

TOXICITY STUDY OF THREE ROOTS GRANULE MEDICINE

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Abstract

Purpose: To study the effect of Three roots granule medicine with compound medicines ingredient of *Asparagus cochinchinensis*, *Polygonatum odoratum* and *Polygonatum sibiricum* on acute toxicity test and genotoxicity tests.

Methods: In toxicity study of Three roots granule medicine, by acute toxicity test were observed general status of animals, body weight changes, signs of poisoning and death for 14 day and determined the maximum tolerated dose, by Ames test, mouse bone marrow polychromatic erythrocyte micronucleus test and mouse sperm deformity test were determined genotoxicity effect. The data were analyzed through SPSS 19.0.

Results and Conclusions: In the result of toxicity study, three roots granule medicine was MTD>15g/kg, no acute toxic activity, did not induce mutagenic effect in Ames test and was negative in mouse bone marrow polychromatic erythrocyte micronucleus test and mouse sperm deformity test. Three roots granule medicine has no acute toxicity effect, no genotoxicity effect and safety. We as regard as in future can continuously study to the other pharmacology study of three roots granule medicine.

Key words: San Gen granule medicine, acute toxicity test, Genotoxicity test

Introduction

Three roots granule medicine (TRGM) is new medicine ingredient with three components of *Asparagus cochinchinensis*, *Polygonatum odoratum* and *Polygonatum sibiricum*. In Mongolian traditional medicine, those three plants include in medicines classification of increase to body nutrition and commonly used to improve nutrient in human body, to increase the strength and to remove renal disease with cold and wind quality etc. [1]. Through research reports, single medicine substances of *Asparagus cochinchinensis*, *Polygonatum odoratum* and *Polygonatum sibiricum* are abundant contain chemical compounds with bioactive and has many pharmacology effects, such as non toxicity, improve immune function, anti-aging, antioxidant, anti-inflammation, anti bacterial, anti cancer etc. [2-6]. Therefore, *Asparagus cochinchinensis*, *Polygonatum odoratum* and *Polygonatum sibiricum* are as can be regard as safety, non toxicity and with benefits pharmacology effects. So we studied effect of Three roots granule medicine with compound medicine ingredient of *Asparagus cochinchinensis*, *Polygonatum odoratum* and *Polygonatum sibiricum* on acute

toxicity test and genotoxicity test. This study can important source for future pharmacology study of three roots granule medicine.

Materials and methods:

Preparation of San Gen granule medicine:

The medicinal raw materials were provided by the Mongolian Institute of Pharmaceutical Engineering Technology of Inner Mongolia and the three roots granule medicine produced in Beijing Hyde Run Pharmaceutical Group Co., Ltd. The final product is granules, brown particles, specification 7.5g/bag,. lot number: 20160613,. Human body recommended dose: 15g/60kg daily. Sample was two kind of type, such as dry cream powder and brown powder. Dry powder recommended dose: 6g/60kgBW daily. Store in a cool and dry place. Shelf life was 24 months for testing purposes. The following report "sample" was dry cream powder.

Animals:

In experiment used Kunming mice of Beijing Vital-China Laboratory Animal Technology Co., Ltd. The test animals were developed in the shielding environment of temperature of 22-24°C and humidity of 60-80%.

Acute toxicity test in mice:

Kunming male and female 20 mice with 18 g-22 g have taken for testing. In mice administered 15.00 g/kg TRGM, twice a day, with interval 6h. During the experiment, the animals freely food intake and drink water, and to administered the test substance by gavages. The general status of animals, body weight changes, signs of poisoning and death were observed for consecutive 14 day and determined the maximum tolerated dose (MTD).

Ames test:

Four strains such as TA97, TA98, TA100, and TA102 of qualified *Salmonella typhimurium* histidine-deficient were used for test and get on standard plate incorporation method test with and without rat liver S9. S9 prepared according to evaluation of technical specifications and Evaluation of Health Food (2003 Edition) as well as guiding book of Ames test work center (CIHFBUU- ZYZSD02-08-2005). In the result, we checked in sterilized condition and to measured by Lowry method the protein content (<40 mg/ml) and determined biological activity as *in vitro* metabolic activation system. Test groups received five doses of the TRGM, equivalent to the doses of 8 µg/plate, 40 µg/plate, 200 µg/plate, 1000 µg/plate, 5000 µg/plate. Weigh 1.00 g sample plus sterile water to prepare 20mL liquid, sterilized for 20min at 0.103 MPa. Then, it was incubated for 24h at 37 °C and grown in sterilized condition. Based on highest concentration 50 mg/ml (ie 5000 µg/plate) to determined dose concentration and by sterile water to diluted with 5 times same ratio. Also to established positive control, solvent (sterile water) control, solvent (sterile DMSO) control, untreated control. Water and DMSO as solvents were sterilized at 0.103 MPa for 20 min. The positive control without S-9 was acted kexon (50.0 µg/plate applicable strains TA97, TA98, TA102), sodium azide (1.5 µg/plate applicable strain TA100), and the positive control with S-9 was acted 2-aminofluorene (10.0µg/plate, applicable strains TA97, TA98, TA100), 1,8-dihydroxy anthraquinone (50.0µg/plate, applicable strain TA102). At the top agar added 0.1ml bacteria liquid of increase test strain, 0.1ml test substance solution and 0.5ml of the S-9 mixture. Mixed after 3 plates from each dose pour into the bottom culture plate.

Then incubated at 37 ± 1 °C for 48h and reverse mutated colonies were counted. When the reverse-mutated colonies number more than twice, it is positive. The test is repeated twice under the same conditions.

Mouse bone marrow polychromatic erythrocyte micronucleus test:

Fifty mice (25-35g) were randomly divided into 5 groups such as positive control group of cyclophosphamide, solvent control group of sterile water, low, middle and high dose group of three roots granule medicine with 5 males and 5 females mice in each groups. Mice in treatment groups were administered orally 2.5, 5, 10g/kg TRGM respectively, twice daily, with 24h interval, in positive group was administered orally 40mg/kg cyclophosphamide once daily and solvent group was administered orally 20 ml/ kg saline solution once daily. Six hours after the last administered, animals were sacrificed by cervical dislocation. Take sternal bone marrow, was diluted with calf serum, fixed in methanol, stained with Giemsa and read in double-blind method. In optical microscope, were counted about 1000 of total polychromatic erythrocytes (PCE) of each animal and calculated to rate of micronucleated cells and rate of micronucleus-containing PCE (1000). At the same time, were counted about 200 of total polychromatic erythrocytes and normochromatic erythrocyte (NCE), and the PCE / NCE ratio was calculated.

Mouse sperm deformity test:

Fifty matured male mice (25-35g) were randomly divided into 5 groups such as positive control group of cyclophosphamide (40mg/kg), solvent control group of sterile water and low, middle, high dose (2.5, 5, 10g/kg) groups of TRGM with male 5 mice in each groups. Mice in positive group and solvent group were administered 20 ml/kg saline solution and mice in treatment groups were administered 2.5, 5, 10g/kg San Gen granule medicine respectively, twice daily for 5 day and then sacrificed on day 30 with cervical dislocation. Take epididymides, stained with eosin. In microscopy, was counted about 1000 of total sperm and calculated to rate of sperm deformity.

Statistic analysis:

Datas of experimental result are expressed as mean±standard deviation, and statistical

significance between the groups was determined by one-way ANOVA, Dunnett's T test and Duncan of. SPSS 19.0.

Result : Effect of Three roots granule medicine on acute toxicity test

Table 1.

Effect of Three roots granule medicine on acute toxicity test

Animal strains	Gender	Dose (g/kg)	Number of animals	Initial weight ($\bar{x}\pm SD$)	Final weight (g)	Number of deaths (g)	MTD (g/kg)
Kunming mice	male	30.00	10	20.4±1.1	39.6±1.7	0	>15
	female	30.00	10	20.4±1.0	34.4±1.5	0	>15

As can be seen from the results of Table 1, the mice of two genders were administered twice a day with the dose of 30.00 g/kg. During the 14 days of observation, the animals were in normal condition without any signs of toxicity or death. On the 15th day, the animals were sacrificed for

gross anatomy, and were didn't observe obvious abnormalities in the main organs. In female and male mice, administered maximum tolerated dose (MTD) was higher than 15g/kg. Three roots granule medicine was no acute toxic activity.

Effect of Three roots granule medicine on genotoxicity test

Table 2.

Effect of Three roots granule medicine on first times Ames test

Group		Dose(μ g / plate)	TA97 ($\bar{x}\pm SD$)	TA98 ($\bar{x}\pm SD$)	TA100 ($\bar{x}\pm SD$)	TA102 ($\bar{x}\pm SD$)
Solvent control (sterile water)	-S9	0	109±1	40±6	146±6	262±12
Solvent control (sterile water)	+S9	0	111±8	37±3	144±17	281±20
Solvent control (sterile water, DMSO)	-S9	0	104±1	44±2	131±4	273±13
Solvent control (sterile water, DMSO)	+S9	0	121±14	37±2	151±7	267±6
Untreated control	-S9	0	118±3	39±4	152±19	267±14
Untreated control	+S9	0	125±8	43±2	167±8	264±9
Test substance	-S9	8	122±4	44±2	146±22	285±13
		40	126±11	38±7	152±6	264±8
		200	118±12	41±3	136±8	271±18
		1000	118±6	37±3	156±16	284±5
		5000	113±19	43±6	156±11	259±10
Test substance	+S9	8	133±6	37±5	146±11	276±12
		40	126±19	39±2	139±12	270±15
		200	116±11	42±4	134±8	287±7
		1000	123±11	38±7	153±20	270±13
		5000	113±12	39±5	151±20	283±20
Dexon	-S9	50.0	1847±178	805±79		803±63
Sodium azide	-S9	1.5			872±24	
2-aminofluorene	+S9	10.0	1443±56	5156±153	1014±106	
1,8-dihydroxy anthraquinone	+S9	50.0				802±74

Note#: The values are the mean \pm standard deviation of three plates.

Table 3.

Effect of Three roots granule medicine on second times Ames test

Group		Dose (µg/plate)	TA97 ($\bar{x} \pm SD$)	TA98 ($\bar{x} \pm SD$)	TA100 ($\bar{x} \pm SD$)	TA102 ($\bar{x} \pm SD$)
Solvent control (sterile water)	-S9	0	125±7	36±5	138±14	268±25
Solvent control (sterile water)	+S9	0	109±7	41±3	144±23	285±7
Solvent control (sterile water, DMSO)	-S9	0	122±11	42±1	153±21	267±21
Solvent control (sterile water, DMSO)	+S9	0	120±14	41±3	152±7	284±16
Untreated control	-S9	0	121±11	36±5	150±22	255±6
Untreated control	+S9	0	131±7	45±1	144±12	273±15
Test substance	-S9	8	128±8	39±6	145±25	267±13
		40	121±4	43±5	148±20	283±13
		200	116±20	38±7	141±13	264±13
		1000	113±4	40±4	146±23	276±20
		5000	117±7	42±4	138±14	274±21
Test substance	+S9	8	155±12	35±3	143±16	281±24
		40	110±9	41±4	138±9	275±1
		200	120±13	38±2	147±8	178±6
		1000	122±6	41±1	153±19	289±2
		5000	123±12	38±5	143±17	284±16
Dexon	-S9	50.0	1749±131	773±65		801±75
Sodium azide	-S9	1.5			794±88	
2-aminofluorene	+S9	10.0	1500±133	2152±286	869±85	
1,8-dihydroxy anthraquinone	+S9	50.0				745±64

Note#: The values are the mean ± standard deviation of three plates.

The results are shown in Table 2 and Table 3. In each test substances group, reverse-mutated colonies number were no more than twice compared to solvent control group and untreated control group and was no dose-response

relationship. For Three roots granule medicine with and without liver microsomal enzyme activation system, the result of Ames test was negative and did not induce mutagenic effect.

Effect of Three roots granule medicine on mouse bone marrow polychromatic erythrocyte micronucleus test

Table 4.

Effect of Three roots granule medicine on mouse bone marrow polychromatic erythrocyte micronucleus test of female mice

Group	Negative control group (n=5)	Low dose group (n=5)	Middle dose group (n=5)	High dose group (n=5)	Positive control group (n=5)
Dose (g/kg)	0	2.5	5.00	10.00	0.04
PCE number	5X1000	5X1000	5X1000	5X1000	5X1000
Number of micronucleated PCE	11	11	10	12	54
Micronucleus rate (%) ($\bar{x} \pm SD$)	2.2±1.3	2.2±1.1	2.0±0.7	2.4±0.9	10.8±2.8

P value	¾	1.000	1.000	0.839	# 0.000
PCE number	1000	1000	1000	1000	1000
NCE number	885	878	837	862	885
PCE/NCE ($\bar{x}\pm SD$)	1.13±0.06	1.14±0.05	1.20±0.06	1.16±0.08	1.13±0.07

Note #: significant difference between the positive control group and the solvent control group (P <0.01)

Table 5.

Effect of Three roots granule medicine on mouse bone marrow polychromatic erythrocyte micronucleus test of male mice

Group	Solvent control group (n=5)	Low dose group (n=5)	Middle dose group (n=5)	High dose group (n=5)	Positive control group (n=5)
Dose (g/kg)	0	2.5	5.00	10.00	0.04
PCE number	5X1000	5X1000	5X1000	5X1000	5X1000
Number of micronucleated PCE	10	9	12	10	59
Micronucleus rate (%) ($\bar{x}\pm SD$)	20±0.7	1.8±0.4	2.4±0.9	2.0±1.0	11.8±2.8
P value	¾	1.000	0.677	1.000	# 0.000
PCE number	1000	1000	1000	1000	1000
NCE number	867	852	837	881	882
PCE/NCE ($\bar{x}\pm SD$)	1.16±0.09	1.18±0.07	1.20±0.07	1.14±0.08	1.14±0.08

Note #: significant difference between the positive control group and the solvent control group (P <0.01)

Results are shown in Table 4 and Table 5. In each doses group of the two sexes was no significant inhibitory effect on the proliferation of bone marrow cells and the PCE/NCE ratio in three doses group of test substance was no cytotoxicity. In each dose group, micronucleus rate of bone marrow polychromatic erythrocytes of two sexes mice was no significant difference

compared to the solvent control group (P> 0.05). The micronucleus rate of bone marrow polychromatic erythrocytes in the positive control group was significantly higher than that in the solvent control group (P <0.01). Bone marrow polychromatic erythrocyte micronucleus test of Three roots granule medicine in mice was negative.

Effect of Three roots granule medicine on mouse sperm deformity test

Table 6.

Effect of Three roots granule medicine on mouse sperm deformity test

Group	Negative control group (n=5)	Low dose group (n=5)	Middle dose group (n=5)	High dose group (n=5)	Positive control group (n=5)
Dose (g/kg)	0	2.5	5.00	10.00	0.04
Number of sperm	5X1000	5X1000	5X1000	5X1000	5X1000
Deformity number	78	87	72	74	289
Deformity rate (%)	1.56±0.11	1.74±0.34	1.44±0.09	1.48±0.48	5.58±0.79
P value	¾	0.530	0.681	0.745	# 0.000

Note #: significant difference between the positive control group and the solvent control group (P <0.01)

As can be seen from Table 6, in each doses group, rate of sperm deformity was no significant difference compared to the solvent control group (P> 0.05). In positive control group was rate of

sperm deformity was significantly higher than the solvent control group (P <0.01). Mouse sperm deformity test of Three roots granule medicine in mice was negative.

Discussion: Single medicine substances of *Asparagus cochinchinensis*, *Polygonatum odoratum* and *Polygonatum sibiricum* are has shown a variety of pharmacological effect and was determined non toxicity on pharmacology study of safety and toxicity. Through the safety study of *Polygonatum sibiricum*, it was had LD50 > 10g/kg, non toxic effect, chromosome fracture effect in bone marrow cell, no mutagenic effect of sperm cell and no mutagenic effect on 4 strain of bacteria [6]. And in sub-chronic toxicity test of *Asparagus polysaccharide* (ACP), ACP under 1.60 g/kg with intraperitoneal injection was no obvious change in Alanine transaminase activity, Aspartate Transaminase activity, creatinine and the level of urea nitrogen and was no visible pathological changes on the liver, spleen and kidney histology [7]. In toxicity studies of *Rhizoma Polygonati Odorati*, by the acute toxicity test *Polygonati Odorati* did not induce any toxicity sign or death in mice and did not induce abnormalities in hemology, blood biochemistry, autopsy, histopathology, appearance, behaviors and body weight by the chronic toxicity test. In Ames tests did not induce a mutagenetic effect. In the bone marrow micronucleus test and sperm aberration test, did not increase the rate of micronucleus cells, the number of aberrant sperms and the rate of sperm aberration of mice. [8]. Therefore those three plants are safety and have no toxicity.

In the result of our study, three roots granule medicine with compound medicines ingredient of *Asparagus cochinchinensis*, *Polygonatum odoratum* and *Polygonatum sibiricum* was MTD>15 g/kg, no acute toxic activity, did not induce mutagenic effect in Ames test and was negative in mouse bone marrow polychromatic erythrocyte micronucleus test and mouse sperm deformity test. Three roots granule medicine has no acute toxicity effect, no genotoxic effect and safety. We as regard as in future can continuously study to other pharmacology study of Three roots granule medicine.

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Уншин танилцаж, нийтлэх санал өгсөн:
ЭЗУ-ы доктор, дэд профессор Э.Сэлэнгэ