

## RESEARCH ARTICLE

## STUDY ON THE EFFECT OF RHODIOLA EXTRACT ON LIVER STARCH AND EXERCISE FATIGUE MORPHOLOGY OF LIVER CELL FOR TABLE TENNIS PLAYERS

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## ARTICLE DETAILS

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## ABSTRACT

**Objective:** To inspect the effect of rhodiola rhizome extract to the table tennis time and content of liver starch and liver cells morphology of sports fatigue. **Methods:** Using weight loading table tennis to determine table tennis time, using kits to determine liver starch, using transmission electron microscope to observe the diversity of liver cells morphology construction. **Results:** To compare with negative control group, the rhodiola rhizome extract can obviously extend survival time of table tennis; increase the content of liver starch. **Conclusions:** Rhodiola rhizome extract can raise the staying power of sports fatigue, strengthen sport ability and play a part in anti-fatigue by increasing the content of liver starch and protecting liver cells of sports fatigue for table tennis players.

## KEYWORDS

Rhodiola, Rhizome, morphology

## 1. INTRODUCTION

For the past few years, Traditional Chinese medicine has great effects on adjusting blood fat metabolism and preventing atherosclerosis and coronary heart disease. Rhodiola sacra extract from the integripetal rhodiola herb on highland. Many people carried on clinical tests and animal experiment give full proof of the fact that those Rhodiola sacra can reduce blood fats and prevent coronary heart disease. It is used widespread in preventing and treating coronary artery disease, angina pectoris, myocardial ischemia and other disease of blood stagnation in clinic. The therapy has been proved effective and adverse reactions haven't been found. But the mechanism of Rhodiola sacra extract in treating hyperlipidaemia need further investigation. In 1990 year, Issemann find a member of the steroid hormone receptor superfamily by peroxisome proliferators. It called Peroxisome proliferators-active receptor (PPAR). Some fatty acids and Peroxisome proliferators can activate PPAR. PPAR have diverse biological functions and play an important role in lipid metabolism, inflammatory process, and immunologic reaction and so on, which is the focus of much of the researches at present. PPAR-s have three subtypes: PPAR- $\alpha$ , PPAR- $\gamma$  and PPAR- $\beta$ . PPAR- $\alpha$ s expressed most in liver, then kidney, heart and skeletal muscle. PPAR- $\gamma$  is expressed most in adipose cell. Peroxisome proliferators-activated receptors (PPARS) are members of the nuclear receptor superfamily. It may induce adipocyte differentiation and regulate the metabolism of lipids. It may be the physical sensor of lipid and play great effects on the dynamic equilibrium of lipids metabolism. In this research, we fed male Wistar rats with high fat diet to make hyperlipidaemia rat model (Dement et al., 1987; Bi et al., 2008). To objective the effect of integripetal rhodiola herb extract on the expression of PPAR- $\alpha$ , PPAR- $\gamma$  in Liver, in order to interpret the mechanism of the integripetal rhodiola herb extract on lipid metabolism (Bogdashin et al., 1990; Vasileva et al., 2016).

Fatigue fracture is known as one of the main failure modes in metal components of engineering structures. In order to prevent fatigue failure,

numerous empirical law and theoretical analysis methods have been proposed to predict the fatigue life since the middle of the 19th century. Over the past few decades, continuing advances in modern machine, aerospace, ocean engineering have driven a substantial increase in the size of engineering structures as well as reduction in weight. Fatigue fracture of metal structures under high speed, high temperature and high loading conditions is a recurring challenge in modern industry. The investigations about the fatigue failure are always a great concern of scholars and engineers. The fatigue life of structures is predictable under give crack growth rates. Thus, most of the research attention about fatigue crack growth has been focused on the crack propagation speed before the crack reaches the critical failure length. The stress intensity factor ranges at a crack tip are proposed by Paris as governing parameters for fatigue crack growth rate in 1961. This landmark development established the theoretical basis for estimation of damage tolerance and fatigue life of engineering structures (Getova et al., 2014; Wong et al., 2014). However, series of experimental investigations in the past few decades have shown that the linear-elastic-fracture based Paris law is not capable of describing the fatigue crack propagating processes that dependents on the shape, size and the stress distributions of the plastic zone, such as the effects of load ratio, overload retardation, the transverse stress on biaxial fatigue as well as the propagation of physically short crack emanating from notch root (Niu et al., 2014).

Fatigue is a very complicated problem for the structure and machinery. And fatigue researches contain a quantity of empirical laws and contradictory views. There are many influencing factors on fatigue strength and fatigue life. At present, most influencing factors can't quantitatively describe fatigue phenomenon with physical models or mathematical models. And there are big differences between test conditions and practical applications (Kang et al., 2015; Titomanlio et al., 2014). Although a lot of theory results and test data have been obtained, accurate prediction and analysis for the fatigue strength and fatigue life can't realized better. One hand, fatigue destroy can be prevented efficiently with fatigue research results and taking related measures in

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actual engineering application. On the other hand, though the same principles guide the same applications, fatigue destroy accidents still can't be prevented. All facts show that fatigue problem still can't be solved completely on theories and practice. With the more requirements of rapid development diversity in society, fatigue more and more has been an important problem on academic circles and industry circles in home and abroad. The paper mainly studies the fatigue problem of the engineering machinery members. The engineering machinery is one of important tools in the human society application. With the development of modern science technology, more and more high demands are put forward for the engineering machinery. Not only high bearing capacity is demanded in order to decrease the material use, but also long fatigue life is requested for structure. The researches of machinery strength valuation, design and fatigue life estimation are paid more attention and developed. When the researches are done with the existing theories, the shape characteristics and case environments of machinery members become important influencing factors. Besides, calculation steps and use methods are also very different (Zdanowski et al., 2014; Qi et al., 2015).

## 2. INTRODUCTION

Today's sports have the increasingly high demand for athletes' physical fitness. Common dietary protein quality cannot meet the needs of training and competition. The intensity of competitive sports is growing. After the large amount of exercise training, the athletes will have all kinds of fatigue, so we need to be eliminated by scientific means. For sports fatigue problem, in addition to constantly improving training methods, how timely and effective supplement energy substances to improve athletic ability, eliminating sports fatigue as soon as possible, is attracting increasing attention of domestic and foreign scholars. Sports or work, the body is in a high-intensity stress state. All parts of physical or physiological response positively in order to maintain or balance "movement" needs. In movement, physiological changes are mainly manifested in energy consumption of materials, water loss, electrolyte loss, accumulation of lactic acid, free radical damage and internal environment disorder, etc.

Rhodiola extract is a kind of grain used as food and medicine, with relieving summer heat, detoxification, reducing blood fat, cholesterol, soften blood vessels and other effects. Rhodiola extract are rich in protein and amino acid variety. Wherein the essential amino acid lysine content is up to 6.9% and the content of essential amino acids is higher than the FAO / WHO recommended value. Rhodiola extract is also rich in dietary fibre, carbohydrates, vitamins and trace elements, and tannin, Coumadin, alkaloids, sapiens and flavonoids and other bioactive substances.

$\alpha$ -lactalbumin is an excellent source of essential amino acids and branched chain amino acids, and is the only whey protein ingredients having the capable of binding to the metal element and calcium. Recent studies have also found that it may have anti-cancer function. In addition lactalbumin and human milk are very similar in amino acid ratio structure, as well as the features. Clinical studies have shown  $\alpha$ -lactalbumin enriched infant formula is safe.

$\alpha$ -lactalbumin is about 25% of whey protein, but also is an excellent source of essential amino acids and branched-chain amino acids. It and bovine serum albumin are rich in cysteine residues, can security through the digestive tract and blood into the cell membrane, reduced to two cysteine to synthesize glutathione, maintaining the best glutathione (GSH) levels of cells and tissues. The latter substance is required by the antioxidant defense system, the immune system cells and skeletal muscle cells.  $\alpha$ -lactalbumin is the only whey protein component which can combine with the metal, and is the most stable whey protein for thermally. Recent studies have found that it is capable of regulating mammary lactose synthesis and anti-cancer function.

$\beta$ -lactoglobulin is the main component of whey protein, accounting for about 50% of whey protein. It has the best amino acid ratio, high content of branched-chain amino acids. Branched chain amino acids leucine is essential, including leucine, valine, and isoleucine. Wherein leucine has strong biological effects, could be the substrate material of glutamine, can also be made directly to the fuel cell. Study confirmed that branched chain amino acid plays an important role in promoting protein synthesis and reducing protein breakdown. Leucine and its oxidation products inhibit proteolytic activity, enabling muscle protein synthesis, increased lean body mass. In endurance during exercise, branched chain amino acid tryptophan into the brain to block the barrier, thereby enhancing the central resistance to fatigue. According to recent research of Southeast Dairy Research Center University of North Carolina,  $\beta$ -lactoglobulin can pre-bind soluble nutrients, such as vitamin A and vitamin E, so as to promote the absorption of people.

Serum protein comes from the blood. The SA separated from milk and the SA in blood have the same chemical and immunological properties. SA synthesizes in the liver with blood concentration of 30 ~ 40g/L; it may transfer into the milk via the mammary cells through specific receptors. SA can be combined with milk thyroxine, zinc and copper. However, SA and IgG can compete newborn pups gastrointestinal carrier, reducing the absorption of IgG of pups.

The average concentration of lactoferrin in the milk of 0.01g/100ml. In whey protein product at a concentration of up to 30 ~ 100mg/l of sweet whey. Lactoferrin content was low, but high biological activity, which activity comprises: anti-bacterial, inhibiting the formation of free radicals, regulate body iron transfer, promote cell growth and enhance immunity, promote the proliferation of bifidobacteria, and as an anti-oxidant. Figure 1 shows the normal liver cells.

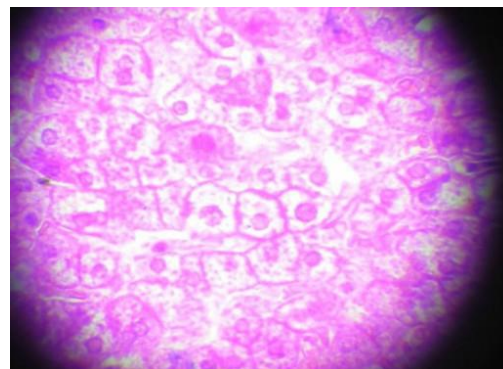


Figure 1: The normal liver cells.

## 3. METHODS AND MATERIALS

### 3.1 The Methods and Model

Fatigue is the phenomenon in which a crack develops and extends under cyclic stressing conditions, accounts for the vast majority of service failures. All types of materials are liable to fatigue fracture, but present-day knowledge is largely associated with crystalline solids, particularly metals and alloys. Consequently, the subject has been studied in depth by both engineers and metallurgists; the former being concerned with the endurance or lifetime of components under given fluctuating stresses, and the latter investigating the structural changes involve. Fatigue is progressive and localized structural damage that occurs when a material is subjected to cyclic loading and is one of the primary causes for catastrophic failure in structural materials. Enhancement of fatigue life is desired for improving utility of the materials.

The application of a stress, in surplus of the local yield value, to a crystalline material results in the production and movement of dislocations, which form slip bands or slip lines where they intersect the surface of the specimen. On repeated stressing the subsequent deformation is concentrated into certain of these slip steps, which become broader and deeper until eventually a micro-crack initiates. Incomplete reversibility of slip accounts for this process, but the precise dislocation mechanisms involved have not yet been unequivocally elucidated. Very thin folds of material known as extrusions are pushed out of the surface, and they are generally associated with small crevices, termed intrusions penetrating along the slip planes of maximum resolved shear stress. A stage I fatigue crack is simply an enlarged intrusion, and so any demarcation between initiation and stage I growth would appear unrealistic. The intrusion-extrusion mechanism may perhaps be regarded as the "classical" process of fatigue-crack initiation, although there are several other common sites at which cracks develop. These include interfaces of all types, and grain boundaries, particularly at high strain amplitudes and/or elevated temperatures. While intrusions and extrusions are being created at the surface, changes are also taking place within the interior of the specimen. A stable dislocation arrangement is set up, which is largely influenced by the cyclic stress amplitude and the stacking-fault energy of the material. A high value of the latter favours a continuous sub-grain structure, whereas in materials in which cross slip is difficult, islands of very high dislocation density occur in an essentially dislocation free matrix.

30 adult, healthy, clean, male Wistar rats, 16~20 weeks old, 250~300g weight, were purchased from experimental animal center and acclimatized for 2 weeks. Animals were randomly divided into normal control (NC) group, high-fat diet (HF) group. NC group (n=10) were fed with basic diet. The calorie constitution is that of calories from 65.5% for the carbohydrate, 10.3% for the fat, 24.2% for the protein; high-fat diet

(HF) group was fed high-fat diet. The calorie constitution is that of 20.1% for the carbohydrate, 59.8% for the fat, 20.1% for the protein. The oil, butter all came from experimental animal centre. At the end of 4th weeks, HF group were random divided into two equal groups: rats without rhodiola sacra extract treatment, that is hyperlipidaemia group (HL), and rats with rhodiola sacra extract, that is hyperlipidaemia and integripetal rhodiola herb extract group (HLR). Integripetal rhodiola herb extract 1 g•kg<sup>-1</sup>•d<sup>-1</sup> were given orally to HLR. Equal amount normal saline was given orally to HLR group. Animals were fed for 8 weeks. Body weight (BW), systolic blood pressure (SBP), serum total cholesterol (TC), serum triglyceride (TG), serum free fatty acids (FFA) were observed every 4 weeks. Animals were put to death at the 8 weeks. And the level of PPAR- $\alpha$ , PPAR- $\gamma$  RNA in Liver was measured by reverse transcription polymerase chain reaction technique (RT-PCR). The correlation among the FFA, TC, TG, and the level of PPAR- $\alpha$ , PPAR- $\gamma$  in Liver was studied.

### 3.2 The Algorithm

$S_{HM}$  solution vectors are randomly generated according to the variable range for each variable. Namely,

$$HMV = \begin{bmatrix} x^1 & f(x^1) \\ x^2 & f(x^2) \\ \vdots & \vdots \\ x^{S_{HM}} & f(x^{S_{HM}}) \end{bmatrix} = \begin{bmatrix} x_1^1 & x_2^1 & \cdots & x_N^1 & f(x^1) \\ x_1^2 & x_2^2 & \cdots & x_N^2 & f(x^2) \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ x_1^{S_{HM}} & x_2^{S_{HM}} & \cdots & x_N^{S_{HM}} & f(x^{S_{HM}}) \end{bmatrix} \quad (1)$$

Hence, we have equation (2):

$$x_i^{new} = \begin{cases} x_i^j, & j \in \{1, 2, \dots, S_{HM}\}, \text{ if } rand < HMCR \\ \bar{x}_i \in X_i, & \text{otherwise} \end{cases} \quad (2)$$

As for  $x_i^{new}$  from harmony memory, we have equation (3):

$$x_i^{new} = \begin{cases} x_i^{new} + rand * BW, & \text{if } rand < PAR(\text{Continuous}) \\ (k + \lambda) * x_i^{new}, \lambda \in [-1, 1], & \text{if } rand < PAR(\text{Discrete}) \\ x_i^{new}, & \text{otherwise} \end{cases} \quad (3)$$

The worst harmony is replaced with the new harmony, i.e.,

$$x^{worst} = x^{new}, \text{ if } f(x^{new}) < f(x^{worst}) \quad (4)$$

According to the analysis and description of routing problem in express delivery, the constraints can be expressed as:

(1) Distribution route length does not exceed the maximum value, i.e.,

$$\sum_{k=1}^n d(k-1, k) + d(n, 0) \leq MD \quad (5)$$

(2) The mathematical model of route programming can be defined as

$$L = \min \left( \sum_{k=1}^n d(k-1, k) + d(n, 0) \right) \quad (6)$$

Based on the gradient descent method, node center and base width parameter are:

$$w_j(k) = w_j(k-1) + \eta(y(k) - y_m(k))h_j + \alpha(w_j(k-1) - w_j(k-2)) \quad (7)$$

$$\Delta c_{j,i} = (y(k) - y_m(k))w_j \frac{x_j - c_{j,i}}{b_j^2} \quad (8)$$

$$c_{ij}(k) = c_{ij}(k-1) + \eta \Delta c_{ij} + \alpha(c_{ij}(k-1) - c_{ij}(k-2)) \quad (9)$$

Jacobian matrix algorithm is shown as follows:

$$\frac{\partial y(k)}{\partial u(k)} \approx \frac{\partial y_m(k)}{\partial u(k)} = \sum_{j=1}^m w_j h_j \frac{c_{ij} - x_i}{b_j^2} \quad (10)$$

Where  $x_i = u(k)$ .

### 4. DISCUSSION

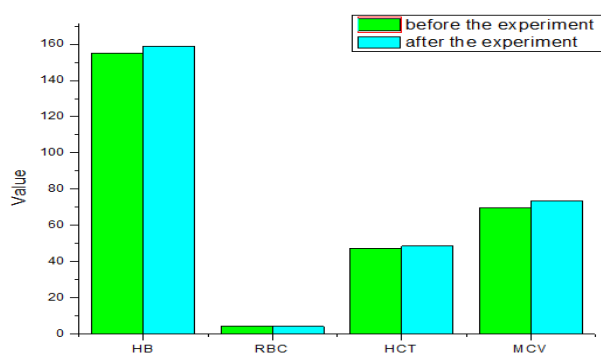
Fatigue includes sports fatigue and mental fatigue in two ways. For the cause of exercise-induced fatigue, there are many theories, but the most important reason is the free radical damage during exercise. Radical theory that the free radicals generated a lot in body tissue due to ischemia is an important cause of exercise-induced fatigue. free radicals directly affect Ca-Mg-ATP enzyme dehydrogenation, causing cytosolic Ca<sup>2+</sup> + concentration increases, thereby inducing a large number of mitochondrial Ca<sup>2+</sup> + uptake, accumulation, inhibition of cell respiration, reduce the rate of ATP resynthesis for energy, leading to fatigue. free radicals affect the cell membrane, so that the occurrence of lipid peroxidation of phospholipid microenvironment changes, liquid membrane fluidity changes, increased permeability. Fatty acids containing double bonds can generate peroxide malondialdehyde, enabling the formation of cross-linking and polymerization between the membrane elements, and thus the basic characteristics of the film such as allosteric, ion transport, enzyme performance changes, affecting the body's normal physiological metabolism and lead to fatigue.

From Table 1, before the experiment, the difference of HB, RBC, HCT and MCV of the control group and the nutrition group was not statistically significant ( $P > 0.05$ ). But after the experiment, the difference of HB, RBC, HCT and MCV of the control group and the nutrition group was statistically significant ( $P < 0.05$ ). The MVC of the control group has significant difference ( $P < 0.05$ ) before and after the experiment, and the difference comparing with the the nutrition group after the experiment was statistically significant ( $P < 0.05$ ). Figure 2 shows the results of control group before and after experiment and figure 3 shows the results of nutrition group before and after experiment.

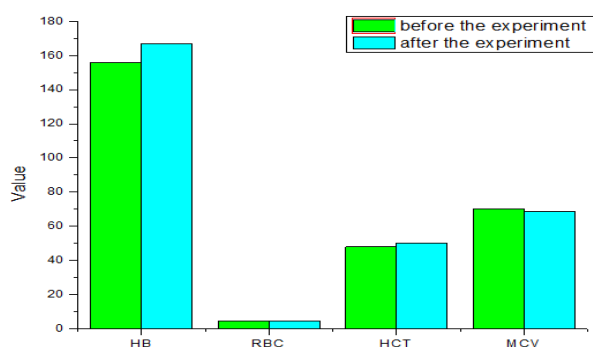
**Table 1:** Experimental Results

| Group           | Time              | HB       | RBC       | HCT        | MCV        |
|-----------------|-------------------|----------|-----------|------------|------------|
| control group   | before experiment | 155±2.88 | 4.20±0.28 | 47.12±2.13 | 69.36±1.57 |
|                 | after experiment  | 159±2.56 | 4.01±0.35 | 48.35±3.11 | 73.44±1.90 |
| nutrition group | before experiment | 156±2.59 | 4.22±0.37 | 47.85±2.18 | 70.18±2.10 |
|                 | after experiment  | 167±3.50 | 4.57±0.31 | 50.22±2.64 | 68.55±2.34 |





**Figure 2:** The Results of Control Group Before and After Experiment



**Figure 3:** The Results of Nutrition Group Before and After Experiment

From the experiment, we can get the following results:

1. Body weight in HF group increased, but there were not difference than that of in NC group ( $p>0.05$ ) after 4 weeks. Body weight in HLR group didn't increase obviously, showed no difference between NC groups ( $p>0.05$ ) and no difference than of in NP group ( $p>0.05$ ) after 8 weeks.
2. Blood pressure in HF group increased obviously, there were significantly difference compared with NC group ( $p<0.05$ ) after 4 weeks. Blood pressure in HL group increased obviously, there were significantly difference compared with NC group ( $p<0.01$ ) after 8 weeks. Blood pressure in HLR group decreased obviously, showed significantly difference between NP groups ( $p>0.05$ ) but no than of in NC group ( $p<0.01$ ) after 8 weeks.
3. TC, TG in HF group increased obviously, there were significantly difference than that of in NC group ( $p<0.05$ ) after 4 weeks. TC and TG in HL group showed significantly difference than of in NC group ( $p<0.01$ ) after 8 weeks. TC and TG in HLR group decreased obviously, showed no difference between NC groups ( $p>0.05$ ) but significantly difference than of in NP group ( $p<0.01$ ) after 8 weeks.
4. FFA increased significantly in NF groups compared with that in NC group after feeding for 4 week ( $p<0.01$ ). FFA in HLR and NC group can obviously lower than that in HL group ( $p<0.01$ ) at 8 week, but there were no difference among HLR and NC group ( $p>0.05$ ).
5. The level of PPAR- $\alpha$ mRNA in HL group rat liver is obviously lower than in NC group after 8 weeks. The level of PPAR- $\gamma$ mRNA in HL group rat liver showed no difference between NC groups ( $p>0.05$ ) after 8 weeks. The level of PPAR- $\alpha$ mRNA and PPAR- $\gamma$ mRNA was markedly increased in all experimental groups compared with the NC group ( $p<0.01$ ) after 8 weeks.
6. The level of PPAR- $\alpha$ mRNA in Liver was negatively correlated with FFA, TC, TG and BP. The level of PPAR- $\gamma$ mRNA in Liver showed no obvious correlation with FFA, TC, TG and BP.

So we can get the following conclusions:

1. Respectively fed with high fat diet displayed hypertriglyceridemia, hypercholesteremia and high blood pressure; so Wistar rats with hyperlipidemia were induced successfully.

2. FFA, TC, TG and BP in HL group increased obviously, there were significantly difference compared with NC group ( $p<0.01$ ) after 8 weeks. FFA, TC, TG and BP in HLR group decreased obviously, showed significantly difference between NP groups ( $p>0.05$ ) but no than of in NC group ( $p<0.01$ ) after 8 weeks.

3. The level of PPAR- $\alpha$ mRNA in HL group rat liver is obviously lower than that of in NC group after 8 weeks. The level of PPAR- $\gamma$ mRNA in HL group rat liver showed no difference between NC groups ( $p>0.05$ ) after 8 weeks. The level of PPAR- $\alpha$ mRNA and PPAR- $\gamma$ mRNA was markedly increased in HLR groups compared with the NC group ( $p<0.01$ ) after 8 weeks. 4. The level of PPAR- $\alpha$ mRNA in Liver was negatively correlated with FFA, TC, TG and BP. The level of PPAR- $\gamma$ mRNA in Liver showed no obvious correlation with FFA, TC, TG and BP. These results suggested that the change of the level of PPAR- $\alpha$ mRNA was related closely with hyperlipidemia.

The anti-oxidative activity of *R. rosea* extract (0,10,20,30,40,50,60 mg/L) and salidroside were compared, using strong antioxidant VC or 2,6-di-tert-butyl-4-methylphenol (BHT) as control, by OH radical clearance rate, DPPH free radical scavenging rate, H<sub>2</sub>O<sub>2</sub> clearance rate, oleic acid antioxidant capacity and Fe<sup>3+</sup> reduction ability test. *R. rosea* extract and salidroside could clear up ·OH radical, H<sub>2</sub>O<sub>2</sub> and DPPH free radical to different extents, with the clearance strength order as *R. rosea* extract>VC>salidroside; when the concentration of them was 60 mg/L, clearance rates of them to OH radical, H<sub>2</sub>O<sub>2</sub> and DPPH free radical were 92%, 81.33% and 79.51%; 83.76%, 72.69% and 57.29%; 43.70%, 37.30% and 28.53%, respectively. In oleic acid antioxidant capacity test, antioxidant capacity was in descending order as *R. rosea* extract>BHT>salidroside. In Fe<sup>3+</sup> reduction ability test, reduction ability of *R. rosea* extract was better than salidroside under the condition of same concentration; Fe<sup>3+</sup> reduction rate of *R. rosea* extract and salidroside were 43.63% and 16.18% when their concentration reached 60 mg/L. Both *R. rosea* and salidroside show good antioxidant activity in vitro, but the antioxidant activity of *R. rosea* extract is better than that of salidroside, indicating other components of *R. rosea* extract may play a leading antioxidant effect besides salidroside.

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