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Poria ameliorates the side effects of rhubarb in pair treatment

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Abstract: To investigate the effect of Poria and effective constituents on gastrointestinal injury animals in the area of the side effects which caused by Rhubarb. Mice were administered *i.g.* with Rhubarb until the induction of diarrhea followed by gastrointestinal injury. The gastrointestinal injured mice were treated with high, medium and low doses of poria water extract and it's subfractions for 5 days. All indexes were determined to evaluate the action of poria in the pair treatment. The results showed that the higher dose of poria water decoction was discovered to be the most effective dose to treat gastrointestinal injury induced by rhubarb. Body weight, thymus and spleen indexes, the small intestinal propulsion rate and D-xylose absorption in mice with diarrhea and intestinal injury were analyzed to reveal the significant difference with the model group ($P<0.01$). EAF (Ethyl Acetate Fraction), PEF (Petroleum Ether Fraction) and CPF (Crude Polysaccharide Fraction) not only increase the levels of AMS, GAS and VIP significantly but also ameliorate diarrhea and intestinal injury situation compared with the model group ($P<0.01$). EAF, PEF and CPF were the most effective components to alleviate diarrhea and gastrointestinal injury induced by rhubarb.

Keywords: Poria, rhubarb, diarrhea, fraction, gastrointestinal injury.

INTRODUCTION

Rhubarb (or *Dahuang* in Chinese), the dried roots and rhizome of *Rheum officinale* Baill (Pharmacopoeia of the people's republic of china, 2015), was firstly recorded in the herbal medicinal treatise of *Shen Nong Ben Cao Jing* and has been used in Chinese folks for several thousand years (The Divine Farmer's Materia Medica, 1998). Anthraquinone glycosides are responsible for it's purgative action (Nonaka *et al*., 1973). However the long term use of rhubarb could injure the gastrointestinal tract and it's digestive and absorptive function may be disturbed, even led to melanosis coli and the over dosage can cause nausea, vomiting and diarrhea etc (Steer *et al*., 1975). Rhubarb is always been used as a drug to induce spleen deficiency in the animal model of traditional Chinese medicine (TCM) due to its purgative and inhibitory in immune function (Qu *et al*., 2001). To avoid it's side-effects, rhubarb is always used together with drugs having "tonifying spleen and eliminating dampness" properties (Li *et al*., 2009).

Poria (or *Fuling* in Chinese), the dried sclerotium of *Poria cocos* (Schw.) Wolf (Pharmacopoeia of the people's republic of china, 2015) belongs to family Polyporaceae, has been used medicinally for about 2,000 years in China. In TCM it has the properties of tonifying spleen and calming mind, promoting diuresis and clearing damp (Yeung *et al*., 2012; Xiao *et al*., 2015; son *et al*., 2008). Thus, poria and rhubarb are used concurrently as pair in combined treatment strategy. Such as "Tiao Zhong Tang" from <Pu Ji Fang>, "Da Huang Xie Re Tang" from <Qian Jin Fang> and "Ping Wei San" from <Yi Fang Ji Cheng>. Various studies have been reported for the diuretic and immunomodulating properties of poria (Wu *et al*., 2014; Ma *et al*., 2010), but there is no article on it's beneficial effects in combined therapy especially with rhubarb. So we carried out this study to scientifically elucidate the use of rhubarb with poria as purgative agent but with minimized side effects when used concomitantly and to explain experimentally the compatibility principle of TCM for poria-rhubarb combined use. As purgative status of mice induced by rhubarb is similar to the symptoms of spleen deficiency, thus our results can also elucidate the invigorating spleen substance of poria.

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MATERIALS AND METHODS

Instrument and reagents

Multimode microplate reader (Tecan Group Ltd. Austria), Freeze-drying machine (Labconco Corporation England), Centrifuge (Thermo Electron Corporation Germany), Sonicator model KQ-250DE (Ultrasonic Instrument Co. Kunshan, China), water-bath model HH-S (Yuhua Co. Gongyi, China), Acculab analytical balance (Sartorius group Germany), incubator model HPP-9272 (Zhisheng Scientific and Technological Co. Ningbo, China), and high speed homogenate model XHF-D (Zhisheng Scientific and Technological Co. Ningbo, China).

Rhubarb was bought from Anhui Yuhetang Pharmaceutical Co. Ltd, China (Batch No.20120703) and Poria was purchased from Yunnan, Tengchong, China (Batch No.20121101). Both of them were identified as the roots and rhizomes of *Rheum officinale* Baill and the sclerotium of *Poria cocos* (Schw.) Wolf, respectively, by professor Wang Bing, Liaoning University of Traditional Chinese Medicine and their voucher specimens were

deposited at the herbarium of Liaoning University of Traditional Chinese Medicine. Other chemicals used during the study include Smecta tablet (Beaufour Ipsen Pharmaceutical Co. Ltd. Batch No.E18188, Tianjin, China), Saline (Heilongjiang Kelun Pharmaceutical Co. Ltd. Batch No.12060201-2, China), D-xylose (Tianjin Bodi Chemical Industrial Co. Ltd. Batch No.20090916, China), Activated Carbon (Shenyang Xinxing Chemical Reagent Factory Batch No.20110619, China); Gum Arabic powder (Kermel Chemical Co. Ltd Batch No.20120410, China), HPLC grade Methanol (Tiedi Chemical Co., Ltd. USA), Assay kit of D-xylose and amylase (AMS) (Nanjing Jiancheng Bioengineering Institute Batch No.20130325, 20131203, China), Elisa assay kit of vasoactive intestinal peptide (VIP) and gastrin (GAS) (R&D Systems Batch No.201306 and 201306, USA). All other chemical reagents were purchased from Kermel Chemical Co., Ltd. (China).

Preparation of herbal extracts

Rhubarb decoction

Rhubarb, dried roots of *Rheum officinale* Baill, was pulverized and boiled with water for 10 min, 3 times, and then the filtrates were combined and evaporated in vacuum to 1.0 g/mL.

Preparation of poria extract and it's subfractions

Poria was pulverized and decocted with water for 2 hours, twice. After filtration, both filtrates were combined and evaporated in vacuum. Three types of dose strength were administered to animals 1.25 g/mL as high dose of poria, 0.625 g/mL as medium dose and 0.25 g/mL as low dose.

The poria extracts and its sub-fraction was prepared according to our method (Xiao *et al*., 2015). Multi-mode separation methods were employed to split the different fractions of poria to yield EAF, PEF, CPF, WEF (Water Eluated Fraction), AEF (Alcohol Eluated Fraction) and RPF (Refined Polysaccharide Fraction). The mice were orally administered of the water decoction and different fractions of Poria, purified water was used as control. Preparation of EAF, PEF, CPF, each fraction have two types of dose that were administered to animals 1.25 g/mL as high dose of poria, 0.625 g/mL as medium dose of poria.

Animals

Both male and female healthy KM mice, weighed 20.0±2 g, were purchased from laboratory animal center of Changsheng Bio-Technique Co. Ltd, qualified number SCXK (Benxi, Liaoning, China) 2010-0001. All mice were maintained with free access to pellet food and water in plastic cages at 25±2℃, relative humidity 40±10%, and kept on a 12 h light/dark cycle. The study complied with the current ethical regulations for animal research of Liaoning University of Traditional Chinese Medicine and was approved on Nov.1, 2012.

Preparation of the reference reagents

5% carbon powder suspension: 3.5 g powder of gum acacia was added to 30 mL of purified water, and boiled until the solution became transparent, then 1.75 g activated carbon powder was added in the above solution, boiled for 3 times, concentrated to 35 mL after the solution get cold. 5% D-xylose solution: 2 g of D-xylose dissolved in 40 mL of purified water to prepare 5% D-xylose solution.

Administration of poria and rhubarb

Each of the mice was administered *i.g.* with 20 g/kg of 100% rhubarb decoction for 8 days. The mice showed symptoms, such as weight loss, sluggish, loosed stools and skin tarnished. Poria water decoction and subfractions were administered *i.g.* to mice. Blood samples were drawn from orbital sinus for AMS and GAS tests. The body, thymus and spleen weight of mice were also weighted to know the body weight influence and immune organs index. Furthermore, the length of the small intestines and VIP were measured to know the improvement effect in diarrhea.

Test 1: The 60 mice were divided into 6 groups (N=10 per group) randomly. They were administered *i.g.* rhubarb decoction with a daily dose of 20 g/kg for a week. The dosage was determined from the product of highest daily dose (60g/70kg) of the Chinese Pharmacopoeia and the equivalent dose coefficient. After grouping, each group was administered *i.g.* in the following manner: Group 1 (control group), 2 (model group): 0.2mL/10g body weight of water; Group 3 to 5: 5g/kg, 12.5g/kg, 25g/kg body weight of poria decoction respectively. Group 6 was fed standard drug Smecta with a daily dose of 1.5 g/kg. Organ indexes were measured after 5 days and each administered *i.g.* mouse was tested for designated properties.

Test 2: According to the results of test 1, the high dose of poria (1.25g/mL) was selected.The mice were divided into 10 groups (N=10 per group). After grouping, each group was administered *i.g.* for 5 days in the following manner: Group 1 (control group), 2 (model group): 0.2 mL/10g body weight of 1% tween-80 steamed water solution; Group 3: 25g/kg body weight of poria decoction; Group 4: 0.4225g/kg body weight of WEF respectively; Group 5: 0.0702g/kg body weight of AEF respectively; Group 6: 0.02312g/kg body weight of EAF respectively; Group 7: 0.004892g/kg body weight of PEF; Group 8: 0.1650g/kg body weight of CPF respectively; Group 9: 0.03571g/kg body weight of RPF respectively; all the fractions were dissolved in 1% tween-80 steamed water solution, Group 10: 1.5g/kg body weight of smecta montmorillonite powder. Mice were tested for the designated properties.

Test 3: The 90 mice were divided into 9 groups (N=10 per group) randomly. After grouping, each group was administered *i.g.* for 5 days in the following manner: Group 1 (control group), 2 (model group): 0.2 mL/10g body weight of 1% tween-80 steamed water solution; Group 3: 0.1156g/kg body weight of EAF; Group 4:0.2312 g/kg body weight of EAF respectively; Group 5: 0.002446 g/kg body weight of PEF respectively; Group 6: 0.004892 g/kg body weight of PEF respectively; Group 7: 0.0825 g/kg body weight of CPF; Group 8:0.1650 g/kg body weight of CPF respectively; all the fractions were dissolved in 1% tween-80 steamed water solution, Group 9: 1.5g/kg body weight of smecta montmorillonite powder. Mice were tested for the designated properties.

Measure the biochemical indexes

Measurement of the spleen and thymus index

The experiment was based on previous publications (Lin *et al*., 1995) and performed as follows. After weighing mice weight and the mice were sacrificed, the spleen and thymus of mice were taken out, the wet weight of the spleen and thymus of mice were weighed by the electronic analytical balance, spleen and thymus index were calculated respectively.

$$\text{Spleen index} = \frac{\text{Wet weight of spleen (mg)}}{\text{Body weight (g)}} \times 100\%$$

$$\text{Thymus index} = \frac{\text{Wet weight of thymus (mg)}}{\text{Body weight (g)}} \times 100\%$$

Measurement of the small intestinal propulsion rate

After fasting for 24 hours, and during this time, water wasn't banned. The mice were administered *i.g.* with 0.2mL/10g body weight of 5% carbon powder suspension. The mice were sacrificed 30 min after the administration, the next experiment was performed according to the previously described methods (Han *et al*., 2008).

$$\text{The ratio of small intestinal propulsion} = \frac{\text{Carbon propulsion distance (cm)}}{\text{The full length of the small intestine (cm)}} \times 100$$

Measurement of D-xylose contents

After the last administration, fasting for 24 hours and water was given, the mice were administered *i.g.* with 0.2mL/10g body weight of 5% D-xylose solution. One hour later, blood samples were drawn from orbital sinus for D-xylose test, the blood was left to stand for 30 min at room temperature, it was centrifuged at speed of 3000 rpm/min for 15 min, preparation of serum before usage. D-Xylose was measured using D-Xylose kit following the manufacturer's protocol, to determine the serum content of mice in each group by the colorimetric assay.

Measurement of AMS and GAS contents

After the last administration, fasting for 24 hours and water was given. Blood samples were drawn from orbital sinus for GAS and AMS tests, the blood was left to stand for 30 min at room temperature, and then centrifuged at speed of 3000 rpm/min for 15min, preparation of serum before usage. GAS and AMS were measured via ELISA kits according to the manufacturer's protocol.

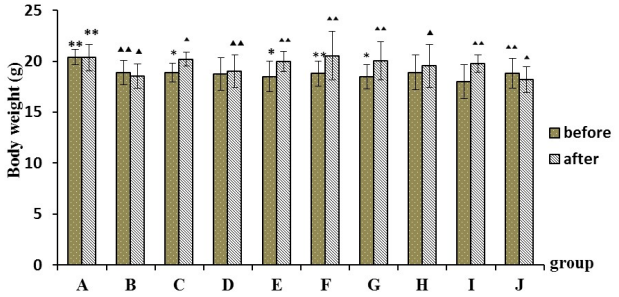


Fig. 1: Effect on body weight of poria subfractions fed mice. control group (A); model group (B); water decoction(C); WEF(D); AEF(E); EAF(F); PEF(G); CPF(H); RPF(I); positive drug group(J); ▲P<0.05, ▲▲P<0.01 compared with control group; *P<0.05 , ** P<0.01 compared with model group

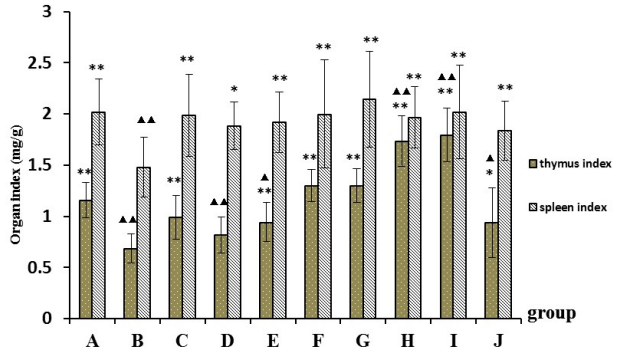


Fig. 2: Effect of poria subfractions on mice immune organ index. control group (A); model group (B); water decoction(C); WEF(D); AEF(E); EAF(F); PEF(G); CPF(H); RPF(I); positive drug group(J); ▲P<0.05, ▲▲P<0.01 compared with control group; *P<0.05, ** P<0.01 compared with model group

Measurement of VIP content

After fasting for 24 hours, and during this time, water wasn't banned. And then mice were sacrificed. Remove the colon tissue and wash the contents with saline, dry the water to weigh. Add saline (1:9, v/v), the colon tissue of each mouse was homogenized, and it was centrifuged at speed of 3000 rpm/min for 20 min, the supernatants were collected, stored at -20 °C until analysis. VIP level was measured using VIP ELISA kit following the manufacturer's protocol.

The histopathology effect

After fasting for 24 hours, and during this time, water wasn't banned. And then the mice were sacrificed. Remove the liver, spleen, stomach, colon tissue and washed with PBS. After fixed and stained, they were

observed under an electron microscope and photographed (Zhang *et al*., 1997).

STATISTICAL ANALYSIS

Statistical evaluation was performed by using SPSS 19.

RESULTS

Comparisons of the body weight and the dilution rate (fecal water content)

As shown in figs. in the supplementary material. After the last administration, the body weight of mice in each group was measured by electronic balance. Essentially all models were developed to observe the changes in weight loss, sluggish, loosed stools and skin tarnished. Subsequently, all poria and subfractions fed mice data were compared standard model symptoms. This approach has proved useful to study animal models of diarrhea and intestinal injury.

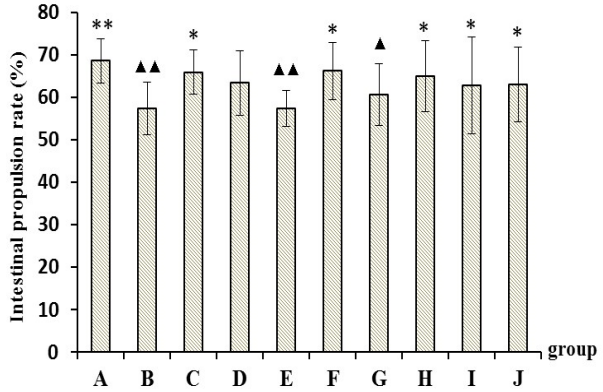


Fig. 3: Effect of poria subfractions on mice intestine propulsion. control group (A); model group (B) ; water decoction(C); WEF(D); AEF(E); EAF(F); PEF(G); CPF(H); RPF(I); positive drug group(J); ▲P<0.05, ▲▲P<0.01 compared with control group; *P<0.05, ** P<0.01 compared with model group

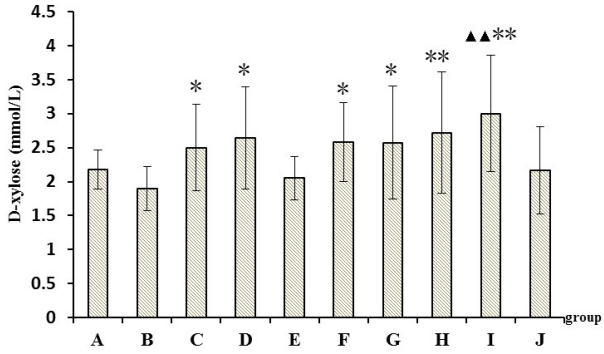


Fig. 4: Effect of poria subfractions on serum D-xylose content. control group (A); model group (B); water decoction(C); WEF(D); AEF(E); EAF(F); PEF(G); CPF(H); RPF(I); positive drug group(J); ▲P<0.05, ▲▲P<0.01 compared with control group; *P<0.05, ** P<0.01 compared with model group

Effect on mice body weight with poria water decoction and subfractions

The results shown in figs. in the supplementary material indicated that the high dose of poria water decoction has obvious improvement on spleen deficiency diarrhea animal. As shown in fig. 1, we also find out that each group has significant difference compared with control group (P<0.05). But only model and positive drug group eventually has significant difference (P<0.01) after 5 days treatment by poria split components, while others has no statistical significance (P<0.05). Treatment groups of weight were increased to a certain degree especially with WEF, AEF, EAF, PEF are more obvious.

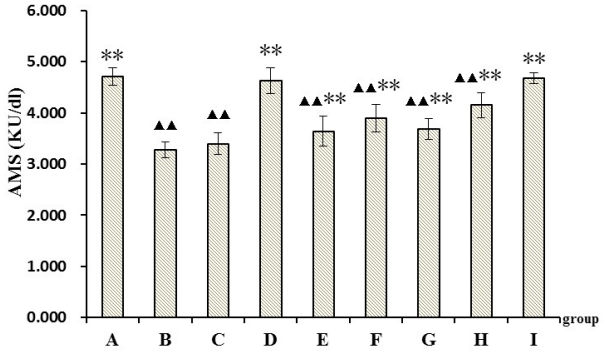


Fig. 5: Effect of poria subfractions on AMS. control group (A); model group (B); medium dose of EAF(C); high dose of EAF(D); medium dose of PEF(E); high dose of PEF(F); medium dose of CPF(G); high dose of CPF(H);positive drug group(I); ▲P<0.05, ▲▲P<0.01 compared with control group; *P<0.05, ** P<0.01 compared with model group

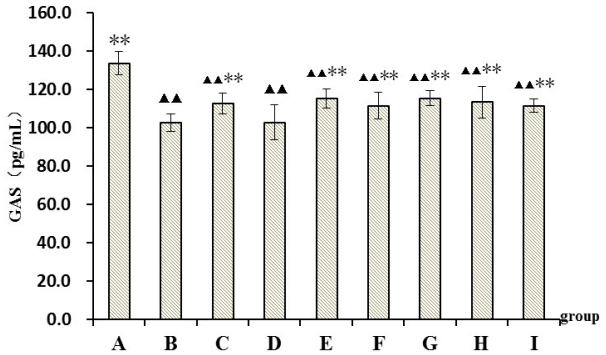


Fig. 6: Effect of poria subfractions on serum gastrin. control group (A); model group (B); medium dose of EAF(C); high dose of EAF(D); medium dose of PEF(E); high dose of PEF(F); medium dose of CPF(G); high dose of CPF(H); positive drug group(I); ▲P<0.05,▲▲P<0.01 compared with control group; *P<0.05 , ** P<0.01 compared with model group

Effect of poria water decoction and subfractions on mice immune organ index

The results shown in figs. in the supplementary material indicated that the high dose of poria water decoction has

obvious improvement. As shown in fig. 2, the thymus index in AEF, EAF, PEF, CPF and RPF groups are all significantly higher than that in model group (P<0.01). To spleen index, every groups decreased compared with control group (P<0.01). Index in the poria water decoction, AEF, EAF, PEF, CPF and RPF group all higher significantly than that in model group (P<0.01). Therefore, we can conclude that the poria water decoction and its subfractions *i.e.* AEF, EAF, PEF, CPF and RPF has improved mice immune organ index significantly.

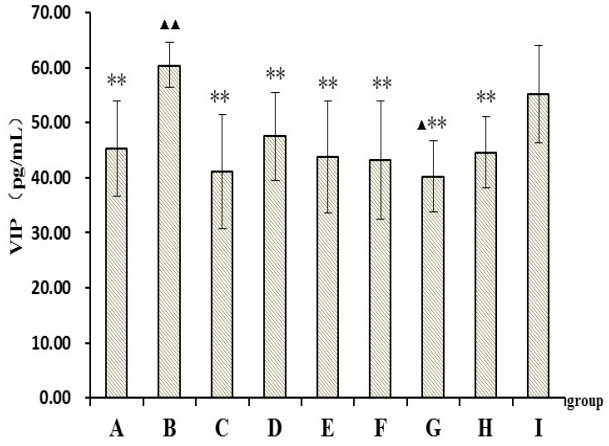


Fig. 7: Effect of poria subfractions on VIP. control group (A); model group (B); medium dose of EAF(C); high dose of EAF(D); medium dose of PEF(E); high dose of PEF(F); medium dose of CPF(G); high dose of CPF(H); positive drug group(I); ▲P<0.05,▲▲P<0.01 compared with control group; *P<0.05 , ** P<0.01 compared with model group

Effect of poria water decoction and subfractions on mice intestine propulsion

We can conclude that high dose of poria water decoction and medium dose group has obvious improvement on spleen deficiency diarrhea animal, however the response of higher dose is more significant (P<0.01) than medium dose group (figs. in the supplementary material). As shown in fig. 3, model group was lower than that in control group (p<0.01); the poria water decoction, EAF, CPF and RPF group all higher than that in model group (P<0.05), which demonstrated that its subfractions *i.e.* EAF, CPF and RPF has obvious improvement on spleen deficiency diarrhea animal.

Effect of poria water decoction and subfractions on serum D-xylose content

The results shown in figs. in the supplementary material indicated that the high dose of poria water decoction has obvious improvement. As shown in fig. 4, model group was lower than that in control group, RPF group was higher than that in control group (p<0.01); the poria water decoction, WEF, EAF, PEF group all higher than that in model group (P<0.05), CPF and RPF group all higher significantly than that in model group (P<0.01). Therefore, we can conclude that its subfractions *i.e.* EAF, CPF, RPF and PEF have obvious improvement on spleen deficiency diarrhea animal.

Effect of poria subfractions on AMS

Compared with control group the level of serum amylase (AMS) is significantly lower (P<0.01) in model group, medium dose of EAF group, high and medium dose of PEF and CPF group. In comparison with model group, high dose of EAF, high and medium dose of PEF and CPF, the AMS level is significant (P<0.01). In other words, we can say that higher dose of EAF improve AMS level in blood. PEF and CPF have greater effect on the AMS level at medium dose and while the high dose of EAF has most significant effect.

Effect of poria subfractions on GAS

All groups with split components and model group have lower content of GAS than control group (P<0.01). After the model cause of spleen deficiency, content of GAS drop down more conspicuously. Medium dose of EAF, high and medium dose of EAF and CPF has significantly difference compared with model group (P<0.01), while high dose of EAF and PEF effect is less obvious (P>0.05). Medium dose of EAF, PEF and CPF has higher activity than high dose, though high dose of EAF will have less influence on GAS which can greatly convince that these components has bidirectional influence on GAS regulating function in diarrhoea mice and on the contrary higher dose can restrain the secretion of GAS (fig. 6).

Effect on VIP with poria different components

The VIP content in model and medium dose of CPF group is lower than that in control group (P<0.01 and P<0.05) while medium dose of EAF, high and medium dose of PEF and CPF has more significant difference compared with model group (P<0.01). Medium dose of EAF has higher effect on VIP content than high dose. However, various concentrations of PEF and CPF play a more important role in VIP regulation in colon *i.e.* can decrease VIP content and promote colon movement better (fig. 7).

DISCUSSION

Drug compatibility is an important characteristic in TCM pair herb therapy with the aim either to increase/reinforce useful effects or ameliorate adverse effects. In present work, the poria/rhubarb pair treatment in an animal model was explained. Here an animal model of gastrointestinal diarrhea injury induced by rhubarb (Wu *et al*., 2013) was established to evaluate the tonifying principles of poria decoction and subfractions, *i.e.* EAF, PEF and CPF. The results showed that the body weight, immune-organ index, D-xylose absorption and intestinal propulsion rates were dramatically increased in the mice administered with a high dose of water decoction of poria.

SUPPLEMENTARY MATERIAL

The selection of doses

According to pharmacopoeia of China, the doses of poria (10-15g), the pharmacopoeia dosage as 1 times dose, conversion of mice dosage is 2.5g/kg and set 2 times (low dose = 5g/kg) and 5 times (medium dose = 12.5g/kg), 10 times (high dose = 25g/kg) the dose. The standard weight of per mice is 20g, to fill the stomach with 0.4mL/day, solution concentration were 0.25 g/mL, 0.625 g/mL, 1.25 g/mL. The fractions of poria are made in the laboratory, the recovery rate of the fractions in poria can be seen in the table 1.

Table 1: The recovery rate of the fractions

	WEF	AEF	EAF	PEF	CPF	RPF
Recovery rate (%)	1.69	0.28	0.09	0.02	0.66	0.14

Comparisons of the body weight and the dilution rate (fecal water content)

As shown in Fig. 5 and Fig. 6, after the last administration, the body weight of mice in each group was measured by electronic balance. Essentially all models were developed to observe the changes in weight loss, sluggish, loosed stools and skin tarnished. Subsequently, all poria and subfractions fed mice data were compared standard model symptoms. This approach has proved useful to study animal models of diarrhea and intestinal injury.

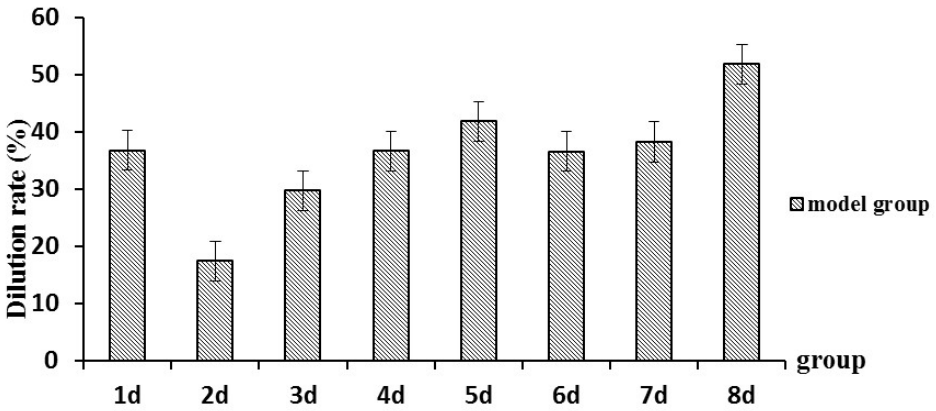
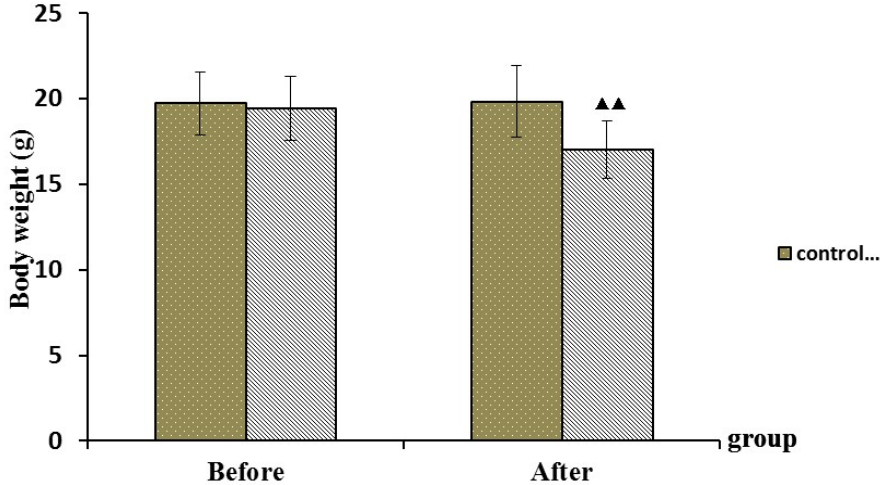


Fig. 5: Dilution rate in model group



Note: ▲ indicates that P<0.05 compared with the control group; ▲▲ indicates that P<0.01 compared with the control group

Fig. 6: Body weight of the diarrhea and gastrointestinal injured mice model

Effect on mice body weight with poria water decoction

As shown in Fig. 7, we measured the body weight in all groups and find out that each groups were all significantly lower than that in control group ($p < 0.01$).We also supplemented a regular dose of poria water decoction in model groups for 5 days and tested out groups with highest dose has the significant difference with model group ($p < 0.05$) whereas there was no significant difference in low and medium dose group. However, there is still a significantly difference in model and lower dose groups compared with control group (P<0.01), which can explain cogently that high dose of poria water decoction has obvious improvement on spleen deficiency diarrhea animal.

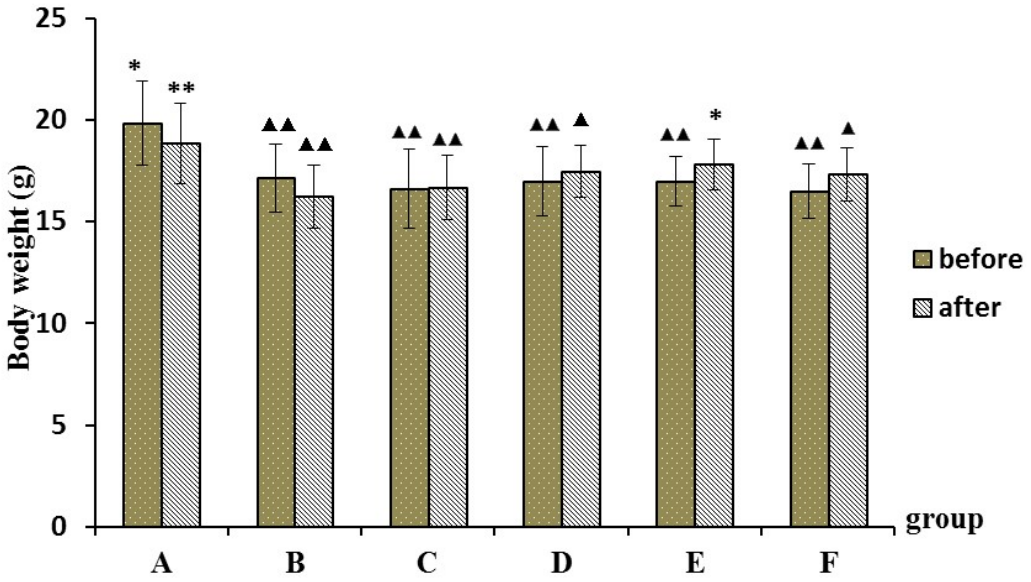


Fig. 7: Effect on mice body weight with poria water decoction. control group (A); model group (B); high dose group(C); medium dose group(D); low dose group(E); positive drug group(F); ▲P<0.05,▲▲P<0.01 compared with control group; *P<0.05 , ** P<0.01 compared with model group

Effect of poria water decoction on mice immune organ index

As shown in fig. 8, compared with control group, thymus index is decreased significantly in every groups (P<0.01) except for high dose and positive drug group. We supplemented a regular dose of poria water decoction in model groups for 5 days and tested out groups with highest dose has the significantly difference with model group (p<0.01) whereas there was no significant difference in medium and low dose group, which can explain cogently that high dose of poria water decoction has obvious improvement.

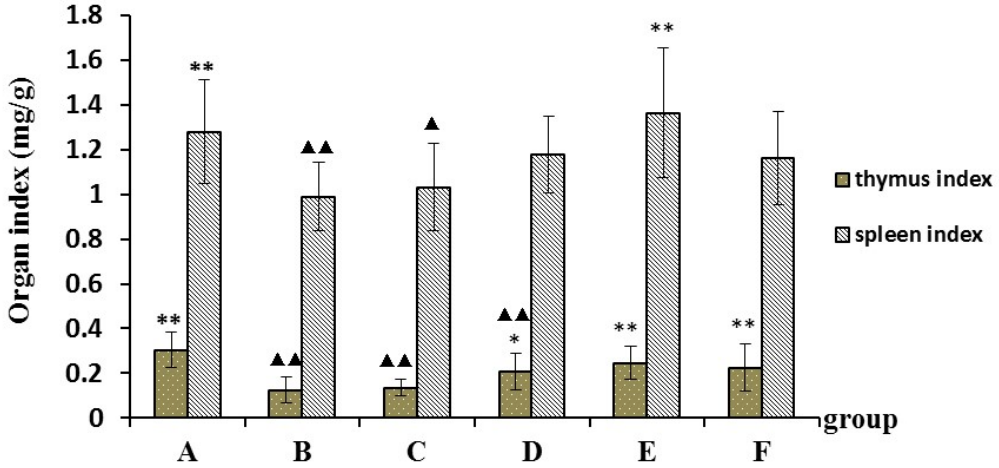


Fig. 8: Effect of poria water decoction on mice immune organ index. control group (A); model group (B); high dose group(C); medium dose group(D); low dose group(E); positive drug group(F); ▲P<0.05,▲▲P<0.01 compared with control group; *P<0.05 , ** P<0.01 compared with model group

Effect of poria water decoction on mice intestine propulsion

As shown in Fig. 9, model group and medium dose group were lower than that in control group ($p < 0.01$); medium dose and high dose group were all significantly higher than that in model group ($P<0.01$) .Therefore, we can conclude that high dose of poria water decoction and medium dose group has obvious improvement on spleen deficiency diarrhea animal, however the response of higher dose is more significant ($P<0.01$) than medium dose group.

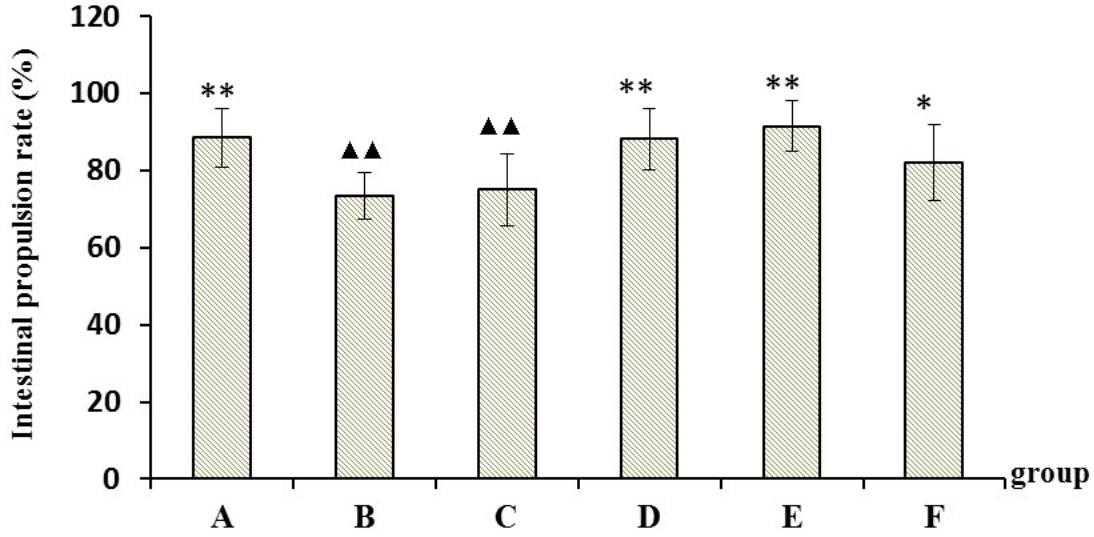


Fig. 9: Effect of poria water decoction on mice intestine propulsion. Control group (A); model group (B); high dose group (C); medium dose group (D); low dose group (E); positive drug group (F); ▲P<0.05,▲▲P<0.01 compared with control group; *P<0.05 , ** P<0.01 compared with model group

Effect of poria water decoction on serum D-xylose content

As shown in fig. 10, model group was lower than that in control group ($p < 0.01$); medium dose group was higher than that in model group ($P<0.05$), high dose group and positive drug group were all significantly higher than that in model group ($P<0.01$), which can explain cogently that high dose of poria water decoction has obvious improvement on spleen deficiency diarrhea animal.

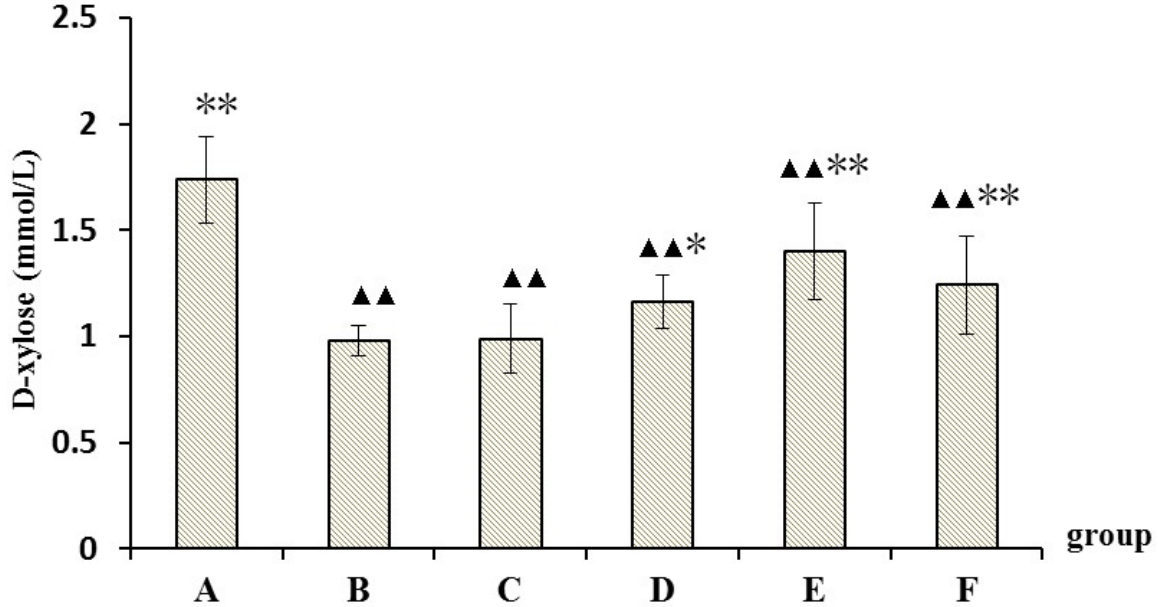


Fig. 10: Effect of poria water decoction on serum D-xylose content. Control group (A); model group (B); high dose group (C); medium dose group (D); low dose group (E); positive drug group (F); ▲P<0.05,▲▲P<0.01 compared with control group; *P<0.05 , ** P<0.01 compared with model group

Our study concluded that the gastrointestinal function indicators were decreased in the rhubarb induced gastrointestinal injury in mice model. After administration of poria *i.g*, the injured gastrointestinal functions were recovered and higher dose of poria water decoction was discovered to be the most effective dose to treat gastrointestinal injury induced by rhubarb. Immunochemical assays indicated that immunity was strongly improved with 5 subfractions of poria (AEF, EAF, PEF, CPF and RPF). EAF, CPF, RPF could increase intestinal propulsion rate as compared with model group. The absorption of D-xylose via the small intestine was significantly improved with WEF, EAF, PEF, CPF and RPF treatment, which indicated a remarkable healing power of small intestine′s injury. The immune function, intestinal motility and absorption function of mice were regulated and recovered by EAF, PEF, CPF and RPF, this endorses these four fractions the most active fraction from poria that can improve gastrointestinal function.

Based on the fact that the active components in poria can improve the gastrointestinal function, we may consider the AMS, GAS, VIP and other aspects for further mechanism study. The AMS and GAS were dramatically increased in EAF, PEF and CPF administered mice while VIP was decreased. The gastrointestinal abnormality was improved because of EAF, PEF and CPF healing effect that led to improving digestion, increased gastrin, stimulated secretion of gastric acid, pepsin and trypsin; and elevated contraction of gastric antrum. The reduction in colon VIP might be caused by tension and contraction in colon. EAF, PEF and CPF had shown no significant effect on AMS level with the increased in the dose, but had slight significant regulating effect on GAS and VIP, especially when the dose reached 25 g/kg of EAF showed no increase in GAS. In other words, EAF, PEF, CPF has bidirectional regulation effect on GAS and VIP *i.e.* agonistic for one concentration and antagonistic for other. This point may be more elaborated that GAS and VIP are regulated by a variety of feedback mechanisms. Briefly, the water decoction of poria was separated into fractions as PEF mainly composed of phthalic acid bis-(2-ethylhexyl) ester, dibutyl phthalate and other Liposoluble compounds; EAF composed of pachymic acid, dehydropa chymic acid and other triterpenoids; AEF from macro porous resin mainly composed amino acid; WEF from macro porous resin composed of monosaccharides and inorganic salt; CPF mainly composed of polysaccharides (Li *et al*., 2015; Hu *et al*., 2014; Lin *et al*., 2013). Above all, EAF, PEF, CPF were the most effective components to alleviate diarrhea and gastrointestinal injury induced by rhubarb and thus may be the effective substances to minimize the adverse effect of rhubarb.

CONCLUSION

The higher dose of poria water decoction was discovered to be the most effective dose to treat gastrointestinal injury induced by rhubarb. Based on the experiment that the fractions of poria (WEF, AEF, EAF, PEF, CPF and RPF), the EAF, PEF, CPF can improve the gastrointestinal function. Poria and effective constituents (EAF, PEF and CPF) have an effect on gastrointestinal injury animals in the area of the side effects which caused by Rhubarb. EAF, PEF, CPF were the most effective components to alleviate diarrhea and gastrointestinal injury induced by rhubarb and thus may be the effective substances to minimize the adverse effect of rhubarb.

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