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A STUDY ON THE EFFECT OF ETHANOL EXTRACT OF *RADIX RHAPONTICI* ON ERYTHROCYTE IMMUNE FUNCTION IN RATS

Xiang Yan¹, Hong Zhao^{1*}, Yin Guan¹, Yanbin Song², Jing Meng¹

¹Medical Oncology Department of PLA General Hospital, BeiJing, China, ²Experimental Teaching Center of Biochemistry and Molecular Biology of Southern Medical University, Guang Zhou, China

*E-Mail: zh3011@163.com

Abstract

This paper mainly studied the effect of ethanol extract of *Radix rhapontici* on erythrocyte immune function in SD rats with acute blood stasis. The methods used the effect on erythrocyte immune function. After intragastric administration of suspension of ethanol extract of *Radix rhapontici* to SD rats for 3 weeks, on the 21st day from intragastric administration, SD rats were made into blood stasis model and bloods were collected to determine the C3b, C3bRR, RFIR, and RFER in erythrocyte immune function. Meanwhile, serum total antioxidant activity (TAA), superoxide dismutase (SOD) activity, and serum malondialdehyde (MDA) level of rats were determined, and experimental results were analysed with analysis of variance and Q test. The results showed that the ethanol extract of *Radix rhapontici* had a very good effect on enhancement of erythrocyte immune function in SD rats.

Key words: *Radix rhapontici*; immune; TAA; SOD; MDA.

Introduction

Radix rhapontici is the dried root of *Rhaponticum uniflorum* (L.) DC., which belongs to the Asteraceae family. It is mainly grown in places such as Hubei, Liaoning and Shanxi. It is collected in spring and autumn. Residual stems and fibrous roots are removed; soil is washed off, and the remaining is thoroughly moistened, cut into thick slices, dried in the sun, and placed in dry and ventilated place. Chemical constituents of plants in genus *Rhaponticum* mainly include ecdysterones, flavonoids, triterpenoid saponins, volatile oil, and organic acids. Pharmacology studies have shown that the extract of *Rhaponticum uniflorum* (L.) DC., a plant in this genus, has multiple effects such as antifungal, anti-atherosclerotic, anti-lipid peroxidation and immune function improvement. A lot of monomeric compounds have also been isolated from *Radix rhapontici*, including arctinal, arctinol, oleanolic acid, B-sitosterol, stigmasterol, oleanolic acid, palmitic acid, etc. (Jin et al, 2011; Li et al, 2007; Liu et al, 1989; Zhang et al, 2010). This paper mainly studies the effect of *Radix rhapontici* extract on erythrocyte immunity in rats, thus laying the foundation for future development of valuable drugs for immunity improvement.

Materials and Methods

Materials

The specimen of *Radix rhapontici* (2012-2.233) was identified by Professor Wang HH, and it was placed in the pharmacy centre. 60 healthy male SD rats, weighing (280 ± 20) g, provided by the Center for SPF Laboratory Animals of PLA General Hospital (2012-2002-12) were used. Dried powder of ethanol extract of *Radix rhapontici* (1g of dried powder is equivalent to 2.752 g of crude drug) was self-prepared. Yeast and complement sensitised yeast are from the Immunology Laboratory of PLA General Hospital. LG-B-190 RBC deformation / aggregation tester, Beijing Steellex Scientific Instrument Company were also used.

SOD, TAA, and MDA assay kits were provided by Nanjing Jiancheng Bioengineering Institute. Lyophilised tumour cell reagents, lyophilised complement sensitised and non-sensitised yeast polysaccharide reagents were purchased from the Immunology Laboratory of Shanghai Second Military Medical University. All experimental procedures were approved by the Animal Research Ethics Committee.

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Establishment of animal model

Slight change was made to the existing literature in the development of animal model used in this experiment. Animals were randomised into six groups (n = 10); intragastric administration schedule: normal control group: distilled water 10 ml/kg; blood stasis model group: distilled water 10 ml/kg; *Radix rhapontici* ethanol extract high-dose group: 15 g/kg; medium-dose group: 7.5 g/kg; low-dose group: 3 g/kg. In accordance with the above doses, the extract was prepared in the same volumes with distilled water and intragastrically administered once a day for two weeks. After establishing "blood stasis" model on the 14th day, rats were lightly anaesthetised with ether, and 6 ml of blood was collected from abdominal aorta.

Preparation of drug (Hou et al, 2007)

Referring to the literature, the optimum extraction process was adopted to extract active components from *Radix rhapontici*. 50 g of *Radix rhapontici* was weighed, ground into powder, and passed through a 20 mesh sieve. The optimum extraction conditions were 3 times of extraction using 70% ethanol, with extraction time of 1.5 h using 8-fold amount of ethanol for 1st extraction, extraction time of 1.0 h using 6-fold amount of ethanol for 2nd extraction, and extraction time of 1.0 h using 4-fold amount of ethanol for 3rd extraction. Then, the extracts were combined, ethanol was removed under reduced pressure, followed by vacuum drying at 65 °C, and *Radix rhapontici* ethanol extract was obtained. *Radix rhapontici* ethanol extract was dissolved in distilled water and diluted to 3 concentrations; final concentrations of the drug were 15, 1.5, and 0.15 mg/ml respectively.

Determination of erythrocyte immune function indicators (Li et al, 2012; Shi, 2008)

Slight modification was made to the test method in the reference literature to determine the erythrocyte C3b receptor rosette rate (E-C3bRR), erythrocyte immune complex rosette rate (E-ICR), rosette forming enhancing rate (RFER), and rosette forming inhibitory rate (RFI). Kits were used to determine serum total antioxidant activity (TAA), superoxide dismutase (SOD) activity, and serum malondialdehyde (MDA) level of rats. All experimental results were analysed using analysis of variance and Q test.

Results

Effect of *Radix rhapontici* ethanol extract on erythrocyte immune function in rats

Compared with the control group, the indicators with decreased activity were RFER and E-C3bRR, and immune indicators with increased activity were E-ICR and RFI in model group. The data of *Radix rhapontici* ethanol extract groups showed that the high-dose group had obvious changes compared with the low-dose group, and had statistically significant differences compared with the model group. Of which E-ICR value of the high-dose group was around 6.82, RFI indicator was around 51.03; RFER indicator of high-dose group was around 84.31; E-C3bRR indicator was around 18.61.

Compared with the model group, high-dose group all had significant differences (P<0.05); low-dose group and medium-dose group, when compared with the model group, had no significant differences (P>0.05), as shown in Table 1.

Table 1: Effect of *Radix rhapontici* ethanol extract on erythrocyte immune function in rats ($\bar{X} \pm S$)

Group	E-ICR	RFER	E-C3bRR	RFI
Control group	5.45±2.03	92.81±10.58	19.93±2.51	44.27±8.11
Model group	9.58±2.39 ^b	67.32±12.73 ^b	15.14±2.47 ^b	75.42±7.54 ^b
High-dose	6.82±1.97 ^a	84.31±12.38 ^a	18.61±2.34 ^a	51.03±6.22 ^a
Medium-dose	7.72±2.63	73.16±13.62	16.04±1.83	63.32±6.85
Low-dose	8.12±2.45	68.35±12.90	15.27±2.18	73.41±6.07

^aP < 0.05, significantly different compared with the model group

^bP < 0.05, significantly different compared with the control group

Table 2: Effect of *Radix rhapontici* ethanol extract on SOD, TAA, MDA in rats ($\bar{X} \pm S$)

Group	Number	SOD (NU/ml)	TAA (U/ml)	MDA (nmol/ml)
Control group	10	2819±411.42	7.63±0.65	4.81±0.68
Model group	10	1735±331.52 ^b	6.35±0.59 ^b	5.93±0.47 ^b
High-dose	10	2726±359.23 ^a	7.15±0.34 ^a	5.72±0.81 ^a
Medium-dose	10	2607±327.84 ^a	7.54±0.52	5.51±0.72
Low-dose	10	2415±361.72	7.76±0.75	5.13±0.64

^a P < 0.05, significantly different compared with the model group

^b P < 0.05, significantly different compared with the control group

Model group, when compared with the control group, all had statistically significant differences. High-dose group and part of the low-dose group, when compared with the model group, also had statistically significant differences. Effect of ethanol extract of *Radix rhaponticis* on erythrocyte SOD activity and serum TAA, MDA levels in blood stasis model rats: the results are shown in Table 2. Compared with the rats in control group, the erythrocyte SOD activity and serum TAA level both significantly decreased in rats of the model group, indicating that the experimental model was successfully established. Compared with the model group, erythrocyte SOD activity and serum MDA level in treatment groups were both significantly elevated (P<0.01); while serum MDA level decreased significantly (P>0.05).

Discussion

As a kind of traditional Chinese medicine, *Radix rhapontici* also has a very wide range of pharmacological effects and clinical applications. In clinical practice, it is commonly used in the treatment of liver cancer, soft tissue trauma, idiopathic nephrotic syndrome, psoriasis and other diseases, and has shown very good efficacies (Li et al, 2011). The *Radix rhapontici* ethanol extract mentioned in this paper is mainly the extract of substances with greater polarity. A lot of active substances cannot be obtained by simple water extraction process. Therefore, ethanol extract was used in this experiment. In fact, *Radix rhapontici* water extract has a very good *in-vitro* antioxidant effect, which can concentration-dependently scavenge hydroxyl radicals, and inhibit H₂O₂- and Fe²⁺-induced hepatic mitochondrial lipid peroxidation (Liu et al, 2012). There has been a study which used a mice hyperlipidemia model established using 75% egg yolk emulsion to demonstrate very significant increase in HDL-C (P<0.01) level in mice of *Radix rhapontici* water extract low-dose group. Lipid-lowering effect of *Radix rhapontici* water extract is stronger than that of *Radix rhapontici* ethanol extract (Wang et al, 2012). *Radix rhapontici* water extract can also exert a good effect on influencing the activity of immune function. Erythrocytes has multiple effects such as identification, adhesion, concentration, and antigen killing, and can produce a variety of immune factors, as the blood cell having the highest content in blood circulation. The immune-related functions of erythrocytes are equally important as the functions of leukocytes on immune system. Therefore, looking for active ingredients from traditional Chinese medicine *Radix rhapontici* to improve the body's erythrocyte immune function is of very important significance.

We know that many of traditional Chinese medicine polysaccharides have an immunity improving effect, such as ginseng polysaccharides, seaweed polysaccharides, and *Lycium barbarum* polysaccharides all have a promoting effect on body fluids, cells, and erythrocyte immunity. They can promote the functioning of monocyte-macrophage cells, NK cells, plaque-forming cells, dendritic cells and cytokines, at the same time, play an active role in receptor expression, signal transduction, and nerve-endocrine-immune network (Zhang et al, 2012). Due to the *Radix rhapontici* ethanol extract extracted by us exhibited a very good erythrocyte immunity improving efficacy, such extract must be rich in polysaccharides. It also implies that the extraction, isolation and targeted activity assay on polysaccharides from *Radix rhapontici* is also a very valuable research subject.

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