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The Study on the Toxicology of FRC001 （ China No.1 Tian Xian Liquid ）

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ABSTRACT: According to the estimation index, content and method of toxicologic study, we investigated the acute toxicity test, cumulative toxicity test, polychromatic erythrocytes micronucleus test of mouse, aberration test of sperm and Ames test of FRC001. The results showed that the acute toxicity test, cumulative toxicity test and Ames test of FRC001 were normal, whereas the micronucleus rate of polychromatic erythrocytes and aberration rate of sperm of FRC001were higher than control group. These results reflected the safety level of Edfrnn and provided some data of dosage and administration route for the use of FRC001.

Key Words:FRC001; LD50; mutagenicity; aberration rate;micronucleus rate

BACKGROUND

Toxicology mainly studys the relations between the toxicity of drugs and their ingredients and physiochemical property, the rule of absorption, distribution, conversion, cumulation and excretion of drugs. There are two sorts of toxicologic tests,I, e. test in vitro and test in vivo. The former invludes acute toxicity test, cumulative toxicity test ,aberration test, mutagenicity test on whole animals, while the latter includes mutagencity test on microorganism. In order to evaluate the toxicity of FRC001 in clinical application, we studied the toxicdogy of FRC001.

MATERIALS AND METHODS

1.Materials

1.1 Subject:FRC001,provided by China-Japan Feida Union Co., Ltd.

1.2 Exper imental Animals:

1.2.1Mouse, Kunming species, healthy,2~3 months old, male or female

1.2.2 Rat, Wistar species, healthy,3~4 months old, 180~220g, male or female

Mice and rats were provided by Experimental Animal Center of Tongji University of Medical Science.

1.3Experimental Strain:Histidine dystrophic of Salmonella typhimurium TA98,TA100 and TA102 were provided by Ames Laboratory of California.

2.Methods:

2.1Acute toxicity test:According to Horn's method. Fifty mice were randomly divided into five groups:one control group and four dose groups(21.5,10.0,4.64 and 2.15g/kg. bw) . Each group had ten rats with half male and half female. FRC001 was diluted by distilled water. The rats were fed with drugs endogastricly, one time per day for successive two weeks.

2.2 increasing dose method. Thirty-two rats were randomly divided into two groups:control and experiment group. Each group had 16 rats with half male and half female. The rats of experiment group were fed with FRC001 endogastricly beginning at 3.0g/kg. bw, then increasing the dosage daily to 5.26 LD50 for successive 30days. The control group were fed with normal saline the same way as experiment group.

Micronucleus test:Fifty mice were randomly divided into five groups:one negative control group, one positive group and three dose groups (4.3,10.8,21.5 g/kg. bw). Each group had ten mice with half male and half

2.1 female. The negative group were fed with distilled water 5g/kg.bw endogastricly, the positive group were injected with cyclophosphamide 100mg/kg. bw intraperitoneally and they were killed after 30hrs. The other groups were fed drugs endogastricly one

time daily for successive four days, then killed at the fifth day. The sterna were separated and crushed to prepare the smear of bone marrow polychromatic erythrocytes. The polychromatic erythrocyte and micronucleus cell were calculated.

2.2 Sperm aberration test: Forty male mice were randomly divided into five groups:negative group, positive gorup and three drug groups (4.3,10.8,21.5 g/kg. bw) Each group had eight mice. The mice of negative group were fed with distilled water 21.5g/kg. bw(I. G), the mice of positive group were fed with cyclophosphamide 21.5 g/kg. bw,and the mice of drug groups were fed with FRC001. All of them were fed intragastricly one time daily for successive five days, and killed after 30 days of normal feeding. The epididymides were separated, put into five ml normal saline, cut into pieces, then put into 36.5 ℃ water bath for 15 min. The total sperms and active sperms in 200 sperms were counted. The sperm aberration rate was calculated by staining smear.

2.3 Ames test: FRC001 was diluted by DMSO into five concentrations:1:50,1:200,1:500,1:2000 and 1:5000. DMSO was the negative control subject, while Dexon and 2,7-diaminofluorene were the positive control subjects. Dexon was used as direct mutagen and 2,7- diaminofluorene was used as indirect mutagen. To see the detail in reference(4)

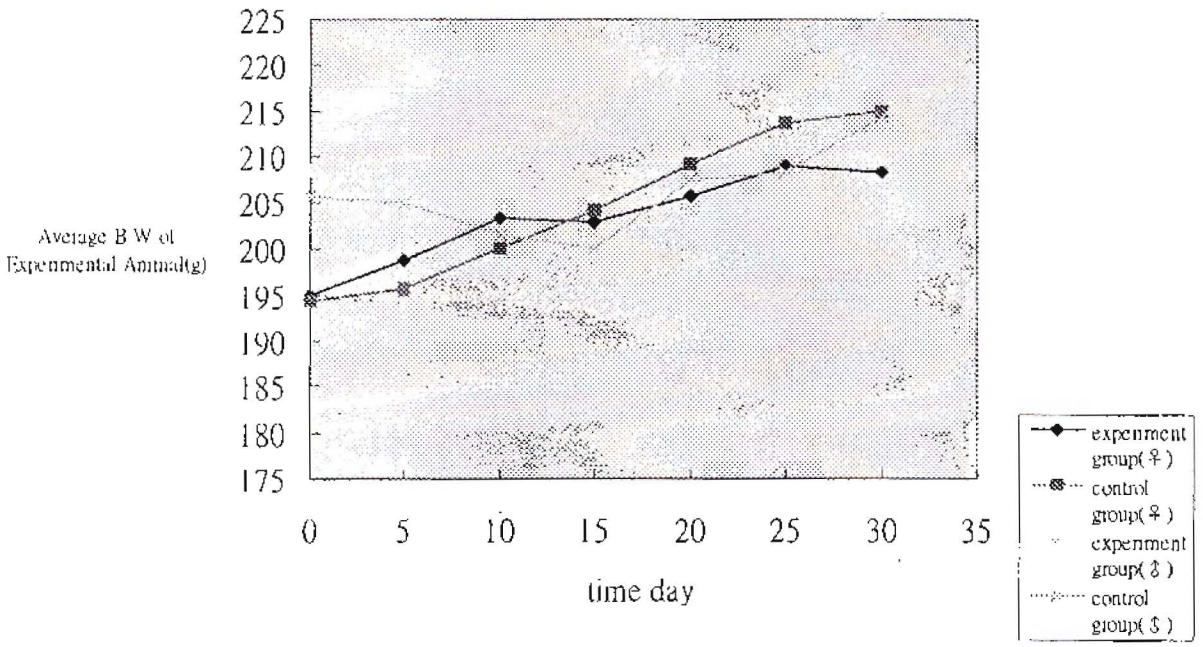
RESULTS AND DISCUSSION

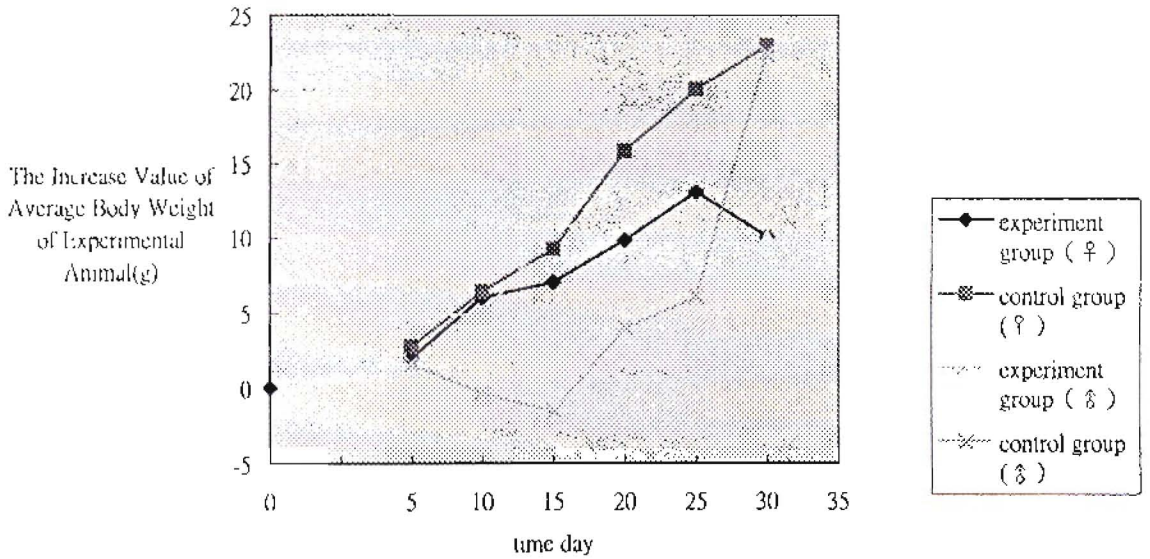
1.Acute toxicity test of FRC001 :

To do the test according to the method of acute toxicity test. After two week's observation, all animals fed with FRC001 had no abnormal manifestation, were normal food-intake and water-intake, activity. The body weight has no obvious change. No animal died during test period. LD50 of FRC001 by oral administration is more than

21.5g/kg.bw.

2.Cumulative toxicity test of Edfrnn





2.2The influence on main organ coeffeicents of rats (Table 1)

Table 1 The Influence of FRC001 on Main Organ Coefficients of Wistar Rats

Group	Number	Organ Coefficients(X ± SD)				
		Heart	Liver	Spleen	Lung	Kidney
Control	16	0.45 ± 0.04	3.96 ± 0.60	0.38 ± 0.07	0.96 ± 0.30	0.85 ± 0.08
Experiment	16	0.44 ± 0 05	4.14 ± 0.39	0.46 ± 0.16	1.03 ± 0.26	0 88 ± 0.06

Difference between the two groups was not significant,P>0.05

According to Table 1 and Figure 1,2,FRC001 had no obvious effects on the body weight and the increase value of average body weight with successive 30 days of intragarstric feeding. The food-intake and activity were normal. No toxic reaction was fourd. Organs were normal by pathological examination. The difference between control group and experimental group was not significant (P>0.05). The cumulative dose ws up to 5.26 LD50 in 30 days and no animal died. These results showed that FRC001 had no obvious cumulative toxicity.

3.The influence of FRC001 on bone marrow cell micronucleusrate of mice (Figure3,4)

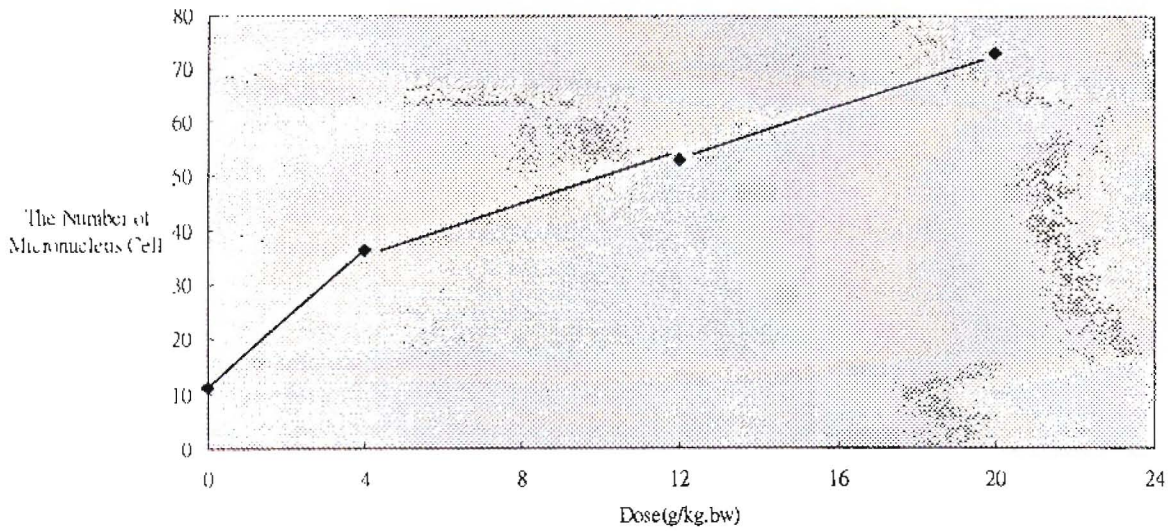


Figure 3 The Relation between the Dose of FRC001
and the Number of Micronucleus Cells

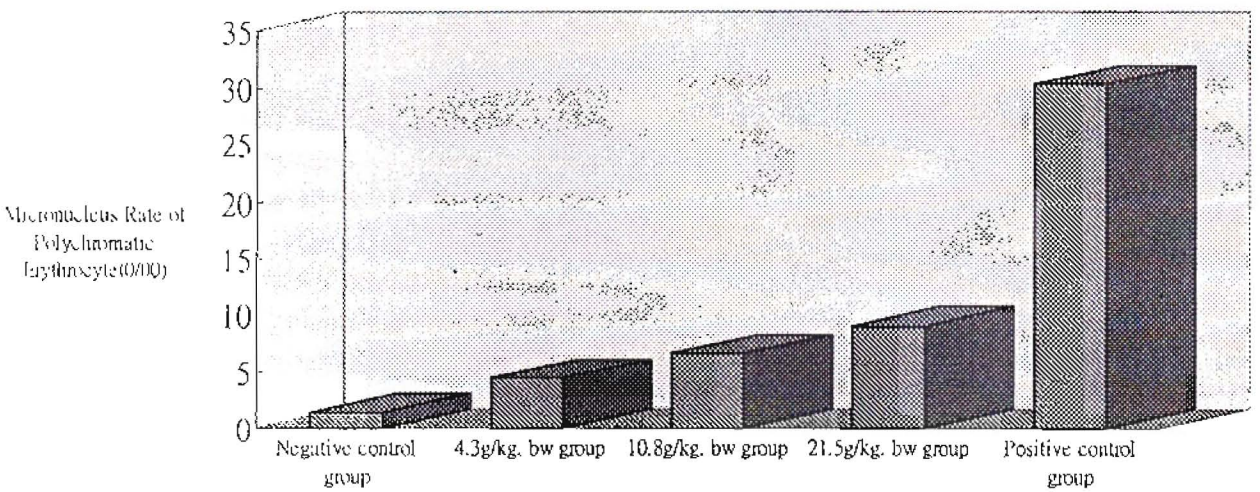


Figure 4 The Micronucleus Rates of All Groups

Generallyk in the micronucleus test, the micronucleus rate of cyclophosphamide (positive control group) is 0~3%, the micronucleus rate of negative cotnrol group is lower than 3%. According to Figure 3,4, the micronucleus rate of positive control group and negative control group were 30.3% and 1.4%,respectively. The results showed that this

test was dependable. The micronucleus rate of low, middle ad high dose of FRC001 were 4.5%、6.6%、9.0%, respectively. The difference between Edfmn groups and negative control group were significant. These findings showed that FRC001 had mutagenic action.

4.The influence of FRC001 on sperm aberration of mice (Figure 5 and Table 2)

Table 2 The Influence of FRC001 on Sperm Aberration Rate of Mice

Groups	Number	The Number of Sperm	No. of Active Sperm	Sperm Aberration Rate
Negative	8	237.1	158.9	9.3
4.3g/kg.bw	8	156.3	109.7	11.3
10 8 g/kg.bw	8	253.0	177.5	19.4
21.5 g/kg bw	8	212.0	139.2	27.9
Positive	8	378.9	263.1	55.1

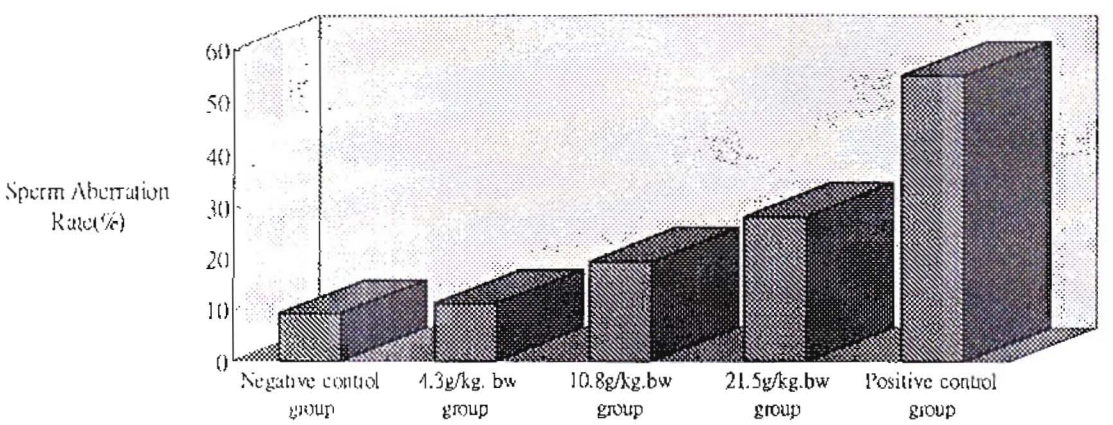


Figure 5 The Influence of FRC001 on Sperm Aberration Rate of Mice

Accroding to Table 2, the sperm aberratin rate of three doses of FRC001 and postive control group were higher than that of negative control group. Generally, the sperm aberration rate doubles that of negative control group shows that the sperm aberration rate is positive. In this test, the sperm aberration rate of positive control group was 6 times of negative control group, while that of low middle and high dose of FRC001 were 1.2, 2.1 and 3 times of negative control group. The difference were significant ($P<0.05$, $P<0.01$) . These findings showed that FRC001 had some harmful and toxic action on the heredity of germ cells.

5.Ames test of FRC001 (Table 3)

Table 3. The Results of Ames Test of FRC001

Concentration of FRC001 (ug)	MR(Rt/Rc)							
	TA97		TA98		TA100		TA102	
	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9	+S-9	-S-9
1/50	1.02	0.80	0.78	1.26	0.96	1.04	0.94	0.90
1/200	1 18	0.85	1.00	1.24	0.91	1.14	1.16	1.00
1/500	1 02	0.86	1.12	1.04	1.00	1.09	1.12	0.86
1/2000	1.08	1.02	1.25	1.13	1.02	1.13	1.29	1.26
1/5000	1 11	1.03	1.06	1.10	1.07	1.08	1.18	1.13
Dexon(50 ug/)	_	11 30	_	15.70	_	4.33	_	3.55
2-AF	3 35	_	5.32	_	5.07	_	2.10	_
DMSO	_	1 14	_	0.74	_	0.96	_	0.84

According to Table 3, using incorporatin method to do Ames test of FRC001,the ratio(MR) of mutagenic colony (Rt) and spontaneous reverse mutation colony (Rc) were lower than two.The results showed that FRC001 had no obvious mutagenicaction. The MR of Dexon and 2,7-diaminofluorene were higher than two showed that Ames test ws positive and they had mutagenic action. The MR of DMSO was lower than two showed that it had no mutagenic action. These results also showed the test was dependable.

In a conclusion, in the toxicology test of FRC001, the actue toxicity test, cumulative toxicity test and Ames test were normal while the micronucleus test and sperm aberration test were abnormal. It is generally thought that the occurance ofmicronucleus in interphase caused by mutagen, i, e, the micronucleus consists of the pieces of chromosome after the acting of mutagen. The micronucleus rate is closely related to chromosomal aberration. FRC001 induced the increase of sperm aberration rate of mice showed that it had potential damage action on the heredity of male germ cells. According to the estimation procedure and method of toxicology of food and drug issued by government ;the subject can not be used as food and drug if there are two positive in heredity toxicity test and it has apparent toxicity in short term feeding test; if the short term feeding test is suspicious positive, the application is determined by the importance and the potential in -take of the subject. The positive of micronucleus test and sperm aberration test of FRC001 may be related to its ingredients, the interaction of ingredients

and /or the formation of new products during the preparation of FRC001. We suggest that the application departments should think over the mutagensis of FRC001 while they can try to use it in clinic. Whether the toxicity of FRC001 can be decreased by controlling the dose or small amount and repeated administration is awaiting further study.

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