

1270 '03 MAR 19 P2:25

Attachments 3
Analytic and Testing Centre
Nanjing Railway Medical College

REPORT ON QUALITY TEST

(food) Test No. 11

Name of Sample: Xiwang capsule

Delivered by: Department of Leaf Protein, Hefei Economy and Technology College

Produced by: Department of Leaf Protein, Hefei Economy and Technology
College

Date of Report Release: March 28, 1997

Specifications of Report on Quality Test

1. If there is any disagreement, please appeal to Analytic and Testing Center during 15 days from the date of report release.
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Analytic and Testing Centre of Nanjing Railway Medical College
REPORT ON QUALITY TEST

(food) Test No. 11

Report No. 970328

Name of Sample: Xiwang capsule

Test Type: Identification

Delivered by: Department of Leaf Protein, Hefei Economy and Technology College

Telephone: 0551-3416033, extension 237

Address: Hefei, Anhui Province

Postcode: 230000

Sampling place: delivered sample

Sample Lot Number: 961103

Date of acceptance: Nov. 24, 1996

Date of Sampling: Nov. 24, 1996

Sample character: capsule with orange powder Sample amount: 6,000 g

Aims and items of test

Aim: The test is to assess the safety of the delivered sample using food toxicological testing procedures and methods.

Items:

1. Acute toxicity test in mice
2. Ames test
3. Bone marrow micronucleus test in mice
4. Sperm aberration test in mice
5. 90-day feeding test
6. Traditional teratogenesis test

Results and Evaluation:

1. The LD₅₀ (oral) of Xiwang capsule in mice was more than 10.0 g/kg.bw, therefore, Xiwang capsule falls into the category of "virtually non-toxic", based on the classification of acute toxicity.
2. The results of Ames test were negative, which demonstrates that Xiwang capsule has no mutagenic effects.
3. The results of bone marrow micronucleus test in mice were negative, which shows Xiwang capsule has no cytotoxic and mutagenic effects.
4. The results of sperm aberration in mice were negative, which demonstrates that

Xiwang capsule has no mutagenic effects to reproductive cells.

5. In 90- day feeding test in rats, there were no abnormal findings in general observation, body weight, food efficiency, and clinical chemistry. In hematology tests, blood platelet count of females in the high-dose group was lower than the controls; In males, the high-dose group in the middle of the test, and medium and high-dose groups at the end of the test, blood platelet counts were lower than the controls; The relative liver weight of females in the medium and high-dose groups was higher than the controls; Different degrees of liver watery degeneration was observed in female or male animals in the medium or high-dose groups, and the degree of degeneration increased as the doses increased. The NOEL of this test was 0.363 g/kg b.w. (equivalent to 72.5 µg/kg b.w. selenium) in male rats and 0.448 g/kg b.w. (equivalent to 89.7 µg/kg b.w. selenium) in female rats.
6. There were no abnormal findings in teratogenic test; therefore, Xiwang capsule has no teratogenic effects.

Prepared by:

Reviewed by:

Signed and issued by:

Seal of testing laboratory

March 28, 1997

Analytic and Testing Centre of Nanjing Railway Medical College
Report on acute toxicity in mice

(food) Test No. 11

Report No. 970328

Delivered by: Department of Leaf Protein, Hefei Economy and Technology College
Address: Hefei, Anhui Province Delivered by: Zhang Jinshong
Telephone: 0551-3416033, extension 237 Name of Sample: Xiwang capsule
Date of acceptance: Nov. 24, 1996 Sample character: capsule with orange powder

Animals and Methods:

The Horn's method was used. Fifty Kunming mice (25 males and 25 females) were purchased from the Experimental Animal Center of Nanjing Railway Medical College. Animal certificate number: Su animal (quality 95036); Environment certificate number: Su environment (environment 95102). Animals were randomly divided into four treatment groups and a negative control group.

Results:

1. LD50: Females, > 10,000 mg/kg b.w. and males, > 10,000 mg/kg b.w.
 2. No abnormal observations were found.
-

Notes: LD50, which shows the toxic nature and degree of toxicity of the tested sample, can not be used as the only basis of safety evaluation, but only as the reference for further toxicological tests.

Tested by:

Reviewed by:

Signed and issued by:

Seal of testing laboratory

March 28, 1997

Analytic and Testing Centre Nanjing Railway Medical College
Report on Ames test

(food) Test No. 11

Report No. 970328

Delivered by: Department of Leaf Protein, Hefei Economy and Technology College

Name of Sample: Xiwang capsule

Date of acceptance: Nov. 24, 1996

Materials and Methods:

Standard plate incorporation assay was used. Strains and S-9 were obtained from Shanghai Institute of Occupational Diseases Prevention.

Results:

Table 1. Ames test of Xiwang capsule

Group	Dose ($\mu\text{g}/\text{plate}$)	TA 97		TA 98		TA 100		TA 102	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Xiwang	10	101.0 \pm 6.00	106.3 \pm 7.37	37.7 \pm 4.04	44.0 \pm 7.06	127.3 \pm 12.7	134.3 \pm 15.3	214.0 \pm 9.17	238.3 \pm 10.5
	50	96.0 \pm 7.51	110.3 \pm 11.6	36.3 \pm 5.00	43.3 \pm 7.77	121.0 \pm 10.0	126.0 \pm 14.5	220.7 \pm 8.02	233.3 \pm 6.81
	200	96.7 \pm 4.51	113.5 \pm 9.87	36.3 \pm 4.51	42.3 \pm 8.37	109.7 \pm 10.3	121.3 \pm 9.50	224.3 \pm 13.0	233.3 \pm 6.31
	1000	101.7 \pm 6.02	112.7 \pm 13.9	36.1 \pm 2.00	40.0 \pm 7.05	112.3 \pm 5.51	118.0 \pm 11.27	230.3 \pm 4.93	225.0 \pm 8.72
Negative control		97.0 \pm 4.58	103.0 \pm 5.29	33.7 \pm 6.02	30.0 \pm 2.00	124.0 \pm 12.3	130.0 \pm 17.5	233.0 \pm 23.1	232.3 \pm 12.7
Positive control*		1024.7 \pm 32.0	1100 \pm 28.7	616.3 \pm 54.0	675.3 \pm 63.0	870.7 \pm 53.1	941.0 \pm 51.1	1103.0 \pm 14.3	1137.7 \pm 137.4

* Positive controls: TA 97 (-S9), ICR-191; TA98 (-S9), TA97 (+S9), TA98 (+S9), 2,7-aminofluorene; TA100 (-S9): sodium azide; TA100 (+S9): cyclophosphamide; TA102 (-S9): mitomycin C; TA102 (+S9): 1,8-quinizarin.

Conclusions:

The number of revertant colonies in all Xiwang-treated groups, whether with S9 or without S9, were less than two times of the spontaneous revertant colonies, and no dose-response was found. The results showed that Xiwang capsule has no mutagenic effects.

Tested by:

Reviewed by:

Signed and issued by:

Seal of testing laboratory

March 28, 1997

Analytic and Testing Centre of Nanjing Railway Medical College

Report on bone marrow cell micronucleus test in mice

(food) Test No. 11

Report No. 970328

Delivered by: Department of Leaf Protein, Hefei Economy and Technology College

Name of Sample: Xiwang capsule Date of acceptance: Nov. 24, 1996

Sample character: orange powder Treat method: diluted with distilled water

Animal: Kunming mice

Animal source: Experimental animal center of Nanjing Railway Medical College

Animal certificate number: Su animal (quality 95036)

Environment certificate number: Su environment (environment 95102)

Gavage amount: 2ml/100 g b.w.

Marrow source: sternal bone Animal body weight: 25-29 g

Results:

Table 1 Effects of Xiwang capsule on the frequency of micronucleated cells in mice bone marrow

Group	Sex	Dose (g/kg)	Micronucleus			PCE/RBC		
			No. of observed cell	No. of micronucleate d cell	Frequency of micronucleated cell (%)	PCE number	RBC number	PCE/RB C
Treatment	Male	200	5×1000	12	2.40	5×200	1136	0.88
	Female		5×1000	11	2.20	5×200	1108	0.90
	Male	1000	5×1000	14	2.80	5×200	1123	0.89
	Female		5×1000	12	2.40	5×200	1089	0.92
	Male	5000	5×1000	13	2.60	5×200	1187	0.84
	Female		5×1000	14	2.80	5×200	1027	0.97
Negative control (distilled water)	Male		5×1000	13	2.60	5×200	1050	0.95
	Female		5×1000	12	2.40	5×200	1079	0.93
Positive control (cyclophosph amide)	Male		5×1000	109	21.80	5×200	1514	0.66
	Female	40	5×1000	116	23.20	5×200	1489	0.67

Notes: PCE: polychromatic erythrocytes; RBC, red blood cells

Conclusions:

Micronuclei rate in the positive control group was significantly higher than the other groups. No significant differences were found in Xiwang capsule-treated groups as compared with the negative control group in micronuclei rate and PCE/RBC. Xiwang capsule, therefore, has no mutagenic effects.

Tested by:

Reviewed by:

Signed and issued by:

Seal of testing laboratory

March 28, 1997

Analytic and Testing Centre of Nanjing Railway Medical College

Report on Sperm Aberration test in mice

(food) Test No. 11

Report No. 970328

Delivered by: Department of Leaf Protein, Hefei Economy and Technology College

Name of Sample: Xiwang capsule Date of acceptance: Nov. 24, 1996

Sample character: orange powder Treat method: dilution with distilled water

Animal: Kunming mice

Animal source: Experimental animal center Nanjing Railway Medical College

Animal certificate number: Su animal (quality 95036)

Environment certificate number: Su environment (environment 95102)

Gavage amount: 2ml/100 g b.w.

Animal treated: killed on the 35th day after the first gavage Animal body weight: 25-29 g

Results:

Table 1 Effects of Xiwang capsule on sperm aberration rate in mice

Group	Dose (mg/kg b.w.)	number of mice	number of sperm	number of aberration	aberration rate(%)
Xiwang	1250	5	5000	131	2.62
	2500	5	5000	118	2.36
	5000	5	5000	144	2.88
Negative control		5	5000	137	2.74
Positive control (cyclophosphamide)	40	5	5000	343	6.86

Conclusions:

The aberration rate in the negative control group was 2.62%, which was in the normal aberration range of mice (normal aberration rate is 1.2-3.4%). The aberration rate in the positive control group was 6.86%, which was significantly higher than the negative control group ($P < 0.01$).

The aberration rate in the three Xiwang-treated groups were 2.36%, 2.88% and 2.74%, respectively. No significant difference was found as compared with the negative control group ($P > 0.05$) and no dose-response was found. Xiwang capsule, therefore, has no mutagenic effects on mice sperms.

Tested by:

Reviewed by:

Signed and issued by:

Seal of testing laboratory

March 28, 1997

Analytic and Testing Centre of Nanjing Railway Medical College
Report on 90-day oral toxicity test of Xiwang capsule

(food) Test No. 11
Report No. 970328

Comissioned by the Department of Leaf Protein, Hefei Economy and Technology College, a 90-day feeding test in rats on Xiwang capsule were conducted from November 1996 to March 1997. The aim of this study was to provide the basis for toxicological safety assessment on this product.

1. Materials

1.1 Sample

Xiwang capsule was provided by the Department of Leaf Protein, Hefei Economy and Technology College. The compound is an orange powder, and the selenium concentration was 200 $\mu\text{g/g}$ (the analytical data was provided by the Department of Leaf Protein, Hefei Economy and Technology College).

1.2 Animals

SD rats were obtained from the Experimental animal center, Nanjing Railway Medical College. Animal certificate number: Su annial (quality 95036), Envionment certificate number: Su envionment (envionment 95102).

2 Methods

2.1 Animal treatments

Eighty weaning SD rats, 50-70 g body weight, were randomly divided into 4 groups. Each group consisted of 10 males and 10 females. Group 1, the basal diet group; Group 2, 0.50% Xiwang capsule + 99.50% basal diet; Group 3, 1.50% Xiwang capsule + 98.50% basal diet; Group 4, 3.00% Xiwang capsule + 97.00% basal diet (equivalent to 0.0, 1.0, 3.0, or 6.0 μg selenium/g diet). Basal diet was made according to the regular formula. Each rat was kept in one cage and free access to food and water. The animals were observed daily for 90 days.

2.2 Parameters

2.2.1 General observation: The behavior and fur color of animals were observed daily.

2.2.2 Body weight and growth: Body weights were measured weekly.

2.2.2 Food consumption and efficacy: Diets were added and recorded for every two days.

2.2.4 Hematology tests: The tests were performed in the middle of the study and at the end of the study. Blood samples were withdrawn from the tail vein. The hematological tests carried out were as follows: hemoglobin, red cell count, total white cell count,

platelet count, reticulocyte count and white cell differential count.

2.2.4 Analysis of Clinical chemistry tests: At the end of the study, blood was obtained from femoral artery and serum was separated. Model RA-XT autoanalyzer was used to analyze alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLO), albumin/globulin (A/G), urea nitrogen (BUN), creatinine (CRU), glucose (GLU), and total cholesterol (T-Che).

2.2.6 Organ weights and histopathological examination:

At the end of the experiment, animals were sacrificed by bleeding from femoral artery. Rats were dissected for macroscopic examination first, then liver and kidneys were measured to calculate the organ and body weight ratio (relative organ weight); tissues including heart, liver, kidney, and intestine were fixed in formaldehyde solution for subsequent processing, i.e. embedded in paraffin wax and tissue sections were stained with Hematoxylin-Eosin.

3 Results

3.1 General condition: The animals in all groups were in good condition. No overt signs or toxic effects were observed and no animal died during the study.

3.2 Body weight: The mean body weight gains were: females, 188.8 ± 13.5 g; males, 332.9 ± 19.4 g. Analysis of variance: females, $F = 1.17$, $P > 0.05$; males, $F = 1.03$, $P > 0.05$. There were no significant differences among different groups (Table 1).

3.3 Food efficiency: There were no significant differences in food efficiency between the Xiwang capsule treated groups and the control group (Table 2).

3.4 Hematology: Platelet count in female rats of high-dose group was lower than the control. In male rats of high-dose group in the middle of the study, and medium dose and high dose groups at the end of the study, platelet counts were lower than the control. No significant differences were observed between Xiwang capsule treated groups and the control group in the other variables (Table 3 and 4).

3.5 Clinical chemistry:

The results of serum TP, ALB, GLO, A/G, BUN, ALT, AST, CRE, GLU, and T-Che were within normal range, and no significant differences were observed between Xiwang capsule treated groups and the control group (Table 5-1 and 5-2).

3.6 Organ weight and body weight ratio (relative organ weight)

No significant differences were observed in kidney and body weight ratio and liver and body weight ratio of male rats among different groups. Liver and body weight ratio in male rats of group 3 and group 4 was higher than group 1 (Table 6).

3.7 Histopathology

No relevant histopathologic lesions were observed in heart, kidney and intestine of all rats. In group 3 and group 4, different degrees of watery degeneration were observed in female and male rats, and the degeneration worsened as the Xiwang capsule concentration in the diet increased (Table 7).

4 Conclusions:

There were no abnormal findings in general conditions, body weight, food efficiency and blood clinical chemistry. However, Platelet count in female rats of high-dose group, male rats of high-dose group in the middle of the study, and medium dose and high dose groups at the end of the study, were lower than the controls. Liver and body weight ratio in male rats of group 3 and group 4 was higher than group 1. In group 3 and group 4, different degrees of watery degeneration were observed in female and male rats, and the degeneration worsened as the Xiwang capsule amount in the diet increased.

Group 2 was the no-observed-effect-level (NOEL) group, and the intake of Xiwang capsule in group 2 was shown in Table 8. The NOEL of this test is 0.363 g/kg b.w. (equivalent to 72.5 µg/kg b.w. selenium) in male rats and 0.448 g/kg b.w. (equivalent to 89.7 µg/kg b.w. selenium) in female rats.

Table 1. Effects of Xiwang capsule on body weight (g) in rats

Group	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
1	65.3±4.0	103.4±4.7	141.0±12.1	147.2±12.8	170.0±15.5	183.7±16.0	196.5±20.8	203.5±24.7	212.2±20.5	222.4±20.3	229.7±22.4	237.3±22.3	247.7±19.0	255.0±20.4
2	65.2±4.1	103.8±4.3	142.6±8.3	149.3±9.7	172.4±10.9	184.9±12.8	199.0±16.2	206.7±20.3	214.2±18.4	226.1±16.8	233.4±14.7	240.2±13.9	248.9±13.2	258.6±12.3
3	65.3±4.5	104.2±4.9	143.4±7.4	153.8±10.2	173.5±12.6	184.5±13.5	198.3±17.4	205.3±19.1	213.9±20.8	224.7±23.6	230.6±24.8	236.4±24.6	244.8±25.5	251.3±26.9
4	65.3±3.9	104.7±5.1	144.2±6.3	156.3±8.7	179.2±10.6	188.5±14.8	201.4±17.4	208.3±21.7	217.4±25.4	229.4±27.3	233.9±23.6	240.0±22.0	246.1±21.3	251.4±20.0
1	68.4±6.2	129.5±19.2	174.3±38.6	204.7±38.3	248.6±43.6	281.3±46.6	284.1±60.3	302.1±56.2	333.8±59.4	353.4±47.3	385.0±44.8	382.7±47.5	394.4±51.3	405.0±54.5
2	68.5±7.4	130.2±18.4	177.5±26.7	201.3±39.4	237.5±42.6	279.4±59.3	286.4±54.6	305.8±52.9	331.7±41.8	350.7±46.2	378.4±42.5	381.3±43.4	390.1±44.3	398.6±46.5
3	68.1±5.3	137.8±9.7	172.5±21.3	202.2±33.5	241.7±47.2	282.8±44.5	283.3±43.9	310.7±57.2	333.8±53.5	351.6±55.2	380.5±49.1	388.3±51.7	390.4±52.8	394.7±54.6
4	68.3±6.5	133.5±17.3	170.6±22.7	198.4±34.1	234.5±39.5	275.8±47.9	282.7±51.6	309.2±51.2	334.8±43.9	347.2±41.8	389.3±42.0	397.1±36.2	407.2±36.5	406.5±38.4

Table 2 Effects of Xiwang capsule on food efficiency^a in rats

Sex	Group	Body weight increase(g)	Food consumption(g)	Food efficiency(%)
Female	1	189.7±34.3	1647.0±104.7	11.5
	2	193.4±41.8	1524.6±202.5	12.7
	3	189.0±21.7	1686.3±123.9	11.3
	4	186.1±36.4	1632.5±99.4	11.4
Male	1	336.6±27.1	1954.8±126.9	17.2
	2	330.1±42.8	1826.1±214.7	18.1
	3	326.6±33.3	1855.7±174.5	17.6
	4	338.2±28.3	1989.4±107.3	17.0

a: g of body weight increase/100 g food

Table 3. Effect of Xiwang capsule on hematological determinations

Day	Sex	Group	Hemoglobin (g/L)	Red cell (10 ¹² /L)	Total white cell (10 ⁹ /L)	Platelet (10 ⁹ /L)	Reticulocyte (10 ⁹ /L)
45	Female	1	148.7±21.4	6.5±0.7	20.9±4.5	760.0±149.6	36.1±8.1
		2	163.3±16.5	7.8±1.0	19.8±7.3	802.5±89.6	39.0±11.2
		3	139.5±7.9	7.6±0.8	28.3±8.0	751.0±218.6	28.1±8.1
		4	145.4±9.2	7.6±1.5	30.1±15.0	436.7±105.1**	27.8±16.2
	Male	1	142.3±8.7	6.6±0.3	20.8±2.4	867.5±132.5	38.6±12.3
		2	139.7±35.1	7.4±1.4	19.2±5.4	737.5±81.0	30.3±11.2
		3	146.2±7.7	7.2±0.9	20.0±12.0	765.05149.5	45.4±7.1
		4	152.7±16.2	7.4±0.7	24.3±11.0	574.8±185.3*	30.1±9.1
90	Female	1	150.4±3.29	8.6±1.6	20.7±4.6	952.0±202.9	16.4±7.1
		2	167.2±22.1	9.5±2.1	22.9±2.4	769.0±233.7	17.8±7.2
		3	153.4±6.2	9.6±1.4	30.7±6.2	566.2±153.7	16.3±8.3
		4	163.4±9.9	9.3±0.9	28.9±10.6	489.0±215.0**	18.0±7.0
	Male	1	163.5±4.9	9.4±1.7	28.2±6.6	750.5±115.0	14.2±6.1
		2	156.3±7.6	9.4±0.3	35.9±6.9	749.3±140.5	20.8±8.2
		3	154.7±10.4	9.3±1.2	30.4±5.9	620.2±180.4*	15.9±6.8
		4	161.4±12.2	9.5±1.2	28.5±9.15	478.8±88.1**	20.6±11.0

* P<0.05, as compared with the control

** P<0.01, as compared with the control

Table 4. Effect of Xiwang capsule on white cell differential count

Day	Sex	Group	Neutrophil (%)	Basophilic (%)	Eosinophil (%)	Lymphocyte (%)	monocyte (%)
45	Female	1	8.5±5.2	-	0.50±0.53	90.5±4.78	-
		2	11.4±3.4	0.20±0.44	1.30±0.70	87.4±3.5	-
		3	10.2±5.9	-	1.50±0.90	89.4±6.3	-
		4	11.7±3.4	0.11±0.33	0.40±0.70	87.8±3.2	-
	Male	1	8.0±2.2	0.25±0.50	0.50±0.58	91.0±2.6	0.25±0.50
		2	15.0±3.6	0.25±0.50	1.00±0.80	83.8±8.5	0.25±0.50
		3	11.2±2.5	-	0.70±0.80	88.1±2.4	-
		4	14.5±5.8	0.13±0.35	0.63±0.74	84.7±6.1	-
90	Female	1	9.4±5.8	-	3.0±3.4	87.6±4.9	-
		2	10.8±2.6	-	1.2±1.1	87.2±2.2	0.8±1.1
		3	8.3±4.1	-	2.6±3.2	89.1±4.8	-
		4	12.6±7.1	0.20±0.40	2.3±1.2	84.7±7.0	0.2±0.7
	Male	1	9.0±2.9	-	4.3±3.0	80.7±4.1	-
		2	7.7±2.1	0.35±0.60	1.3±0.6	90.4±2.5	0.3±0.6
		3	9.3±5.2	-	2.2±1.1	88.5±4.8	-
		4	10.9±6.8	-	2.4±2.6	85.4±1.2	1.3±1.2

Table 5-1. Effects of Xiwang capsule on blood clinical chemistry assays

Sex	Group	TP(g/L)	ALB(g/L)	GLO(g/L)	A/G	BUN(mmol/L)
Female	1	74.24±1.10	33.32±2.90	41.0±1.73	0.84±0.09	8.41±1.35
	2	73.84±1.13	34.88±0.95	39.2±1.48	0.90±0.07	8.17±1.13
	3	71.00±3.18	32.70±2.81	38.3±2.45	0.87±0.12	7.41±0.63
	4	73.12±0.60	33.23±1.77	40.0±2.00	0.84±0.07	7.57±1.01
F value		1.004	0.046	0.428	0.447	0.527
Male	1	71.90±1.71	31.40±1.15	40.8±0.96	0.78±0.05	7.86±1.69
	2	69.10±1.13	31.63±1.06	37.7±1.15	0.87±0.06	8.05±0.53
	3	71.83±1.64	30.70±1.59	41.3±2.31	0.75±0.07	7.87±0.66
	4	70.28±1.51	31.35±1.52	39.0±1.41	0.80±0.08	7.55±1.03
F value		0.625	0.029	1.038	0.742	0.639

Table 5-2. Effects of Xiwang capsule on blood clinical chemistry assays (continued)

Sex	Group	ALT(U/L)	AST(U/L)	CRE(μ mol/L)	GLU(mmol/L)	T-Che(mmol/L)
Female	1	51.8 \pm 14.2	198.4 \pm 19.0	39.02 \pm 6.10	8.46 \pm 2.14	1.63 \pm 0.33
	2	39.0 \pm 13.8	159.8 \pm 29.0	34.08 \pm 4.47	6.38 \pm 0.55	1.64 \pm 0.16
	3	33.0 \pm 8.12	174.3 \pm 49.8	32.90 \pm 4.17	6.08 \pm 1.53	1.57 \pm 0.26
	4	41.0 \pm 15.0	163.9 \pm 14.3	34.86 \pm 4.81	6.52 \pm 0.93	1.73 \pm 0.39
F value		1.853	0.854	1.006	0.406	0.012
Male	1	43.0 \pm 9.13	141.8 \pm 14.8	35.55 \pm 10.6	5.91 \pm 1.24	1.65 \pm 0.15
	2	51.3 \pm 8.80	196.3 \pm 18.2	32.10 \pm 2.45	6.86 \pm 0.49	1.31 \pm 0.16
	3	47.2 \pm 6.66	153.7 \pm 11.1	33.53 \pm 2.80	6.03 \pm 1.36	1.54 \pm 0.16
	4	41.9 \pm 6.66	165.6 \pm 20.3	35.06 \pm 6.35	6.49 \pm 1.00	1.60 \pm 0.17
F value		1.472	1.113	0.741	0.228	0.093

Table 6. Effects of Xiwang capsule on organ and body weight ratio^a

Sex	Group	liver/bw %	kidney/bw %
Female	1	2.96 \pm 0.23	0.71 \pm 0.057
	2	3.37 \pm 0.44	0.70 \pm 0.039
	3	3.51 \pm 0.48*	0.68 \pm 0.059
	4	3.73 \pm 0.49**	0.73 \pm 0.081
Male	1	3.17 \pm 0.28	0.68 \pm 0.077
	2	3.39 \pm 0.27	0.72 \pm 0.092
	3	3.47 \pm 0.12	0.55 \pm 0.058
	4	3.24 \pm 0.07	0.53 \pm 0.061

* F=9.072, P<0.05, as compared with the control group

** F=10.974, P<0.01, as compared with the control group

Table 7. Effects of Xiwang capsule on rat liver histopathology

Sex	Group	No. of rats	Normal	Low-grade watery degeneration	Medium-grade and high-grade degeneration
Female	1	10	10	0	0
	2	10	10	0	0
	3	10	0	6	4
	4	10	0	3	7
Male	1	10	10	0	0
	2	10	10	0	0
	3	10	0	6	3
	4	10	0	3	7

Table 8. Intake of Xiwang capsule in rats of Group 2

Sex	Mean body weight (g)*	Mean food consumption	Xiwang capsule consumption (g/kg.b.w)	Equivalent to selenium ($\mu\text{g}/\text{kg.b.w.}$)
Female	188.95	16.94	0.4483	89.66
Male	279.8	20.29	0.3626	72.52

* means of mean body weight of each week

Tested by:

Reviewed by:

Signed and issued by:

Seal of testing laboratory

March 28, 1997

Analytic and Testing Centre of Nanjing Railway Medical College
Report on teratogenicity test of Xiwang capsule

(food) Test No. 11

Report No. 970328

Comissioned by the Department of Leaf Protein, Hefei Economy and Technology College, a teratogenicity test in rats on Xiwang capsule were conducted from November 1996 to March 1997. The aim of this study is to provide the basis for toxicological safety assessment on this product.

1. Materials

1.1 sample

Xiwang capsule was provided by Department of Leaf Protein, Hefei Economy and Technology College. The compound is an orange powder, and the selenium concentration was 200 µg/g (the analytical data was provided by the Department of Leaf Protein, Hefei Economy and Technology College).

1.2 Animals

SD rats were obtained from the Experimental Animal center, Nanjing Railway Medical College. Animal certificate number: Su annial (quality 95036), Envionment certificate number: Su envionment (envionment 95102).

2. Methods

Forty-eight female SD rats, 200-250 g body weight, and twenty-four matured male rats were paired in the propotion of two females to one male. Pudental bolts were observed every morning and the day of pudental bolts observed was set as Day 0 of gestation. Pregnant rats were randomly divided into 4 groups. Each group consisted of 12 rats. Group 1, the basal diet group; Group 2, 0.50% Xiwang capsule + 99.50% basal diet; Group 3, 1.50% Xiwang capsule + 98.50% basal diet; Group 4, 3.00% Xiwang capsule + 97.00% basal diet (equivalent to 0.0, 1.0, 3.0, or 6.0 µg selenium/g diet). Basal diet was made according to the regular formula. Rats consumed the above diet from Day 7 to the Day 16 of gestation. Each rat was kept in one cage and free access to food and water. The animals were killed on the Day 20 by cervical dislocaiton and dissected to obtain uterus and fetus.

3. Parameters

- 3.1 **Female rats reproductive function:** uterus weight, implantation number, early absorbed fetus number, delayed death fetus and alive fetus number.
- 3.2 **Alive fetus examination:** fetus weight, fetus body length, fetus appearance.
- 3.3 **Fetus skeleton examination:** One-half fetus were fixed in Alizarin red solution and stained after skinned and visceral discarded. Skull, temporal bone, occipital bone, cervical vertebrae, thoracic vertebrae, lumbar vertebrae, caudal vertebrae and bones of limbs were examined.
- 3.4 **Fetus visceral examination:** One-half fetus were fixed in Bouins solution and examined following serial sectioning.

4. Results

- 4.1 **Female reproductive function:** The results were shown in Table 1. There were no significant differences in all the parameters among different groups (Chi-square test, $X^2=2.949$, $P>0.05$). Therefore, there were no Xiwang capsule-treated effects on rat gestation.
 - 4.2 **Alive fetus examination:** There were no abnormal effects on fetus appearance. The results of fetus weight and body length were shown in Table 2. There were no significant differences among different groups by variance analysis ($P>0.05$).
 - 4.3 **Fetus skeleton examination:** There were no effects on skull, temporal bone, occipital bone, cervical vertebrae, thoracic vertebrae, lumbar vertebrae, caudal vertebrae and bones of the limbs.
 - 4.4 **Fetus visceral examination:** No abnormal observations were found on fetus visceral in all groups.
5. **Conclusion:** There were no Xiwang capsule-treated effects on all the parameters, therefore, Xiwang capsule was not teratogenic in SD rats.

Table1 Effects of Xiwang capsule on gestation of rats

Group	Fetus number	Uterus weight (g)	Implatation number(%)	Early absorbed fetus(%)	Delayed death fetus (%)	Alive fetus (%)
1	12	60.4±3.05	11.6±1.38	1.44(2)*	0	137
2	12	60.2±4.75	12.0±1.41	1.39(2)	0	142
3	12	58.7±3.89	11.8±1.60	2.13(3)	0	138
4	12	56.4±7.53	11.4±1.13	5.11(7)	0	130

* The number in the bracket were the number of absorbed fetus

Table 2 Effects of Xiwang capsule on gestation of rats

Group	Fetus weight (g)	Fetus body length (g)
1	2.94±0.97	3.82±1.11
2	3.24±1.03	4.41±1.18
3	3.11±0.76	3.93±0.91
4	2.89±0.94	3.98±0.97

Tested by:

Reviewed by:

Signed and issued by:

Seal of testing laboratory

March 28, 1997

Notes:

Main instruments used:

Olympus microscope, Model TCS-500A animal balance, super clean workplace, Hachi 7060 autoanalyzer.

Test conditions:

Temperature: 18-20 °C

Relative humidity: 50-60%