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Research Article
Studies on the Effective Materials of Baizhu for Immuno-Enhancing Action

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Abstract

To study the effective material basis of Baizhu for immuno-enhancing action, multi-mode separation methods were applied to split the chemical components of Baizhu and each fraction was characterized by HPLC with analysis of similarity degrees. The serum hemolysin against Sheep Red Blood Cell (SRBC), the indexes of the clearance rate of carbon particles and Peripheral Blood Lymphocyte (PBL) transformation assays were used to explore the immuno-enhancing components of Baizhu. Volatile Oil Fraction (VOF), Petroleum Ether Fraction (PEF), Water Eluated Fraction (WEF), Alcohol Eluated Fraction (AEF), Crude Polysaccharide Fraction (CPF), Refined Polysaccharide Fraction (RPF) were obtained. The similarity degrees between any two fractions, except CPF and RPF were calculated to be less than 0.1. The minimal effective dose of Baizhu Water Decoction (WD) for its immuno enhancing action was examined to be 8 g kg^{-1} according to crude drug. The immuno-modulating experiments showed that CPF, RPF and PEF of Baizhu could increase the level of serum hemolysin, raise the PBL transformation and improve the phagocytic indexes in the clearance of carbon particle assays. The HPLC analysis indicated that atractylenolides and polyacetylenes are the main components of PEF and atractylon is for VOF. Atractylenolides, polyacetylenes and polysaccharides are the main effective components for improving the function of specific immunity and nonspecific immunity. The VOF showed, to some extent, to damage the immune organ due to its inhibitory effect on spleen index.

Key words: *Atractylodes macrocephala* koidz, Baizhu, atractylenolides, atractylon, similarity degrees, immune

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Baizhu, the rhizome of *Atractylodes macrocephala* Koidz, compositae (Chinese Pharmacopoeia Commission, 2010) is a generally-used Chinese drugs and possesses various pharmacological activities, such as invigorating qi and strengthening spleen and tocolysis etc. (Shang, 1986; Xi and Zhu, 1987; Li, 1977). Su Wen, the earliest classical treatise on traditional Chinese medicine stated "If vital qi exit in body, the exogenous evil could be resisted" (Zheng, 2007). So the qi in Traditional Chinese Medicine theory has the similar function of immune system to protect the organisms from diseases. The function of invigorating qi in Baizhu is also related to immune enhancement. So far, only Baizhu decoction or some separated fractions for immuno-enhancing action were engaged. For example, Baizhu decoction and polysaccharide had obviously immune enhancing effect on cyclophosphamide-injured mice and volatile oil of Baizhu can stimulate the macrophages activity on normal mice (Chang *et al*., 2003; Sun *et al*., 2011; Guan *et al*., 2001).

Owing to the lower content of individual compounds in plant body, they are hard to be accumulated enough amount to take a test for further various pharmacological actions. As a plant living organisms of metabolism, it encompassed thousands of components, just the pharmacological actions of a few individual compounds could not represent the whole plant. Thus, crude extracts overcome the above disadvantages to reflect the efficacy of herbal medicine. However, it brings up another problem that a component dismissed in several separated extracts by a single separated mode can lead to the missing of some active compounds in the screening experiments. To elucidate the effective material basis of Baizhu, multi-mode splitting methods consisted of solvent partition method and chromatography methods to give 5 fractions and the CPF was further purified to eliminate protein and yield the RPF so as to exhibit the activity of polysaccharides. This study deals with the fraction separations and their immuno-modulating assays, so as to elucidate the material basis for immuno-enhancing effect of Baizhu.

MATERIALS AND METHODS

Drugs and reagents: Baizhu was collected in Yuqian, Zhejiang province, November 2012, which were identified by Professor Wangbing (Liaoning University of Traditional Chinese Medicine) as rhizomes of *Atractylodes macrocephala* Koidz. The vouder specimen (No. 20121101) was deposited in Liaoning University of Traditional Chinese Medicine. Sheep red blood and serum complements were obtained from Dalian Medical University. Petroleum ether (analytical grade) was obtained from Jinfen Chemicals Ltd. (Tianjin, China); methanol and acetonitrile (HPLC grade) were obtained from Oceanpak Alexative Chemical Ltd. (Sweden). Normal saline was obtained from Kelun Pharmaceutical Ltd. (Heilongjiang, China). The PHA was obtained from Zhurui Bio-Technique Co. Ltd. (Shanghai, China); Wright-Giemsa Stain was obtained from Gibco Ltd. (USA). Indian ink was obtained from Solar bio Ltd. (Beijing, China). Other reagents were all of analytical grade.

Animals: Eight week old (18-22 g) male Kunming mice, qualified number SCXK Benxi, Liaoning, China 2010-0001 were housed at Laboratory Animal Center of Liaoning University of Traditional Chinese Medicine (Dalian, Liaoning, China) until surgery and maintained with free access to food and water on a 12 h light/dark cycle at 23°C. Animals were housed for one week prior to the experiments. The experimental protocols were approved by the Guide for the Care and Use of Laboratory Animals Published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All studies were carried out in accordance with the ethics regulations of Liaoning University of TCM (131/2010).

Fraction splitting of Baizhu: Baizhu contains various compounds, which are accumulated through the rigid biosynthetic approach in plants, leading directly to a series of compounds which are bound to have similar chemical property. Chromatography and solvent distribution applied by the chemical affinity with components to separate or purify target components. However single separation method could not achieve the results that components bearing similar chemical property were separated into the same fraction as possible. So multi-mode split methods were adopted to separate Baizhu fractions and the yield of every fraction was showed in Table 1 and the separation procedure was shown in Fig. 1.

Chromatographic conditions: All the analysis of each fractions were performed on a Kromasil C_{18} column (4.6×250 mm, 5 μm) using high performance liquid chromatography system (HPLC, Agilent, USA). The mobile phase was composed of acetonitrile (B) and water (A) with a gradient elution composed of 3% A in 0-5 min, 3-10% A in 5-10 min, 10-40% A in 10-25 min, 40-60% A in 25-40 min, 60-100% A in 40-50 min, 100-3% A in 60-70 min. The flow rate of the mobile phase was set at 1.0 mL min^{-1} and the temperature was maintained at 25°C. Detector wavelength was at 242 nm.

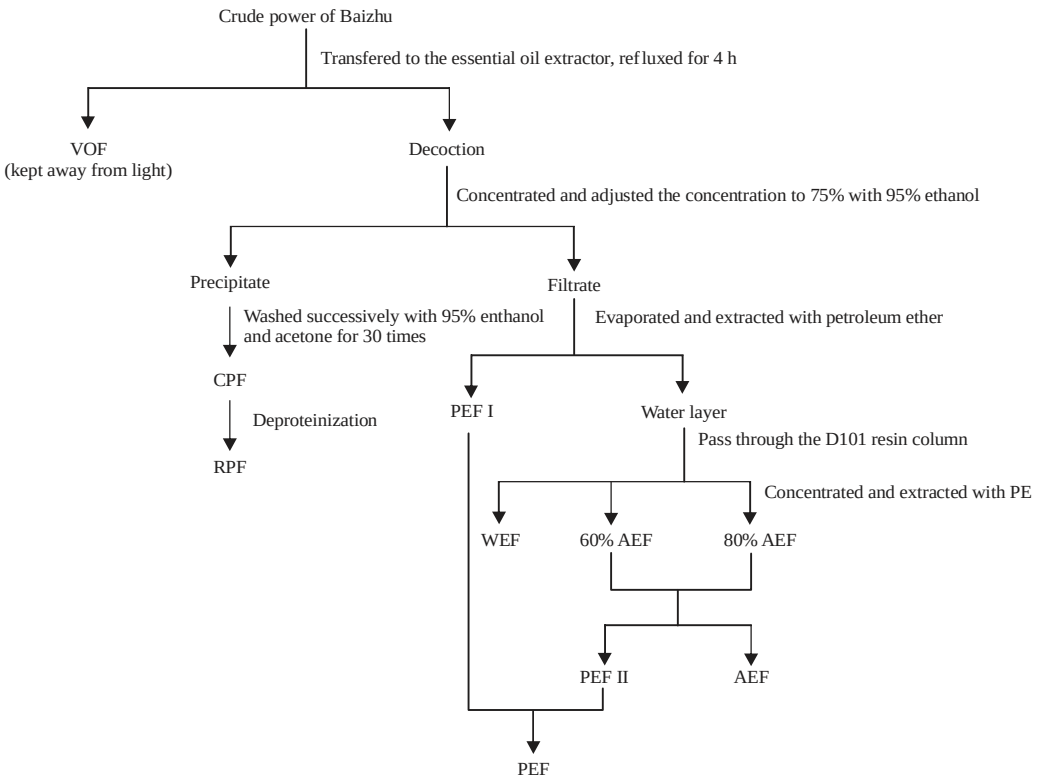

Fig. 1: Separation procedure of Baizhu fractions

Preparation of sample solutions: Took a certain quantity of the separated fractions of Baizhu, respectively and dissolved 10.20 mg VOF in normal hexane-ethyl ether (10:1), 50.51 mg AEF in 15% methanol, 20.10 mg PEF in methanol, 50.10 mg WEF in ultrapure water to a 5 mL volumetric flask precisely and then mixed up, filtrated with 0.45 μm microporous membranes before HPLC injection.

Preparation of standard solutions: The components of separated fractions of Baizhu were further isolated with silica gel column chromatography, ODS column chromatography and preparative high performance liquid chromatography to furnish atractylone, atractylenolide, atractylenolideII, atractylenolideIII and (4E, 6E and 12E)-tetradeca-4, 6, 12-triene-8, 10-diyne-1, 3 and 14-triol. And their structures were identified by comparison of their NMR data with references (Huang and Yuan, 2010; Yu *et al*., 2010). All the standards were dissolved in methanol before analysis in HPLC.

Preparation of 8 g kg^{-1} Baizhu water decoction: Thousand gram of Baizhu crude drug was decocted with water for 2 times. The first time, 12 L water was used to extract for 1 h, then filtered and the residue was decocted 1 h with additional 10 L water for the second time. The filtrates was collected and evaporated in vacuum to 0.8 g mL^{-1} on water bath under 65°C.

Analysis of similarity degree of split fractions of Baizhu: To evaluate the repeatability of the multi-mode split methods for components of Baizhu, the split procedure of Baizhu was repeated 10 times. By the similarity evaluation system for chromatographic fingerprint of TCM (Zhu *et al*., 2008), the VOF, PEF, WEF and AEF were analyzed and then the similarities between any two fractions were obtained.

Test of serum hemolysin content against Sheep Red Blood Cell (SRBC): The minimal effective dose of Baizhu water decoction for serum hemolysin content against SRBC on Kunming mice was investigated with 8 g kg^{-1} crude herbs. The assay was performed in accordance with references (Liu *et al*., 2007) with a modification. Simply, the Kunming mice were administered i.g. at 8:00 in the morning for consecutive 7 days with decoction or correspondent separated fractions according to 8 g kg^{-1} crude drug. All the fractions were dissolved in 1% tween-80 steamed water solution. Control group was administered 1% tween-80 steamed water solution

with 0.2 mL/10 g. On the 4th day of administration, the animals were sensitized with 0.2 mL 5% SRBC. On the seventh day, after 1 h of administration, blood was taken from retroocular venous plexus of the mice. Serum was separated by centrifugation at 3000 rpm for 10 min. Added 1 mL serum diluted 1000 fold with normal saline, 0.5 mL 5% SRBC diluted 20 fold by normal saline and 1 mL 10% serum complements diluted 10 fold by normal saline into 15 mL graduated centrifuge tubes, then kept the tubes of thermal cultivation at 37°C for 30 min and terminated the reaction at 0 at ice bath for 5 min, centrifuge these tubes at 1500 rpm min^{-1} for 10 min. The absorbance of the supernatant was detected with UV-2100 (UNICO Co. Ltd., Shanghai) at 540 nm.

Rest of Peripheral Blood Lymphocyte (PBL) transformation: The minimal effective dose of Baizhu water decoction for PBL transformation on Kunming mice was investigated with 8 g kg^{-1} crude herbs. The assay was performed in accordance with references (Xu, 1982). Thus the doses of split fractions of Baizhu were determined according to the yield rates of separation procedure. Simply, Kunming mice received intramuscular injection of PHA for 3 days. In the second day, every morning at 8:00, the 6 groups of Kunming mice were administered i.g., the decoction and separated fractions respectively, which were dissolved in 1% tween-80. Control group was administered i.g., 0.2 mL/10 g 1% tween-80 simultaneously. In the 5th day, the number of lymphoblast and transitional cells from per 100 lymphocytes were observed and the PBL transformation rate was calculated.

Test of the indexes of the clearance rate of carbon particles: The minimal effective dose of Baizhu water decoction for the indexes of the clearance rate of carbon particles on Kunming mice was also investigated with 8 g kg^{-1} crude herbs. The assay was performed in accordance with references (Pei *et al*., 1993; Ding *et al*., 1994) with a modification. Every morning at 8:00, the 6 groups of Kunming mice were administered i.g., with the decoction and separated fractions respectively, which were dissolved in 1% tween-80. Control group was administered 0.4 mL 1% tween-80. One hour after the 7th day administration, weighted the Kunming mice, then blood was taken from retroocular venous plexus of the mice at the precise time of 2 and 10 min, with initiating the time of intravenous injection of Indian ink diluted 4 fold by normal saline. Took 20 μL blood in time before blood clotted, then added the blood to 2 mL 0.1% Na_2CO_3, mixed. The absorbance of the mixture was detected with UV-2100. Mice were executed to get entire tissues of spleens, livers and thymus. Weight these organs after their tissue fluid and blood were cleaned by filters. The formulas of K value for clearance of carbon particles, α value for the macrophage phagocytic index and organ index were calculated as following equation:

$$K = \frac{\text{LogOD}_2 - \log \text{OD}_{10}}{\text{T10} - \text{T2}} = \frac{\text{LogOD}_2 / \text{OD}_{10}}{8}$$

Where:

Organ index = Organ weight/body weight

α = Body weight/Organ weight × $K^{1/3}$

RESULTS

Result of multi-mode splitting method of Baizhu: The VOF, PEF, AEF, WEF, CPF and RPF were separated in accordance with the procedure in Fig. 1. Meanwhile the yield rates of the separated fractions in Baizhu were obtained (Table 1). The RPF and WEF were main parts of Baizhu decoction, which were obviously physiological essential components of the plants and other separated fractions should be secondary metabolites.

Analysis of similarity degrees of separated fractions of Baizhu: The chromatographic fingerprints of the VOF, PEF, AEF and WEF fractions were well established (Fig. 2-5). The similarity degree of their chromatographic fingerprints can also be termed crossing degree of the fractions, so 1-S means the difference between any two fractions and was named nonsimilarity degree (NS). As a result, NS indicated the unoverlappping property of any two fractions. The 0.099 is the maximal S value between PEF and AEF and 0 is for the minimal value of S between VOF and AEF, VOF and WEF (Table 2). Correspondingly, NS values were calculated as in Table 3.

Table 1: Yield rates of Baizhu separated fractions $\bar{X} \pm S$, n = 10

Group	Yield rate (%)
VOF	0.864±0.03
PEF	0.2045±0.00
AEF	0.858±0.10
WEF	32.70±2.00
CPF	36.66±2.23
RPF	32.65±0.40

VOF: Volatile oil fraction, PEF: Petroleum ether fraction, AEF: Alcohol eluated fraction, WEF: Water eluated fraction, CPF: Crude polysaccharide fraction, RPF: Refined polysaccharide fraction

Table 2: The S values of Baizhu split fractions

	VOF	PEF	WEF	AEF
VOF	1	0.008	0	0
PEF	0.008	1	0.003	0.099
WEF	0	0.003	1	0.022
AEF	0	0.099	0.022	1

VOF: Volatile oil fraction, PEF: Petroleum ether fraction, WEF: Water eluated fraction, AEF: Alcohol eluated fraction

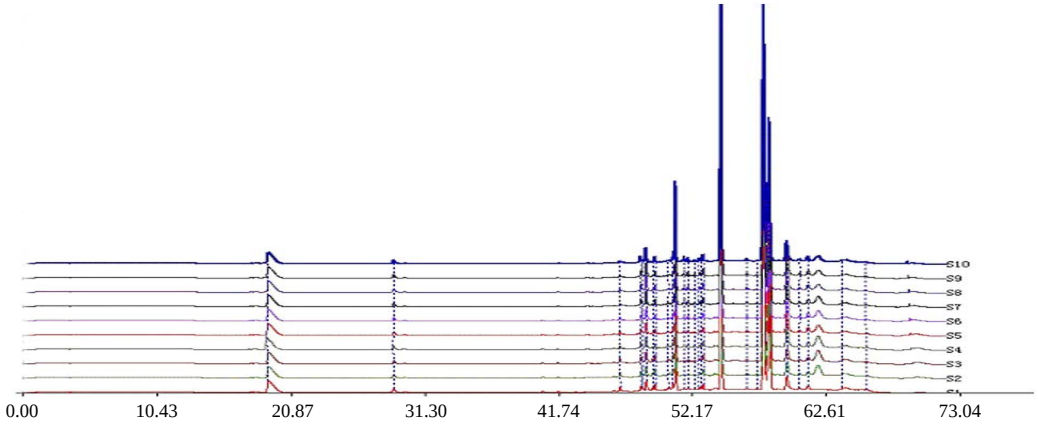

Fig. 2: Chromatographic fingerprints of VOF (n = 10)

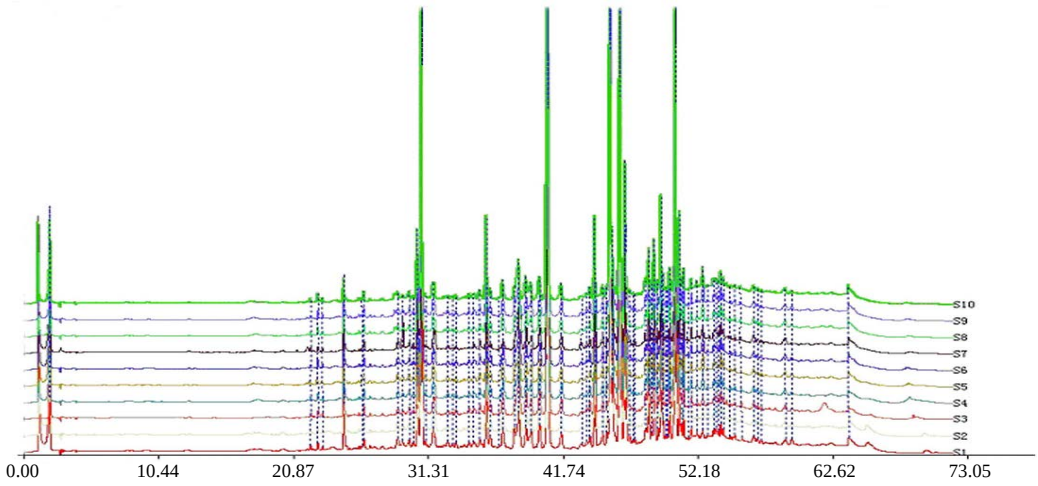

Fig. 3: Chromatographic fingerprints of PEF (n = 10)

Table 3: The NS values of Baizhu split fractions

	VOF	PEF	WEF	AEF
VOF	0	0.992	1	1
PEF	0.992	0	0.997	0.901
WEF	1	0.997	0	0.978
AEF	1	0.901	0.978	0

VOF: Volatile oil fraction, PEF: Petroleum ether fraction, WEF: Water eluated fraction, AEF: Alcohol eluated fraction

Thus, the NS degree between any two separated fractions is over 0.9, which revealed the feasibility of multi-mode separation on splitting fractions of Baizhu.

Effects of serum hemolysin against Sheep Red Blood Cell (SRBC): In Fig. 6, there was no difference between the level of serum hemolysin in VOF and control group and the other groups showed obvious difference with control group. In addition, owing to the maximal average value of serum hemolysin of CPF group, other separated fractions were also compared with it. Nonsignificant difference among PEF, CPF and RPF and significant difference were found among AEF, WEF when compared with CPF group. This showed the main effective split fractions of Baizhu for serum hemolysin stimulation were PEF, CPF and RPF of Baizhu.

The PBL transformation rate of PHA-stimulated T lymphocytes elevated to the highest level on the 6th day after the final intramuscular injection with PHA (Fig. 7). Compared with control group, there was no significant difference between VOF group and AEF group from the 3rd day to the 7th day behind the final intramuscular injection with PHA.

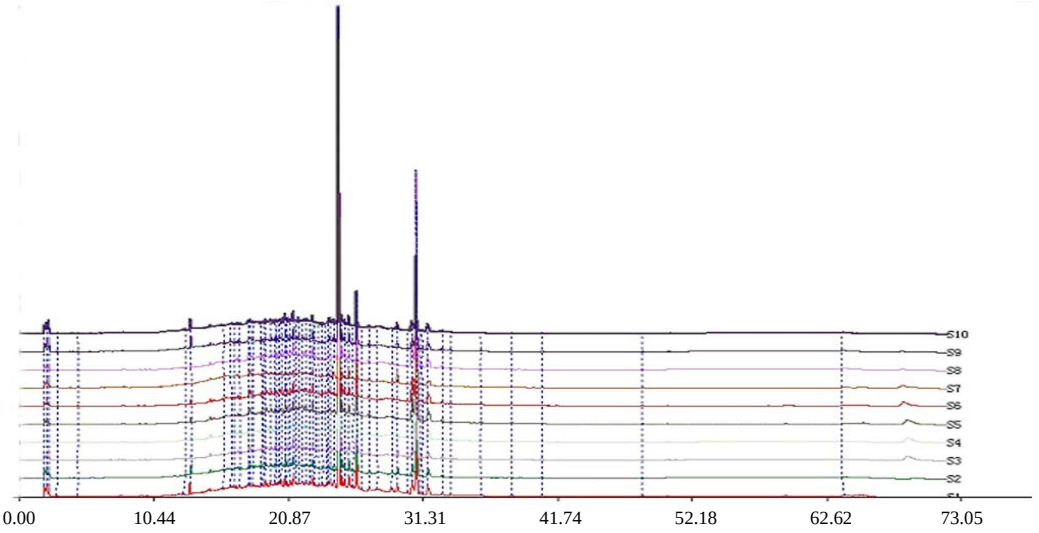

Fig. 4: Chromatographic fingerprints of AEF (n = 10)

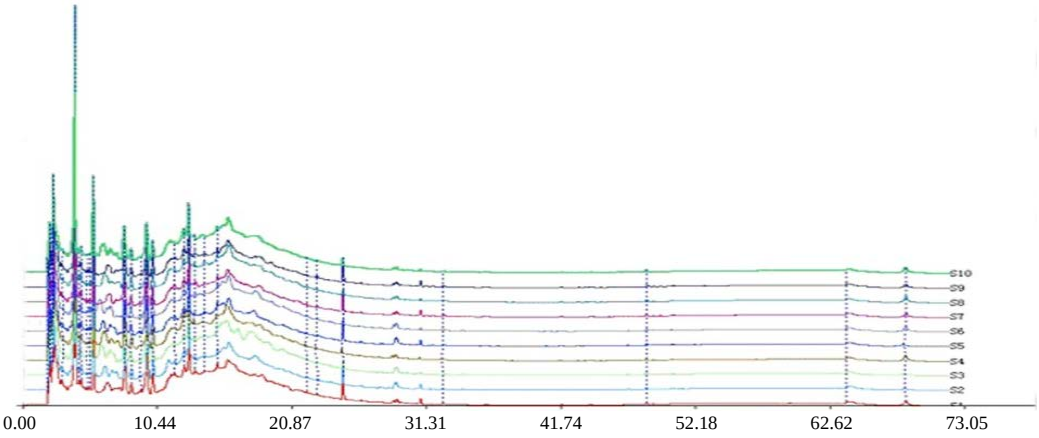

Fig. 5: Chromatographic fingerprints of WEF (n = 10)

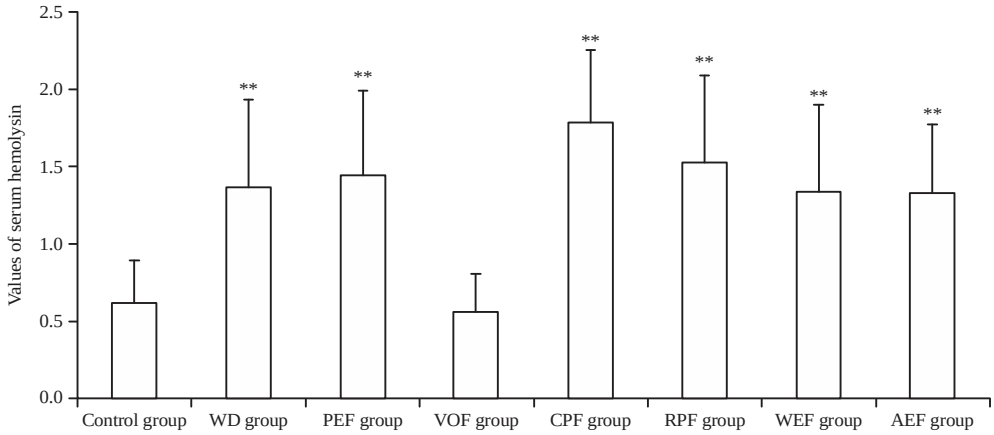

Fig. 6: Effects of serum hemolysin against Sheep Red Blood Cell (SRBC), values are t in LSD analysis, *p<0.05, **p<0.01 compared with control group, ^p<0.05, ^^p<0.01 compared with CPF group

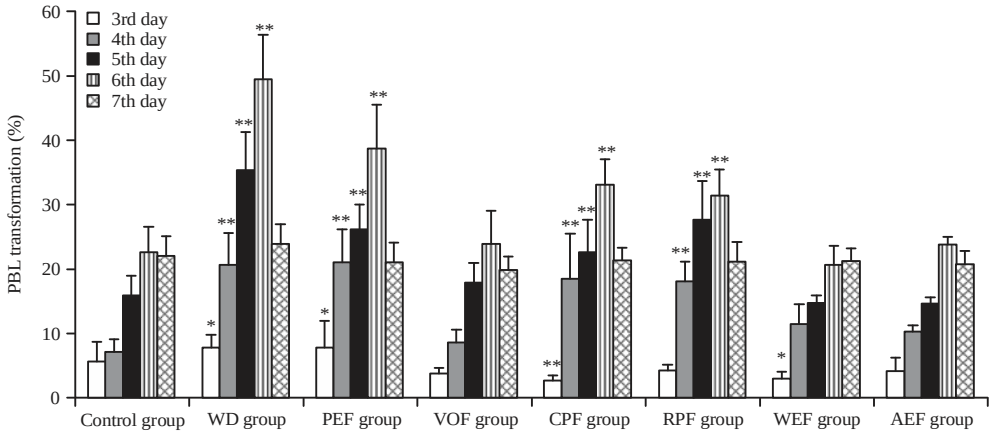

Fig. 7: Effects of Peripheral Blood Lymphocyte (PBL) transformation, values are t in LSD analysis, *p<0.05, **p<0.01 compared with control group, ᵃp<0.05, ᵃᵃp<0.01 compared with CPF group

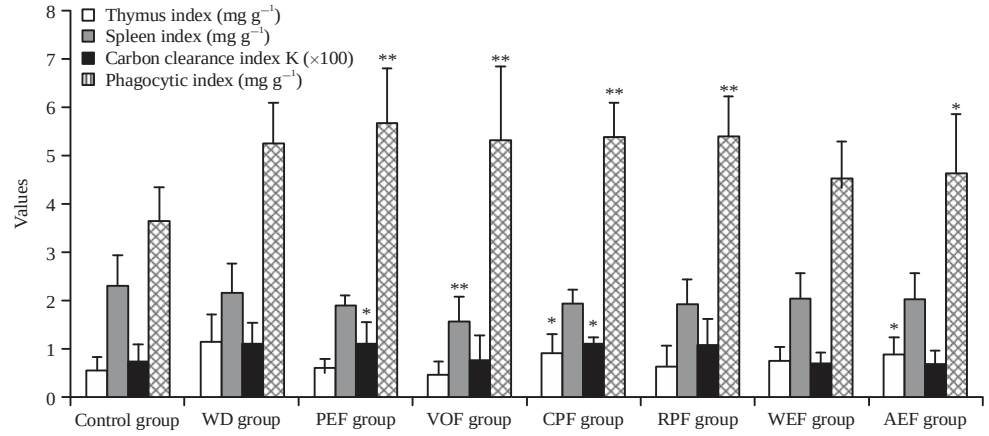

Fig. 8: Effects of indexes of the clearance rate of carbon particles, values are t in LSD analysis, *p<0.05, **p<0.01 compared with control group, ᵃp<0.05, ᵃᵃp<0.01 compared with CPF group

On the 3rd day after the final intramuscular injection, the PBL transformation of WEF group merely come into effect (p<0.05). The PBL transformation rates of PEF and CPF groups from the third day to the 7th day behind the final intramuscular injection with PHA, took action but RPF did from the fourth day to the 7th day behind the final intramuscular injection with PHA. Thus PEF, CPF and RPF are the effective components of Baizhu for promoting the PHA-stimulated T-lymphocyte transformation.

In the experiments, compared with control group, there was significant difference in thymus index among CPF group and AEF groups (Fig. 8). And the spleen index of VOF group was significantly less than control group.

Compared with control group, there was significant difference in K value for PEF group and CPF group, indicating that PEF and CPF strengthening phagocytic function of the mononuclear phagocyte system significantly.

Phagocytic index was calculated with K value, thymus and liver weight. Compared with control group, there was significant difference for VOF, AEF, PEF and CPF groups. The inhibitory effect of VOF group on spleen index and its almost the equal K value with control group, resulted in the increase of its phagocytic index. Thus if the VOF of Baizhu can promote the function of nonspecific immune is worth further researching.

Characterization of the active fractions by HPLC: According to retention time of standard substance separated, the components in VOF included atractylone (Fig. 9), the components in PEF were mainly atractylenolides and polyacetylenes (Fig. 10).

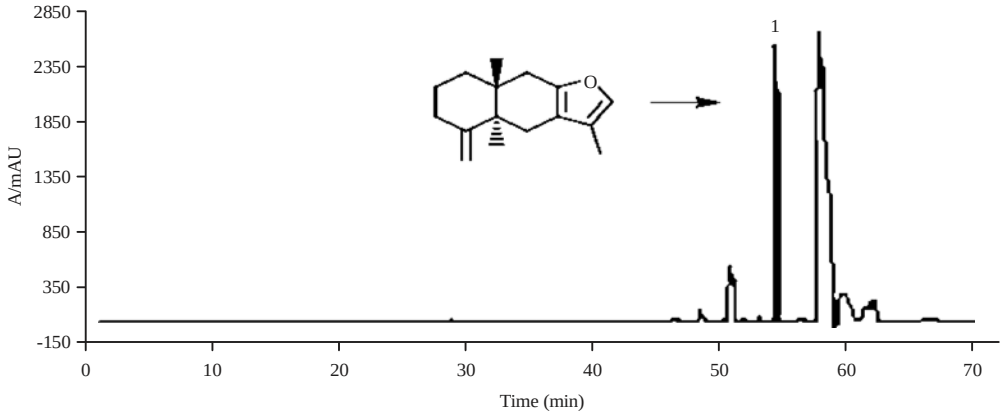

Fig. 9: HPLC fingerprint of VOF A, VOF 1, atractylone

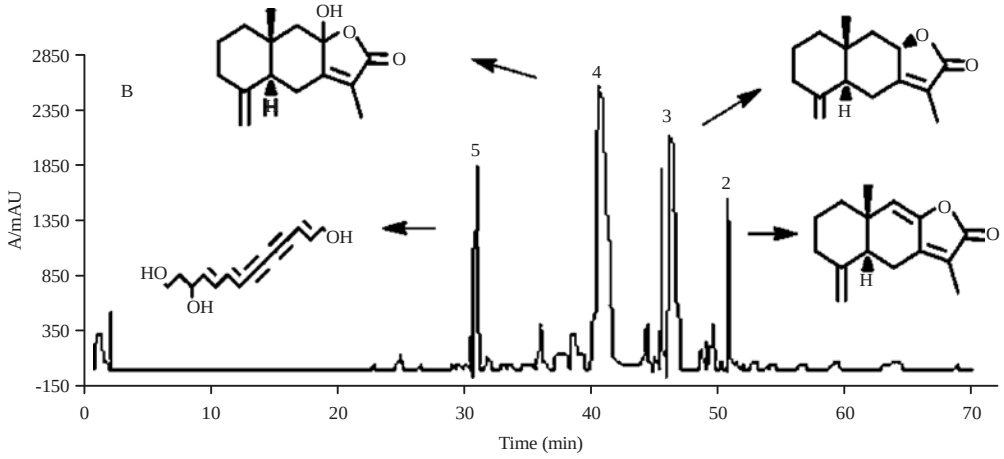

Fig. 10: HPLC fingerprint of PEF B, PEF 2, atractylenolide I 3, atractylenolide II 4, atractylenolide III 5 (4E, 6E and 12E)-tetradeca-4, 6,12-triene-8,10-diyne-1, 3,14-triol

DISCUSSION

Baizhu a generally-used herbal medicine, firstly recorded in "ShenNong's Herbal Classic" about 2000 years ago, which stated that it possessed warm property, sweet and bitter taste (Sun and Sun, 2006). It is vital drug for invigorating qi and strengthening spleen, which was given a high-esteem by the successive classical works of TCM (Shang, 1986; Xi and Zhu, 1987; Li, 1977). The TCM theory holds that qi can resist the exogenous evil and spleen as the biggest peripheral immune organ, plays an important role in immunomodulating action on human body. So the correlational research between invigorating qi and strengthening spleen and the immune-enhancing effect is worth to be explored.

Up to now, most of researches on immune-enhancement of Baizhu focus on its crude extract (Sun *et al*., 2011; Guan *et al*., 2001). Generally water decoction of Baizhu was used in TCM clinical practice. As reported, VOF of Baizhu were effective in the immune experiments (Guan *et al*., 2001) but its dose is highly exceed the conventional amount of Baizhu if calculated by the extraction yield. Possibly, the method is an effective way for new drug discovery but could not reflect the effective material of Baizhu. In this study, the effective materials of Baizhu were elucidated as follows. The minimal dose of Baizhu water decoction for immune-modulation was firstly examined, thereof the doses of the split fractions were determined with the corresponding yields. By comparison of the pharmacological action of the split fractions of Baizhu as the same dosage of crude drug, the effective fractions for its immune-enhancement were determined. Furthermore, the effective materials which represent the efficacy of Baizhu were elucidated.

Innate immune, also called nonspecific immune, was the oldest defense mechanism possessed prevalently in

multicellular life. The capability of the nonspecific immune was obtained at the beginning of living, which quickly stimulated immune response against antigens. It encompasses physiological barrier, nonspecific immunocyte and immune molecules. Among them, mononuclear phagocyte plays an important role in nonspecific immune and its phagocytic function reflects the ability of nonspecific immune system, which is often indicated by the indexes of the clearance rate of carbon particles. Acquired immunity, also called specific immune, contains the humoral immune mediated by B cell and cellular immunity mediated by T cell. In the process of immune response mediated by B cells stimulated by antigen, they can activate, proliferate and differentiate to plasma cells that produce antibody to eliminate antigens. Serum hemolysin against SRBC is one of the antibodies secreted from B lymphocytes, so the model of serum hemolysin against SRBC is examined for the evaluation on B cells function. In the process of immune response mediated by T cells, activated T cells (Th1 cells and CLT cells) can remove foreign antigens and its own cells (Liu, 2009). The PBL transformation on PHA-stimulated animal experiment is often used to test the ability of T cells transformation which can reflect the level of cellular immunity.

In our experiments, CPF showed a better pharmacological activity than RPF. This indicated that the proteins in CPF may also contribute their actions for immuno-stimulating effect. The mechanism research needs to be further studied. The VOF showed a better effect of enhancing activity of macrophage phagocytosis on normal mice administrated for 15 g kg^{-1} of VOF (Guan *et al*., 2001) and this dose was far more than the one administered in our experiments. Our research showed VOF possessed inhibitory effect on spleen, which caused false positive results on phagocytic index α for the smaller values of its spleen index. So the different doses of Baizhu volatile oil caused the opposite pharmacological action needs further to be explored.

CONCLUSION

The main components of VOF is atractylone, the components in PEF were mainly atractylenolides and polyacetylenes. Therefore, our research indicated that polysaccharides, atractylenolides and polyacetylenes of Baizhu, are the main active components on promoting the nonspecific immune and specific immune and atractylone processes inhibitory effect of immune system. As the doses of split fractions were administrated as the basis of the same level of crude drug, our results can reflect the real material basis of Baizhu for immune-enhancing action. thus, polysaccharides, atractylenolides and polyacetylenes are the effective materials of Baizhu for immune-stimulating action.

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REFERENCES

Chang, Y.T., J.L. Sun, S.C. Qiu, Z.Q. Wang and J. Zhou, 2003. Effect of *Atractylodes macrocephala* (AM) on the immune function of mice. J . Binzhou Med. Coll., 26: 350-351.

Chinese Pharmacopoeia Commission, 2010. Chinese Pharmacopoeia. China Medical Science Press, Beijing, China, pp: 95-96.

Ding, Y., P. Chen, S. Xing and X.B. Ru, 1994. Effect of *Epimedium polysaccharide* against inhibitory action of azidothymidine on mouse hemopoietic and immune functions. Chin. J. Pharmacol. Toxicol., 8: 203-205.

Guan, X.H., X. Qu, Z.P. Yang, W.L. Huang and H.X. Sun, 2001. Effect of *Atractylodes macrocephala* Koidz on immune function of mice. J. Beihua Univ. (Nat. Sci.), 2: 122-124.

Huang, Y.P. and P. Yuan, 2010. [Study on RP-HPLC fingerprint of deep-fried rhizoma atractylodis macrocephalae]. J. Chin. Med. Mater., 33: 1554-1556, (In Chinese).

Li, S.Z., 1977. Compendium of Materia Medica. People's Medical Publishing House, Beijing, China, Pages: 734.

Liu, J., M. Gao, H. Yu, Y. Wang, K. Wu, H. Yuan and C. Zhu, 2007. Efects of wild lactarius volemus from Guizhou province on immunological function in mice. Chin. J. Public Health, 23: 291-292.

Liu, W.T., 2009. Medical Immunology. Chinese Medicine Press, Beijing, China, Pages: 101.

Pei, R.J., L.H. Qi and X.J. Liu, 1993. Effects of triptonide on mouse immune functions. Acta Pharmacologica Sinica, 14: 238-242.

Shang, Z.J., 1986. Proofreading and revision, Liang Tao Hong-jing's works. MING YI BIE LU, People's Medical Publishing House, Beijing, pp: 22.

Sun, W.P., F.S. Li, C. Chen, H. Luo, G. Yang and H. Liu, 2011. Immunomodulation of atractylodis polysaccharides in mice. Chin. J. Microecol., 23: 881-886.

Sun, X.Y.Q. and F.Y. Sun, 2006. Sheng Nong's Herbal Classic. Science and Technology Literature Publishing House, Beijing, China, pp: 12-13.

Xi, Y.M. and Z.H. Zhu, 1987. Proofreading and revision. Qing Huang, G.X. BEN CAO QIU ZHEN, pp: 6-8.

Xu, S.Y., 1982. Methodology of Pharmacological Experiment. People's Medical Publishing House, Beijing, China, Pages: 952.

Yu, L.L., T.Z. Jia and Q. Cai, 2010. Research on the chemical references of *Atractylodes macrocephala* Koidz. Asia-Pac. Tradit. Med., 6: 36-39.

Zheng, H.X., 2007. Basis of Traditional Chinese Medicine. People's Medical Publishing Sun, Beijing, Pages: 17.

Zhu, L.L., D.W. Qian, J.A. Duan and J. Zhang, 2008. Study on the feature and similitude of HPLC fingerprint of *Chrysanthemum morifolium* Ramat. Chin. J. Pharmaceut. Anal., 28: 1131-1135.