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# Research Article
# Bidirectional Effective Components of Atractylodis Macrocephalae Rhizoma on Gastrointestinal Peristalsis

Jing Chen, XiaLiu and Deqiang Dou

College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian 116600, China

## Abstract

To find the bidirectional effective components of Atractylodis Macrocephalae Rhizoma (AMR) on gastrointestinal peristalsis (GIP). Multi-mode separation methods (solvent partition method and macroporous adsorptive resins) were used to split AMR component; HPLC and GC-MS were used to analyze the main compounds of fractions; the indexes included gastric residual rate and intestinal propulsive rate were used to observe the effects of AMR and its fractions on GIP; ELISA was used to examine the level of Vasoactive Intestinal Peptide (VIP) and P substance (SP) in antrum and ileum. The Water Decoction (WD) of AMR was separated into five fractions, namely, Volatile Oil Fraction (VOF), Petroleum Ether Fraction (PEF), Alcohol Eluate Fraction (AEF), Water Eluate Fraction (WEF) and polysaccharides (CPF); the GIP was promoted in mice with dose of 1.0 g $kg^{-1}$ WD, VOF, CPF and WEF. However, GIP was inhibited in mice with dose of WD (10.0 g $kg^{-1}$), PEF and AEF. The AMR had bidirectional regulation effects on gastrointestinal function; the VOF (contained monoterpenes and sesquiterpenes components), WEF (contained 5-hydroxymethyl furfural and small molecular sugar) and CPF (contained inulin-type oligosaccharides) are the fractions of AMR for promotion effects on GIP and PEF (contained sesquiterpene lactone) and AEF (contained polyacetylene) of AMR played opposite action; the underlying action mechanism maybe relate to the SP and VIP levels.

**Key words:** Atractylodis macrocephalae rhizoma, fraction, vasoactive intestinal peptide, P substance

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**Corresponding Author:** Deqiang Dou, College of Pharmacy, Liaoning University of Traditional Chinese Medicine, 77 Life 1 Road, Dalian 116600, China
Tel: +8641187586014, Fax: +8641187586078



**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

Atractylodis macrocephalae rhizoma (Baizhu in Chinese), the rhizome of *Atractylodes macrocephala* Koidz, is a commonly used drug in Traditional Chinese Medicine (TCM). The AMR has been described as warm, bitter and sweet and tropism to the heart, spleen and stomach meridians and can invigorate the spleen and cure patients with splenic asthenia, anorexia, oedema, excessive perspiration and abnormal fetal movement (NPC., 2010). Generally, AMR has been regarded as a vital drug to invigorate the spleen, suggesting that the main action of AMR is to modulate the digestive functions of the body. Evidence has shown that GIP was promoted in low dose of AMR water decoction and was inhibited in high dose of AMR water decoction (Wu *et al*., 2005). The volatile oil of AMR exhibited promotion effect and lactone of AMR presented inhibition effect on GIP (Chen *et al*., 2009; Zhang *et al*., 1999). Research indicated that sesquiterpenoid-type compounds are the major components of AMR and contributed to most of the pharmacological functions, especially, the regulation of gastric emptying time (Wu *et al*., 2005; Xing *et al*., 2003). However, the chemical components of AMR responsible for bidirectional effects on GIP have not been reported systematically in previous reports. Recently, the fraction-splitting of water decoction of AMR were studied (Li *et al*., 2014). In addition, the main components of CPF were identified as in ulin-type oligosaccharides by HILIC-MS Lin *et al*. (2015). Therefore, the effects of ARM and fractions on GIP were studied systematically to clarify the effective components of AMR in the study.

## MATERIALS AND METHODS

**Drugs and reagents:** The AMR was collected from Yuqian, Zhejiang, November, 2012 (Batch No. 20121101), where is the geo-authentic producing area and were identified by Professor Wang Bing (Liaoning University of Traditional Chinese Medicine) as rhizomes of *Atractylodes macrocephala* Koidz. Domperidone tablets (Batch No. 120913731, Xian-Janssen Pharmaceutical Ltd.). The P substance Elisa Kit (Batch No. 201310, Shanghai lianshuo Biological Technology Co., Ltd.) and Vasoactive Intestinal Peptide (VIP) Elisa Kit (Batch No.201310, Shanghai lianshuo Biological Technology Co., Ltd.). Other reagents were of analytical grade.

**Animals:** Male Kunming mice (18-22 g) were purchased from Liaoning Changsheng Biotechnology Co., Ltd [SCXK (Liao) 2010-0001]. All mice were maintained with free access to food and water in plastic cages at 22±2, relative humidity 50-60% and kept on a 12 h light/dark cycle. 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).

**Preparation of splitted fractions:** One hundred gram of AMR was pulverized and immersed in water for 1 h, boiled twice and the filtrates were combined and concentrated in vacuum to 0.5 g crude drug $mL^{-1}$ and then stored in 4°C for the water decoction of AMR. Further, according to the polarity of components of AMR, the water decoction of AMR was split as reported in reference (Li *et al*., 2014). Briefly, the water decoction of AMR was concentrated and the 95% ethanol was added to regulate the alcohol concentration to 75%, standstill under 0~5°C overnight and then the supernatant was poured out to obtain the precipitate and the supernatant. The precipitate was washed three times by 95% ethanol and acetone respectively to furnish the Crude Polysaccharides Faction (CPF) after lyophilization. Then, the supernatant was evaporated on water bath at 50°C to eliminate the alcohol and the resulting water layer was extracted eight times with 60~90°C petroleum ether until there is no color in layer of petroleum ether to give Petroleum Ether Fraction (PEF). The resulting water layer was subjected to a column of macroporous adsorption resin D101, washing with distilled water, 60% ethanol and 80% ethanol successively. The water eluate was concentrated at 50°C and lyophilized to give Water Eluted Fraction (WEF) and the 60 and 80% ethanol elutes were collected and evaporated in vacuum to give the Alcohol Eluted Fraction (AEF).

**Analysis of the main compounds in VOF, PEF, AEF and WEF:** The GC-MS analysis was performed using an Agilent HP 5975 Series instrument combined with an Agilent HP 7890 Mass Selective Detector. Separation of VOF was performed using a polar capillary column (DB-5MS) with 30 m×0.25 mm×0.25 μm film thickness. The carrier gas was helium and the flow rate was 1.0 mL $min^{-1}$. The temperature program was optimized to separate quickly as follows: initial temperature 70°C, held for 2 min, increased at 10°C $min^{-1}$ to 280°C for 20 min. The injection volume was 1 μL and the split ratio was 1/50. The injector temperature was 260°C. The mass spectrometer was operated in the electron impact

ionization mode at 70 eV. The ionization source and transfer line temperature were kept at 230 and 260°C. Identification of the constituents of each sample was achieved by matching their MS and with the fragmentation pattern of their mass spectra with those in NIST 05.LIB.

Various chromatographic columns, including silica gel, ODS column and HPLC methods, were used to isolate and purify the chemical compounds of AMR. The chemical compounds of AMR were identified by the analysis of their spectroscopic data in comparison with reference compounds. The fingerprint of each of the separated chemical fractions was established using an HPLC method. A HPLC Agilent system 1260, with a DAD detector and a Kromasil$C_{18}$ (250×4.6 mm, 5 μm) column was used. Conditions were as follows: Column temperature 25°C, flow 1.0 mL $min^{-1}$, running time 70 min; the solvent were acetonitrile and B water; gradient: 0-5 min, 3% A→5-10 min, 3-%-10% A→10-25 min, 10%-40% A→25-40 min, 40-60% A→40-50 min, 60-100% A→50-60 min, 100% A→60-70 min, 100-3% A. Detection at 242 nm provided the best level information for all compounds. The main peaksin every fraction were identified by the retention time of the individual compounds isolated.

**Preparation of semi-solid paste:** According to the literature method (Xing *et al*., 2003),10 g CMC-Na was dissolved with 250 mL distilled water and mixed with 10 g milk powder, 8 g sugar, 8 g starch and 2 g active carbon powder, respectively. About 300 g black semi-solid paste was obtained and stored at 4°C.

**Effects of water decoction of AMR on GIP:** The slight modification of earlier methods was carried out to evaluate the gastric residue and intestinal propulsion rates (Xing *et al*., 2003). Sixty Kunming mice, half male and half female, were randomly divided into six groups with 10 mice for each group. Each group was administered i.g for 7 days as follows. Control group, 0.2 mL/10 g of the purified water, Positive control group, 0.01 g $kg^{-1}$ domperidone, 0.1 g $kg^{-1}$ water decoction group, 0.1 g $kg^{-1}$ WD group, 0.1 g $kg^{-1}$ water decoction; 1 g $kg^{-1}$ water decoction group 1 g $kg^{-1}$ WD group, 1 g $kg^{-1}$ water decoction, 2 g $kg^{-1}$ water decoction group 2 g $kg^{-1}$ WD group, 2 g $kg^{-1}$ water decoction, 10 g $kg^{-1}$ water decoction group 10 g $kg^{-1}$ WD group, 10 g $kg^{-1}$ water decoction. Before the experiment, the mice were deprived of food but not water for 24 h. The mice were i.g with 0.4 mL/10 g semi-solid paste at day 8. Then the mice were sacrificed after 20 min and the gastric residue and intestinal propulsion rates were evaluated. The methods of evaluation as follow:

$$\text{Gastric retention rate} = \frac{\text{A-B}}{\text{C}} \times 100\%$$

$$\text{Intestinal propulsive rate} = \frac{\text{D}}{\text{F}} \times 100*$$

where, A is total gastric weight of mice, B is gastric weight, C is the weight of the administration semi-solid paste in mice, D is the length of black small intestine and F is the length of small intestine.

**Effects of the fractions of AMR on GIP:** The experiment protocol is similar to the water decoction of AMR and the mice were randomly divided into 14 groups with 10 mice for each group. Each group was orally administered i.g as follows. Control group: the purified water 0.2 mL/10 g, positive control group: 0.01g $kg^{-1}$ domperidone, 1g $kg^{-1}$ WD group, 1 g $kg^{-1}$ water decoction; the low dosage of 5 splitted fractions groups: corresponding extracts according to 1 g $kg^{-1}$ crude herbs; 10 g $kg^{-1}$ WD group: 10 g $kg^{-1}$ water decoction and the high dosage of 5 splitted fractions groups: corresponding extracts according to 10 g $kg^{-1}$ crude herbs. Before the experiment, the mice were deprived of food but not water for 24 h. The mice were i.g with 0.4 mL/10 g semi-solid paste at day 8. Then the mice were sacrificed after 20 min and the gastric residue and intestinal propulsion rates were evaluated. The methods of evaluation as follow:

$$\text{Gastric retention rate} = \frac{\text{A-B}}{\text{C}} \times 100\%$$

$$\text{Intestinal propulsive rate} = \frac{\text{D}}{\text{F}} \times 100*$$

where, A is total gastric weight of mice, B is gastric weight, C is the weight of the administration semi-solid paste in mice, D is the length of black small intestine and F is the length of small intestine.

**Determination of VIP and SP in gastric antrum and ileum:** After the evaluation of fractions on GIP, gastric antrum and ileum of the mice were isolated and homogenized for the determination of VIP and SP levels.

**Statistical analysis of data:** All data were expressed as Mean±Standard Deviation (SD). The statistical analysis of the results was performed by one-way analysis using the Statistical Package for Social Science program (SPSS 20.0, Chicago, IL, USA). The $p<0.05$ was considered to indicate a statistically significant difference.

## RESULTS

**Analysis of the main compounds in VOF, PEF, AEF and WEF:** In this study, 18 main compounds were isolated and analyzed as follow: atractylone which was obtained from VOF; Juniper camphor, atractylenolide, taraxeryl acetate, (4E, 6E, 12E)-tetradeca-4, 6, 12-trien-8, 10-diyne-1, 3, 14-triol, 3β-acetoxy-atractylenolide, stigmasterol, atractylenolide, Isoatractylenolide, sitosterin, atractylenolide, dibutyl phthalate and diisobutyl phthalate were obtained from PEF; 14. atractyloside A; 3 β-acetoxy-atractylenolide, caprolactam, 5-hydroxymethyl furfural ether and (4E, 6E, 12E)-3, 14-dihydroxytetradeca-4, 6, 12-trien-8, 10-diyn-1-yl acetate were obtained from AEF; 5-Hydroxymethyl furfural and small molecular sugar were obtained from WEF. In combination with the HPLC chromatogram, it is founded that VOF mainly contains atractylone, PEF mainly contains sesquiterpene lactone, AEF mainly contains polyacetylene and WEF mainly contains 5-hydroxymethyl furfural and small molecular sugar. In the Table 1, 37 compounds were identified and analyzed. The results indicated that the content of atractylone was highest than others and most of compounds belong to terpene and sesquiterpenoids.

**Effects of water decoction (WD) of AMR on GIP in mice:** Results (Fig. 1) indicated the gastric retention rate in 0.1 g $kg^{-1}$ WD group and 1 g $kg^{-1}$ WD group were significantly decreased than control group; the intestinal propulsion rate in 1 g $kg^{-1}$ WD group and 2 g $kg^{-1}$ WD groups was significantly increased than control group. However, the gastric retention rate in 10 g $kg^{-1}$ WD group was significantly increased than control group; the intestinal propulsion rate in 2 g $kg^{-1}$ WD group and 10 g $kg^{-1}$ WD group was significantly decreased than control group.

Table 1: GC-MS analysis of VOF

| $t_R$ $min^{-1}$ | Molecular formula | Compound name | Percentage composition |
|---|---|---|---|
| 10.394 | $C_{12}H_{20}$ | 1,5-dimethyl-2,6-bis(methylene)-cyclooctane | 0.483 |
| 10.605 | $C_{15}H_{24}$ | 2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydro-naphthalene | 0.192 |
| 10.606 | $C_{15}H_{24}$ | (-)-aristolene | 0.063 |
| 11.103 | $C_{15}H_{24}$ | 6S-2,3,8,8-tetramethyltricyclo[5,2,2,0(1,6)]undec-2-ene | 0.437 |
| 11.189 | $C_{15}H_{24}$ | (+)-calarene | 0.743 |
| 11.262 | $C_{15}H_{24}$ | 1,4-dimethyl-7-(1-methylethenyl)-1,2,3,5,6,7,8,8a-octahydro-azulene | 0.104 |
| 11.368 | $C_{15}H_{24}$ | (-)-cyperene | 0.58 |
| 11.623 | $C_{15}H_{24}$ | γ-neoclovene | 0.469 |
| 11.751 | $C_{15}H_{24}$ | (+)-Ledene | 2.016 |
| 11.870 | $C_{15}H_{24}$ | caryophyllene | 3.586 |
| 11.934 | $C_{15}H_{24}$ | γ-elemene | 1.255 |
| 12.064 | $C_{15}H_{24}$ | α-caryophyllene | 0.548 |
| 12.296 | $C_{15}H_{24}$ | γ-himachalene | 0.399 |
| 12.302 | $C_{16}H_{26}O$ | 3,6,8,8-tetramethyl-2,3,4,7,8,8a-hexahydro-1H-3a,7-methanoazulene | 0.95 |
| 12.409 | $C_{13}H_{20}O$ | 4-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)- 2-butanone | 0.95 |
| 12.508 | $C_{15}H_{24}$ | eudesma -4(14),11-diene | 5.204 |
| 12.512 | $C_{15}H_{24}$ | β-humulene | 0.292 |
| 12.575 | $C_{15}H_{24}$ | 4a,8-dimethyl-2-(1-methylethenyl)-1,2,3,4,4a,5,6,8a-octahydro-naphthalene | 1.575 |
| 12.896 | $C_{15}H_{24}$ | γ-neodovene | 1.575 |
| 12.906 | $C_{15}H_{24}$ | (+)-valencene | 0.333 |
| 13.149 | $C_{15}H_{24}$ | 6-ethenyl-6-methyl-1-(1-methylethenyl)-3-(1-methylidene)-6-cyclohexene | 5.246 |
| 13.231 | $C_{15}H_{24}$ | (+)-aromadendrene | 0.661 |
| 13.303 | $C_{15}H_{24}$ | β-panasinsene | 6.981 |
| 13.435 | $C_{15}H_{24}$ | 4a,8-dimethyl-2-(propan-2-ylidene)-1,2,3,4,4a,5,6,8a-octahydro-naphthalene | 2.429 |
| 13.485 | $C_{15}H_{24}$ | 4,5-dehydro-isolongifolene | 0.206 |
| 13.693 | $C_{15}H_{26}O$ | 2-nerolidol | 2.334 |
| 14.250 | $C_{15}H_{24}$ | β-elemene | 0.234 |
| 14.628 | $C_{15}H_{24}$ | β-vatirenene | 1.055 |
| 14.684 | $C_{15}H_{24}$ | 8,9-dehydro-neoisolongifolene | 0.644 |
| 15.043 | $C_{15}H_{24}O$ | (-)-spathulenol | 4.566 |
| 15.203 | $C_{15}H_{22}O$ | atractylone | 26.302 |
| 15.454 | $C_{15}H_{26}O$ | 1,4a-dimethyl-7-(1-methylethylidene)-decahydro-1-naphthalenol | 0.356 |
| 15.550 | $C_{15}H_{24}O$ | ledene alcohol | 0.94 |
| 15.688 | $C_{15}H_{22}O$ | 3,8-dimethyl-4-(1-methylethylidene)-2,4,6,7,8,8a-hexahydro-azulenone | 0.685 |
| 15.738 | $C_{15}H_{22}O$ | 4a,5-dimethyl-3-(1-methylethylidene)-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone | 0.479 |
| 15.779 | $C_{15}H_{20}O_2$ | 7R,8R-8-hydroxy-4-isopropylidene-7-methylbicyclo[5,3,1]undec-1-ene | 0.126 |
| 18.737 | $C_{15}H_{20}O_2$ | 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-5,6-azulenedicarboxaldehyde | 0.446 |

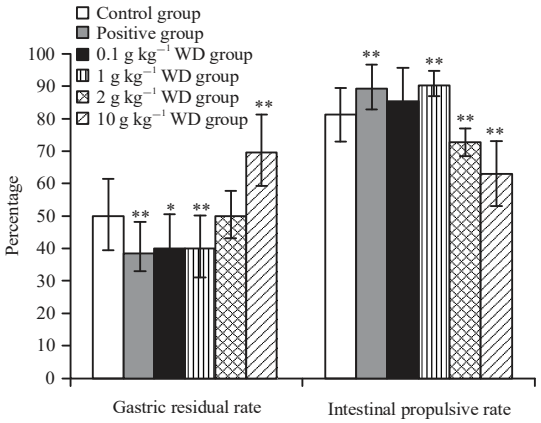


Fig. 1: Effects of water decoction of AMR on GIP in the mice. Cumulative values are reported as Mean±SD for 10 rats in each group. *p<0.05 and **p<0.01 compared to controls using LSD analysis

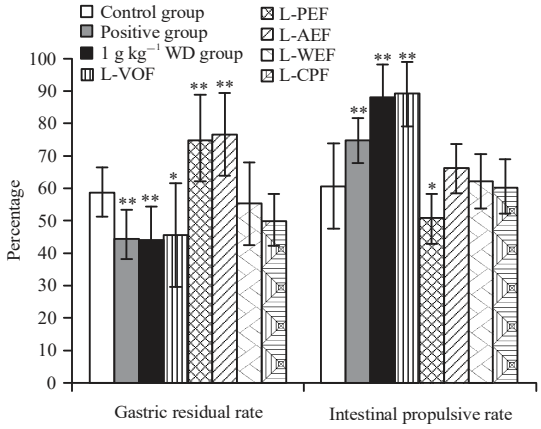


Fig. 2: Effects of fractions of low dose of water decoction (1 g kg$^{-1}$ water decoction) on GIP. Cumulative values are reported as Mean±SD, for 10 rats in each group. *p<0.05 and **p<0.01 compared to controls using LSD analysis

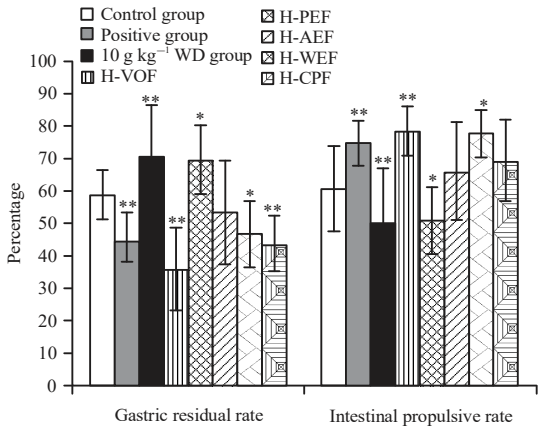


Fig. 3: Effects of fractions of high dose of water decoction (10 g kg$^{-1}$ water decoction) on GIP. Cumulative values are reported as Mean±SD, for 10 rats in each group. *p<0.05 and **p<0.01 compared to controls using LSD analysis

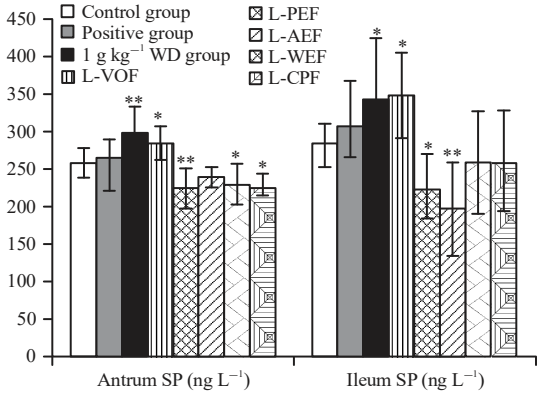


Fig. 4: Effects of fractions of low dose of water decoction (1 g kg$^{-1}$ water decoction) on the level of SP in antrum and ileum. Cumulative values are reported as Mean±SD for 10 rats in each group, *p<0.05 and **p<0.01 compared to controls using LSD analysis

**Effects of fractions of AMR on GIP in mice:** Figure 2 indicated the gastric retention rates of mice in low dose of VOF (L-VOF) group were significantly decreased than control group; however, the gastric retention rates of mice in low dose of PEF (L-PEF) and low dose of AEF (L-AEF) groups were significantly increased. The intestinal propulsion rates of mice in low dose of VOF (L-VOF) group were significantly increased than control group, whereas low dose of PEF (L-PEF) group were significantly decreased.

Figure 3 indicated the gastric retention rates of mice in high dose of VOF (H-VOF), high dose of WEF (H-WEF) and high dose of CPF (H-CPF) groups were significantly decreased than control group; however, the gastric retention rates of mice in high dose of PEF (H-PEF) group were significantly increased. The intestinal propulsion rates of mice in high dose of VOF (H-VOF) and high dose of WEF (H-WEF) group were significantly increased than control group, whereas high dose of PEF (H-PEF) group were significantly decreased.

**Effects of AMR and its fractions on the level of SP and VIP:** Figure 4-7 showed that the VIP and SP levels of mice in 1 g kg$^{-1}$ WD and L-VOF groups were significantly increased than control group. The gastric antrum and ileum SP and ileum VIP levels in L-PEF group were significantly decreased

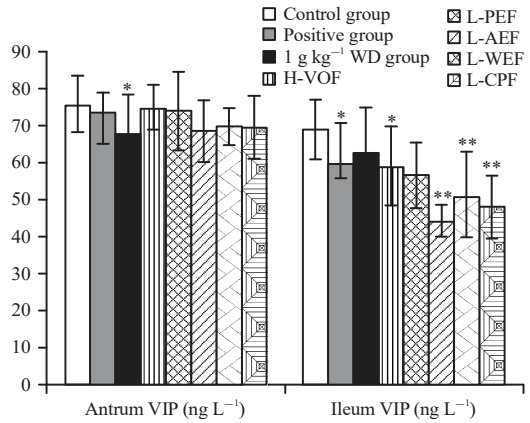


Fig. 5: Effects of the fractions of low dose of water decoction (1 g $kg^{-1}$ water decoction) on the level of VIP in antrum and ileum. Cumulative values are reported as Mean±SD for 10 rats in each group, *p<0.05 and **p<0.01compared to controls using LSD analysis

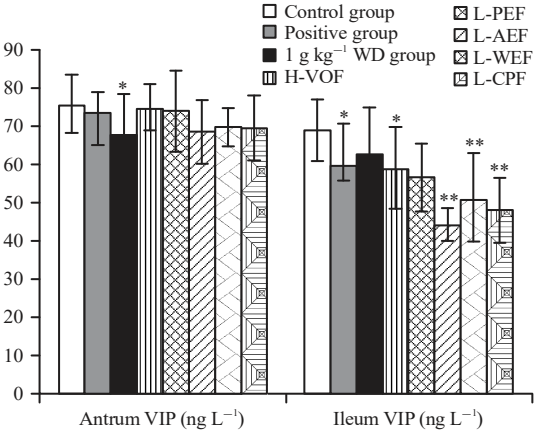


Fig. 6: Effects of the fractions of high dose of water decoction (10 g $kg^{-1}$ water decoction) on the level of VIP in antrum and ileum. Cumulative values are reported as Mean±SD for 10 rats in each group, *p<0.05 and **p<0.01 compared to controls using LSD analysis

than control group. The ileum SP and VIP levels in L-AEF and H-AEF group were significantly decreased than control group. The gastric antrum SP and ileum VIP levels in H-WEF and H-CPFgroups were significantly decreased than control group. Gastric antrum and ileum VIP levels in10 g $kg^{-1}$ WD group were significantly increased than control group. The gastric antrum and ileum VIP and gastric antrum SP levels in H-VOF group were significantly decreased than control group but ileum SP level was increased than control group. The gastric antrum SP and ileum VIP levels in H-PEF and H-AEF groups were significantly increased than control group. The gastric antrum SP, gastricantrum and ilium VIP levels in H-WEF groups

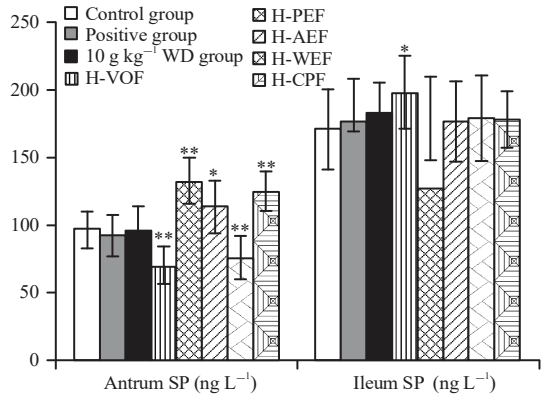


Fig. 7: Effects of the fractions of high dose of water decoction (10 g $kg^{-1}$ water decoction) on the level of VIP in antrum and ileum. Cumulative values are reported as Mean±SD for 10 rats in each group, *p<0.05 and **p<0.01 compared to controls using LSD analysis

were significantly increased than control group. The gastric antrum SP and VIP levels in H-CPF groups were significantly increased than control group.

## DISCUSSION

Since the plant living organisms contains thousands of components, just a few individual compounds always could not represent the whole pharmacologic effects. In this study, we attempted to use splitted fraction obtained from the crude extract to overcome this disadvantages. In order to reflect the real effective material of AMR, in this study, the main components of the fractions were identified by HPLC and GC-MS chromatography. The results indicated that the main components of VOF were monoterpenes and sesquiterpenes; the main components of PEF were sesquiterpene lactone; the main components of AEF were polyacetylene; the WEF mainly contained 5-hydroxymethyl furfural and small molecular sugar. In the same time, the research (Lin *et al*., 2015) indicated inulin-type oligosaccharides were the main component of CPF.

Our results of GIP experiments indicated the low dose of water decoction exhibited promotion effects, moreover, the effects of 1.00 g $kg^{-1}$ water decoction were strongest than others; the high dose of water decoction exhibited inhibition effects, moreover, the effects of 10.00 g $kg^{-1}$ water decoction were strongest than others. Thus, AMR possessed bidirectional regulation effects on GIP. As for the dosage, in order to reflect the real effective material of AMR, in this study, effects of the fractions of 1.00 g $kg^{-1}$ water decoction and10.00 g $kg^{-1}$ water decoction on GIP were firstly explored. The results indicated

that L-VOF, H-VOF, H-WEF and H-CPF exhibited promotion effect, however, L-PEF, H-PEF and L-AWF exhibited inhibition effect. And the effect order as follow: promotion effect: H-VOF>L-VOF, H-WEF>L-WEF, H-CPF>L-CPF; inhibition effect: L-PEF>H-PEF, L-AEF>L-AEF. It indicated that promotion effects of fractions were VOF, WEF and CPF and that VOF was most important; promotion effect of fractions were PEF and AEF and that PEF was most important. Moreover, the promotion effect of H-WEF and H-CPF and the inhibition effect of L-AEF were found for the first time; promotion effect of VOF and inhibition effect of PEF were consistent with the reference (Chen *et al*., 2009; Liu, 2009). In combination with components of fractions, we can know that the real effective material of promotion effect were monoterpenes, sesquiterpenes, 5-hydroxymethyl furfural, small molecular sugar and in ulin-type oligosaccharides; the real effective material of inhibition effect were sesquiterpene lactone and polyacetylene.

Further, in the study the effect of relative mechanism were explored. The GIP was mainly regulated by gastrointestinal nerve and brain-gut peptide (Ma, 2006). The SP, as an important gastrointestinal peptide, was widely distributed in the enteric nerve system and the gastrointestinal tract (Maake *et al*., 1999). Moreover, SP was the main excitatory neurotransmitter in the control of regulating GIP; when it exhibited excitatory effect on gastrointestinal tract, it showed double contraction effects on longitudinal gastrointestinal muscle and circular muscle and the effects contain direct-short term effects and indirect-long term effects (Lordal *et al*., 1997; Wheatley *et al*., 1999). In the same time, the research indicated that the effects of AMR were related to the SP levels (Zhao *et al*., 2008). The VIP, a non-adrenergic inhibition neurotransmitter, distributed in the gastrointestinal tract and the central nervous system, could inhibit gastrointestinal motility owing to its effects of relaxing gastrointestinal smooth muscle, increasing gastrointestinal fluid and electrolyte secretion(Grider, 1993; Ljung and Hellstrom, 1999). Thus, the SP and VIP in gastric antrum and ileum were selected as index to explored relative mechanism. In combination with the effects of WD and its fractions on GIP, WD, VOF and CPF had promotion effects on gastrointestinal function through up regulated SP and down regulated VIP; PEF had inhibition effects on gastrointestinal function through up regulated VIP; AEF had inhibition effects on gastrointestinal function through down regulated SP but promotion effects of WEF on gastrointestinal function had no relation to up regulated SP or down VIP. Thus, the effects of AMR maybe related to the level of SP and VIP and the fractions had different effect on the level of SP and VIP and that the effects of AMR were related significantly to VIP level for the first time. The AMR possessed bidirectional regulation effects on GIP. The real effective materials of promotion effect were monoterpenes, sesquiterpenes, 5-hydroxymethyl furfural, small molecular sugar and inulin-type oligosaccharides; the real effective materials of inhibition effect were sesquiterpene lactone and polyacetylene. The relative mechanism may be related to the SP and VIP levels. All the conclusions in the study have role significance in studying the effects of AMR on GIP at later.

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## REFERENCES

Chen, Z., Q. Xia, Z.Q. Huang, C.L. Ge and D.J. Xu, 2009. Effect of essential oils of *Atractylodes macrocephala* koidz on gastrointestinal function of mice. Chin. J. Exp. Tradit. Med. Formulae, 15: 66-68.

Grider, J.R., 1993. Interplay of VIP and nitric oxide in regulation of the descending relaxation phase of peristalsis. Am. J. Physiol., 264: G334-G340.

Li, L.H., X.K. Ran, Y.B. Xu, B. Li and D.Q. Dou, 2014. A method for evaluation of non-similarity degrees between splitting fractions of atractylodis macrocephalae rhizoma. Chin. J. Anal. Chem., 42: 343-348.

Lin, Z., Y.F. Liu, Y. Qu, L.Y. Shi, D.Q. Dou and H.X. Kuang, 2015. Characterisation of oligosaccharides from Baizhu by HILIC-MS. Natl. Prod. Res.: Formerly Natl. Prod. Lett., 13: 1194-1200.

Liu, X.L., 2009. [Effects of different processed products of *Atractylodis macrocephalae* rhizomaon gastrointestinal motility in mice]. Henan J. Anim. Husbandry Vet. Med., 30: 1-5, (In Chinese).

Ljung, T. and P.M. Hellstrom, 1999. Vasoactive intestinal peptide suppresses migrating myoelectric complex of rat small intestine independent of nitric oxide. Acta Phsiol. Scand., 165: 225-231.

Lordal, M., E. Theodorsson and P.M. Hellstrom, 1997. Tachykinins influence interdigestive rhythm and contractile strength of human small intestine. Dig. Dis. Sci., 42: 1940-1949.

Ma, D., 2006. Gastrointestinal Motility and Clinical. Military Medical Science Press, New York, USA.

Maake, C., W. Kloas, M. Szendefi and M. Reinecke, 1999. Neurohormonal peptides, serotonin and nitric oxide synthase in the enteric nervous system and endocrine cells of the gastrointestinal tract of neotenic and thyroid hormone-treated axolotls (*Ambystoma mexicanum*). Cell Tissue Res., 297: 91-101.

NPC., 2010. Pharmacopoeia of the people's republic of China (2010 Edition). China Pharmaceutical Science and Technology Publishing House, National Pharmacopoeia Committee (NPC), Beijing, China, pp: 95-96.

Wheatley, J.M., J.M. Hutson, C.W. Chow, M. Oliver and M.R. Hurley, 1999. Slow-transit constipation in childhood. J. Pediatr. Surg., 34: 829-832.

Wu, H., L. Zhu and Y. Ma, 2005. Study on the effect of *Atractylodes macrocephala* on mouse gastrointestinal activities. Jiangsu J. Tradit. Chin. Med., 26: 66-67.

Xing, J.F., W. Feng and J. Hou, 2003. Investigation of the experimental methods for observing mouse gastric emptying and small intestine propulsion. J. Beijing Univ. Tradit. Chin. Med., 26: 50-52.

Zhang, Y., S. Xu and Y. Lin, 1999. [Gastrointestinal inhibitory effects of sesquiterpene lactones from *Atractylodes macrocephala*]. J. Chin. Med. Mater., 22: 636-640, (In Chinese).

Zhao, C.F., M. Su and P. Yu, 2008. Analysis of promoting of Rhizoma *Atractylodis macrocephalae* on digestive function. J. Qigihar Med. Coll., 29: 913-914.