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ORIGINAL ARTICLE

Effects of Retinoic Acid-Induced Osteoporosis on Pharmacokinetics and Tissue Distribution of 2,3,5,4′-Tetrahydroxy Stilbene-2-O-β-D-Glucoside and β-Ecdysterone in Rats

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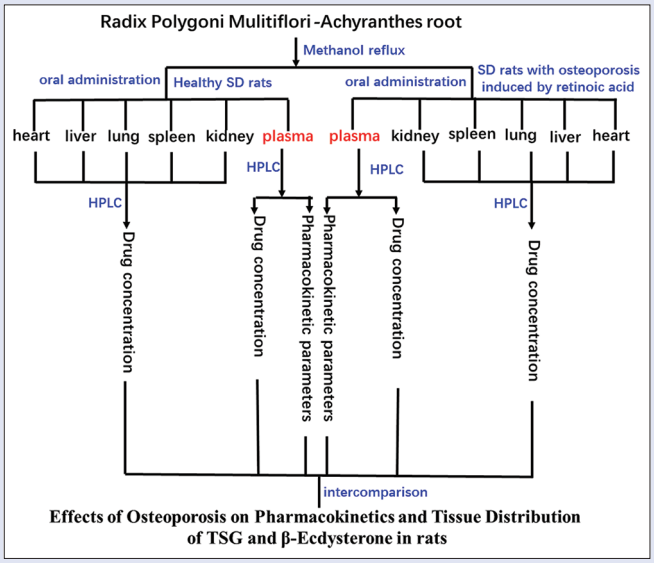
ABSTRACT

Background: The compatibility of *Radix Polygoni multiflori-Achyranthes* (RPMA) root is a Traditional Chinese medicine pair frequently used in the clinic and has good anti-osteoporosis (OP) effects. **Materials and Methods:** The extract of the compatibility of RPMA root was given orally in this study. The pharmacokinetics and the distribution in the tissues of tetrahydroxystilbene glucoside and β-ecdysterone were considered by high-performance liquid chromatography in OP rats induced by retinoic acid and healthy rats. Quercetin was employed as the internal standard. According to the above results, the effects of Osteoporosis on the process of β-ecdysterone and 2,3,5,4′-tetrahydroxy stilbene 2-O-β-D-glucoside in rats were analyzed. **Results:** The results displayed that the peak time of β-ecdysterone in plasma of OP model rats was earlier and the plasma concentration and pharmacokinetic parameters of tetrahydroxystilbene glucoside in the model group were suggestively different from those in the normal group. The concentration distribution of the two components in each tissue of the model group was significantly diverse from that of the normal group. In particular, the concentrations of the two components in kidney tissue of the model group were inferior than those of the normal group at most time points. **Conclusion:** It is ventured that the two components may be convoluted in the treatment of OP. This experiment can deliver the reference for the further pharmacodynamic study of the compatibility of RPMA root and the correlation analysis of pharmacodynamics and the content of active ingredients *in vivo*.

Key words: 2,3,5,4′-tetrahydroxy stilbene-2-O-β-D-glucoside, osteoporosis, pharmacokinetics, tissue distribution, β-ecdysterone

SUMMARY

- The *Radix polygoni multiflori-Achyranthes* (RPMA) is a Traditional Chinese medicine pair frequently used in the clinic and has good anti-OP effects. β-ecdysterone and 2,3,5,4′-tetrahydroxy stilbene-2-O-β-D-glucoside (TSG) are the foremost active ingredients of RPMA, and they all have good anti-OP effects. To study the effect of OP on the distribution of β-ecdysterone and TSG in the plasma and tissues of rats, the research group employed retinoic acid to replicate the rat OP model, and the high-performance liquid chromatography method was used to study the pharmacokinetics and distribution differences of TSG and β-ecdysterone in healthy rats and model rats after intragastric administration of the extract of RPMA. The results disclosed that the peak time of β-ecdysterone in plasma of OP model rats was earlier and the plasma concentration and pharmacokinetic parameters of TSG in the model group were considerably different from those in the normal group. The concentration distribution of the two components in each tissue of the model group was knowingly different from that of the normal group. It is gambled that the two components may be involved in the treatment of OP. This experiment can deliver a reference for the further pharmacodynamic study of the RPMA and the correlation analysis of pharmacodynamics and the content of active ingredients in the organism.



Abbreviations used: RPMA: *Radix polygoni multiflori-Achyranthes* root; OP: Osteoporosis; TSG: 2,3,5,4′-tetrahydroxy stilbene-2-O-β-D-glucoside; SD: Sprague-Dawley; AIC: Akaike's information criterion; IS: internal standard.

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INTRODUCTION

The compatibility of *Radix polygoni multiflori-Achyranthes* root (RPMA)[1] is a traditional Chinese medicine pair clinical employed. *Radix polygoni multiflori* is the dried root of Polygonaceae plant *Polygonum multiflorum* Thunb. and *Achyranthes* root is the dry root of Amaranthaceae plant

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Achyranthes bidentata Bl.[2] *Radix polygoni multiflori combined* with Achyranthes root can significantly strengthen muscles and bones and largely used for the treatment of numbness of limbs, weakness of waist and knee,[3] and both have noteworthy anti osteoporosis (OP) effects.[4-6] β-ecdysterone and tetrahydroxystilbene glucoside (2,3,5,4'-tetrahydroxy stilbene-2-O-β-D-glucoside [TSG]) are the key active ingredients of *Radix polygoni multiflori* and *Achyranthes* root,[3] and they all have good anti-OP effects.[7-10] OP comprises primary OP and secondary OP. The primary type mainly contains postmenopausal and senile OP and the secondary type mainly denotes to OP induced by taking certain drugs or suffering from certain diseases. The common methods of constructing OP include ovariectomy,[11,12] retinoic acid induction,[13,14] and glucocorticoid injection.[15,16] Retinoic acid, also known as Vitamin A acid, is an active metabolite of Vitamin A. Some studies[17,18] have publicized that a certain concentration of retinoic acid can encourage the differentiation of osteoblasts and promote OP, while a high concentration of retinoic acid can quicken bone metabolism and lead to OP. These results designate that retinoic acid is operative in inducing OP and its modeling operation is simple and fast. Therefore, retinoic acid is nominated as the inducer to replicate the OP model.

The pharmacokinetics and tissue distribution of TSG in *Radix polygoni multiflori* showed that the TSG could be spotted in rat plasma, liver, lung, and other tissues after gavage.[19-21] The results exhibited that β-ecdysterone could be noticed in plasma and tissues of rats or mice after gavage with *Achyranthes* root extract[22] or intravenous injection of β-ecdysterone.[23] The experimental animals in the above pharmacokinetic experiments were all healthy rats (mice). To study the effect of OP on the distribution of β-ecdysterone and TSG in the plasma and tissues of rats, the research group employed retinoic acid to replicate the OP model,[24,25] and the high-performance liquid chromatography (HPLC) method was used to study the pharmacokinetics and distribution differences of TSG and β-ecdysterone in healthy rats and model rats after intragastric administration of the extract of RPMA. This study can clarify the effect of OP on the *in vivo* process of the two components and deliver a reference for the study of the relationship between the content of effective components in rats and the anti-OP effect.

MATERIALS AND METHODS

Materials and reagents

Radix polygoni multiflori and *Achyranthes* root were procured in Bozhou Medicinal Material Market, Anhui Province, China, and they were recognized as the roots of *P. multiflorum* Thunb. and *A. bidentata* Bl. by Professor Zhai Yanjun of Liaoning University of Traditional Chinese Medicine. Experimental research on plants (either cultivated or wild), comprising the collection of plant material, complied with the *IUCN Policy Statement on Research Involving Species at Risk of Extinction* and the *Convention on the Trade in Endangered Species of Wild Fauna and Flora*. Reference substances of β-ecdysterone, TSG, and quercetin were acquired from the Chinese Institute of Food and Drug Verification, and the purity was more than 98% (Beijing, China). Retinoic acid was obtained from Beijing Solebo Technology Co., Ltd. (Beijing, China). Acetonitrile and methanol of HPLC grade were purchased from Komeo Chemical Reagent Co., Ltd. (Tianjin, China).

Male Sprague-Dawley (SD) rats (age, 10–12 weeks; weight, 200–220 g) were achieved from the Liaoning Changsheng Biotechnology Co., Ltd. (Benxi, China). Temperature, humidity, and light conditions in the rat environment were kept persistent, with food and water delivered *ad libitum*. All rats were familiarized to the laboratory for at least 1 week before the experiment. Before testing, the animals have abstained overnight with free drinking water.

All animal experiments were carried out by the Guidelines for the Care and Use of Laboratory Animals and were permitted by the Animal Ethics Committee of Liaoning University of Traditional Chinese Medicine (license: SYXK (辽) 2013-0009).

Euthanasia of rats imitates to the group standard of the Chinese Society of Experimental Animals (T/CALAS 31-2017) after intraperitoneal injection of pentobarbital sodium (dose 50 mg/kg) for 15 min. Until the respiratory frequency of rats diminished, the main respiratory rule was abdominal respiration, the eyelid reflex vanished, the corneal reflex was weak, and no harmful reflex was caused to severe stimulation. Fix the rat on the lid of the feeding box, grasp the tail of the mouse with one hand, pull back with a little force, press down the head with the thumb and index finger of the other hand, and make the cervical vertebra dislocate quickly with two hands, thus causing the disconnection of the spinal cord and the brain marrow. Six rats were treated each time.

Sample preparation

The powder of the same quality of *Radix polygoni multiflori* and *Achyranthes* root were mixed and five times different extraction solvents were added. After reflux extraction for 6 h, the solvent was filtered and dried. Residues were added to purified water to form a solution at a concentration of 0.8 g/mL for the pharmacokinetic and tissue distribution experiment.[21-23]

The concentration of mixed standard solutions containing β-ecdysterone and TSG was 0.58, 1.18 mg/mL by adding methanol. The methanol solution of quercetin with a concentration of 0.11 mg/mL was prepared. Retinoic acid was accurately weighed and added with saline to prepare a solution of 0.03 g/mL concentration.[24,25]

The establishment of osteoporosis model

Eighty SD rats were arbitrarily alienated into two groups (male and female half). One of the groups was induced OP by retinoic acid through intragastric administration for 14 days and the other group was given the same amount of normal saline. Retinoic acid was administered at a dose of 70 mg/kg.[13,26,27]

Chromatography conditions

The samples were injected into an 1100 HPLC system (Shimadzu, Japan) with an SPD-10AVP UV detector. To correct the conditions of liquid chromatography, we explored the absorption wavelength and mobile phase, respectively. The chromatographic conditions were measured as follows: chromatographic column was Agilent Eclipse XDB-C_{18} (5 μm, 4.6 mm × 250 mm, USA), phase A was acetonitrile, phase B was water, and gradient elution procedure is shown in Table 1. The absorption wavelength was 252 nm and the injection volume was 10 μL. The temperature of the column incubator was 30°C.

Treatment schedule, sample collection, and preparation

Animals received a single dose of 12 g/kg[28] (the dosage of the two drugs is planned as 50%* the dosage of *Radix polygoni multiflori*[29] +50%* the dosage of *Achyranthes* root)[30] by gavage and were euthanized 0, 0.5, 1,

Table 1: Gradient elution procedure

Time (min)	A (%)	B (%)
0-5	5	95
5-10	17	83
10-23	25	75
23-30	40	60
30-50	85	15

2, 3, 4, 6, and 8 h after treatment. The heart, liver, lungs, kidneys, and spleen were composed from five animals at a time. We also collected roughly 500 μL of blood from the orbital sinuses in polypropylene tubes for validation assays.

The blood samples were centrifuged at 15,000 rpm and −15°C for 20 min with the adjustable high-speed dispersing device (Jiangsu Jintan Splendor Equipment Manufacture Co., Ltd., Jiangsu, China). The different tissues were sensed in saline and blotted dry with filter paper and then weighed for wet weight and homogenized in ice-cold physiological saline solution. The preparation of the experimental samples was similar to that of the calibration standards and quality control samples. The tissue homogenates and the plasma extractions were deposited at −20°C.

Liquid-liquid extraction was accomplished before HPLC analysis. The sample was extracted with 3 mL ethyl acetate by vortex for 5 min. After centrifugation at 5000 rpm for 20 min, a 2.5 mL organic layer was collected and vacuum dried at 25°C. The residue was resuspended in 50 μL of mobile phase by vortex for 2 min and analyzed by HPLC.

Pharmacokinetic assays

We employed the 3P97 software (Chinese Pharmacology Society: Beijing, China, 1987) to estimate the pharmacokinetic parameters.[31] We also selected the appropriate pharmacokinetic model based on the lowest Akaike's information criterion value, lowest weighted squared residuals, lowest standard errors of the fitting parameters, and then dispersion of the residual under an equal weight scheme.[32]

RESULTS

Validation

We had used the blank plasma and tissue to draw the standard curve by using the internal standard (IS, Quercetin) method. Sample volume (A) was used for the abscissa and the ratio of β-ecdysterone, TSG, and IS's peak area (C) as the vertical axis. The resulting standard curve, revealed in Table 2, showed a good linear relationship and all the correlation coefficient (r) >0.9. After testing, the precision, accuracy, recovery, stability, and freeze–thaw times of this experiment were in line with the applicable requirements. At the same time, the recovery rate of IS was also inspected, and the results met the requirements of *in vivo* drug analysis.

Pharmacokinetics

The mean plasma concentration–time profiles of β-ecdysterone and TSG in rats are shown in Figure 1. The data were fixed using the two-compartment model, and the corresponding pharmacokinetic parameters are potted in Tables 3 and 4. The results of the plasma drug concentration–time curve presented that the concentration changes of the two components in the model group and the normal group were knowingly different and the peak time of the plasma concentration was different. The pharmacokinetic parameters of β-ecdysterone in the model group had slight difference from that in the normal group. However, the pharmacokinetic parameters of TSG in the model group were suggestively dissimilar from those in the normal group. Such as, C_{max} (59.667 ± 6.437 ng/mL) and AUC_{0-8} (699.252 ± 69.567 ng. h/L) in the model group were expressively higher than those (C_{max} × 48.801 ± 8.638 ng/mL and AUC_{0-8} 235.519 ± 45.467 ng. h/L) in the normal group. The levels of *V*/*F* (1.642 ± 0.255 mL) and *CLs*/*F* (1.629 ± 0.478 L/h/kg) in the model group were much lower than those (*V*/F 29.199 ± 4.424 ml and *CLs*/*F* 7.116 ± 1.253 L/h/kg) in the normal group.

Tissue distribution

The grades of tissue distribution of β-ecdysterone and TSG in rats at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 h are accessible in Figures 2 and 3. From the distribution of β-ecdysterone in the model group and the normal group, the content of β-ecdysterone was different at each time point. The distribution trend of β-ecdysterone in the liver and lung was similar, the distribution difference in spleen and heart was larger, and the difference in kidney was the largest. According to the distribution of TSG in the model group and the normal group, the content of TSG in each tissue was diverse at different time points. The content of TSG in the liver, lung, and spleen was similar, but the content in the heart and lung was fairly different.

DISCUSSION

Selection of internal standard

It was found that quercetin could not be sensed in the water extract of the RPMA in the preliminary experiment. The results of HPLC showed that there was no noteworthy interference between quercetin and the extract. The recovery of IS was more than 85%. Hence, quercetin was nominated as the IS.

Analysis of pharmacokinetic results

The data of β-ecdysterone and TSG were fitted with the two-compartment model. The drug concentration of β-ecdysterone in the model group touched the peak earlier. There was no momentous difference in drug concentration and other pharmacokinetic parameters between the model and the normal groups. The marks of the drug time curve of TSG showed that the drug concentration of the model group reached the peak later, but the drug concentration was higher. The pharmacokinetic parameters of TSG such as AUC_{0-8}, *V*/*F*, and *CLs*/*F* were meaningfully different between the model and the normal group.

Table 2: The acylation-stimulating protein standard curve and linear range

Sample	Chemical composition	Standard curve line	The linear range (μg/ml)	*r*
Plasma	β-ecdysterone	C=2.5499X+0.1511	0.116-0.58	0.9513
	TSG	C=0.2022X+0.1007	0.112-0.59	0.9476
Lung	β-ecdysterone	C=0.3253A−0.0174	0.232-1.16	0.9970
	TSG	C=0.0582A−0.0184	0.472-2.36	0.9822
Liver	β-ecdysterone	C=0.1578A+0.0063	0.232-1.16	0.9866
	TSG	C=0.0754A−0.0117	0.472-2.36	0.9842
Spleen	β-ecdysterone	C=0.2216A+0.0058	0.232-1.16	0.9683
	TSG	C=0.0506A−0.011	0.472-2.36	0.9245
Kidney	β-ecdysterone	C=0.0803A+0.0112	0.118-0.59	0.9609
	TSG	C=0.0758A+0.0092	0.472-2.36	0.9688
Heart	β-ecdysterone	C=0.2001A+0.0158	0.232-1.16	0.9638
	TSG	C=0.0328A+0.0098	0.472-2.36	0.9882

TSG: 2,3,5,4'-tetrahydroxy stilbene-2-O-β-D-glucoside

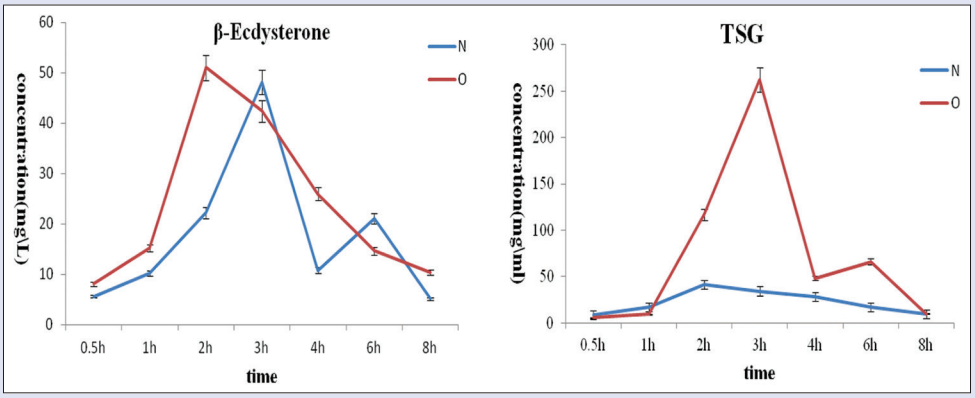

Figure 1: Plasma content-time profiles of β-ecdysterone and 2,3,5,4′-tetrahydroxy stilbene-2-O-β-D-glucoside (*n* = 3) (N: Normal control group; O: OP model group)

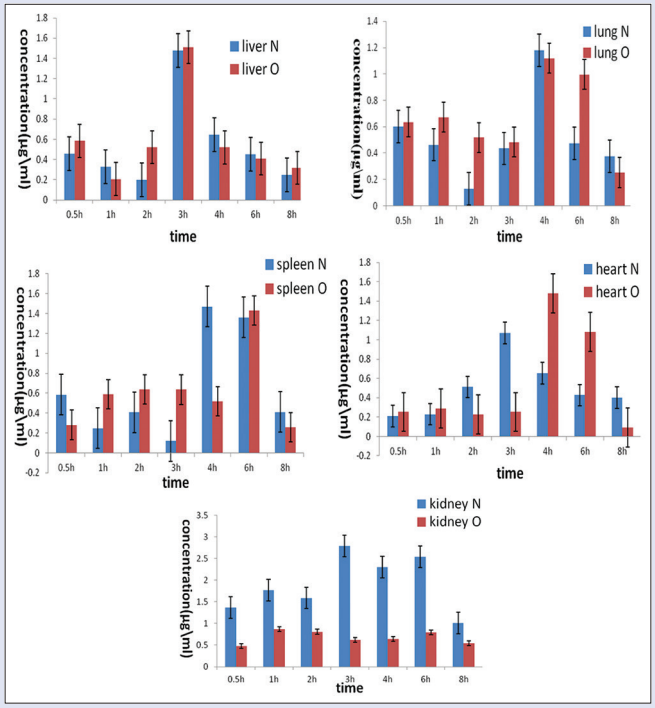

Figure 2: Tissue content-time profiles of β-ecdysterone (*n* = 3, mg/ml) (N: Normal control group; O: OP model group)

Table 3: The main pharmacokinetic parameters of β-ecdysterone

Parameter	Unit	Mean±SD	
		Value N	Value O
k_e	h^{-1}	0.401±0.032	0.567±0.087
$t_{1/2\beta}$	h	6.086±1.057	6.303±0.728
$t_{1/2\alpha}$	h	1.965±0.442	1.398±0.336
AUC_{0-8}	ng h/L	374.033±37.532	312.908±58.227
T_{max}	h	3.222±0.214	2.675±0.357
C_{max}	ng/ml	149.258±26.324	157.557±7.225
V/F	ml	9.545±2.104	9.388±2.616
CLs/F	L/h/kg	3.830±0.514	5.325±1.071

k_e: Elimination constant; $t_{1/2\beta}$: Elimination phase half-life; $t_{1/2\alpha}$: Distribution phase half-life; AUC_{0-8}: Area under the concentration–time curve from 0 to 8 h; T_{max}: Time to achieve C_{max}; C_{max}: Maximum concentration; *V*: The volume of distribution; *CL*s: Systemic clearance; F: Bioavailability; 3 samples per time point; N: Normal control group; O: OP model group

Table 4: The main pharmacokinetic parameters of 2,3,5,4′-tetrahydroxy stilbene-2-O-β-D-glucoside

Parameter	Unit	Mean±SD	
		Value N	Value O
k_e	h^{-1}	0.395±0.015	0.411±0.024
$t_{1/2\beta}$	h	6.472±1.424	5.853±0.995
$t_{1/2\alpha}$	h	2.171±0.762	1.786±0.126
AUC_{0-8}	μg h/L	290.673±45.467	781.207±69.567
T_{max}	h	2.468±0.428	3.012±0.357
C_{max}	ng/ml	144.801±28.638	269.667±6.437
V/F	ml	14.979±4.424	3.729±0.255
CLs/F	L/h/kg	5.923±1.253	1.533±0.478

k_e: Elimination constant; $t_{1/2\beta}$: Elimination phase half-life; $t_{1/2\alpha}$: Distribution phase half-life; AUC_{0-8}: Area under the concentration–time curve from 0 to 8 h; T_{max}: Time to achieve C_{max}; C_{max}: Maximum concentration; *V*: The volume of distribution; *CL*s: Systemic clearance; F: Bioavailability; 3 samples per time point; N: Normal control group; O: OP model group; SD: Standard deviation

Results of analysis of tissue distribution

From the tissue distribution results of β-ecdysterone, it can be noticed in all tissues within 0.5–8 h. On the whole, β-ecdysterone content in the kidney of normal rats was low. The content of the model group was significantly different from that of the normal group. It can be seen that the OP has a substantial effect on the distribution of β-ecdysterone. The concentrations in different tissues were exaggerated. The peak time of the drug in different tissues was altered; the peak time of drug concentration of the model group was about 1 h earlier in the liver, spleen, heart, and kidney. Changing the peak time of drug concentration in numerous tissues, such as liver, spleen, heart, kidney, and other tissues, the peak time of drug components was about 1 h previous than that of the normal group.

From the consequences of tissue distribution, we could see that TSG can be spotted from 0.5–8 h. The content of TSG was ominously different between the OP model and normal groups in different tissues. It can be seen that OP has a significant effect on the distribution of TSG in rats. OP upsets the distribution of drug components in different tissues. At most time points of different tissues, the content of TSG of the model

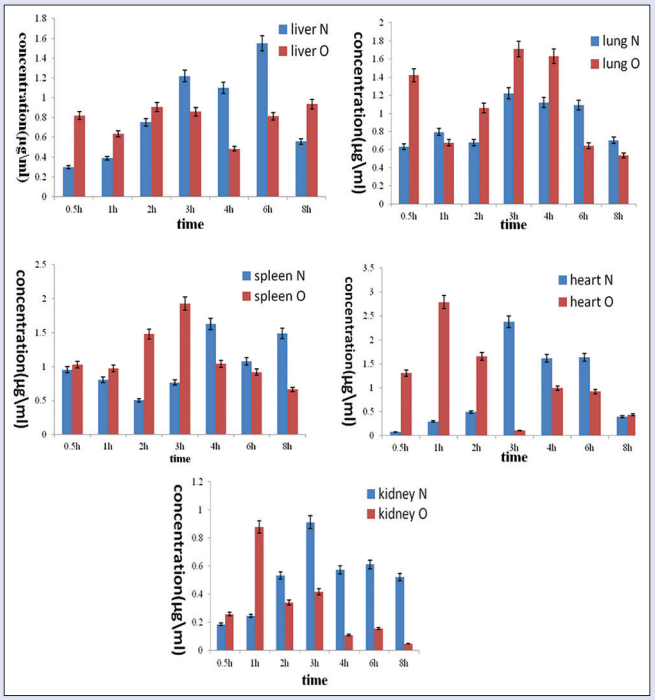

Figure 3: Tissue content-time profiles of 2,3,5,4′-tetrahydroxy stilbene-2-O-β-D-glucoside (*n* = 3, mg/ml) (N: Normal control group; O: OP model group)

group was lower. The peak time of drug concentration in different tissues was former.

CONCLUSION

It is recommended that the effect of OP on the pharmacokinetics of β-ecdysterone is that the concentration of β-ecdysterone influences the maximum in advance. The effect of OP on the pharmacokinetics of TSG is to upsurge the concentration of TSG in plasma and hasten the elimination rate. It can be seen that OP has a momentous effect on the distribution of β-ecdysterone and TSG in rats. In particular, the concentrations of the two components in kidney tissue of the model group were lower than those of the normal group at most time points. It is ventured that the two components may be involved in the treatment of OP.

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Conflicts of interest

There are no conflicts of interest.

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