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# Pharmacokinetics and tissue distribution of ergosterol in rats

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**Abstract:** *Phellinus ellipsoideus* is a wood-rotting fungus used medicinally as sanghuang in the north-central area of Fujian Province, China. Ergosterol (ergosta-5,7,22-trien-3β-ol), one of the best-known bioactive steroids, has been identified as the active constituent of *P. ellipsoideus*. The objective of this study was to in-vestigate the pharmacokinetics and tissue distribution of ergosterol after oral and intravenous administration in rats using high performance liquid chromatography with an external standard. The isolation was achieved with a C18 column using a methanol/water (98:2, v/v) solvent system with a flow rate of 0.8 mL/min at 25ºC and a maximum absorption wavelength of 283 nm. The pharmacokinetic profiles of the two routes of administration revealed that ergosterol was absorbed rapidly into the systemic circulation and was widely distributed throughout the body, followed by a rapid elimination phase. The highest concentration of ergosterol was found in lung tissue, followed by spleen and liver. These results will be useful for clinical applications or further study of ergosterol from fungi.

**Key words:** Ergosterol; Pharmacokinetics; Tissue distribution; HPLC.

## Introduction

Fungi are used in traditional medicines in Asia, Europe and America. There are various classes of primary and se-condary metabolites in mushrooms that exhibit biologi-cal activity. Steroids are a major chemical component of fungi (1-4) and have been reported to possess anticancer (5,6), antimicrobial (7), diuretic (8), antioxidant (9) and immunosuppressive activities (10).

Ergosterol (ergosta-5,7,22-trien-3β-ol) is one of the best-known bioactive steroids and exists widely in medi-cinal fungi, including *Phellinus ellipsoideus* (11), *Phelli-nus yamanoi* (5), *Hypholoma fasciculare* (12), *Tricholo-ma mongolicum* (13) and *Lycoperdon pyriforme* (14).

While screening for antitumor agents from fungi in our previous studies, the petroleum ether extract of dried *P. ellipsoideus* fruiting bodies was found to induce signi-ficant cytotoxicity in tumor cell lines. Chromatography identified the active constituent as ergosterol (11). *Phelli-nus ellipsoideus* has been used as the traditional medicine sanghuang in the north-central area of Fujian Province, China, and was first identified scientifically by Cui and Dai and collected from Wanmulin Natural Reserve, Fujian Province (15).

The objective of this study was to describe the phar-macokinetic profile of ergosterol, including its absorption, distribution, metabolism and elimination in plasma, and tissue distribution, in Wistar rats (16). Furthermore, a fast and sensitive method was established to measure ergoste-rol in plasma and tissue samples using high-performance liquid chromatography (HPLC).

## Materials and Methods

***Chemicals and Materials***The standard of ergosterol (Fig. 1a) was isolated by our laboratory from *F. ellipsoidea* with 97% purity as determined by HPLC. The procedure isolation and pu-rification of ergosterol was published before (11). Its structure was characterized by chemical and spectrosco-pic methods (1HNMR, 13CNMR and MS) and compared with literatures (17). Vitamin D2 (Fig. 1b) was used as external standard which was purchased from Jinsui Co. (Shanghai China). High-pressure liquid chromatography (HPLC)-grade methanol and dichloromethane were pur-chased from Yuwang Co. (Shandong China). Water was purchased from Wahaha Co. (Hangzhou China). Poloxa-mer was purchased from BASF Co. (Germany).

### Rats

Wistar rats (Equal numbers of males and females, 200-220g) were supplied by the experimental animal research center of Jilin University. The animals were kept in an en-vironmentally controlled breeding room on a 12 h light/12 h dark cycle (light on at 8:00 am) at ambient temperature (23-25°C) and 60% relative humidity with unlimited ac-cess to food and water for 7 days before starting the expe-riment. They were fasted for 12 h and had free access to water before drug administration.

### *Instrumentation*

Analysis was performed with an Agilent 1100 series HPLC system equipped with an UV spectrophotome-tric detector, a quaternary pump and a thermostatically controlled column apartment.

Chromatographic separations were performed with a 5μm ZORBAX Eclipse XDB-C18 (4.6×150 mm) main-tained at 25°C. The mobile phase was a 98:2 (v/v) mixed solution of methanol and water at the flow rate of 0.8 mL/ min. 10μL were injected into HPLC system for analysis. The system pressure was 46 bar. The monitoring wavelen-gth was 283 nm.

### *Preparation of standard samples*

The stock (120μg /mL) and working solutions (12μg / mL) of ergsterol were prepared by dissolving an accura-tely weighed quantity

of ergsterol in dichloromethane and serial dilution with the same solvent. The stock (150μg / mL) and working solution (15μg /mL) of vitamin D2 were prepared in the same way. All the solutions were stored at 4°C.

***Calibration Curve***

The calibration curve was constructed by plotting the peak area versus the concentration of the calibration stan-dard. Calibration samples were prepared by spiking plas-ma samples and tissue mixtures with ergosterol standard solution. The final concentrations of ergosterol in the ca-libration samples were 0.009, 0.037, 0.150, 0.600, 1.200 and 2.400μg /ml. Quality control (QC) samples containing 0.009 (low), 0.150 (medium) and 2.400μg /mL (high) of ergsterol were also prepared in the same way. To each plasma samples (40μL), dichloromethane (240μL) was added. and tissue samples (0.2g) was homogenized with dichloromethane (2 ml). Then mixture was vortex mixed for 1 min and centrifuged at 10,000rmp for 5 min. A 10μL portion of the substratum was subjected to HPLC analysis.

***Precision and accuracy***

The intra-day and inter-day accuracy and precision were evaluated by replicative analysis of five sets of samples spiked with QC samples at three concentration le-vels of ergosterol (0.009, 0.150 and 2.400μg /mL) within a day or during three consecutive days. The accuracy was expressed as bias, obtained by calculating the percentage of difference between the measured and spiked concen-tration over that of the spiked value, whereas the preci-sion was denoted by using the relative standard deviation (RSD%).

***Extraction recovery***

The absolute recovery of the extraction was deter-mined by comparing the peak area obtained from the samples with peak areas obtained by the direct injection of pure ergosterol standard solutions in the mobile phase at three different concentration levels. The quantification of the chromatogram was performed by using peak area ratios of ergosterol to standard.

***Pharmacokinetic Study***

Experiments were performed on 60 rats that were ran-domly divided into 12 groups (n=5). For plasma samples preparation, ergosterol aqueous solution was intrave-nously injected by vena caudalis of rats in 3 groups with three dose (25mg /kg, 12.5mg /kg and 6.25mg /kg) and orally administered in 3 groups with three dose (50mg / kg, 25mg /kg and 12.5mg /kg) in average. Tissue samples were prepared in 6 groups by intravenous injection at a single dose (12.5 mg /kg) and oral administration at a dose of 25 mg /kg.

Blood samples (approximately 0.3 mL) were collected from orbital vein at control (0 min) and 0.08, 0.25, 0.50, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24 h after the administration. The sample was transferred to a 1.5mL centrifuge tube with heparin sodium and centrifuged at 10000rpm for 10 min immediately. The plasma was separated into another clean tube and frozen at -20°C until analysis. Next 240μL dichloromethane was added into the plasma sample and vortex mixed for 1min. Then the mixture was centrifuged at 10000rpm for 5 min. At last, 10μL of the substratum was injected into the HPLC for analysis with external standard method.

Rats in three groups were sacrificed at 10min, 1h and 3h (0.5h, 1h and 3h) respectively after the intravenous administration (oral administration). Subsequently, the brain, heart, lung, liver, spleen and kidney were imme-diately removed, washed in normal saline and blotted dry with filter paper. An accurately weighed amount of the soft tissue samples (0.2g) was individually homogenized with dichloromethane (2 ml). Then the mixture was centrifuged at 10000rpm for 5 min. At last, 10μL of the substratum was injected into the HPLC for analysis.

The concentrations of ergosterol in plasma samples and tissue samples were expressed as mean ± standard deviation (SD). The mean concentration-time curve was constructed by the sample data. The pharmacokinetic pa-rameters for ergsterol were evaluated by analyzing the data of plasma concentration-time profiles, which was cal-culated by the pharmacokinetic software, 3P97 (Chinese Pharmacological Society, China). The absolute bioavai-lability of ergosterol expressed as F. $F = AUCpo \cdot Div / AUCiv \cdot Dpo \times 100\%$ .

**Results**

***Isolation and purification of ergosterol***

The purity of ergosterol was above 97% as determined by HPLC in our published paper (11). The characteristics of ergosterol are: white needles from petroleum ether, mp. 153-155°C,1H NMR(CDCl3, 500 MHz) 3.64(1H, m, H-3), 5.57(1H, dd, H-6), 5.38(1H, s, H-7), 0.63 (3H, s, H-18), 0.95 (3H, s, H-19), 1.04 (3H, d, H-21), 5.17 (1H, dd, H-22), 5.46 (1H, dd, H-23), 0.92 (3H, s, H-25), 0.83 (3H, s, H-27), 0.82 (3H, s, H-28); 13C NMR (CDCl3,125 MHz) 39.09 (C-1), 32.00 (C-2), 70.46 (C-3), 38.38 (C-4), 139.78 (C-5), 119.59 (C-6), 116.29 (C-7), 141.35 (C-8), 46.26 (C-9), 37.03(C-10), 21.25 (C-11), 40.40 (C-12), 42.38 (C-13), 54.56 (C-14), 22.99 (C-15), 28.27 (C-16), 55.75 (C-17), 12.04 (C-18), 16.28 (C-19), 40.80 (C-20), 21.10 (C-21), 135.56 (C-22), 131.98 (C-23), 42.83 (C-24), 33.09 (C-25), 19.64 (C-26), 19.94 (C-27), 17.59 (C-28).

***Optimization of the chromatographic conditions***

The HPLC parameters were compared to develop ac-curate, valid and optimal chromatographic conditions, including: mobile phase (methanol, methanol-water and acetonitrile-water solutions); column type (Agilent ZOR-BAX Eclipse XDB-C18 column 4.6 × 150 mm, 5 μm, Agilent ZORBAX Eclipse XDB-C18 column 4.6 × 250 mm, 5 μm and Agilent Venusil MP-C18 column 10 × 250 mm, 10 μm); wavelength (265 and 283 nm); column tem-

(a) (b)

**Figure 1**. Chemical structures of ergosterol (a) and vitamin $D_2$ (b).

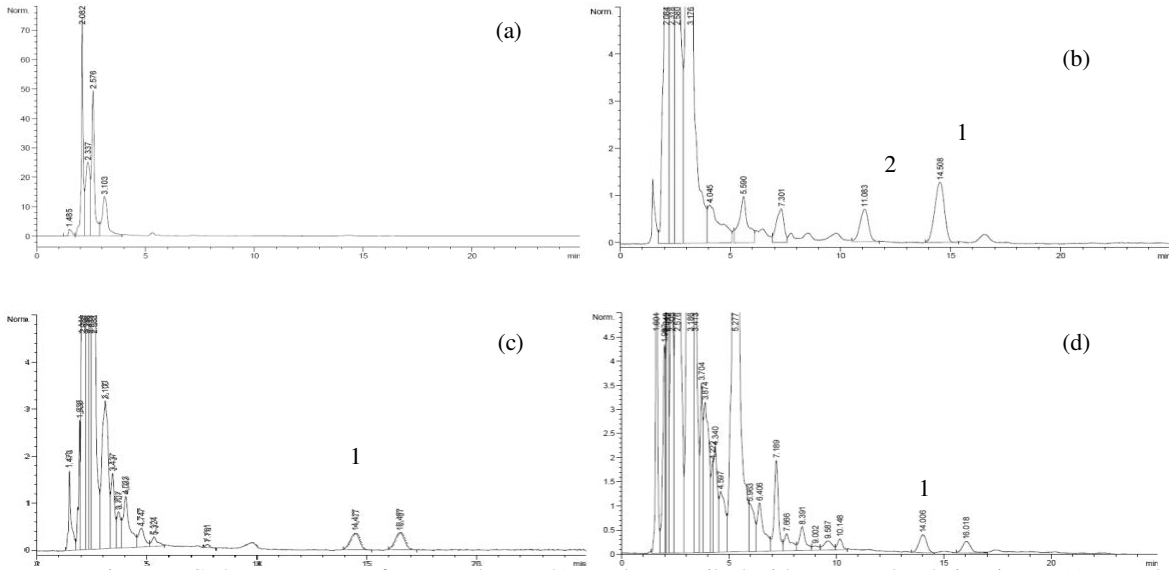


**Figure 2**. Representative HPLC chromatograms of (a) rat plasma; (b) rat plasma spiked with ergosterol and vitamin $D_2$; (c) a rat plasma sample obtained 15 min after intravenous injection of ergosterol at a dose of 6.25 mg /kg; and (d) a rat plasma sample obtained 15 min after oral adminis-tration of ergosterol at a dose of 50 mg /kg. Peak identifications: 1 ergosterol, 2 vitamin $D_2$.

**Table 1**. Calibration curves, correlation coefficients and linear ranges of ergosterol in rat plasma and tissue samples (n = 3).

| Biological samples | Calibration curves | Correlation coefficients (r2) | Liner ranges (μg /ml) |
|---|---|---|---|
| Plasma | y = 15.680x + 2.978 | 0.999 | 0.009-2.400 |
| Liver | y = 9.604x + 2.145 | 0.996 | 0.009-2.400 |
| Heart | y = 7.496x + 2.392 | 0.997 | 0.009-2.400 |
| Spleen | y = 9.986x + 2.450 | 0.996 | 0.009-2.400 |
| Lung | y = 11.150x + 2.245 | 0.995 | 0.009-2.400 |
| Kidney | y = 9.398x + 1.922 | 0.996 | 0.009-2.400 |
| Brain | y = 8.390x + 2.607 | 0.997 | 0.009-2.400 |

perature (20, 25 and 30℃); and flow rate of the mobile phase (0.5, 0.8 and 1.0 mL/min). Based on these comparisons, the samples were separated by HPLC using an Agilent ZORBAX Eclipse XDB-C18 column (4.6 × 150 mm, 5 μm) with a methanol/water (98:2, v/v) solvent system at a flow rate of 0.8 mL/min at 25℃ and a maximum absorption wavelength of 283 nm. Optimum peak shapes and high responses were obtained for ergosterol and Vitamin D2 under these conditions. Vitamin D2 was chosen as the external standard for its similarity in structure with the analyte (Fig. 1).

***Selectivity***

Representative chromatograms from the blank rat plasma, blank rat plasma spiked with the analyte and external standard and plasma sample at 15 min after intravenous injection of 6.25 mg/kg of ergosterol are shown in Figure 2c, and the plasma sample at 15 min after oral administration of 50 mg/kg of ergosterol is shown in Figure 2d. Endogenous plasma did not interfere with the elution of ergosterol and vitamin D2. Peak identification: (1) ergosterol, (2) vitamin D2. The retention times of ergosterol and vitamin D2 were 14.3 and 11.1 min, respectively, with a total run time of less than 20 min.

***Calibration curves***

The representative regression equation for the calibration curve (y = ax + b) was determined as the peak area (y) versus analyte concentration (x). Linearity was determined using linear regression weighted by 1/x2. The results of the calibration curves, correlation coefficients and linear ranges of ergosterol in plasma and tissues are listed in Table 1.

***Accuracy, precision and extraction recovery***

The intra- and inter-day precision (RSD, n = 5) for the ergosterol-spiked control samples at 0.009, 0.150 and 2.400 μg/mL varied from 1.57 to 9.94%. The correspon-

**Table 2**. The recovery, precision and accuracy (n = 5) of the assay method.

| Tissues | Concentration (μg /mL) | Absolute recovery (Mean±SD, %) | Intra-day | | Inter-day | |
|---|---|---|---|---|---|---|
| | | | Precision (RSD%) | Accuracy (bias%) | Precision (RSD%) | Accuracy (bias%) |
| Plasma | 0.009 | 95.40±1.59 | 6.29 | 7.91 | 7.36 | -0.21 |
| | 0.150 | 94.79±2.51 | 4.54 | 4.78 | 4.85 | 5.26 |
| | 2.400 | 94.14±2.12 | 8.05 | -4.46 | 3.56 | -2.06 |
| Liver | 0.009 | 96.96±1.66 | 8.88 | -1.76 | 4.11 | 7.34 |
| | 0.150 | 96.58±2.07 | 7.73 | -0.07 | 3.36 | 4.27 |
| | 2.400 | 96.49±1.98 | 8.29 | 0.93 | 8.65 | 1.63 |
| Heart | 0.009 | 95.58±2.06 | 7.72 | 6.69 | 3.48 | 3.75 |
| | 0.150 | 96.21±2.21 | 7.74 | 0.96 | 1.85 | 3.01 |
| | 2.400 | 95.35±2.57 | 8.15 | 3.74 | 4.87 | 0.37 |
| Spleen | 0.009 | 96.17±1.17 | 6.40 | 9.95 | 3.65 | 9.05 |
| | 0.150 | 95.91±3.15 | 4.57 | 4.91 | 4.88 | 8.21 |
| | 2.400 | 97.19±2.41 | 9.42 | 5.20 | 4.51 | 4.39 |
| Lung | 0.009 | 96.28±1.65 | 5.71 | -4.78 | 8.14 | 8.27 |
| | 0.150 | 96.58±2.55 | 6.80 | 0.94 | 3.49 | 7.70 |
| | 2.400 | 95.82±1.97 | 8.08 | -6.72 | 9.03 | 2.16 |
| Kidney | 0.009 | 95.15±2.16 | 7.70 | 7.02 | 3.12 | 5.01 |
| | 0.150 | 96.44±2.55 | 6.66 | -0.42 | 5.46 | 5.37 |
| | 2.400 | 95.38±2.95 | 6.56 | 1.45 | 7.63 | -1.77 |
| Brain | 0.009 | 96.44±2.42 | 9.16 | 6.09 | 3.19 | 3.75 |
| | 0.150 | 97.35±1.63 | 9.45 | 4.05 | 4.34 | 3.01 |
| | 2.400 | 95.48±2.57 | 9.94 | 2.95 | 1.57 | 2.46 |

ding intra- and inter-day accuracy (bias, n = 5) ranged from −4.78 to 9.95% (Table 2). The absolute recoveries of ergosterol from plasma were greater than 95%, indica-ting that most of the ergosterol in the plasma samples was extracted with no obvious interferences in the chromato-gram.

***Pharmacokinetics***

The HPLC method was applied successfully to deter-mine the pharmacokinetics of ergosterol following intra-venous injection of 6.25, 12.5 or 25 mg/kg and oral admi-nistration of 12.5, 25 or 50 mg/kg. The pharmacokinetic parameters were calculated with 3P97 software using a compartmental analysis. The plasma concentration-time profile of ergosterol is shown in Figure 3. The corres-ponding pharmacokinetic parameters are presented in Tables 3 and 4. The distribution processes were fitted to a two-compartment model.

The results indicated that ergosterol was eliminated ra-pidly after intravenous injection; the half-lives (t1/2) were 0.27, 0.26, and 0.23 h for doses of 6.25, 12.5 and 25 mg/ kg, respectively (Table 3). After oral dosing, ergosterol concentrations increased rapidly within the first hour, and decreased substantially from 1 to 3 h, followed by a gra-dual decrease thereafter. At doses of 12.5, 25 and 50 mg/ kg, the maximum plasma concentrations (Cmax) were 0.23, 0.46 and 0.91 μg/mL with times of peak concentration ($T_{3).max}$) of 0.84, 0.84 and 0.86 h, respectively (Table 4; Fig.

***Tissue distribution***

Tissue distribution of ergosterol was evaluated 10 min, 1 h and 3 h after intravenous dosing (12.5 mg/kg) and 0.5, 1 and 3 h after oral dosing (25 mg/kg). The results (Table

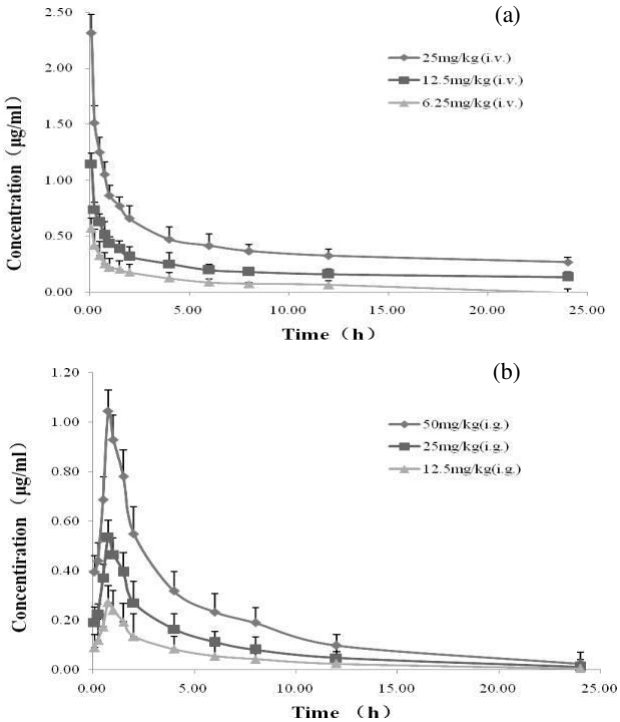


**Figure 3**. (a) Plasma concentration-time profile after intravenous injec-tion of ergosterol at the dose of 25, 12.5 or 6.25mg /kg (n = 5). (b) Plas-ma concentration-time profile after oral administration of ergosterol at the dose of 50, 25 or 12.5mg /kg (n = 5).

5; Fig. 4) indicated that ergosterol was rapidly and widely distributed throughout the body, including the liver, heart, spleen, lung, kidney and brain. Ergosterol was transported to most organs within 10 min of intravenous administra-tion. The ergosterol concentration in rat

**Table 3**. The pharmacokinetic parameters of ergosterol in rats following intravenous injection (n = 5).

| Parameters | 6.25 mg /kg | 12.5 mg /kg | 25 mg /kg |
|---|---|---|---|
| $t_{1/2}$ (h) | 0.23±0.04 | 0.26±0.03 | 0.27±0.05 |
| $V_2$(L/mg) | 13.59±2.39 | 19.84±4.24 | 20.20±4.02 |
| $CL_2$(L/mg/h) | 15.21±2.62 | 16.32±2.79 | 15.72±3.18 |
| AUC $_{0-t}$ (μg/ml*h) | 1.76±0.07 | 4.62±0.16 | 9.34±0.29 |
| AUC $_{0-inf}$ (μg/ml*h) | 1.83±0.11 | 5.56±0.13 | 11.37±0.26 |

**Table 4**. The pharmacokinetic parameters of ergosterol in rats following oral administration (n = 5).

| Parameters | 50 mg /kg | 25 mg /kg | 12.5 mg /kg |
|---|---|---|---|
| $T_{max}$ (h) | 0.86±0.07 | 0.84±0.02 | 0.84±0.04 |
| $C_{max}$ (μg/ml) | 0.91±0.11 | 0.46±0.07 | 0.23±0.02 |
| $t_{1/2}$ (h) | 0.53±0.05 | 0.52±0.03 | 0.52±0.04 |
| AUC $_{0-t}$ (μg/ml*h) | 4.60±0.13 | 2.23±0.06 | 1.13±0.03 |
| AUC $_{0-inf}$ (μg/ml*h) | 4.95±0.10 | 2.36±0.05 | 1.20±0.02 |
| $V_2$/F (L/mg) | 51.86±4.73 | 48.86±3.82 | 49.70±4.47 |
| $CL_2$/F (L/mg/h) | 15.73±1.43 | 15.29±2.15 | 15.33±1.76 |

**Table 5.** The concentration (μg /g) of ergosterol in rat tissues after administration (n=5).

| Biological samples | | Liver | Heart | Spleen | Lung | Kidney | Brain |
|---|---|---|---|---|---|---|---|
| Intravenous administration | 10 min | 10.82±1.24 | 1.62±0.30 | 6.04±0.91 | 161.27±3.43 | 1.89±0.18 | 0.97±0.71 |
| | 1 h | 4.43±0.48 | 0.68±0.35 | 5.14±0.24 | 96.12±3.03 | 0.92±0.13 | 0.20±0.58 |
| | 3 h | 1.24±0.40 | ND | 2.46±0.48 | 7.81±0.39 | ND | ND |
| Oral administration | 0.5 h | 1.72±0.23 | 1.37±0.16 | 3.75±0.18 | 25.35±1.88 | 0.14±0.17 | 0.07±0.33 |
| | 1 h | 13.32±1.01 | 3.47±0.33 | 5.87±0.34 | 11.76±0.93 | 1.82±0.39 | 0.40±0.42 |
| | 3 h | 3.01±0.55 | 0.04±0.69 | 0.22±0.07 | 0.39±0.37 | 0.63±0.38 | ND |

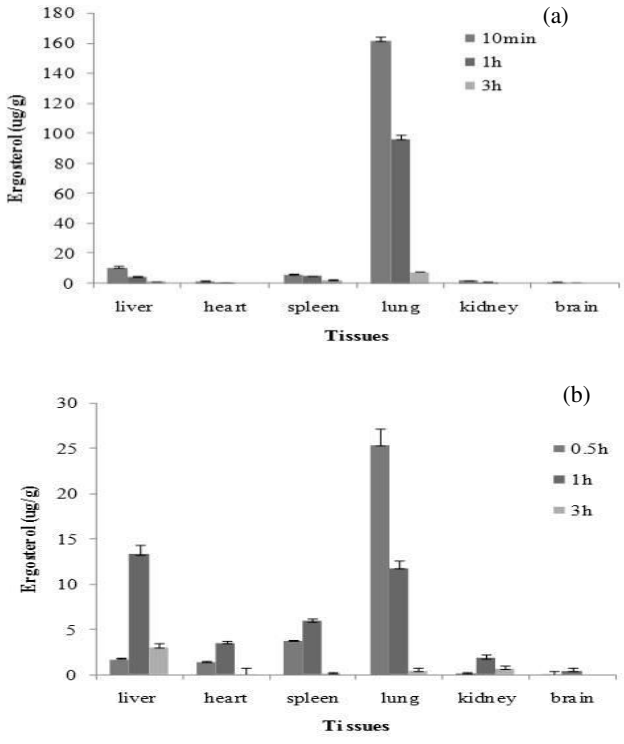


**Figure 4**. Tissue distribution of ergosterol in rats after intravenous injection (a) at dose of 12.5 mg /kg and oral administration (b) at dose of 25 mg /kg (n = 5).

in other tissues, followed by spleen and liver. The elimi-nation of ergosterol was rapid. Following oral adminis-

tration, ergosterol was absorbed rapidly and its concen-tration in lung peaked at 30 min, while concentrations in other organs peaked at 60 min. Samples collected 3 h after administration indicated that ergosterol cleared gradually. The results suggested that ergosterol, a small, fat-soluble molecule, was easily absorbed into the blood stream and that the tissue concentration of ergosterol was related to blood circulation. First, ergosterol in the blood flowed into the right atrium through the liver, then it completed pulmonary circulation and finally redistributed into other tissues, such as the spleen. Since lungs receive the largest blood flow volume, the highest concentration of ergosterol was detected in lung tissue 10 min and 0.5 h after intra-venous and oral administration, respectively, followed by liver and spleen.

**Discussion**

The HPLC method was established successfully and used to study the pharmacokinetics of ergosterol in Wistar rats. Ergosterol is a steroid and one of the major bioactive constituents of fungi. The HPLC method described pro-vides higher resolution, baseline stability and ionization efficiency. By developing this method further, the ergos-terol assay could be adapted for use with other biological samples, such as urine and feces.

Ergosterol is found in all fungi and its biological ac-tivity, in particular antitumor activity, has been reported in the literature (5-10). However, only two studies were

found on its pharmacokinetics (18,19), which reported a Tmax in rat plasma of about 8 h, while we demonstrated a $T_{max}$ of about 0.8 h. By examining the $T_{max}$ and $t_{1/2}$ of ergosterol, our results indicate that ergosterol is absorbed and eliminated rapidly in plasma and its concentration in plasma is dose-dependent. The absolute bioavailability of ergosterol was 24.46%. In a tissue distribution study, Yi reported (20) that *Flammulina velutipes* sterols, consis-ting of ergosterol and others, were distributed mostly in the liver and spleen and eliminated rapidly. However, we found that ergosterol was rapidly and widely distributed in rat tissues, including liver, heart, spleen, lung, kidney and brain, but especially lung tissue. These results will be use-ful for clinical applications and further study of ergosterol extracted from fungi. This is the first report on the phar-macokinetics of ergosterol in rats using both intravenous and oral routes of administration. Further experiments will be conducted that combine enzymes or receptors in rats with ergosterol and examine the mechanisms of its antitumor activity.

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