Mechanism of Pharmacological Action of Chromones in Radix Saposhnikovia Based on Biological Significance of Secondary Metabolites

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Abstract

According to origin of species, both animals and plants come from a common ancestor, so they all possess similar/same metabolic activities. Plants adapt to stress conditions by eliminating reactive oxygen species (ROS) partly with secondary metabolites, which are often the medicinal ingredients. With this, the mechanism of pharmacological action and biological action of secondary metabolites may be the same. *Radix saposhnikoviae* the root of *Saposhnikovia divaricata*, has antipyretic, analgesic and anti-inflammatory effects. It was analyzed that the correlation between fever, analgesia and inflammation and the activities of POD, CAT, the H_2O_2 contents in serum before and after intravenous administration for cimicifugin, an active component. For the antipyretic, the correlation coefficient between the body temperature and the H_2O_2 contents was 0.9689 in the model group and 0.5221 in the treatment group, indicating that fever was closely related to the H_2O_2 . In the presence of electron donor, POD can eliminate ROS, the correlation coefficient between POD activities and H_2O_2 content before and after administration were 0.8085 and -0.5070, respectively, indicating that cimicifugin can eliminate ROS through POD. At 39.5 °C, POD activity was about 1.47 times that of normal body temperature, and the scavenging efficiency of POD was 2.94 times that of CAT, which indicated that cimicifugin enhanced the elimination of H_2O_2 mainly through POD. For the analgesic and anti-inflammatory, the correlation coefficients of POD activity with analgesic and anti-inflammatory effects were 0.6685 and 0.4466, respectively, indicating that they were closely related to the H_2O_2 . In this paper, we found the ROS is an important factor of fever, pain and inflammation, the pharmacological actions of *Radix Saposhnikoviae* also is elimination of ROS through the synergistic action of chromone and POD, the mechanism of pharmacological action of Chromones being the same as the biological action of secondary metabolites.

Introduction

Radix saposhnikoviae, the dried root of *Saposhnikovia divaricata* (Turcz.) Schischk, with antipyretic, analgesic and anti-inflammatory effects^[1], and is the most common herbal medicine in many Asian countries.

Drought, coldness, high salinity, metal toxicity, ultraviolet radiation and pathogen invasion are common stress for plants^[2,3]. Under stress conditions, the equilibrium of fixed CO_2 and O_2 in chloroplast of plant cells is collapsed, which result in the accumulated O_2 being reduced to $O_2^{\cdot-}$, and then transformed into other Reactive oxygen species (ROS)^[4,5], an oxygen-containing free radicals or superoxide which form free radicals easily, such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\cdot OH$), etc., with an extremely high activity^[6,7]. Due to strong oxidation ability of ROS, the secondary and tertiary structures of proteins (including enzymes) are usually constructed by the -S-S- groups between two non-adjacent amino acids, so ROS have the ability to regulate the activities of enzymes^[8,9], further, regulate various metabolism. However, too much of ROS can also do damage to organic macromolecules such as enzymes, interfere with normal metabolism, and affect the normal growth of plants^[10]. Antioxidant

enzymes, exist in animals, plants, fungi cells, and other organisms^[12], mainly include superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), etc.^[11], responsible to eliminate excessive ROSs^[13]. However, antioxidant enzymes are also protein, can be destroyed by excessive ROS^[14]. It has been proved that excessive ROS under severe stress conditions can reduce the activities of SOD, CAT, and POD^[15], further increase the destructive force of ROS, and result in damage to cell membrane, DNA, and protein^[16], even making cells to death. Animals have the ability to avoid over-production of ROS by dodging harsh environment, the survival of individuals mainly depends on the food competition among species, not harsh environment, but plants cannot move, it is certain that more ROS are produced because of more stress^[17], with this, it is difficult to eliminate too much ROS only by antioxidant enzymes, therefore, plants evolve additional secondary metabolism system to reduce production or the harm of ROS besides antioxidant enzymes^[18,19,20].

Under stress conditions, plants can regulate the equilibrium of ROS by increasing the activities of secondary metabolites and antioxidant enzymes to improve adaptability to the various stress. Phenolic compounds (flavonoids, tannins, hydroxycinnamates, and lignin) are all secondary metabolites of plants, exist in almost all kinds of plants, with high antioxidant properties^[21], and can scavenge ROS and inhibit lipid peroxidation^[22]. Their antioxidant activities depend on the number and position of hydroxyl groups (-OH) or methoxy group (-OCH₃) substituents. The -OH at C5, C6 and C7 positions on A-ring and C3', C4' and C5' positions on B ring are the main sites influencing the activity^[23,24]. The activities are also significantly enhanced when hydroxyl group is introduced into B ring and double bond is introduced into C ring^[25]. The high-quality product areas of *Radix saposhnikoviae* are semi-arid grasslands. Cimifugin, Prim-O-glucosylcimifugin and 4-O-β-D-glucosyl-5-O-methylvisamminol are the main secondary metabolites of *Saposhnikovia divaricata*. However, chromones from *Saposhnikovia divaricata* contain methoxy-substituted (-OCH₃), instead of -OH, while the activity of -OCH₃ is far lower than -OH. In this case, only presence of POD can chromones get the best out of them. POD is an enzyme related to adversity, only under stress condition can POD exhibit higher activity. POD eliminating H₂O₂ must have an electron donor, and chromones exactly is what POD need^[26]. It has been proved that POD activity of *Saposhnikovia divaricata* increased under the stress conditions^[26]. The structures of chromones from *Radix Saposhnikovia* is shown in Figure 1.

According to origin of species, animals and plants have a common ancestor^[27], so they all possess similar/same cell structure, biological enzyme, genetic material, and metabolism, et al.. But in the face of different environments, they have evolved their own strategies over hundreds of millions of years^[28,29]. Plants adapt to stress conditions by eliminating excessive ROS with secondary metabolites^[30,31], which are often the medicinal ingredients^[32,33]. It has been proved that animals will also produce much of ROS when they are exposed to radiation, hyperbaric oxygen, cigarette smoke, air pollution, heavy metals, pesticides, pathogens and other external factors^[34,35]. All kinds of diseases of animals have an inseparable relationship with ROS. For example, ROS is involved in the pathological process of cancer, diabetes, inflammation, pain, hypertension, atherosclerosis, diabetic angiopathy, and ventricular

remodeling after myocardial infarction^[36,37]. *Radix saposhnikoviae* has definite antipyretic, analgesic and anti-inflammatory effects^[38,39]. The biological mechanism of the ecological action and pharmacological action of chromone of *Radix saposhnikoviae* may be the same.

Materials And Methods

Instruments and Reagents

UV-160A UV/VIS spectrophotometer (SHIMADZU, Japan) K3 Microplate Reader (Thermo Fisher Instruments Co., Ltd., China); BSA224S-CW Electronic analytical balance (Sartorius Scientific Instruments Co., Ltd) A11618 Electronic thermometer (Guang Dong Tonze Electric Co., Ltd) XL-90 Low temperature centrifuge (Beckman, USA) Micropipette (Thermo Fisher Instruments Co., Ltd, China) YLS-6B Intelligent hot plate instrument (Jinan Yiyang Science and Technology Development Co., Ltd, China); HH-2 Digital Display Constant Temperature Water Bath (Jiangsu Jintan ronghua Instrument Manufacturing Co., Ltd, China); KQ2200E Ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd, China); Milli-Q Integral 5 Ultra-pure water system (Merk Millipore, German); FP240h Constant temperature drying oven (Binder, German). High activity dry yeast (Product license number SC2115042600158 Angel Yeast Co., Ltd.). Cimifugin Batch number: S-007-150421, purchased from Chengdu Herbpurify Co., Ltd., with purity of 98% above). Protein quantitative Kit (A045-2), POD Kit (A084-1), CAT test Kit and H₂O₂ test Kit (purchased from Nanjing Jiancheng Bioengineering Research Institute). Sodium chloride injection Batch number: 170109D1, Harbin Medisan Pharmaceutical Co., Ltd.). Methanol, xylene, vaseline and other reagents are analytical pure.

Animals

KM mice, with weight of 18-22g, half male and half female (Animal Certificate No: SYXK (Hei) 2010001); SPF level SD rats, male, with body weight of 180-220g (license No: SCXK (Hei) 2019-0006), all purchased from Safety Evaluation Center of Heilongjiang University of Traditional Chinese Medicine. All the experimental animals were fed in the laboratory for 3 days, keeping the experimental environment quiet, good ventilation, cage clean, with room temperature (22 ± 2) °C and humidity (60 ± 10)%. The experimental protocol were reviewed and approved by the Ethical Committee of Heilongjiang University of Chinese Medicine (approval number: HUCM2018-08598) and was conducted according to the principles expressed in the Declaration of Helsinki.

Antipyretic Effect of Cimifugin

Animal Screening and Modeling. The basic body temperature of SPF male SD rats was measured after 3 days of adaptive feeding. The rats whose body temperature was more than 39.0 °C or whose twice body temperature fluctuations were more than 0.5°C were eliminated. Finally, 20 SD rats were selected as qualified experimental animals for modeling.

Temperature monitoring of rats. The qualified experimental animals were divided into experimental group (15 animals) and model group (5 animals). The rats were forbidden to feed but not drink for 12 h before the experiment, and the average temperature was measured at an interval of 15 minutes for three times as the normal temperature before modeling, and the temperature was monitored at an interval of 1 hour from 0 h to 22 h after administration.

Determination of antipyretic effect of Cimifugin. The experimental groups were injected with 0.216mg/kg of Cimifugin solution by tail vein at 0 h and 6 hours, respectively. The model group was given the same volume of normal saline. 20% dry yeast suspension was injected subcutaneously into the back after the first injecting Cimifugin solution half an hour later. The antipyretic effect (Δt) of Cimifugin was calculated with change of the body temperature for 6 h and 8 h after administration.

The antipyretic effect $\Delta T/^{\circ}\text{C} = T_{8\text{h}} - T_{6\text{h}}$

The serum of rats was collected before administrate drug (0 h) and at 8 h after administrate drug for testing POD and CAT activities.

The Relationship between Fever and H_2O_2 in Rats

Five rats of the model group were selected to measure the contents of H_2O_2 and body temperature at 0h, 8h and 22h. Hydrogen peroxide (H_2O_2) content was determined with hydrogen peroxide (H_2O_2) kit.

Determination of CAT and POD activity

CAT activity was detected by Catalase (CAT) kit. The amount of substance (1 μmol) decomposing substrate (H_2O_2) per ml of serum was used as one activity unit (U / ml). POD activity was measured by POD kit, and the amount of substance (1 μg) per mg of tissue protein (serum protein) was used as one activity unit (U / mgprot).

Determination of Optimum Temperature of POD and CAT

Blood was collected from 5 rats, and the serum was mixed equally, the activities of POD and CAT were measured at 37.0 $^{\circ}\text{C}$, 37.5 $^{\circ}\text{C}$, 38.0 $^{\circ}\text{C}$, 38.5 $^{\circ}\text{C}$, 39.0 $^{\circ}\text{C}$, 39.5 $^{\circ}\text{C}$, 40.0 $^{\circ}\text{C}$, 40.5 $^{\circ}\text{C}$, and 41.0 $^{\circ}\text{C}$, respectively.

Analgesic Effect of Cimifugin

Screening of experimental animals and determination of average pain threshold. Before the experiment, 30 KM female mice were screened. The pain threshold was determined by hot plate method. The temperature of hot plate was set at 55 $^{\circ}\text{C}$. Fifteen mice with pain threshold from 5s to 30s were selected as qualified experimental animals. The average pain threshold (s) was measured before the experiment.

Determination of POD activity and analgesic effect. The serum of 15 mice was collected before administration for the determination of POD activities. The pain threshold was measured 1 hour after 0.5mg/kg dosage of cimifugin solution was administrated through tail vein. Each mouse was measured for more than 30s. The average value of three times was used as the indication of the analgesic effect

Analgesic effect (s) = average pain threshold after administration (s) - average pain threshold before administration (s).

Anti-inflammatory Effect of Cimifugin

Serum samples of 15 KM mice, half male and half female, were collected before administration for determination of POD activities. Cimicifugin was given by tail vein at the dose of 0.5mg/kg. After 30 minutes, both sides of the left ear of each mouse was coated 0.2ml xylene, and the right ear was used as control. One hour later, the mice were killed by cervical dislocation. Two ears were cut along the base line of the auricle respectively. Round ear pieces were made on the same part of the left and right ears with an 8mm diameter punch and were weighed. The weight difference between the two ear pieces of the same mouse was taken as the indication of the auricle swelling degree (mg).

Auricle swelling degree (mg)=Weight of left ear(mg)- Weight of right ear(mg)

Data Processing

Taking the activities of serum antioxidant enzymes (POD, CAT) and the contents of H₂O₂ in serum as the X-axis, and the pharmacological action as Y-axis, the correlation between the activities of serum antioxidant enzymes (POD, CAT) and H₂O₂ contents in serum and pharmacological action was analyzed by Microsoft Excel software.

Results

Relationship between fever and H₂O₂ in rats. The body temperature of model group reached 41.4 °C after 8 h of modeling, and then decreased, while the temperature of treatment group was 38.9°C after 8 h and then decreased. The body temperature of the treatment group was significantly lower than that of the model group, indicating that cimicifugin has antipyretic effect, as shown in Figure 2. The basal body temperature of rats was 36.5 °C, and the contents of H₂O₂ in was 47.97~54.49 mmol/L. After 8 hours, the body temperature reached the highest value of 39.9~41.5°C. The H₂O₂ contents increased to 149.95~162.53 mmol/L. After 22 hours, the body temperature dropped to 37.3~37.7 °C, and the H₂O₂ content returned to the normal level of 50.76~61.47mmol/L. The correlation coefficient between body temperatures and H₂O₂ contents in model group was 0.9689 based on above the numerical data, indicating that there was a close relationship between fever and H₂O₂ in rats, as shown in Figure. 3. The correlation coefficient between body temperatures and H₂O₂ contents in the treatment group was 0.5221, indicating that body temperature was also closely related to serum H₂O₂, as shown in Figure 4. Relationship between the antipyretic and the POD, CAT. POD activities were extremely significantly

positive correlated with the contents of H₂O₂ in serum, with a correlation coefficient of 0.8085. However, after administration for cimicifugin, the relationship between the them was reversed, from a significant positive correlation to a significant negative correlation, with a correlation coefficient of -0.5070, indicating that cimicifugin significantly reduced the contents of H₂O₂, as shown in Figure 5. The activities of POD and CAT increased with the increase of body temperature. The activity of CAT was the highest at 37.5 °C, and then decreased sharply. The correlation coefficients between CAT activity and H₂O₂ content in serum before and after administration were 0.3002 and 0.0361, respectively, as shown in Figure 6. The correlation coefficient between the CAT and temperature rise amplitude were 0.5470 and 0.2335 before and after administration, respectively, as shown in Figure. 7 and Figure. 8. But the optimum temperature of the POD and CAT are different. The optimum temperature of the POD is 39.5°C, further still maintain high activity at higher teperature, but the CAT is 37.5°C, decrease sharply as increasing teperature, indicating that CAT played an important role in eliminating H₂O₂ at normal temperature. While POD activity increased rapidly above 38.0 °C and 39.5 °C, with a 1.47 times higher than that of normal temperature, indicating that POD plays an important role in eliminating H₂O₂ under fever condition. At 37.0 °C, the elimination efficiency of POD is 2.07 times that of CAT, and at 39.5 °C, 2.94 times that of CAT, indicating that POD is the main way to eliminate H₂O₂ under fever condition, as shown in Figure. 9.

Analgesic Effect of Cimicifugin According to the results of analgesic effect and POD activity, the linear equation of POD activity and analgesic effect in serum in rats was $y = 1.0956x + 17.769$, ($r = 0.6685$), as shown in Figure 11.

Anti Inflammatory Effect of Cimicifugin The results of serum POD activity and ear swelling degree of mice are shown in Figure 4. The linear equation between the activity of POD in serum and auricle swelling was $y = -22.655x + 37.751$ ($r = 0.4466$), as shown in Figure 12.

Discussion

Antipyretic Effect and H₂O₂

1. H₂O₂ is the essential reason of hyperthermia

ROS can be produced in various pathways, including enzymatic reaction, autoxidation of reductive molecules, and mitochondrial electron transfer chain, participate in cell growth and proliferation, development and differentiation, aging and apoptosis, as well as many physiological and pathological processes. ROS can be reciprocal transformed^[40]. In the antioxidant enzyme system, the O₂⁻ produced by organism firstly generates H₂O₂ through the action of SOD, and then decomposes by CAT and POD. Among many ROS, the half-life of O₂⁻ is about 1 μs^[41], the ¹O₂ is 3μs^[42,43,44], with short life span but strong activity and great destructive power. But the activity of H₂O₂ is weak, with a long life span, high content, can run over long distances, and become the main ROS regulating cell metabolism^[45,46].

Fever is closely related to H₂O₂^[47]. In order to clearly show the correlation between different data in the administration group and the model group, all the correlation data have been reflected in Table 1. Figures 3 and Figures 4 show that the correlation coefficient between body temperature and H₂O₂ content in

model group is 0.9689, indicating that a large amount of H_2O_2 accumulated in various tissues of the body may be the essential reasons of fever reaction. Figure 5 shows that the correlation coefficient between POD activities and H_2O_2 contents is 0.8085 before giving the medicine to rats. This strong positive correlation is related to the characteristics of POD which is an inducible enzyme. Only when cells produce more H_2O_2 under exogenous stimulation, can POD activity be improved by feedback induction [48,49,50]. The mean value of serum POD activity before fever was 4.08U/mgprot, and that of serum POD after administration for 8h (i.e. 7.5h after modeling) was 4.51U/mgprot, with a increase of 10.54%, indicating that the POD activity had been activated and increased under the stimulation of H_2O_2 within 7.5h after being induced fever. That is, POD activity also increases along with the increase of H_2O_2 content. However, in the absence of electron donors, can POD eliminate H_2O_2 [51,52]. Without electron donor, H_2O_2 content must remains at a high level, which result in higher body temperature of rats. The rats were given cimicifugin, a electron donor was supplied, with a result of that the higher the POD activity, the lower the content of H_2O_2 . The significant positive correlation was revered to a significant negative correlation ($r = -0.5070$), as be shown in Figure 5. Figure 2 has also showed that the body temperature decreased significantly after administration, indicating that the fever effect is closely related to the content of H_2O_2 .

2 .POD is the main enzyme to eliminate H_2O_2 in rats

POD and CAT are the main antioxidant enzymes to eliminate H_2O_2 . After administration, the correlation coefficient between the antipyretic effect of cimicifugin and POD was 0.5582, and CAT was 0.2335, shown in Figure and Figure 8, indicating that the antipyretic effect of cimicifugin was closely related to the activity of POD. Figure 9 shows that the optimum temperature of the POD is 39.5°C , further maintain high level at high teperature, but the CAT is 37.5°C , decrease sharply as increasing teperature, Fig-6 also shows that correlation between CAT and H_2O_2 was weak after fever, indicating that CAT is not the main enzyme to remove H_2O_2 at high temperature, while POD has high activity at $38^\circ\text{C} \sim 42^\circ\text{C}$, which is more than 2.5 times higher than that at normal temperature. The POD activity (U / mg prot) is be represented by the mass of catalytic substrate ($1\mu\text{g}$) per milligram of tissue protein (serum protein), and the amount of substance ($1\mu\text{mol}$) of catalyzing substrate (H_2O_2) per milliliter of serum (or tissue protein) is taken as an activity unit (U / ml). With this, it concluded that the ratio of H_2O_2 consumption of POD and CAT of the same protein unit is about 1.47:1, H_2O_2 consumption ratio of POD and CAT of the same active unit at 37°C is about 2.08:1, and that of POD and CAT with the same active unit at 39.5°C is about 2.94:1, as shown in Figure 10. Under the fever condition, the consumption of H_2O_2 by the same POD is almost 3 times that of CAT. CAT is specific to H_2O_2 and can directly eliminate excessive H_2O_2 , but it has poor thermal stability, and its activity is weak when the body temperature is high [53]. POD is a glycoprotein with strong stability, which usually shows high activity under adverse conditions [48,49,50], indicating that POD is the main enzyme to eliminate H_2O_2 , especially when the content of H_2O_2 is high and the body temperature is high.

Pain, Inflammation and H₂O₂

Radix Saposhnikoviae has antipyretic, analgesic and anti-inflammatory effects [38-39], which are interrelated and coexist.

Pain is also closely related to H₂O₂. It has been found that neuropathic pain and inflammatory pain are related to ROS [53,54,55]. ROS in the central nervous system mainly comes from the electron transport chain in the inner membrane of mitochondria. ROS, from Endoplasmic reticulum, induced by virus, can result in neuralgia [56,57], and oxidative scavengers can significantly eliminate mechanical abnormal pain [58]. Figure 11 shows that the correlation coefficient between analgesic effect and POD is 0.6685, indicating that the analgesic effect of cimicifugin is mainly achieved by eliminating H₂O₂ in the body.

Inflammation is the host's defensive immune response to foreign damage factors, and it is also closely related to ROS. When endothelial cells are injured, ROS, as a cell signal molecule, is also an inflammatory mediator [59]. During the occurrence of lithiasis, the main components of the lithiasis can activate over-production of ROS and interact thioredoxin protein in a dose-dependent manner [60,61]. Figure 12 shows that the correlation coefficient between anti-inflammatory effect and POD is 0.4466, indicating that the anti-inflammatory effect of cimicifugin is mainly achieved by eliminating H₂O₂ in the body.

Mechanism of Action of Cimicifugin

The main effective component of Radix Saposhnikoviae is chromone. According to the *Chinese Pharmacopoeia* (Volume I of 2015 Edition), the total content of cimicifugoside and 5-O-methylviadine in Radix Saposhnikoviae should not be less than 0.24%. Because of the high content of cimicifugoside and 5-O-methylviadine, it is regarded as the standard component. The absorption of cimicifugin and 5-O-methylvisamminoside are very small, which can only be detected at high-dosing [62], while the main component in the blood is cimicifugin [63]. Therefore, in order to reduce the impact of intestinal absorption process, intravenous administration was used in this study. The ability of flavonoids to eliminate ROS depends on the amount and location of -OH [64,65]. Cimicifugin lacks -OH, instead of -OCH₃, with much lower activity than -OH. POD present in animal and plant cells widely and is an important enzyme to eliminate H₂O₂, but it needs polyphenols and other electronic donors to play a role [51,52]. The synergism of POD and Cimicifugin can effectively remove H₂O₂ in the cells, and exhibit antipyretic, analgesic, and anti-inflammatory effects.

The components of herbal medicine are very complex. Recent researches on drug action and mechanism mainly focused on the differential metabolites and metabolic pathways. In many cases, although there is correlation, it is not clear whether these metabolites are the upstream or downstream substances leading to the cause of disease, and it is difficult to clear whether it is causation or parallel relations. Darwin's theory of evolution is the three major discoveries of Natural Science in the 19 th century, which greatly promoted the development of life science. According to this theory, the evolution of life has underwent

from inorganic small molecules, to organic small molecules, to organic macromolecules, to independent systems, to primitive life. Animals and plants should have a common ancestor and the same basic life law. The ecological function of chromone of *Saposhnikovia divaricata* is to eliminate the ROS in cells, which is also an important factor of fever, pain and inflammation. The antipyretic, analgesic and anti-inflammatory effects of Radix Saposhnikoviae also eliminate ROS through the synergistic action of chromone and POD, which indicate that the ecological and pharmacological mechanisms of chromone of Radix saposhnikoviae are the same. At present, botany and zoology are two relatively independent research fields. The research results from plant ecology and herbal medicine pharmacology learning from each other can open up new ideas for the researches of plant pharmacology.

Declarations

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Author Contributions

Conceived and designed the experiments: *Xiang-Cai Meng, Si-Si Cao*. Performed the experiments: *Si-Si Cao, Ying Shen, Lei Shi, Jing-Ming Yang*. Analyzed the data: *Xiang-Cai Meng, Si-Si Cao*. Contributed essential reagents: *Lei Shi*. Wrote the manuscript: *Xiangcai Meng, Sisi Cao*. All authors reviewed the manuscript.

Additional Information

Competing financial interest: The authors declare no competing financial interests.

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Tables

Tab. 1 Correlation Analysis of POD, CAT and antipyretic effect in rats before administration

No.	Group	Point of time	Linear Equation		Correlation
1	Model Group	0h~8h~22h	H ₂ O ₂ -Temp.	y = 23.052x - 793.24	r= 0.9689
2	Treatment Group	Before Fever	POD-Antipyretic effect	y = -1.8291x + 4.9484	r = 0.4508
3			CAT-Antipyretic effect	y = -3.0596x + 5.6249	r =0.5470
4			POD-H ₂ O ₂	y = 12.683x + 16.519	r = 0.8085
5		After Fever	POD-Antipyretic effect	y = -3.5706x + 6.0101	r = 0.5582
6			CAT-Antipyretic effect	y = -0.7374x + 6.3771	r= 0.2335
7			H ₂ O ₂ -8hTemp.	y = 38.694x - 1471.6	r= 0.5221
8			POD-H ₂ O ₂	y = -4.2087x + 71.886	r=0.5070

Figures

Cimifugin (R=H)

4'-O- β -D-glucosyl-5-O-methylvisamminol

Prim-O-glucosylcimifugin (R=GLU)

Figure 1

Structure diagram of main chromones in Radix Saposhnikoviae

Figure 2

Changes of fever temperature in rats

Figure 3

Correlation between body temperatures and H2O2 contents in model group

Figure 4

Relationship between body temperatures and H_2O_2 contents in treatment group

Figure 5

Relationship between POD and H₂O₂

Figure 6

Relationship between CAT and H₂O₂

Figure 7

Relationship between POD and antipyretic effect

Figure 8

Relationship between CAT and antipyretic effect

Figure 9

Optimum temperature of POD and CAT in rats

Figure 10

H2O2 elimination of antioxidant enzymes at different temperatures

Figure 11

Correlation of POD and analgesic effect

Figure 12

Correlation of POD and ear swelling

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