Tetrandrine (TET) is a bisbenzylisoquinoline alkaloid isolated from the dried root of Stephania Tetrandra. TET has exhibited broad pharmacological actions, including immunomodulatory, anti-hepatofibrogenic, anti-arrhythmic, anti-hypertensive, anti-cancer, neuroprotective activities, decreasing the radiosensitization and changing multidrug resistance to chemotherapy.\(^1\)\(^-\)\(^3\) Especially in recent years, our research found beneficial effects of TET on reversing the resistance of Candida albicans to azole which is primarily mediated through the inhibition of drug efflux pumps and increases in intracellular azole.\(^4\)\(^-\)\(^6\) In experimental animals and volunteers with vaginal candidiasis or dermatophytosis, synergistic effects are also observed when TET is combined with azole via topical application.\(^7\)\(^-\)\(^9\) Because candidiasis remains a challenging opportunistic infection with high mortality despite current available treatment, TET has been promising and warrant further investigation as the combination of TET and azole antifungal drugs may provide a clinical application with readily-available sources and potentially low toxicity. Female BALB/c mice have been the best choice for studying candidiasis infection in animals. Moreover, with more systemic fungal infection, intravenous exposure to TET has become common, but previous toxicity studies had largely reported about oral or intraperitoneal injection.\(^10\)\(^-\)\(^11\) Few researches have been carried out to assess the toxicity of intravenous TET exposure, especially in the female BALB/c mice. In light of this trend, in the present study, we systematically evaluated the acute and sub-chronic toxicity of TET injected intravenously (i.v.) in female BALB/c mice.

**ABSTRACT**

Objective: To evaluate the acute and sub-chronic toxicity of intravenously administered tetrandrine (TET) in female BALB/c mice. Methods: The median lethal dose (LD\(_{50}\)) of intravenously administered TET was calculated in mice using Dixon's up-and-down method. In the acute toxicity study, mice were intravenously administered with TET at a single dose of 20, 100, 180, 260 and 340 mg/kg, respectively and were evaluated at 14 days after administration. In the sub-acute toxicity study, mice were intravenously administered various doses of TET (30, 90 and 150 mg/kg) each day for 14 consecutive days. Clinical symptoms, mortality, body weight, serum biochemistry, organ weight and histopathology were examined at the end of the experiment, as well as after a 1-week recovery period. Result: LD\(_{50}\) was found to be 444.67 ± 35.76 mg/kg. In the acute toxicity study, no statistically significant differences in body weight, blood biochemistry, or organ histology were observed between the administration and control groups when mice were intravenously administered with single dose at 20, 100, 180, 260 and 340 mg/kg of TET (P > 0.05). In the sub-acute toxicity study, no significant changes in body weight, biochemistry and organ histology were observed with up to 90 mg/kg of TET compared with the control group (P > 0.05), however, in the 150 mg/kg administered group, TET induced transient toxicity to liver, lungs and kidneys, but withdrawal of TET can lead to reversal of the pathological conditions. Conclusions: The overall findings of this study indicate that TET is relatively non-toxic from a single dose of 20, 100, 180, 260 or 340 mg/kg, and that up to 90 mg/kg daily for 14 consecutive days can be considered a safe application dose.

**KEYWORDS** tetrandrine, female BALB/c mice, acute and sub-chronic toxicity, Chinese medicine
The median lethal dose ($LD_{50}$) of TET was estimated using Dixon’s up-and-down method. Animals were sent for necropsy immediately following death to identify its cause, which allowed for determination of the dose ranges for acute and sub-chronic toxicity research so as to found an acceptable dosage of TET that limits adverse events, a step towards utilizing TET in combination with current candidiasis and other therapeutics.

**METHODS**

**Chemicals**

Tetrandrine ($C_{38}H_{42}O_{6}N_{2}$, MW: 622.8 g/mol, purity: 99.6%, batch No. Cac0205) was purchased from Aroma Chemical Company (Hangzhou, China), and its purity was determined by high performance liquid chromatography (HPLC) as previously described.\(^{(2,12)}\) TET powder was dissolved in 0.01 mol/L hydrochloric acid, and the pH was adjusted to 5.5 using 0.01 mol/L NaOH to yield a final concentration of 15 mg/mL. TET was kept at –20°C as aliquots, and drug preparations were made fresh each day of experiments.

**Experimental Animals**

Female BALB/c mice (6–8 weeks old, 17–20 g in weight, specific-pathogen-free grade, certification No. SYXX2010–0106) were obtained from the Experimental Animal Center of Guangdong Province (Guangzhou, China). Each plastic cage held 5 mice and was housed in a ventilated room maintained at 20 ± 2°C and 60% ± 10% relative humidity with a 12 h light-dark cycle. The mice were given water and sterilized food. All animal care and experiments were approved by the Animal Ethics Committee at Shenzhen PKU-HKUST Medical Center (approval No. 2013-005).

**LD$_{50}$ Estimation Using Dixon’s Up-and-Down Method**

LD$_{50}$ was estimated using the up-and-down method as described by Dixon,\(^{(13)}\) which uses an iterative dose-selection algorithm. The first animal receives a dose one step below the best preliminary estimate of the LD$_{50}$. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. Each subsequent dosage was raised or lowered based on the survival of the preceding animal on fixed-time interval (48-h) outcomes. The maximum likelihood estimate for LD$_{50}$ with standard error (SE) was calculated using the following formula: $LD_{50} = \frac{\text{average (X$_i$)} + d}{N \times (A + C)}$. Average (X$_i$) is the average experimental dose for the last N samples, N is the nominal number of samples or total number of samples minus 1 less than the number of identical samples at the beginning of the trial, A and C values are acquired from Dixon’s tables after the series of experiments are performed, and d is the distance between data points. The method assumes that the SD ($\sigma$) is equal to the spacing distance. However, Dixon also gave a method to calculate: $SE = \sigma \times \sqrt{\frac{2}{N}}$. Mortality in this LD$_{50}$ estimation study was recorded and carcasses were sent for immediate necropsy.

**Acute Toxicity Study**

Acute i.v. toxicity was studied in accordance with the procedure for estimating LD$_{50}$. Thirty healthy female BALB/c mice were randomly assigned to 6 groups of 5 each by a random number table such that the weight difference within and between groups did not exceed ± 20% of the sample population. Mice in each group were separately administered i.v. with normal saline (10 mL/kg) or TET at single doses of 20, 100, 180, 260 and 340 mg/kg, respectively. After injection, behavioral manifestations and mortality were observed and carefully recorded daily for 14 days. At the end of the experiment, all animals were sacrificed for subsequent experimental study.\(^{(14)}\)

**Sub-chronic Toxicity Study**

For the TET sub-chronic toxicity study, the choice of doses used was calculated using the following method.\(^{(15)}\) The lowest dose (30 mg/kg) was calculated by reviewing literature and making a best approximation.\(^{(16)}\) The middle (90 mg/kg) and high (150 mg/kg) doses were calculated as 1/5 and 1/3 of LD$_{50}$, respectively. A total of 40 mice were randomly divided into 4 groups, which were administered i.v. with 0, 30, 90 or 150 mg/kg of TET for 14 days, respectively, and given normal saline 10 mL/(kg • day) for 7 days following the termination of the last day of the study (reversibility study). Clinical observations, mortality, body weight, coefficients of organs, blood biochemistry and histopathology were examined at the end of the experiment.

**Body Weight and Coefficients of Organs**

Mice were weighed on days 1, 14 and 21 of TET or saline exposure. At the end of the respective treatment periods, the mice were sacrificed by cervical dislocation and major tissues and organs, including the liver, spleen, kidneys, heart, lung and brain, were