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ORIGINAL ARTICLE

Determination and Tissue Distribution Comparisons of Atractylodin after Oral Administration of Crude and Processed Atractylodes Rhizome

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ABSTRACT

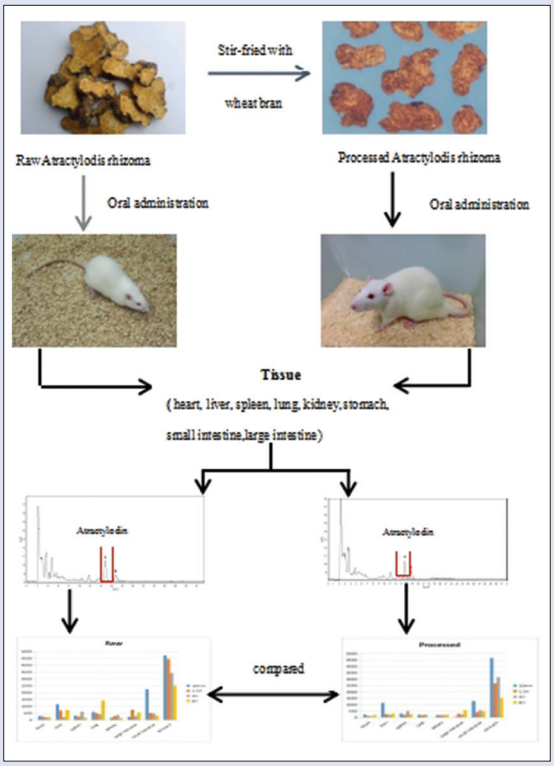
Background: *Atractylodis rhizoma* is one of the most often used drugs in traditional Chinese medicine. Stir frying with wheat bran is the most common processing method. To clarify the principle of processing, an experiment was carried out to compare the tissue distribution of typical constituent after oral administration of raw *A. rhizoma* and processed ones. **Objective:** To compare the tissues distribution of atractylodin after oral administration of raw and processed *A. rhizoma* and clarify the processing principle of *A. rhizoma*. **Materials and Methods:** High-performance liquid chromatogram with ultraviolet detection was developed and validated for the determination of atractylodin in rat tissues. **Results:** The atractylodin in raw and processed *A. rhizoma* was distributed in all tissues involved in this study. **Conclusions:** The concentration of atractylodin in it is the highest in the stomach and small intestine.

Key words: Atractylodin, Atractylodis rhizoma, crude and processed, tissue distribution

SUMMARY

- In this paper, a simple, specific, and rapid reversed phase-high-performance liquid chromatogram method with ultraviolet detection for quantification of atractylodin in rat tissue has been developed for the first time. The result indicates that the concentration of atractylodin in it is the highest in the stomach and small intestine.

Abbreviations used: IS: Internal standard substance; *A. Rhizoma*: *Atractylodis rhizoma*; RSD: Relative standard deviation; HPLC: High performance liquid chromatography.



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INTRODUCTION

Atractylodis rhizoma (Chinese name Cang Zhu) is the dried rhizome of *Atractylodes lancea* (Thunb.) DC. or *Atractylodes chinensis* (DC.) Koidz,[1] which was first recorded in the ancient pharmaceutical book "Shen Nong Ben Tso Ching" and has been used since antiquity to treat rheumatic diseases, digestive disorders, mild diarrhea, and influenza. In clinic, *A. rhizoma* is often processed by stir frying with wheat bran with the aim of reducing its dryness and increasing the function of tonifying spleen.[2,3] It has been reported that *A. rhizoma* contains rich of essential oil including sesquiterpenes and polyethylene alkynes,[4,5] which are the

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main active components. They are the main active components. Recent researches have shown that polyethylene alkynes exhibit various desirable pharmacological effects including anti-inflammatory, antibacterial, and antiarrhythmic activity.[6]

Atractylodin is one of the polyethylene alkyne components and main bioactive components in *A. rhizoma*. In previous researches, our group has studied the pharmacokinetics of atractylodin after oral administration of raw and processed *A. rhizome*.[7,8] The research in this paper is the continuation of previous research and to study the tissue distribution of atractylodin after oral administration of raw and processed *A. rhizoma*. This paper developed a simple high-performance liquid chromatogram (HPLC) method to determinate the concentration of atractylodin in rat tissues and to compare the concentration in different tissues after oral administration of raw and processed *A. rhizoma,* which clarified preliminary the influence of processing on tissue distribution of *A. rhizome*.[9-11]

MATERIALS AND METHODS

Materials and reagents

A. rhizoma (purchased from Herb Planting Base, Hubei, Luotian, China) was identified by Professor Li Feng (Liaoning University of TCM) according to the standards of Chinese Pharmacopoeia 2010. The processed *A. rhizoma* comes from the same batch *A. rhizoma*. The herb was stored in a cool and dry place. Atractylodin (purity, 98%) was supplied by the National Institute for Food and Drug control (Beijing, China). The Internal standard substance (IS) called physcion (purity, 98%) was supplied by the National Institute for Food and Drug control (Beijing, China). Pure water was supplied by Wahaha Company (Hangzhou, China). HPLC grade acetonitrile was purchased from Fisher Scientific Company (New Jersey, USA). Analytical grade ethanol and chloroform were from Baierdi Company (Beijing, China). High-speed homogenizer was purchased from Putian Instrument Manufacturing Co., Ltd. (Changzhou, China).

High-performance liquid chromatography condition

The HPLC system consisted of an LC-10AD Pump, SPD-10A UV spectrophotometric detector (Shimadzu, Kyoto, Japan) with a 20 μL loop (Cotati, CA, USA), and a workstation for data collection. Separation was performed on a Diamonsil C18 analytical column (5 μm, 250 mm × 4.6 mm) from Dikma Technologies (Beijing, China). The mobile phase consisted of acetonitrile and water (75:25, v/v). The detection wavelength was set at 340 nm and flow rate was 1 mL/min. All the measurements were performed at 25°C, and the sample injection volume was 20 μL.

Preparation of Atractylodis rhizoma solution

A. rhizoma (50 g) was crushed into powder and soaked into 600 mL of 95% ethanol for 24h and then percolated at 2 mL/min. After evaporating the solvent under reduced pressure, the residue was redissolved in water and then vortexed. The final concentration of *A. rhizoma* solution was 2 g/mL. The sample was stored in dry and dark place before use.

Processed *A. rhizoma* (50 g) was prepared with same method.

Animals

Healthy cleaning grade Sprague-Dawley rats (290 g ± 10 g) were purchased from the Animal Center of Benxi Chang Sheng Biotechnology Co. Ltd., (Benxi, China) and conventionally raised a week before the experiment. The rats were maintained in an air-conditioned animal quarter at a temperature of 22°C ± 2°C, humidity of 50% ±10%, and 12-h light/dark cycle. Rats were deprived of food overnight before the experiment but were allowed free access to water. All experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals.

In tissue distribution study of atractylodin

Drug administration and tissue sampling

For tissue sample, 45 rats were divided into nine groups (n = 5 per group) randomly. Rats were oral administration raw and processed *A. rhizoma* at a single dose of 20 g/kg. Heart, liver, spleen, lung, kidney, stomach, small intestine, and large intestine were collected at 10 min, 1.5, 4, and 8 h. Tissue samples were weighed 0.2 g rapidly, rinsed with physiological saline to remove the blood or content, blotted on filter paper, and then stored at −20°C and dark place before analysis.

Preparation of tissue sample

For tissue sample, each weighed tissue sample was thawed and then homogenized in ice-cold physiological saline (1 mL). Then, a 200 μL of tissue homogenate (homogenate time 10 s/time, gap 30 s, 3–5 times, ice water bath) was taken and mixed with 20 μL IS. After protein was precipitated with 1000 μL of acetonitrile in 1.5 mL polypropylene tube by vortexing for 2 min, the sample was centrifuged at 8910 ×g for 5 min. The supernatant was transferred into a 5.0 mL tube and added with 1000 μL of chloroform, extract and the under organic phase was transferred to another tube and evaporated to dryness at 40°C with nitrogen. The residue was reconstituted in 50 μL methanol, and centrifugation at 8910 ×g for 5 min, 20 μL supernatant was injected into HPLC system for analysis.

Method validation

Specificity

The selectivity of the method was demonstrated by comparing chromatograms of tissue homogenate spiked with the analytes and IS, and tissue homogenate after an oral dose. All blank tissue homogenates were prepared and analyzed to ensure the absence of interfering peaks.

Calibration curve

The linearity of the method was assessed by plotting calibration curves in tissue homogenate at three concentration levels. The calibration curves were linear over the concentration range of 0.029~5.80 μg/mL in tissue homogenates of atractylodin.

Recovery

The recoveries of atractylodin were determined at low, medium, and high level of sample. The data indicated that the biosample preparation procedure was satisfied and can achieve the acceptable extraction recovery.

Stability

The stock solution stabilities for the atractylodin and the IS and short-term stability were assessed by analyzing samples kept at room temperature for 1 h and 24 h, respectively. Long-term stability was studied by assaying samples following a period of 30-day of storage at −20°C.

Precision

Intraday precision was evaluated by analysis of the five samples with three determinations per concentration at the same day. Interday precision was determined by assaying the standard solutions of the analysis over 3 consecutive days.

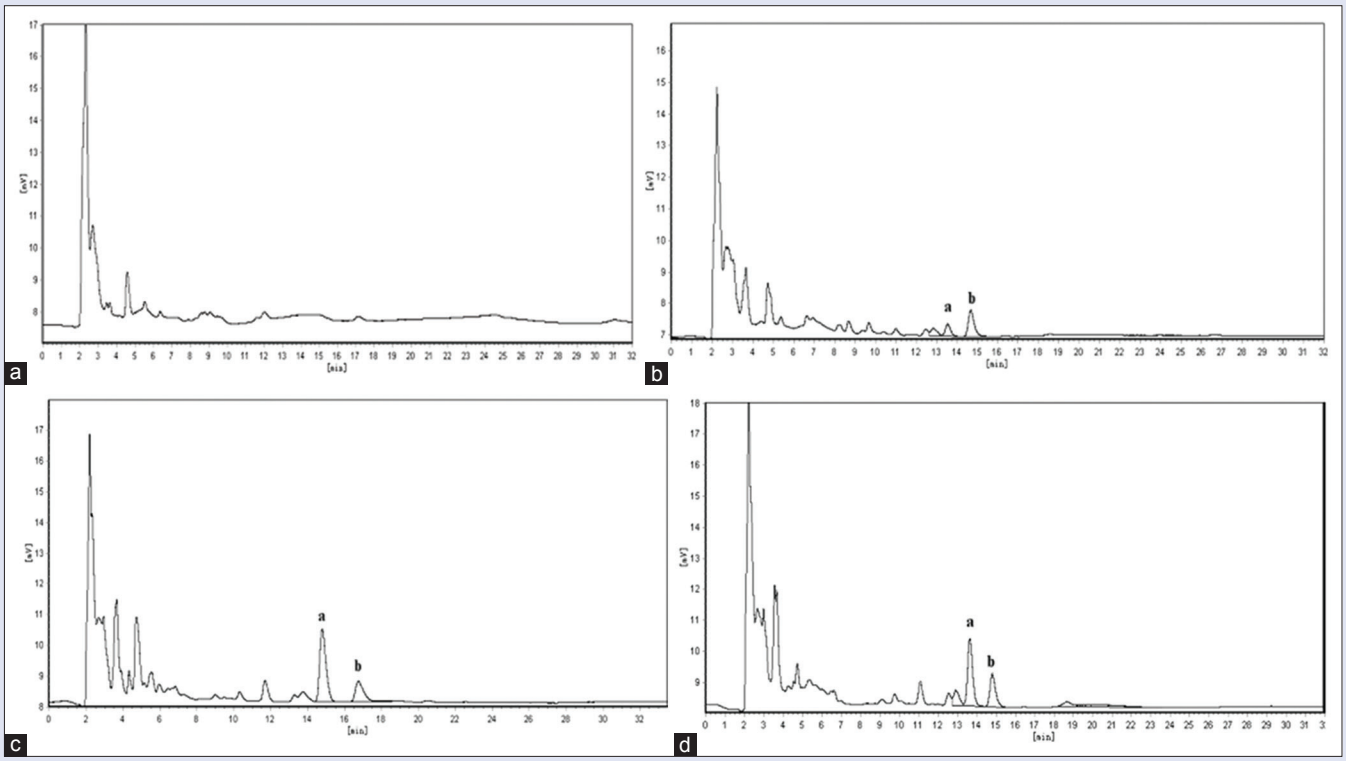

Figure 1: (a) Chromatograms of blank tissue homogenate. (b) Blank tissue homogenate with atractylodin 20 μL and IS 20 μL. (c) Spleen sample (1.5 h) after oral administration of raw *Atractylodis rhizoma* 20 g/kg. (d) Spleen sample (1.5 h) after oral administration of processed *Atractylodis rhizoma* 20 g/kg

Table 1: The linear regression analysis of atracylodin in rat tissue

Tissues	Standard curves	*r*	Linear range(μg/mL)
Heart	Y=2.846X+0.102	0.9955	0.029～5.80
Liver	Y=3.732X+0.207	0.9908	0.029～5.80
Spleen	Y=3.175X-0.140	0.9950	0.029～5.80
Lung	Y=2.965X-0.055	0.9993	0.029～5.80
Kidney	Y=2.286X+0.055	0.9943	0.029～5.80
Large intestine	Y=2.153X+0.428	0.9902	0.029～5.80
Small intestine	Y=3.373X-0.013	0.9966	0.029～5.80
Stomach	Y=2.700X+0.048	0.9932	0.029～5.80

Data analyses

HPLC analysis procedure was applied to analyze tissue distribution of atractylodin.

RESULTS

Method validation

Specificity

Figure 1 shows that no interference peaks from endogenous constituents were detected.

Linearity of calibration curve

The calibration curves were linear over the concentration range of 0.029~5.80 μg/mL in tissue homogenates of atractylodin. The correlation coefficient values of the calibration curve were over 0.9900. Typical linear regression equations and correlation coefficients in each tissue are listed in Table 1.

Recovery and stability

The extraction recoveries of atractylodin ranged from 82.402% to 89.744% in tissue samples. The data are listed in Table 2. The stock solution stabilities for the analyte and IS did not significant differences. The data are listed in Table 3.

Table 2: The recovery of atractylodin in rat tissue (*n*=3)

	Concentration (μg/mL)	Mean(%)	RSD (%)
Heart	0.029	85.635	6.996
	0.580	82.402	3.945
	5.800	89.744	2.111
Liver	0.029	83.266	3.742
	0.580	85.913	2.136
	5.800	85.461	5.174
Spleen	0.029	85.462	3.393
	0.580	85.924	2.242
	5.800	88.027	3.265
Lung	0.029	87.884	1.923
	0.580	85.419	4.327
	5.800	85.202	3.005
Kidney	0.029	85.613	3.485
	0.580	85.064	2.453
	5.800	84.435	1.019
Large intestine	0.029	86.054	3.459
	0.580	85.949	3.944
	5.800	87.814	1.832
Small intestine	0.029	83.529	1.476
	0.580	87.015	3.810
	5.800	88.195	3.648
Stomach	0.029	85.788	2.841
	0.580	85.394	2.390
	5.800	87.915	2.992

Stability of analysis showed no significant sample loss over 24 h at room temperature, three freeze-thaw cycles, and 30 days storage condition. The RSD of three conditions was within ± 15%. The data are listed in Table 4.

Table 3: The stock solution stabilities for atractylodin and the IS (*n*=6)

Time (h)/Peak area (A)	0	4	8	12	24	48	Mean	RSD%
Atractylodin	8803.1	8811.5	8790.2	8781.1	8799.4	8791.3	8796.1	0.12
IS	21745	21721	21733	21716	21672	21659	21708	0.15

Table 4: The stability of atractylodin in rat tissue (*n*=5)

	RSD		
	0.029 μg/mL	0.580 μg/mL	5.800 μg/mL
Heart	7.002	1.730	2.504
Liver	6.170	2.007	2.357
Spleen	3.752	1.361	2.316
Lung	3.232	1.913	1.626
Kidney	4.156	2.445	2.106
Large intestine	3.980	1.386	1.307
Small intestine	2.738	2.383	1.743
Stomach	4.337	1.375	2.067

Table 5: The tissue concentrations of atractylodin after oral administration raw Atractylodis rhizoma at a dose of 20 g/kg to rats (*n*=5)

Tissue (μg/g)	10 min	1.5h	4h	8h
Heart	283.7813	253.1313	202.0188	183.1500
Liver	1137.5563	692.9063	209.1688	728.8438
Spleen	326.3188	245.5688	601.7063	164.2000
Lung	585.9875	502.8125	410.7438	1428.8250
Kidney	153.7438	291.9563	359.7063	138.6938
Large intestine	232.5125	749.4000	290.0313	560.6625
Small intestine	2249.6750	527.9063	462.5938	318.2438
Stomach	4717.8625	4446.9063	3436.4688	2516.8688

Table 6: The tissue concentrations of atractylodin after oral administration processed Atractylodis rhizoma at a dose of 20 g/kg to rats (*n*=5)

Tissue (μg/g)	10 min	1.5h	4h	8h
Heart	216.0563	112.8250	102.3500	180.9875
Liver	1143.7063	271.1500	201.3313	305.9938
Spleen	307.3500	210.3938	505.6938	242.4438
Lung	193.0875	179.6563	220.8188	14.4938
Kidney	189.7125	169.5750	172.5688	211.4938
Large intestine	59.7438	287.4125	227.1813	556.2188
Small intestine	1285.3813	406.8688	557.8250	482.9813
Stomach	4666.1625	2666.6875	3168.8813	1530.4750

Accuracy and precision

Accuracy was assessed by analyzing six aliquots of low, medium, and high concentration samples. Accuracy of atractylodin in tissues ranged from 85.00% to 96.80%. The precision data for atractylodin were not exceed 5%.

Tissue distribution study

The tissue concentrations of atractylodin determined at 10 min, 1.5, 4, and 8 h after oral administration raw and processed *A. rhizoma* a dose of 20 g/kg is shown in Tables 5, 6 and Figures 2, 3.

DISCUSSION

The assay was applied to a tissue distribution experiment in the rat after oral administration of 20 g/kg raw and processed *A. rhizoma*, respectively. The tissue distribution was shown in Tables 5 and 6. The atractylodin in raw and processed *A. rhizoma* was distributed in all tissues, such as heart, liver, spleen, lung, kidney, large intestine, small intestine, and stomach. The concentration of atractylodin in raw and processed *A. rhizoma* is the highest in the stomach and small intestine which proved that the theory of *A. rhizoma* can strengthen spleen-stomach and improve its function of digestion and elimination. The concentration of atractylodin in different tissues after oral processed *A. rhizoma* decreased, the reason needs further research. Atractylodin is one of the main components in volatile oils of *A. rhizoma*. Volatile oils are both "dry" (side effect) components and active components.[12] After being processed, the content of volatile oils was decreased, so the "dry" effects can be weaken while the therapeutic effects can be improved relatively.[6,13] In this study, the IS of rhubarb, emodin, and physcion was studied. Finally, the moderate retention time and no interference peaks from endogenous constituents are physcion.

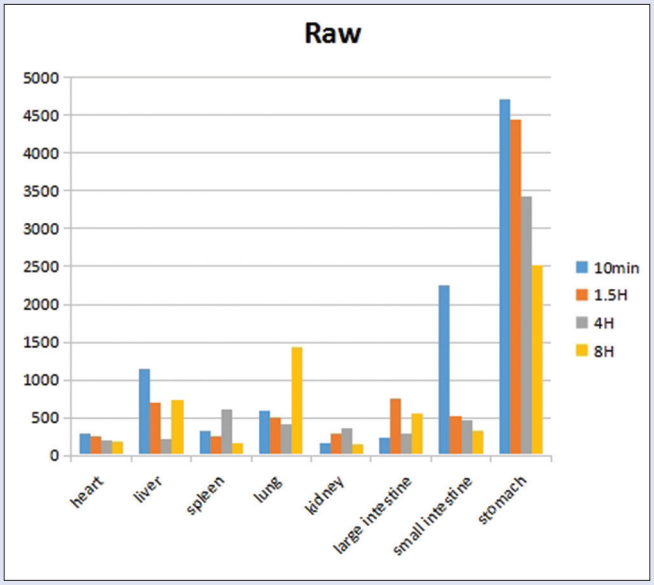

Figure 2: The concentration-time profile of atractylodin after oral administration raw *Atractylodis rhizoma* at a dose of 20 g/kg to rats

CONCLUSIONS

A simple, specific, and rapid reversed phase-HPLC method with ultraviolet detection for quantification of atractylodin in rat tissue has been developed for the first time. It has been successfully applied to a tissue distribution study of atractylodin after oral administration of 20 g/kg raw and processed *A. rhizoma,* respectively. The atractylodin in raw and processed *A. rhizoma* was distributed in all tissues and the concentration of atractylodin is the highest in the stomach and small intestine. The concentration of atractylodin in processed *A. rhzoma* decreased, but its relative concentration is higher in the stomach and small intestine than other tissue.

Acknowledgements

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Nil.

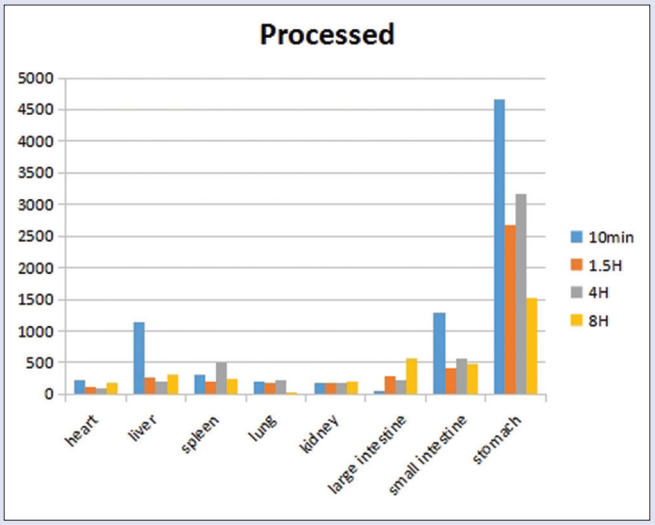

Figure 3: The concentration-time profile of atractylodin after oral administration processed *Atractylodis rhizoma* at a dose of 20 g/kg to rats

Conflicts of interest

There are no conflicts of interest.

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