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Study on Long-Term Toxicity of E-hong Tablets to Rats

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Abstract. To observe the effect of E-Hong tablet on long-term toxicity in rats, sixty rats were randomly divided into three groups, 20 rats in each group. According to the weight of the animals, the drug feed was given once every day, and the dosage was adjusted according to the change of body weight every week. The control group was only given no medicine feed and was fed continuously for 8 weeks, and the weight was weighed once a week, and the animal activity, hair and feces were observed. After treatment, 10 animals were taken from each group (the other animals continued to be kept for 2 weeks), blood samples were collected for routine examination, liver and kidney function, heart, liver, spleen, lung, kidney, stomach, thymus, adrenal gland, a total of 8 organs, fixed with 10% formaldehyde, covered with paraffin. E staining and histopathological examination. Results: the rats in the medication group and the control group had normal activities, active behavior, smooth hair, no abnormal and no death during the 8-week test period. There was no significant difference in blood routine, liver and kidney function test results. The appearance of viscera was normal and no special pathological changes were found in pathological anatomy and histopathology ($P > 0.05$). In conclusion, the toxicity of E-Hong tablet is very small, this experiment provides safe basis for clinical application.

INTRODUCTION

E-hong tablet is the hospital preparation of the 371st Central Hospital of the Chinese people's Liberation Army. The prescription is composed of curcuma, safflower and other drugs. It has the effect of breaking blood stasis and activating blood circulation. It mainly treats ischemic vascular diseases clinically, and has achieved good therapeutic effect. Especially in the treatment of unstable angina pectoris and other coronary insufficiency, the mechanism is related to the inhibition of the release of inflammatory factors such as IL-6, IL-8 and IL-18 [1-2]. In order to ensure the safety of clinical drug use this experiment has carried on the long-term toxicity effect of E-hong tablet on rats.

MATERIALS AND METHODS

Animals

Wistar rats were provided by Experimental Animal Center of Henan University of traditional Chinese Medicine (SYXK) 2005-0001, weighing 130g ±10g, half male and female.

Drugs

E-hong tablet powder (self-made), medicine and feed were mixed to make drug bait. The high dose group was 4.32g/kg/ day. The ratio of powder to feed was 1 : 2.5, which accounted for 28.6% of feed. The daily rat servo drug bait was 24.5 g / kg, accounting for about 33g / kg of food intake. The low dose group was 2.16g/kg/ day and the ratio of powder to feed was 1 / 3, which could ensure the adequate intake of drugs without affecting the nutritional needs of animals.

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Side Test Instrument

COBASTARA II automatic biochemical analyzer made in Switzerland.

Methods

60 10-week-old rats were randomly divided into three groups, 20 rats in each group, half male and half female, observed one week before administration, and no abnormal animal activity, food intake, feces were observed in each group, and then the drug was given. According to the weight of the animals, the dosage was adjusted once a day at 8 am, and the amount was adjusted weekly. After the animals had finished the diet, they were given free drinking and drinking water, while the control group was given only the drug free feed and was fed continuously for 8 weeks. Weigh once a week and observe animal activity, hair, and feces. After medication, 10 animals were taken from each group (the rest were kept for 2 weeks) and blood samples were collected. Blood routine examination, liver and kidney function, heart, liver, spleen, lung, kidney, stomach, thymus, adrenal gland were fixed with 10% formaldehyde, HE staining was embedded in paraffin, and histopathological examination was carried out.

RESULT

The rats in the medication group and the control group were fed during the 8-week test period, with normal activity, lively behavior, smooth hair, no abnormality and no death.

The weight gain of the three groups was basically the same (See table 1), $P > 0.05$.

TABLE 1. Rats weight change by time (week) (g, n=20, $\bar{x} \pm SD$)

Group	Number	0W	1W	2W	3W	4W	5W	6W	7W	8W
Control	Female 10	126.8 ± 4.53	135.7 ± 4.43	144.4 ± 5.24	154.9 ± 5.77	164.6 ± 6.34	176.6 ± 5.7	188.7 ± 6.07	198.9 ± 6.17	210.2 ± 8.38
	Male 10	130.2 ± 5.65	141.5 ± 5.28	153.1 ± 6.35	170.4 ± 8.16	188.5 ± 7.62	208 ± 8.01	225.7 ± 10	248.8 ± 10.66	275.3 ± 9.42
	Total 20	128.5 ± 5.39	138.6 ± 5.67	148.8 ± 7.27	162.7 ± 10.49	176.6 ± 13.85	192.3 ± 17.22	207.2 ± 20.26	223.86 ± 26.43	242.75 ± 33.7
Small Dose	Female 10	127.8 ± 5.34	137.3 ± 5.25	145.9 ± 5.49	155.6 ± 6.23	165.4 ± 6.64	175.5 ± 6.55	187.4 ± 7.75	199.5 ± 7.75	211 ± 7.6
	Male 10	133.3 ± 5.51	143.8 ± 5.27	153.8 ± 6.46	165.5 ± 7.3	186 ± 8.72	205.2 ± 10.6	225.2 ± 11.01	244.9 ± 12.9	274.6 ± 12.71
	Total 20	130.55 ± 6.09	140.55 ± 6.18	149.85 ± 7.18	160.55 ± 8.4	175.7 ± 12.89	190.35 ± 17.22	206.3 ± 21.16	222.2 ± 25.07	242.8 ± 33.48
Large Dose	Female 10	126.8 ± 4.87	135.8 ± 5.84	142.6 ± 4.88	152.9 ± 5.17	162.6 ± 5.68	173.8 ± 6.66	187 ± 7.59	200 ± 9.32	213.3 ± 9.28
	Male 10	132 ± 3.74	141.7 ± 3.63	153.9 ± 4.39	167.5 ± 5.63	185.3 ± 6.44	207.7 ± 8.56	227.9 ± 11.66	248.1 ± 14.92	273.9 ± 15.6
	Total 20	129.4 ± 5.06	138.75 ± 5.69	147.25 ± 8.31	160.2 ± 9.08	173.95 ± 12.8	190.75 ± 18.6	207.45 ± 22.69	224.05 ± 27.07	243.6 ± 32.91

*Proof: Compare with Control Group $P > 0.05$

There was no significant difference in blood routine, liver and kidney function among the three groups. (see Table 2-3), $P > 0.05$.

TABLE 2. Routine blood test result of eight week old rats with feeding E-hong Tables (n=10, $\bar{x} \pm SD$)

Group	Hbg/L	RBC 10^{12}/L	WBC 10^9/L	Sg %	Ly %	Eosino-%	Monocyte %	PC 10^9/L
Control Group	146.4 ± 9.31	7.28 ± 0.72	9.77 ± 2.5	25.4 ± 2.58	70.7 ± 2.49	2.0 ± 0.77	1.8 ± 0.75	294.0 ± 28.1
Small Dose Group	148.5 ± 1.53	7.58 ± 0.39	9.89 ± 1.39	26.7 ± 2.15	69.5 ± 2.06	2.0 ± 0.89	1.8 ± 0.87	300.5 ± 20.43
Large Dose Group	149.7 ± 6.45	7.66 ± 0.39	10.15 ± 2.41	25.9 ± 2.62	70.7 ± 2.42	2.1 ± 1.04	1.8 ± 0.67	306.5 ± 24.6

*Proof: Compare with Control Group P >0.05.

TABLE 3. Effect of E-hong tablet on liver and kidney function in rats for 8 weeks (n=10, $\bar{x}$ ± SD)

Group	GDT's unitu	Bilirubin mmol/L	BUN mmol/L	Creatinine mmol/L
control group	38.911.42	2.41±0.421	4.37±0.49	94.8±7.04
Small dose group	39.16±1.811	2.36±0.40	4.23±0.51	96.04±6.214
high dose group	38.98±1.56	2.46±0.40	4.17±0.41	95.77±6.735

*Proof: compared with the control group, both were P >0.05.

The 8 organs mentioned above in the three groups had normal appearance, and no special pathological changes were found in pathological anatomy and histopathological examination (see Table 4), P >0.05.

TABLE 4. Weight per 100g body weight of E-hong tablet after 8 weeks (n=l0, $\bar{x}$ ±SD)

Visceral Organ Group	Control group	Small dose group 4.32g/kg/ day	High dose group 2.16g/kg/ day
Heart g	0.417 ± 0.011	0.415 ± 0.010	0.415 ± 0.012
Liver g	4.613 ± 0.035	4.619 ± 0.046	4.615 ± 0.039
Spleen g	0.192 ± 0.012	0.191 ± 0.013	0.196 ± 0.013
Lung g	0.768 ± 0.015	0.769 ± 0.016	0.753 ± 0.024
Renal g	0.0995 ± 0.0036	0.1004 ± 0.00498	0.1018 ± 0.00704
thymus gland g	0.124 ± 0.0111	0.124 ± 0.010	0.126 ± 0.0111
Renicapsule mg	30.73 ± 2.24	30.87 ± 1.409	30.37 ± 1.09
Stomach g	1.55 ± 0.16	1.51 ± 0.15	1.56 ± 0.14

*Proof: P >0.05 compared with control group

DISCUSSION

The effective oral dose of E-hong tablet for adults was 3.6 g (powder) per day. The experimental results showed that the drug dosage of rats was 60 and 30 times as much as that of adults. In order to ensure the intake of drugs and avoid interfering with the normal nutritional intake of animals, the oral effective dose of E-hong tablet was 3.6 g / d. The total amount of feed containing drugs per day does not exceed one third of the total intake, so the high dose of 4.32g/kg/ is 2.16 g / kg / day for low dose. According to the principle that the time of long-term toxicity test should be 2-3 times of the clinical course of treatment [3-4], the continuous administration time was determined to be eight weeks. E-hong tablets are 60 and 30 times as much as those used by adults (i.e. 4.32g/kg/ and 2.16g/kg/ days). Sixty rats were fed continuously for 8 weeks, weighing once a week, and observing animal activity and hair and feces. After treatment, 10 animals in each group were collected for blood routine examination, liver and kidney function, and important organs for histopathological examination. Results there was no obvious toxicity. This experiment provided a safe basis for clinical application.

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REFERENCES

1. G.Z. Zhu, S.L. Zhang, S.Q. Wei, X.B. Liu, P. Duan, Q.Y. Dai, Z.H. Zhang, W.L. Chen, R.X. Zhang and H.J. Liu, Med. Inf. *(in Chinese)* **24**, 769 (2011).
2. S.L. Zhang, R.X. Yue, S.Q. Wei, G.Z. Zhu, Q.Y. Dai, Z.H. Zhang and X.B. Liu, Chin. J. Inf. Tradi. Chin. Med., **17**, 15-17 (2010).
3. B.L. Yuan, Chin. J. New Drug **12**, 960-963 (2004).
4. L.D. Li, *Research Guide to New drugs in traditional Chinese Medicine. The first edition*. (Drug Administration Bureau of the Ministry of Health of the people's Republic of China, Beijing, 1993) pp. 205-207.