

SECTION 2

Toxicological tests of *VasoCleaner*TM in Mice and Rats

File#1 provides the toxicological data of *VasoCleaner*TM in Mice and Rats. The toxicological studies were carried out at Beijing Medical University of Chinese Medicine, Medicine Research and Testing Center in 1993. This Center is one of the most reputable testing centers in China. The toxicological data indicated that *VasoCleaner*TM is such a safe formulation that the LD₅₀ could not be established in mice. The maximum tolerance dose in mice is larger than 11.04 g/Kg, which is about 66.24 times of the suggested clinical effective dose in human. The formulation was also tested in rats for 27 weeks at the dosage that is about 209 times higher than that used in human. Neither pathological change nor toxicological response was observed in these animal organs.

File # 1**Toxicological Tests of *VasoCleaner*TM****Abstract**

Acute and chronic toxicity of *VasoCleaner*TM were evaluated in mice and rats respectively. The median lethal dose (LD₅₀) for mice could not be obtained for the experimental setting due to the extremely low level of toxicity. The maximal drug-tolerance dose is 11.04g/kg for mice, which is 66.24 times the clinically effective dose. Chronic toxicity tests in rats showed that both physiological conditions, including body weight, food consumption and activity, and organ weights were normal. Furthermore, pathogenic inspection did not find any abnormality. The toxicity studies indicate that *VasoCleaner*TM is practically non-toxic. It has neither side effects nor delayed toxic reaction.

Key words: *VasoCleaner*TM, toxicological research, mice and rats

1. Acute Toxicity of *VasoCleaner*TM in Mice

Introduction: Since the median lethal dose (LD₅₀) of *VasoCleaner*TM would not be obtained, the maximum drug-tolerance dose was determined instead in mice.

1.1 Aims: Acute toxicity test of *VasoCleaner*TM

1.2 Materials: *VasoCleaner*TM was produced by Jinghai Pharmaceutical Company (Lot# 950088), and the mice were provided by the animal center at the China Academy of Military Medical Science.

1.3 Methods: Twenty Kunming mice (17.5-20g) were orally given *VasoCleaner*TM solution (prepared by dissolving granules in distilled water) of body weight through a feeding needle. All mice were fed with normal mouse chow (refined mouse food) per day. The drinking water for all animals was *ad libitum* during the entire seven-day experiment. Room temperature for the mice was kept from 26 to 28°C.

1.4 Statistical analysis: Results are expressed as mean ±SD (standard deviation). The statistical difference between two groups was determined using Student's t-test. A value of P<0.05 was considered significant.

1.5 Results

The maximum drug-tolerance dose is 11.04g/kg for mice, which is 66.24 times the clinically effective dose. Among the tested mice, there were no side effects. No abnormalities in heart, liver, spleen, lung, kidneys, or intestine were found based on the pathogenic inspections. No significant weight change in mice was observed after the administration of *VasoCleaner*TM (Table 1).

Table 1 Body weight changes in mice (g, $\bar{X} \pm SD$)

N	Before administration	After administration
20	18.80±0.94	22.45±1.32

N represents the number of tested animal.

2. Chronic toxicity of *VasoCleaner*TM in Rats

2.1 Materials and Methods

2.1.1 Materials: *VasoCleaner*TM was produced by Jinghai Pharmaceutical Company (Lot# 950088), and the rats were provided by the animal center at the China Academy of Military Medical Science.

2.1.2 Methods: 120 five-week-old Wister rats (110±10g in body weight) were used for these studies.

Rats were randomized to four groups: (I) High-dose group (30 rats), 34.8g of *VasoCleaner*TM per kilogram of body weight a day; (II) Medium-dose group (30 rats), 25.6g of *VasoCleaner*TM per kilogram of body weight a day; (III) Low-dose group (30 rats), 12.8g of *VasoCleaner*TM per kilogram of body weight a day; (IV) Saline group (30 rats), 2.0ml of physiological saline per 100g of body weight a day. *VasoCleaner*TM granules were dissolved in water and orally administered to rats by feeding needle. And physiological saline was used as control. Drinking water for all animals was *ad libitum* throughout the entire study.

2.1.3 Observations

2.1.3.1 Activity and food consumption: The activity of the rats was observed on a daily basis. The amount of food consumed daily was also recorded.

2.1.3.2 Blood tests: On day 0, which is the very beginning of the experiment, and on day 45, 90, 175, blood was collected from the tail of each rat to examine the function of liver and kidneys through checking the level of glutamic pyruvic transaminase (GPT) and blood urea nitrogen (BUN). The level of the blood sugar, the number of white-blood cells and red-blood cells, the amount of hemoglobin, whole protein and albumen in the serum were also determined.

2.1.3.3 Weight of organs: At the end of the 12th week and the end of the experiment, a certain number (see Table 13 for detail) of rats were sacrificed to determine the weight of the heart, liver, kidneys, spleen, brain, adrenal gland, uterine and testicle. Statistical analysis of the organ to body weight ratio was performed.

2.1.3.4 Pathogenic examination: At the end of the 12th week, six rats were picked randomly from each group and sacrificed. Pathogenic examination of the heart, liver, spleen, kidneys, stomach, and intestine were performed right after. At the end of the experiment, the remaining rats, except those for the continued observation of delayed toxicity, were all sacrificed for the pathogenic examinations of the above-mentioned organs.

2.1.4 Statistical analysis: Results are expressed as mean±SD (standard deviation, $\bar{X} \pm SD$). The statistical difference between two groups was determined using Student's t-test. A value of $P < 0.05$ was considered significant.

2.2 Results

2.2.1 General observation

Throughout the entire experimental period, the general conditions of the rats, including daily activities and other physiological manifestations, were normal after administration of *VasoCleaner*TM.

2.2.2 Food consumption

There was no significant difference in food consumption among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group (Table 2).

Table 2 Effect of *VasoCleaner*TM on rat diet consumption (food weight, g/ body weight, 100g, $\bar{X} \pm SD$)

Month	High-dose group	Medium-dose group	Low-dose group	Saline group
1 st	42.0±4.6	40.0±1.9	45.0±2.6	42.0±3.6
2 nd	51.6±3.7	52.0±4.5	51.2±4.5	50.0±2.8
3 rd	48.5±6.3	47.0±3.7	47.0±2.6	46.5±3.6
4 th	48.6±3.8	48.9±2.4	49.7±3.7	49.0±4.7
5 th	45.1±3.6	45.0±5.9	46.3±4.4	45.0±2.6
6 th	42.8±2.2	43.0±4.6	43.0±5.4	42.5±5.2

2.2.3 Body weight

The body weight of the rats increased with time. However, no significant difference in body weight was found among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group (Table 3,4).

Table 3 Effect of *VasoCleaner*TM on body weight (g, $\bar{X} \pm SD$)

Time	Number of rats	High-dose group	Medium-dose group	Low-dose group	Saline group
Beginning	30	109.6±6.5	109.2±6.3	109.0±6.7	110.6±6.7
2 nd week	30	130.6±6.7	127.5±6.4	128.0±7.9	250.3±7.4
4 th week	30	154.8±8.4	146.2±6.8	144.1±9.1	142.9±8.6
6 th week	30	177.8±8.3	170.2±13.7	157.3±13.2	163.5±12.4
8 th week	30	200.9±10.9	194.8±14.1	180.1±14.6	190.5±15.8
10 th week	30	225.5±11.4	229.0±15.7	199.8±15.1	212.1±15.7
12 th week	30	248.5±12.3	240.6±15.4	221.3±14.7	234.5±15.4
<i>The following measurements were based on 24 rats, because 6 rats were sacrificed for organ weight and pathogenic inspection at the end of the 12th week</i>					
14 th week	24	266.5±12.0	255.2±14.3	242.1±11.1	251.9±12.5
16 th week	24	287.7±13.9	278.1±16.9	269.6±11.3	279.0±13.3
18 th week	24	307.9±14.3	306.3±18.0	299.2±10.1	309.4±14.9
20 th week	24	337.9±16.4	337.5±17.5	329.8±11.2	335.0±15.2
22 nd week	24	364.9±15.4	351.3±17.0	357.5±10.0	357.1±14.1
24 th week	24	383.3±14.1	377.9±13.9	375.4±11.3	374.9±13.0
25 th week	24	390.2±14.1	386.5±13.9	381.0±9.2	380.1±12.0

2.2.4 Blood cells

Blood cells, including red-blood cells and white-blood cells, granulocytes, and lymphocytes were counted from each blood sample drawn from the tail of rat on a certain day. No significant difference in the number of those cells was observed among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group (Table 4-7).

Table 4 Effect of *VasoCleaner*TM on Red blood cells (RBCs)($\times 10^{12}/L$, $\bar{X} \pm SD$)

	Before Treatment	After Treatment		
	N*=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	6.49 \pm 0.49	6.90 \pm 0.50	6.95 \pm 0.81	6.60 \pm 0.61
Medium-dose group	6.47 \pm 0.64	6.45 \pm 0.69	6.93 \pm 0.81	6.94 \pm 0.53
Low-dose group	6.34 \pm 0.66	6.84 \pm 0.63	6.51 \pm 0.59	6.52 \pm 0.65
Saline group	6.41 \pm 0.59	6.70 \pm 0.64	6.22 \pm 0.99	6.79 \pm 0.59

Table 5 Effect of *VasoCleaner*TM on white blood cells ($\times 10^9/L$, $\bar{X} \pm SD$)

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	13.88 \pm 4.40	16.89 \pm 3.6	14.35 \pm 5.99	12.05 \pm 3.71
Medium-dose group	13.78 \pm 3.66	16.86 \pm 3.26	15.07 \pm 3.82	11.24 \pm 3.71
Low-dose group	13.62 \pm 3.52	17.44 \pm 4.90	13.51 \pm 5.30	11.30 \pm 3.30
Saline group	12.48 \pm 2.81	15.70 \pm 3.20	13.59 \pm 5.30	11.30 \pm 3.30

Table 6 Effect of *VasoCleaner*TM on the percentage of granulocytes (%), $\bar{X} \pm SD$)

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	0.23 \pm 0.10	0.23 \pm 0.08	0.28 \pm 0.09	0.23 \pm 0.05
Medium-dose group	0.23 \pm 0.10	0.23 \pm 0.05	0.30 \pm 0.10	0.19 \pm 0.06
Low-dose group	0.23 \pm 0.09	0.22 \pm 0.06	0.26 \pm 0.08	0.18 \pm 0.05
Saline group	0.23 \pm 0.05	0.22 \pm 0.05	0.28 \pm 0.08	0.20 \pm 0.08

Table 7 The effect of *VasoCleaner*TM on the percentage of lymphocytes (%), $\bar{X} \pm SD$

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	0.74±0.13	0.77±0.08	0.72±0.09	0.77±0.05
Medium-dose group	0.77±0.11	0.77±0.05	0.69±0.11	0.81±0.06
Low-dose group	0.76±0.09	0.78±0.06	0.73±0.08	0.82±0.05
Saline group	0.76±0.05	0.77±0.06	0.72±0.08	0.80±0.09

2.2.5 Blood chemical analysis

A series of blood chemical analyses, including the level of Glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), blood sugar, the amount of hemoglobin, total serum protein, and albumin were performed on each blood sample drawn from the rats on a certain day. No significant differences were observed among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group (Table 8-13).

Table 8 Effect of *VasoCleaner*TM on hemoglobin (Hb)(g/L), $\bar{X} \pm SD$

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	128.4±13.3	139.8±5.9	131.4±5.4	136.8±11.6
Medium-dose group	127.7±12.8	134.5±13.4	136.4±7.9	141.2±17.0
Low-dose group	127.2±12.5	140.4±10.6	138.6±9.5	138.0±9.50
Saline group	129.7±12.6	139.9±10.7	142.0±9.0	131.5±9.5

Table 9 Effect of *VasoCleaner*TM on the GPT in blood (U), $\bar{X} \pm SD$

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	31.54±6.56	35.58±8.57	36.11±11.39	36.84±6.87
Medium-dose group	29.20±7.81	34.40±11.61	35.20±7.03	37.95±6.76
Low-dose group	31.85±11.65	31.85±4.49	37.85±8.69	37.10±2.33
Saline group	29.65±5.77	33.10±8.11	36.61±7.14	36.18±8.40

Glutamic-pyruvic transaminase (GPT) assay kit was produced by Shanghai Kexin Bio-Company, Lot 940902

Table 10 Effect of *VasoCleaner*TM on BUN in blood (mmol/L, $\bar{X} \pm SD$)

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	8.12±6.56	7.66±3.59	9.53±2.88	6.88±1.45
Medium-dose group	7.81±3.40	6.99±1.24	10.54±3.15	7.00±1.72
Low-dose group	7.84±2.80	6.50±0.86	8.11±1.68	8.26±2.33
Saline group	8.40±1.68	7.03±1.36	7.94±2.43	8.01±1.51

Urea nitrogen assay kit, Shanghai Kexin Bio-Company, Lot950302.

Table 11 Effect of *VasoCleaner*TM on blood glucose (GLU) (mmol/L, $\bar{X} \pm SD$)

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	8.76±2.67	9.02±2.11	10.87±0.99	7.05±3.40
Medium-dose group	9.35±1.97	9.35±1.56	11.41±0.81	8.20±2.90
Low-dose group	9.43±1.75	8.87±0.57	9.60±0.75	8.10±1.87
Saline group	8.98±1.87	9.16±0.71	10.82±0.82	7.94±3.03

Table 12 Effect of *VasoCleaner*TM on serum total protein (TP)(g/L, $\bar{X} \pm SD$)

	Before Treatment	After Treatment		
	N=30	45 th day (N=10)	90 th day (N=20)	175 th day (N=24)
High-dose group	66.67±6.64	57.40±3.65	71.15±4.48	85.00±8.82
Medium-dose group	62.47±8.88	57.20±4.87	68.10±5.59	75.40±5.34
Low-dose group	62.93±8.31	67.00±2.00	73.00±1.90	75.50±7.06
Saline group	63.60±6.31	61.69±4.87	73.00±3.2	80.40±8.93

Serum total protein assay kit was produced by Beijing Zhongseng Bioengineering INC

Table 13 Effect of *VasoCleaner*TM on the albumen (ALB)(g/L, $\bar{X} \pm SD$)

	Before Treatment		After Treatment	
			45 th (N=10)	90 th day (N=20) 175 th day (N=24)
Time and number of rats N=30				
High-dose group	33.3±4.64	34.40±7.67	34.47±4.32	42.50±6.59
Medium-dose group	34.07±8.05	37.60±8.65	37.33±5.48	42.30±7.78
Low-dose group	37.27±5.53	37.20±5.02	39.50±6.72	43.00±8.35
Saline group	35.80±6.89	37.00±8.06	38.00±5.74	38.00±8.63

Albumin assay kit was produced by Beijing Zhongseng Bioengineering INC.

2.2.6 Organ weight

The wet organ weights of the heart, liver, kidneys, spleen, adrenal glands, uterus, ovary, testicles and brain were measured from rats scarified on a certain day before the pathogenic examination. No significant difference in organ weight was observed among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group (Table 14).

Table 14 Effect of *VasoCleaner*TM on the percent of organ to body weight (g, $\bar{X} \pm SD$)

N of rats	Organ	After Treatment			
		High dose group	Medium dose group	Low dose group	Saline group
15	Heart	0.46±0.03	0.50±0.03	0.48±0.02	0.41±0.02
15	Liver	4.08±0.11	4.18±0.16	4.21±0.21	4.18±0.11
15	Kidney	0.69±0.04e	0.55±0.02	0.60±0.04	0.61±0.03
15	Spleen	0.28±0.02	0.32±0.02	0.30±0.02	0.30±0.03
15	Adrenal glands	0.028±0.003	0.03±0.003	0.03±0.003	0.03±0.002
7	Uterus	0.21±0.02	0.22±0.02	0.24±0.02	0.23±0.01
7	Ovary	0.05±0.005	0.05±0.01	0.05±0.005	0.05±0.009
8	Testicle	1.09±0.04	1.03±0.05	1.06±0.05	1.10±0.04
15	Brain	0.63±0.02	0.63±0.03	0.61±0.02	0.61±0.02

2.2.7 Pathogenic examination

2.2.7.1 Anatomy examination: gross anatomy examination of scarified rats from different groups did not show any difference in rat organs among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group under naked eyes.

2.2.7.2 Pathogenic examination

Pathogenic examination of heart, spleen, kidney, stomach and intestine from scarified rats at the end of the 12th week and end of the whole experiment were performed. No significant difference in pathogenic condition was observed among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group.

At the 12th week, 6 rats (2 rats in the saline group) had slight liver swelling and ecchymosis, 7 rats (1 rat in the saline group) had slight lung inflammation and hemorrhage. At the end of the experiment, 11 rats (4 rats in the saline group) had slight liver swelling. 17 rats (6 rat in the saline group) had slight lung inflammation and hemorrhage. These observed pathogenic conditions do not show any correlation with the dosage of the drug.

2.2.7.3 Continued observation after stopping the administration of drug

Successive observation lasted for two weeks after stopping the drug administration. No significant difference in activity and body weight was observed among all *VasoCleaner*TM groups, or between any *VasoCleaner*TM group and the control group, though the body weight increased with time (Table 15).

Table 15 Body weight change after stopping drug supply (g, $\bar{X} \pm SD$, N=14)

	High-dose group	Medium-dose group	Low-dose group	Saline group
At stopping	371.7±12.8	371.1±11.1	370.7±18.5	370.7±13.0
1 st week	378.0±12.5	379.0±12.7	377.7±14.0	373.0±16.9
2 nd week	383.7±11.3	381.7±12.3	380.7±14.6	378.3±15.0

3. Conclusions

Acute oral toxicity study of *VasoCleaner*TM in mice under the conditions of our study did not generate the value of LD₅₀, which indicates the extremely low level of toxicity of this herbal preparation. The maximum drug-resistant dose is 11.04g/kg, which is 66.24 times the clinical effective dose.

Chronic toxicity studies in rats show that their physiological conditions including activity, food consumption, were normal. The blood chemical analysis does not reveal any abnormality in rats from different experimental groups. The number of blood cells and the wet organ weights also remained consistent in rats among different experimental groups. Gross pathogenic examination of different organs from scarified rats did not show any significant pathogenic difference between the drug-treated groups and the control group. Although there were slight pathogenic changes in the examined rats, these changes do not correlate with drug dose. The continued observation after

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stopping administration of the drug did not show any delayed toxic effect either.

The combined results from both acute and chronic toxicity indicate that *VasoCleanerTM* is practically non-toxic. It does not have side effects or delayed toxic reaction either.