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Research Article

Dietary Toxicity Evaluation of Brown Rice Starter Red Kojic Rice Wine

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Abstract: The present study aims to discuss the reference safety of the Brown Rice Starter Red Kojic Rice Wine (BRSRKRW) and provide scientific basis for rational development and utilization of the wine. In order to evaluate the toxicity of BRSRKRW *in vivo*, acute and subchronic toxicity of BRSRKRW in SD rats by intraperitoneal injections were evaluated. In this acute study, doses of BRSRKRW at 1, 1.5, 2, 2.5, or 31.5, 3 and 6 g/kg·bw, were administered. No adverse effects were observed during a 14-day period and at gross histopathological examination. In the subchronic study, BRSRKRW at 1.5, 3 and 6 g/kg·bw were administered for 30 days. The results showed that BRSRKRW did not affect weight gain in growing rats, in the level of hematological parameters, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, blood urea nitrogen, creatinine, uric acid in blood and histopathological damages of the liver. Overall, the study suggests that the risk of drinking BRSRKRW toxicity to mammals is not negligible.

Keywords: Acute toxicity, brown rice starter red kojic rice wine, food safety, genetic toxicity, subacute toxicity

INTRODUCTION

Brown Rice (BR) is un-milled or partly milled rice, a kind of whole grain. Researchers from the Harvard School of Public Health have just released a study showing that white rice consumption increases the risk of Type 2 diabetes while brown rice consumption actually reduces the risk. As BR is difficult to chew, germinating it may improve texture, palatability and the amount of the bioactive molecules (Patil and Khan, 2011). Brown rice starter is a functional basic material prepared by mixture of honey and brown rice followed by fermentation with dried active yeasts, which is a health food that supplies necessary vitamins, minerals and enzymes and γ-aminobutyric acid (Komatsuzaki *et al*., 2007).

Rice Red yeast rice is a food preparation tradition going back to ca. 300 BC. Red yeast rice is traditionally used in the production of several types of Chinese wine, Japanese sake (akaisake) and Korean rice wine (hongju), imparting a reddish color to these wines. Monascus fermented rice is also widely used as starter culture for brewing red rice wine. It is taken internally to invigorate the body, aid in digestion and revitalize the blood (Erdogrull and Azirak, 2004). The active ingredient in red yeast is the same as the active ingredient in prescription drugs called statins used for high cholesterol. Red yeast rice is a dietary supplement containing monacolins, unsaturated fatty acids and phytosterols capable of lowering Low-Density Lipoprotein (LDL) cholesterol. Some research showed that taking a specific red yeast product for two to three months could significantly lower total and "bad cholesterol" (Low-Density Lipoprotein (LDL) cholesterol) levels and triglycerides (Venero *et al*., 2010; Becker *et al*., 2009). The clinical trials reviewed in the meta-analysis, the only study conducted in the United States reported a 22% reduction of LDL-cholesterol after 12 weeks (Heber *et al*., 1999). The safety of red yeast rice products has not been established and some commercial supplements have been found to contain high levels of the toxin citrinin (Gordon *et al*., 2010) Red yeast contains chemicals similar to the prescription drugs called "statins". Therefore, red yeast might also cause side effects similar to statin drugs, such as liver damage and severe muscle pain and muscle damage. At present, there is no brown rice starter red kojic rice wine safety evaluation research, in order to verify the safety, put it on the market and promote its further research, development and utilization, the acute toxicity test, mouse bone marrow cells in mice and micronuclei sperm malformation test, Ames test and 30 days feeding test were set according to the health food inspection and evaluation technology standard.

MATERIALS AND METHODS

Test substance: BRSRKRW in this study was light yellow liquid, provided by Shenyang normal university, Shenyang, China.

Test animal: SD rats and KM mice were purchased from Shanghai Slac Laboratory Animal Ltd (Shanghai,

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China). Each group of five rats of the same sex was housed in one plastic cage with free access to commercial pellet food and tap water. The animal room was maintained at 20-25°C, 30-70% relative humidity and 12-h light-dark lighting cycle. The rats were weighed and randomly assigned to treatment and control groups.

Acute toxicity: A total of 80 mice of 4 weeks of age, 18-22 g divided into 5 groups, each group consisted of 10 males and 10 females. The mice were fasted for l2 h before dosing. Based on a preliminary dosing experiment, doses of BRSRKRW at 1, 1.5, 2, 2.5, or 3 g/g·bw were administered to mice by lavage. The toxic signs and morbidity were monitored for 14-day. The animal that died during the 14-day observation period was necropsied immediately. All surviving animals were euthanatized and necropsied at the end of the 14-day observation period.

Subchronic toxicity study: A total of 40 rats (5 weeks of age, 80-120 g) were divided into 3 BRSRKRW treatment groups and 1 control group. Each group included 5 males and 5 females, Based on the previous acute toxicity study, 3 dose levels of BRSRKRW were used to administer the animals, 1.5 mg/kg·bw (1/20 LD50), 3 g/kg·bw (1/10 LD50) and 6 g/kg·bw (1/5 LD50). The control animals received only rice wine. After the treatment period of 30 days, all rats were sacrificed.

General observation: All SD rats were weighed on the day of the first treatment and weekly thereafter. Daily observation was conducted on animal fur, movement and behavior. All the premature dead animals were recorded and necropsied immediately. At the end of the 30-day treatment, the weights of the following organs, including brain, thymus, heart, liver, spleen and renal were recorded.

Serum biochemical analysis: All SD rats were fasted for 12 h before blood sample collection. After then, the rats were anesthetized with ether anesthesia and the blood samples were collected from the femoral vein. The serum was obtained by centrifugation of the whole blood at 3000 rpm for 15 min. Liver function was evaluated based on the serum levels of Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Total Protein (TP), Albumin (ALB). Nephrotoxicity was determined by Blood Urea Nitrogen (BUN), Creatinine (Cr), Uric Acid in blood (UA), these biochemical parameters were measured by an automated hematology analyzer (Beckman, USA).

Morphological examination: All the selected organs (liver and kidney) were fixed in 10% formalin embedded in paraffin, sectioned and stained with Hematoxylin-Eosin (HE) for pathological examination under light microscopy.

Statistical analysis: Results were expressed as mean±Standard Deviation (S.D.). Multi-group comparisons of the means were carried out by one-way Analysis of Variance (ANOVA) test. Dunnett's test was used to compare the differences between the experimental groups and the control group. Student's t-test was used to compare the means of each nano-group and the corresponding fine group. The statistical significance for all tests was set at $p<0.05$.

RESULTS

Acute toxicity: The clinical signs observed in the acute toxicity study were excited, gait instability phenomenon in mice after dosing at all dose levels; However, these toxicity signs completely disappeared after 30 min and no further death occurred among the rats that survived during the following 13 days.

From Table 1, it is known that during the test there is no obviously difference on body quality when compared trial group with control group, diet and activity of mice is normal, growth condition is good. There was no obvious pathological change in any of the treated groups at necropsy on the 14th day. BRSRKRW known maximum concentration under the condition of density lavage, MTD more than 30 g/kg·bw, the dose equivalent to the human body the recommended 750 times. The results showed that BRSRKRW is actual non-toxic.

Subchronic toxicity: No deaths were observed in any of the treated groups during the 30-day treatment period. The fur of rats in the high (6 g/g·bw) groups became rough. The rats in the high dose group exhibited excited, phenomenon within the 0.5st h after dosing. However, these behaviors of rats returned to normal 0.5 h after the treatment. From Table 2, it is known that after mice were fed 30 days the different concentration of BRSRKRW, Body weights among different groups were not significantly different ($p>0.05$), indicating that BRSRKRW compared with controls on mice body quality have no obvious influence.

The influence of serum biochemical indices: After 30 day's treatment. The main hematological findings were there was no significant difference between the BRSRKRW and control groups in the serum

Table 1: The result of BRSRKRW through the mouth acute toxicity test

Gender of animals	Dose/ (g/kg·bw)	Number	Mortality (%)	MTD/ (g/kg·bw)
Male	30	20	5	>30
Female	30	20	10	>30

Table 2: The influence of BRSRKRW on SD rats weight

Group of experiments	Initial mass	7 days	14 days	21 days	28 days	30 days
Control group	101±5.12	123±5.32	147±5.76	162±5.33	177±6.45	182±7.04
Low-concentration group	104±5.22	123±6.61	1458±5.03	162±5.46	179±5.65	186±5.56
Medium-concentration group	102±5.41	123±6.23	147±5.25	165±5.36	181±5.65	192±7.85
High-concentration group	99.8±5.23	124±6.22	148±6.45	167±5.33	183±5.67	193±7.31

Table 3: The influence of BRSRKRW on serum biochemical indices in SD rats

Group of experiments	UREA (mmol/L)	Cr (μmol/L)	UA (μmol/L)	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/L)	ALB (g/L)
Control group	5.10±0.45	28.00±1.09	172.00±14.76	36.25±3.57	419.00±8.93	0.50±0.03	63.20±2.23	36.70±3.11
Low-concentration group	5.05±0.48	26.25±4.31	160.00±15.06	42.70±4.73	423.07±11.35	0.60±0.04	64.12±4.56	33.67±2.45
Medium-concentration group	4.27±0.78	25.33±3.68	175.33±12.08	43.45±4.22	421.50±11.05	0.50±0.05	64.00±4.45	34.23±2.34
High-concentration group	4.70±0.20	27.00±2.16	168.67±15.52	41.15±4.75	401.25±12.76	0.50±0.03	73.28±3.56	30.20±2.34

Table 4: The influence of BRSRKRW on SD rats organs indexes

Group of experiments	Brain	Thymus	Heart	Liver	Spleen	Renal
Control group	0.51±0.02	0.08±0.01	0.29±0.02	2.73±0.19	0.21±0.02	0.57±0.020
Low-concentration group	0.67±0.05	0.12±0.01	0.35±0.02	2.57±0.20	0.27±0.02	0.76±0.040
Medium-concentration group	0.73±0.04	0.11±0.01	0.28±0.02	2.72±0.17	0.27±0.01	0.65±0.040
High-concentration group	0.47±0.02	0.07±0.01	0.29±0.02	2.73±0.19	0.21±0.02	0.66±0.020

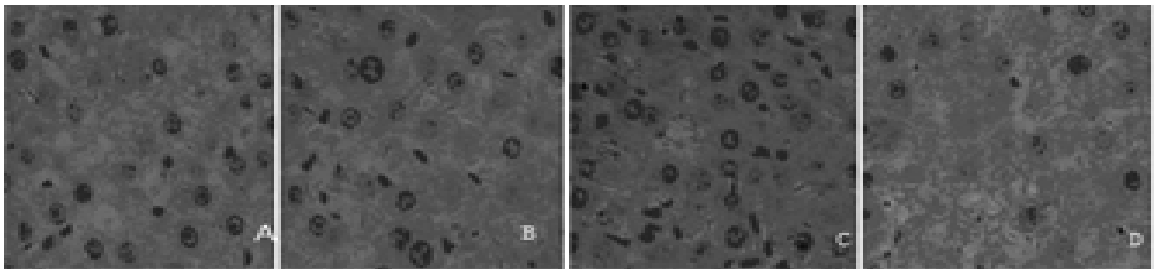

Fig. 1: Histological examination of the liver in the control and BRSRKRW treated rats after 30 days administration (HE×400), (A) shows control group with no observed not observed anomaly (HE×400), (B) shows 1.5 g/kg·bw group with no pathological changes in the liver (H £ E×400), (C) shows 3 g/kg·bw group with no pathological changes in the liver (HE×400), (D) shows 6 g/kg·bw group with no pathological changes in the liver (HE×400)

biochemical indices. From Table 3, it is known that the rats of different concentrations group compared with the blank control group and control group yellow rice wine blood biochemical indicators, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Total Protein (TP), Albumin (ALB). Nephrotoxicity was determined by Blood Urea Nitrogen (BUN), Creatinine (Cr), Uric Acid in blood (UA) have no obvious abnormal changes ($p>0.05$).

The influence of BRSRKRW on organs indexes: None of the other relative weights of organs (brain, thymus, heart, liver, spleen, renal) was significantly different from the control group. After the mice were fed by BRSRKRW feeding mice 30-day, dissected the mouse liver, kidney, spleen, testicular and ovarian. Microscopic observation results show that high dose group and control group and the results are compared to two in normal morphology range, did not see have obvious toxic pathological changes. Each dose group SD rats of brain quality/body quality, thymus quality/body quality, heart quality/body quality, liver quality/body quality, spleen quality/body quality, renal quality/body quality factor and the control group comparison were no significant difference ($p>0.05$), Table 4.

The influence of BRSRKRW on morphological examination of liver tissue: Histopathological exam inations showed that Hepatic cord structure clear, liver cells arranged in neat rows, eosinophilic cytoplasm, cell without obvious swelling, nuclei are round in the central (Fig. 1).

DISCUSSION

In recent years, some researchers suggest that Monascus-fermented rice contains various chemical components, such as Monacolin K. It has been reported to function in lowering of plasma glucose, cholesterol and triacylglyceride (Lin *et al*., 2005; Chang *et al*., 2006). However, the existence of citrinin with the renal toxicity restricts the development of functional red yeast rice. How is the BRSRKRW?

In the present study, we found that in acute toxicity study, the MTD of BRSRKRW is more than 30 g/kg·bw, the dose equivalent to human consumption 1.67 g/kg·bw, human body ecommended dose will amounts to 750 times, which is the actual non-toxic level. So we enter the second phase toxicology test. In the second phase toxicology test of BRSRKRW, we observe the rats cause harmful effect of dose, poison effect nature and target organs, estimate and the

harmfulness of chronic intake and preliminary estimate its biggest no effect dose. The results showed that BRSRKRW did not affect weight gain in growing rats, serum biochemical, organ indexes. There is on obvious pathological histology change on the brain, thymus, heart, liver, spleen, kidney.

Based on the acute and subchronic toxicity results, the acute MTD in mice of BRSRKRW is >30 g/kg·bw and on repeated lavaging every day for 30 days at doses of 1.5, 3 and 6 g/kg·bw, respectively cannot cause hematological changes and serum biochemical indices changes. From the perspective of toxicology, a preliminary conclusion could be drawn that drinking brown rice starter red kojic rice wine in the experiment dose range have no acute toxicity to people. The results is brown rice starter red kojic rice wine can be used as a health food related function research, development and utilization.

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