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Safety assessment of a new multivitamin

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ABSTRACT

A newly created multivitamin possesses many protective health functions. To investigate its safety when applied in medical treatment and when used as a food supplement, we studied its acute oral toxicity and 13-week oral toxicity in mice. The results showed that the oral lethal dose, 50% (LD_{50}) of the biomass of the multivitamin in mice was greater than 2492 mg/kg body weight (BW) and that poisoned mice recovered within 72 h. The no observed effect level (NOEL) of long-term consumption was more than 249.3 mg/kg BW for haematological parameters, clinical chemistry parameters, histopathological examination of organs, food consumption, BW, ratio of organ weight to BW and other physiological parameters and conditions. Therefore, we conclude that dosages of up to 249.3 mg/kg BW/day of this multivitamin do not cause chronic toxicity in animals. Administration of this multivitamin may even improve the resistance of animals to negative environmental factors and may be safe for long-term consumption to enhance the health of individuals in accordance with the prescribed dosage (1.4 ~ 4.2 mg/kg BW/day).

1. Introduction

We invented a new multivitamin consisting of seven kinds of vitamins (vitamins A, B_1, B_2, B_6, C, E and PP) (Tao et al., 2009). This multivitamin possesses many protective health functions, including enhanced disease resistance, anti-senescence, prevention and cure of many chronic diseases, and adjunctive therapy or prevention of skin diseases, cardiovascular disease and tooth disease. The toxicity of each vitamin in the new multivitamin is already known: vitamin A has some toxicity, vitamin E is slightly poisonous and vitamins B_1, B_2, B_6, C and PP have extremely low toxicity (unless their dosages exceed 100 times the recommended nutrient intake [RNI]) (Wang, 2003; Wu and Sun, 2005; Xing and Zhou, 2003). When vitamins are combined to create a multivitamin, researchers must ask themselves the following question (Yan et al., 2010): what level of toxicity does this new multivitamin have? Although many researchers think that multivitamins do not possess any toxicity (Chavarro et al., 2008; Chessex et al., 2005; Ishitani et al., 2008; Lavoie et al., 2007; Neuhouser et al., 2009; Ng et al., 2010; Thompson et al., 2003), reports have indicated that some multivitamins produce side effects in individuals who take certain medications (Stevens et al., 2005; Xing and Zhou, 2003). To ascertain the safety of this new multivitamin, we performed an acute oral toxicity study and a 13-week oral toxicity study. The results show that the multivitamin possesses low acute oral toxicity and no chronic toxicity.

2. Materials and methods

2.1. Study design

The study was conducted according to "The Guidelines of Acute Toxicities of Chinese Traditional Medicines and Crude Medicaments Study of Technical Specifications" and "The Guidelines of Chronic Toxicities of Chinese Traditional Medicines and Crude Medicaments Study of Technical Specifications" issued by the Ministry of Health of China (2005).

2.2. Test substances

The patented multivitamin (Tao et al., 2009) under investigation consisted of vitamin PP (50 mg); vitamin C (50 mg); vitamin E (25 mg); vitamins B_1, B_2 and B_6 (5 mg each); vitamin A (0.8 mg) and medicinal starch (109.2 mg).

2.3. Experimental animals

Health Kunming mice were obtained from the Laboratory Animal Department of Central South University (Changsha, China). At the beginning of the experiments, their body weights (BWs) were 20 ± 2 g, and their ages were 4–5 weeks.

Abbreviations: A/G, albumin to globulin ratio; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; BW, body weight; CHOL, cholesterol; CI, confidence interval; CR, creatinine; GLB, globulin; GLU, glucose; HCT, haemocrit value; HGB, haemoglobin; LD_{50}, lethal dose, 50%; LYM%, lymphocyte percentage; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; NOEL, no observed effect level; PDW, platelet bulk distributing width; PLT, platelet; RBC, red blood cell; RDW, red blood cell bulk distributing width; RNI, recommended nutrient intake; TG, triglyceride; TP, total protein; WBC, white blood cell.

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2.4. Acute oral toxicity study in mice

2.4.1. Preliminary test

To confirm the maximum dose of acute toxicity of the multivitamin in mice, we conducted a preliminary test. The mice were randomly divided into 5 groups of 5 mice. The multivitamin was administered to mice at doses of 16.67 (maximum daily adult dose), 66.68, 266.72, 1066.88 and 4267.52 mg/kg BW. Food, but not water (ad libitum), was withheld for 12 h before the administration of the test article by oral gavage at a dose of 0.4 mL/10 g BW. During the experiments, the animals were monitored daily for 7 days, and the number of animal deaths caused by poisoning was recorded. The maximum dose of acute toxicity was ascertained based on the number of animal deaths caused by poisoning.

2.4.2. Official test

Fifty mice (25 males; 25 females) were randomly assigned to 5 groups of 10 mice (5 males; 5 females). According to the findings of the preliminary test, the maximum dose of acute toxicity was 4267.36 mg/kg BW. The doses among the groups decreased by $1:\sqrt{2}$. The multivitamin was administered to mice at doses of 4267.36, 3017.51, 2133.72, 1508.78 and 1066.88 mg/kg BW. Food was withheld for 12 h before the single-dose administration of the test article (0.4 mL/10 g BW) by oral gavage. During the experiments, the animals were monitored daily for 7 days and were euthanised at the end of the testing to undergo a macroscopic examination.

2.4.3. Data analysis

The lethal dose, 50% (LD_{50}) and the 95% confidence interval (CI) were calculated using the following formula:

$$\lg LD_{50} = Xm - d\left(\sum P - \frac{3 - Pm - Pn}{4}\right),$$

where "*Xm*" is the logarithm of the maximum dose group; *d* is the logarithm of the common ratio; *P* is mortality; and *Pm* and *Pn* are maximal and minimal mortalities, respectively.

$$LD_{50}\ 95\%\ CI = \lg^{-1}\ (\lg LD_{50} \pm 1.96 \times S_{\lg LD50});\ S_{\lg LD50} = i \times \sqrt{\sum \frac{pq}{n}},$$

where *i* is the distance between groups, namely, the logarithm of the common ratio; *p* is mortality; *q* is livability; and *n* is the number of animals.

2.5. Thirteen-week repeated-dose toxicity study in mice

Following a 7-day acclimation period, 80 mice were randomly assigned to 4 groups (3 experimental groups; 1 control group) of 20 mice (10 males; 10 females). The mice in the high-, middle- and low-dose groups were administered the multivitamin at doses of 249.3 (up to 1/10 LD_{50}), 124.6 and 62.3 mg/kg BW/day, respectively. The multivitamin was dissolved in distilled water and was administered orally at concentrations of 6.23, 3.12 and 1.56 mg/mL, respectively, with the mice in the control group receiving the same volume of distilled water (0.4 mL/10 g BW) every morning. Weekly adjustments were made for body weight changes. At approximately the same time each day, clinical observations were made following treatment. The general condition of all animals was checked once a week. Body weights were measured at the initiation of the experiments and at every other week. Throughout the experiments, food was weighed every day, and the average food consumption per animal was calculated at weekly intervals.

2.5.1. Examination at end of 9-week treatment

At the end of the 9th week, 6 mice (3 males; 3 females) were taken from each group, and the body weights of these mice were recorded after an overnight fast (approximately 16 h). Blood samples for haematology and clinical chemistry examinations were taken from the animals via the eyeball aorta. Gross pathological examination (including organ weight and appearance) was then performed on the cadaver and organs. The organs of each mouse were weighed, and the ratio of organ weight to body weight was determined for several organs (heart, liver, spleen, lung, kidneys, ovary and testicle).

2.5.1.1. Haematology examination. Blood samples, collected with EDTA-2K (an anticoagulant), underwent haematological testing with KX-21 fully automated haematology analyzer (Sysmex East Asia, Kobe, Japan). The following parameters were measured: white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB), haemocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT), lymphocyte percentage (LYM%), red blood cell bulk distributing width (RDW), platelet bulk distributing width (PDW) and mean platelet volume (MPV).

2.5.1.2. Clinical chemistry examination. Blood serum samples underwent clinical chemistry testing with NSA-300 fully automated biochemistry analyzer (Shenyang Neusoft Medical Systems, Shenyang, China). The following clinical chemistry measurements were made: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), globulin (GLB), albumin to globulin ratio (A/G), glucose (GLU), cholesterol (CHOL), triglyceride (TG), blood urea nitrogen (BUN) and creatinine (CR).

2.5.2. Examination at end of 13-week treatment

At the end of the 13th week, 8 mice (4 males; 4 females) were taken from each group, and with the exception of the aforementioned examinations, a full histopathological examination was performed on haematoxylin–eosin-stained tissue sections of several organs, such as the liver, kidney, lung, heart, spleen, ovary and testicle, of the mice in the control and high-dose groups. The remaining mice were no longer given the multivitamin.

2.6. Examination of restoration

At the end of the 15th week, restoration examinations (with the above-mentioned examination items) were performed on the remaining animals.

2.7. Statistical analysis

The significance of the difference for each parameter between the control group and treated groups was analysed with 11.5 version of SPSS software (Analysis of variance). The statistical significance of difference among the groups was set at $P < 0.05$ in both studies.

3. Results

3.1. Acute oral toxicity study in mice

After the multivitamin was administered to mice by oral gavage, the animals behaved by closing their eyes, lying still on the ground, arching their backs, crying, responding slowly to stimuli and so on. Animals that died did so within 24 h, whereas those that survived recovered within 72 h and grew normally. The results of the acute oral toxicity experiments conducted with the new multivitamin in mice are presented in Table 1.

The results showed that the number of animal deaths was directly proportional to the dosage of the multivitamin. We anatomised the animals that died by poisoning and those that survived, and did not detect abnormal changes in their hearts, livers, spleens, lungs and kidneys.

3.2. Nine-week repeated-dose toxicity study in mice

At 9 weeks, no mice died or exhibited abnormal physiological signs, such as abnormal changes in weight, appearance, behaviour, urine and fur. A summary of food consumption is presented in

Table 1
The results of acute oral toxicity of the new multivitamin in mice.

Dosage (mg/kg)	No. of mice	No. of deaths	Mortality	LD_{50}[a] (mg/kg)	95% CI[b] (mg/kg)
4267.36	10	8	80%		
3017.51	10	6	60%		
2133.72	10	4	40%	2492.87	2045.17∼
1508.78	10	3	30%		3038.58
1066.88	10	0	0		

[a] Lethal dose, 50%.
[b] Confidence interval.