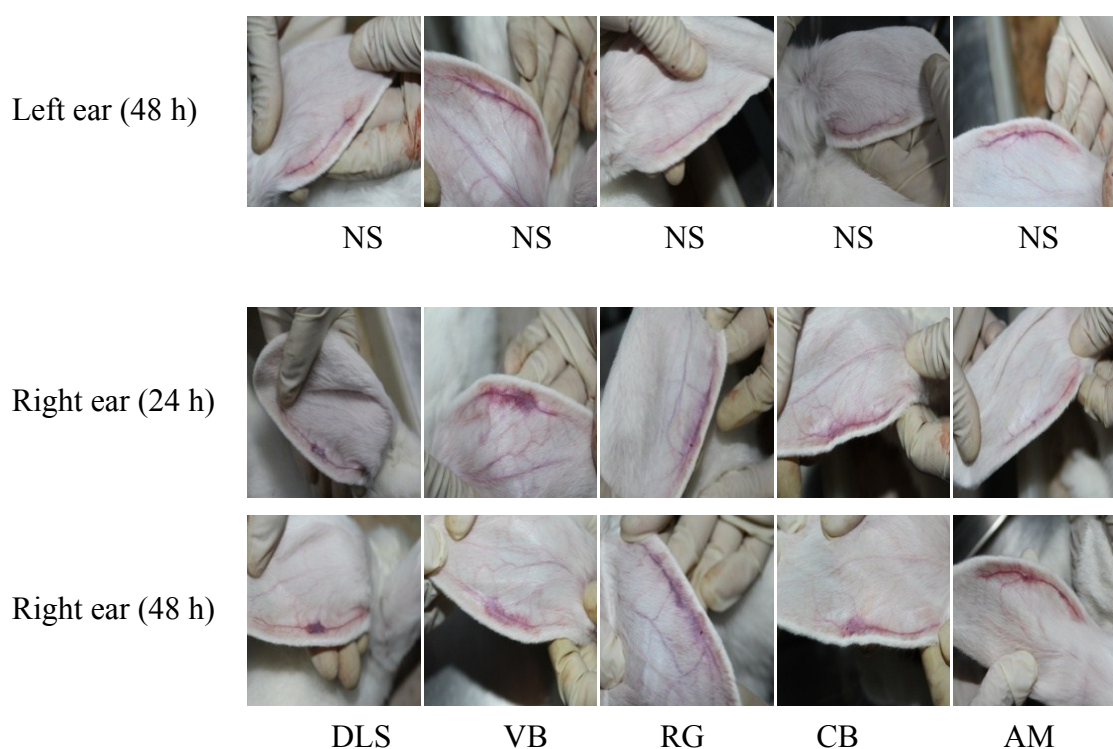
Considering the macroscopic observation and the histopathological results: DLS, VB and CB all exhibited different degrees of irritation on the rabbit ear blood vessels, DLS is the most severity (there are individual differences), followed by VB and CB, The order is DLS> VB>CB.

**Tab. 2 Visual evaluation of the vascular irritation test**

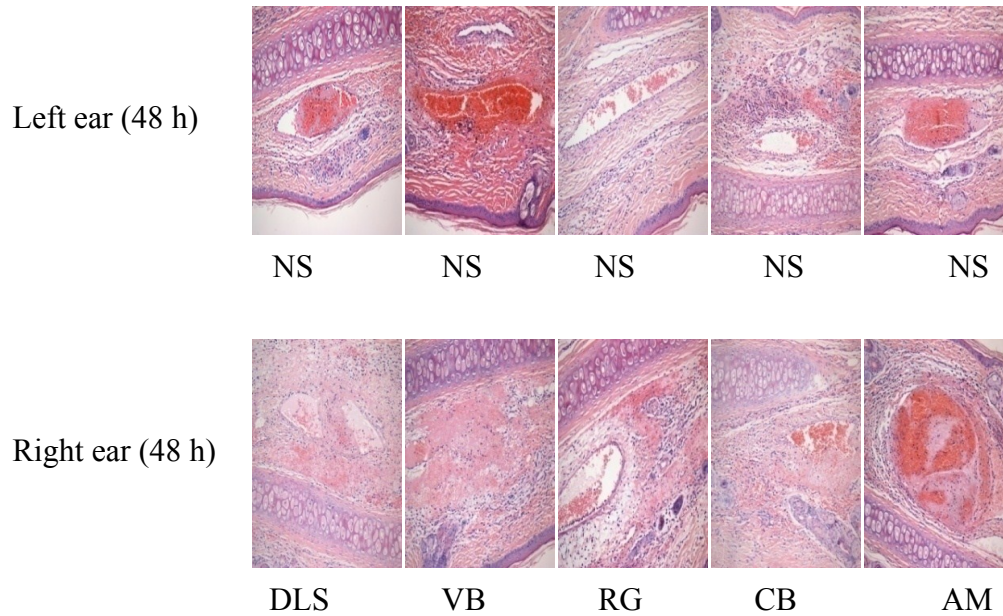
Drugs	Number	Weight(kg)	Sex	The rabbit ear	Groups	Before	24 h	48 h
DLS	1	2.1	♂	left	administration	0	1(++)	1(++)
				right	control	0	0	0
	2	2.5	♀	left	administration	0	1(++)	1(+++)
				right	control	0	0	0
	3	2.0	♀	left	administration	0	1(++)	1(++)
				right	control	0	0	0
4	2.0	♀	left	administration	0	1(+)	1(+)	
			right	control	0	0	0	
CB	5	2.1	♂	left	administration	0	1(+)	1(+)
				right	control	0	0	0
	6	2.3	♀	left	administration	0	1(++)	1(++)
				right	control	0	0	0
	7	2.2	♂	left	administration	0	1(++)	1(++)
				right	control	0	0	0
8	2.0	♀	left	administration	0	1(++)	1(++)	
			right	control	0	0	0	

	9	2.1	♀	left	administration	0	1(+)	1(+)
				right	control	0	0	0
	10	2.3	♀	left	administration	0	1(+)	1(+)
				right	control	0	0	0
RG	11	2.2	♀	left	administration	0	1(+)	1(+)
				right	control	0	0	0
	12	2.0	♂	left	administration	0	1(++)	1(++)
				right	control	0	0	0
	13	2.1	♀	left	administration	0	0	0
				right	control	0	0	0
AM	14	2.1	♂	left	administration	0	0	0
				right	control	0	0	0
	15	2.2	♀	left	administration	0	0	0
				right	control	0	0	0

The right ear received 0.9% sodium chloride injection as control. Symptoms 0 for no obvious irritation; 1 for erythema; 2 for edema; 3 for ulcers; Severity: + mild; ++ obvious; +++ severe.



**Fig. 1 Visual observation of the vascular irritation test**



**Fig. 2 Pathological observation of the vascular irritation test**

**Tab. 3 List of antibodies used in the experiments**

Antibody	Number	Source	Supplier	dilution
TLR2	#13744	Rabbit	Cell Signaling Technology	1:1000
TLR4	#14358	Rabbit	Cell Signaling Technology	1:1000
MyD88	#4283	Rabbit	Cell Signaling Technology	1:1000
p-Erk	#4370	Rabbit	Cell Signaling Technology	1:2000
Erk	#4695	Rabbit	Cell Signaling Technology	1:1000
p-JNK	#4668	Rabbit	Cell Signaling Technology	1:1000
JNK	#9252	Rabbit	Cell Signaling Technology	1:1000
p-P38	#4511	Rabbit	Cell Signaling Technology	1:1000
P38	#8690	Rabbit	Cell Signaling Technology	1:1000
p-NF- $\kappa$ B p65	#3033	Rabbit	Cell Signaling Technology	1:1000
NF- $\kappa$ B p65	#8242	Rabbit	Cell Signaling Technology	1:1000
p-I $\kappa$ B $\alpha$	#2859	Rabbit	Cell Signaling Technology	1:1000
I $\kappa$ B $\alpha$	#4814	Mouse	Cell Signaling Technology	1:1000