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Study on Extraction Technology of Pueraria Flavone and Its Hepatoprotective Effect

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Abstract: Gegen Root Yellow Copper, as a traditional Chinese medicine, contains various active ingredients, such as flavonoids, ginsenosides, etc. It has antioxidant, anti-inflammatory, and liver-protective biological activities. With the increasing intake of toxins, drugs, and alcohol in modern life, the liver's burden has become heavier, and the incidence of liver diseases has gradually increased. Therefore, it has become particularly important to find natural drugs with liver-protective effects. The total flavonoids of Pueraria Montana(Loureiro)Merrill) were extracted by a single factor test combined with response surface method. The total flavonoids of Pueraria were characterized by UV spectrophotometry and high performance liquid chromatography. AGAR diffusion method and DPPH, ABTS+- and total reduction capacity were used to evaluate the physical activity of total flavonoids from Pueraria. The results showed that the optimized extraction process of total flavonoids from Pueraria root was as follows: solid-liquid ratio (m:V.g:mL)1:16, extraction time was 69min, extraction time was 3 times, and the yield of total flavonoids was 7.690%. The total flavonoids of Pueraria root included 9 kinds of common flavonoids, accounting for 91.81% of the total content. Animal experiments showed that Pueraria root flavonoids could significantly reduce the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the content of propyl (MDA) in serum of mice. Increased the activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the liver tissue of mice, and had an obvious hepatoprotective effect. The results can provide a reference for the extraction and application of Gerona.

Keywords: Pueraria flavone, Response surface, hepatoprotective effect

1. Introduction

There are more than 30 kinds of flavonoids, mainly puerarin, fagin, daidzein, daidzein, genistein and coumarin, among which puerarin flavone is a kind of bioactive substance in pueraria, which has many effects such as anti-tumor, anti-oxidation, improving blood circulation, lowering blood sugar, lowering blood lipids and improving immunity. Pueraria montana(Loureiro)Merril), also known as Pueraria Montana, Pueraria and Pueraria, is a leguminous pueraria plant, which has been accepted by the National Health Commission as a medicine and food cognate Chinese medicine. [1] The main active ingredients in pueraria root are pueraria, daidzein, daidzein, genistein and other flavonoids, which have biological activities such as cancer prevention, anti-diabetes, antibacterial and antioxidant.

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Studies have shown that flavonoids are a strong antioxidant, which can effectively remove free radicals in the body and promote blood circulation in the body; Flavonoids are bacteriostatic agents from plants and have good synergistic effect with antibiotics. However, researchers rarely use UV spectrophotometry and high-performance liquid chromatography to analyze the active components of flavonoids in the pueraria root. Therefore, the response surface method was used to optimize the extraction process of total flavonoids from Pueraria, analyze the components and contents of pueraria flavonoids, and conduct a preliminary study on its effects on the ant alcoholic liver, in order to provide a theoretical basis for the comprehensive evaluation and resource development of pueraria.

2. Literature Review

Pueraria Montana(Loureiro)Merril), also known as Pueraria, Kudzu, and Pueraria, is a legume plant that has been accepted by the National Health Commission as a medicine and food cognate Chinese medicine. The main active ingredients in pueraria are flavonoids such as pueraria, daidzein, daidzexitin and genistein, which have biological activities such as cancer prevention, anti-diabetes, antibacterial and antioxidant. Studies have shown that flavonoids are a strong antioxidant, which can effectively remove free radicals and promote blood circulation in the body. Flavonoids are bacteriostatic agents of plant origin, and have a good synergistic effect with antibiotics. The extraction of effective natural antioxidants and bacteriostatic agents from Chinese herbal medicine is a research hotspot, but the analysis of flavonoids in Pueraria by ultraviolet spectrophotometry and high performance liquid chromatography is rarely reported. In this study, response surface method was used to optimize the extraction process of Pueraria flavonoids, analyze the components and contents of pueraria flavonoids, and explore the liver protection and antioxidant activity characterization of pueraria flavonoids, in order to provide a theoretical basis for a comprehensive evaluation and resource development of pueraria.[2]

Flavonoids, also known as biological flavonoids or plant flavonoids, are A class of compounds with C6-C3-Cg structure formed by three carbon atoms connecting two benzene rings (A ring and B ring). According to different components of the benzene ring, flavonoids can be divided into many different types. Including flavonoids, flavonols, isoflavones, chalcones, anthocyanidins, flavanols, etc. 121, flavonoids are one of the main effective components of many traditional Chinese medicines, widely present in some traditional Chinese medicine roots, stems, leaves, flowers, fruits and seeds and other parts. Chinese traditional medicine contains a variety of flavonoids, each with a unique content and a rich pharmacological effect. Modern pharmacological studies have confirmed that flavonoids in traditional Chinese medicine have anti-inflammatory, antioxidant and anti-tumor effects, and have good effects on cardiovascular, neurological, liver and other related diseases. In this paper, the anti-inflammatory, antioxidant and anti-tumor pharmacological effects of traditional Chinese medicine flavonoids and their effects on neurological diseases, cardiovascular diseases, liver diseases and kidney diseases were reviewed, so as to provide a reference for further study of their mechanism of action and development of clinical drugs.[3]

3. Materials and methods

3.1. Materials

3.1.1. Test materials and reagents

Kudzu; 3' -hydroxypuerarin (purity ≥96%), puerarin (purity ≥98%), 3' -methoxypuerarin (purity ≥98%), puerarin -6 "-O-xyloside (purity ≥98%), puerarin apigenoside (purity 298%), ononin (purity 298%) control products; Daixitin (purity: 93.4%), daixitin (purity: 99.3%), genistein (purity: 99.9%) control products; Anhydrous ethanol, chromatic harmonic methanol, potassium persulfate; Water for

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deionized water, Wahaha pure water; 1, 1-diphenyl-2-picrohydrazine free radical (purity >97.0%); 2, 2-hydrazine - bis (3-hexylbenzothiazole-6-transthenic acid) diamine salt (purity is 98%), L-ascorbic acid (purity >99.0%), phosphate buffer (0.1 mol/ l.H6.6), potassium ferricyanide, trichloroacetic acid, ferric chloride.

3.1.2. Instruments

Agilent 1260 High performance liquid chromatograph with DAD detector and reversed phase column 120 HC-C18(4.6 mmx250 mm), Agilent Corporation, USA; UV18000 Ultraviolet-Visible Spectrophotometer, Shimadzu Corporation, Japan; BC-W208 Rotary evaporator, Shanghai Beikai Bio-Chemical Equipment Co., LTD.; ZDHW type electric heating sleeve, Gongyi Yuhua Instrument Co., LTD.; FA2204C Electronic Analytical Balance (S/N:0280736761), Shanghai Tianmei Balance Instrument Co., LTD.; HH-S constant temperature water bath, Jintan Hengfeng Instrument Manufacturing Co., LTD.; Whirlpool Mixing Instrument, Shanghai Huxie Industrial Co., LTD.; Neofuge 15 High speed centrifuge, Lixin Instrument Shanghai Co., LTD.; Infinite M200 PRO multifunctional enzyme labeler, Tecan, Switzerland.

3.2. Methods

3.2.1. Extraction of total flavonoids from Pueraria root

Weigh 20g Pueraria decoction pieces and soak them in deionized water according to a certain ratio of material to liquid for 30 min. Reflux extraction was carried out at 100℃ according to a certain extraction time and extraction times, and then the extracted liquid was pumped and filtered for several times. The filtrate was combined and concentrated at 75℃ under reduced pressure.

3.2.2. Determination of total flavonoids content of Pueraria root

2.50mg pueraria standard product was accurately weighed and dissolved in deionized water to make 0.25 mg/mL mother liquor, and diluted to a concentration gradient of 0, 0.0025, 0.0037, 0.0049, 0.006 1, 0.0074, 0.0086, 0.009 8 mg/mL. The absorbance was measured at wavelength 250 nm, and then the standard curve of pueraria was drawn with concentration (X) as the horizontal coordinate and absorbance (Y) as the vertical coordinate. The regression equation was Y=82.332X-0.005,R2=0.999 7. When the standard concentration of puerarin was 0~0.009 8 mg/ mL, the absorbance showed a good linear relationship with the concentration of pueraria diathesis. 0.5 mL concentrated liquid was precisely measured in a 50 mL volumetric bottle with deionized water, and its absorbance was determined at the wavelength of 250nm. The absorbance was repeated 3 times, and the total flavonoids yield was calculated as 17.8]. The formula is as follows. Total flavonoids yield =C×n×V/m×100% in the formula, c is the detection concentration of total flavonoids of Pueraria root (mg/ mL); n is the dilution of pueraria root extract; V stands for constant total volume (m L) of pueraria root extract; m is the mass of pueraria root (g).

3.2.3. Single-factor experimental design

The effects of solid-liquid ratio, extraction time and extraction times on the extraction rate of total flavonoids were studied. 20 g pueraria root was weighed and soaked for 30min. Other extraction conditions were consistent: (1) The solid-liquid ratio (g:mL) of 5 gradients was set at 1:5, 1:10, 1:15, 1:20, 1:25, extraction time was 80 min, and extraction times were 3; (2) the extraction time of 5 gradients was set at 20, 40, 60, 80, 100 min, the ratio of solid to liquid (g:mL) 1:15, and the extraction times were 3 times; (3) the extraction times of 5 gradients were set at 1, 2, 3, 4 and 5 times, the ratio

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of solid to liquid (g:mL) 1:5, and the extraction time was 80 min; The effects of various factors on the yield of total flavonoids from Pueraria root were investigated.[4]

3.2.4. Response surface experiment design

According to the results of the single-factor experiment, with solid-liquid ratio (A), extraction time (B) and extraction times (C) as independent variables, and total flavonoids yield as response value, 3 levels with better performance of each variable in single-factor experiment were selected, and Design-Expert 8.0.6 response surface software was used. The three-factor, three-level test was designed based on the Box-Behnken principle, and the results were analyzed.

3.2.5. HPLC quantitative analysis of Pueraria flavone

The composition and content of flavonoids in Pueraria root prepared by optimized response surface were determined by high performance liquid chromatography (HPLC) external standard method 111. Chromatographic conditions[5]: Mobile phase A consisted of 85% methanol solution and 15% water; Mobile phase B consisted of 85% water and 15% methanol solution. Elution procedure: 0~15.00 min, mobile phase A 15%, mobile phase B8%; 15.01~60.00 min, mobile phase A40%, mobile phase B 60%; 60.01~75.00 min, mobile phase A60%, mobile phase B40%75.00~75.01 min, mobile phase A15%, mobile phase B85%. The detection wavelength was 254 nm; The flow rate was 1.0 mL/min; Column temperature was 25℃; The sample size was 10μL.

3.3. Data Processing

Origin 9.0 software was used for analysis and mapping of all data, and Design-Expert 8.0.6 was used for response surface analysis. The data were represented by "mean soil standard deviation". 1<P<0.05 indicates a significant difference, and P<0.01 indicates a very significant difference.[6]

4. Results and analysis

4.1. Analysis of results of single factor experiment

4.1.1. Influence of solid-liquid ratio on the yield of total flavonoids from Pueraria root

As shown in Figure 1, with the increase of solvent volume, the yield of total flavonoids increased. When the ratio of solid to liquid (g: mL) reached 1:15, the yield of total flavonoids reached 7.03%. When the solvent volume continued to increase, the extraction rate of total flavonoids fluctuated slightly at about 7.00%, and no longer increased.

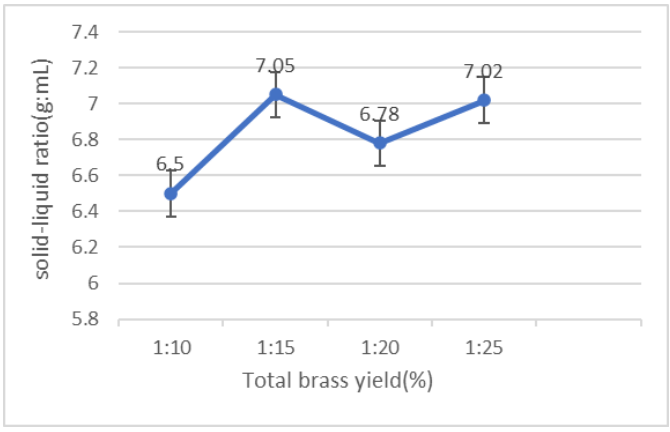

Figure 1: The Effect of Solid-Liquid Ratio on the Yield of Total Genistin in Kudzu Root Extract

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4.1.2. Effects of extraction time on the yield of total flavonoids from Pueraria root

As shown in Figure 2, with the increase of extraction time, the yield of total flavonoids gradually increased, and reached the maximum value of 7.38% when the extraction time was 80 min. As the extraction time extended, the yield of total flavonoids started to decrease. This could be due to the flavonoids continuing to dissolve into the extraction solution, leading to a gradual increase in total flavonoid yield. At the same time, the oxidation of flavonoids and the structural damage caused by continuous high temperature may also occur during the extraction process. When the extraction time is further increased, the yield of flavonoids decreases with the accumulation of oxidation and destruction.

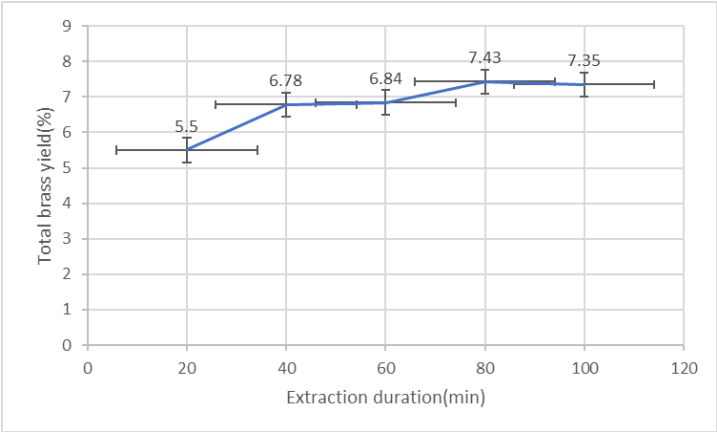

Figure 2: The Effect of Extraction Time on the Yield of Total Genistin in Pueraria Root

4.1.3. Establishment and analysis of regression model

As shown in Figure 3, the yield of total flavone was positively correlated with the number of extraction within 1-3 times. When the number of extraction was 3 times, the yield of total flavone reached the maximum, 7.34%, and then began to decline with the increase of extraction times. This may be because when the extraction times are small, the extraction of flavonoids is insufficient, and the yield increases after multiple extraction and combination. When the extraction time reaches 3 times, the extraction of flavonoids is more adequate. Excessive extraction times greatly increased the concentrated volume, resulting in a long heating time of the extraction solution, which may lead to the loss of flavonoids and reduce the total flavonoids yield; At the same time, it will also bring about a series of problems, such as the overall process time being too long, energy consumption increasing, and the amount of solvent increasing. Therefore, it is recommended to limit the extraction times to a maximum of three.

Table 1: Determination results of MDA contents in sera and SOD.GSH-Px activities in liver tissues of mice(n=10)-

Group	**MDA content/(nmol*mg')**	**GSH-Px activity/(U*ml-')**	**SOD activity/(U*ml-')**
Normal control group	6.62+/-1.12	242.16+/-8.93	899.82+/-90.63
CCL model Group	28.81±3.14a	191.07±6.84a	428.03±24.28a
Diphenyl ate group	25.37+/-2.14	241.15±7.36b	786.46±47.32b
Pueraria flavonoid extract solution experimental group	20.71±1.22b	214.46±8.59c	699.25±36.83

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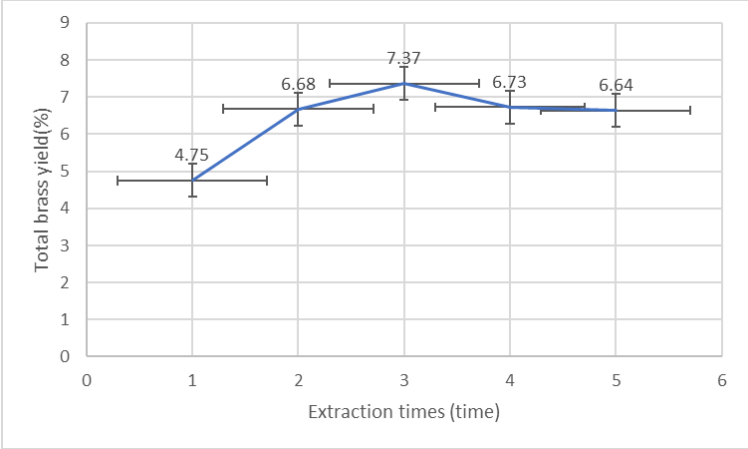

Figure 3: The Effect of Extraction Number on the Yield of Total Flavonoids in Pueraria Root

Variance analysis was performed on the regression model, and the results were shown in Table 4. Model F was 163.68, the difference was extremely significant, the mismatch item F was 2.15, the difference was not significant, and model R2 was 0.995 3,2adj was 0.989 2; This indicates that the model is reliable and can well analyze and predict the yield of total flavone. According to F, the effects of A, B, AB, AC, BC, A^2, B^2 and C^2 on the yield of total flavonoids were extremely significant ($P<0.01$), and the effects of C on the yield of total flavonoids were significant ($P<0.05$). The steepness of the response surface indicated that the factor had a strong effect on the yield of total flavonoids, while the flattening of the response surface indicated a weak effect. The shape of contours can reflect the strength of the interaction effect between the two factors, and the ellipse indicates that the interaction between the two factors is significant, while the circle indicates that the interaction is not significant. As can be seen from FIG. 4, the steepness of the response surface was compared, the solid-liquid ratio and extraction time were greater than the extraction times, indicating that the order of influence of each factor on the yield of total flavonoids was solid-liquid ratio = extraction time > extraction times. Compared with the contours of the interaction of the three factors, the contours of the interaction between the solid-liquid ratio and the extraction time, the solid-liquid ratio and the extraction times were all elliptical, and the denser the alignment, the more significant the interaction was, the steeper the response surface curve was, and the influence on the yield of total flavonoids from pueraria root was extremely significant.[5]

Table 2: Inhibitory activity of puerarin on bacteria

Strain	**Antibacterial zone diameter//mm**			
	Sterile water Yin	**Kanamycin**	**Pueraria total flavonoids**	**MLC**
	Sex contrast	**Positive control**		**mg/mL**
Acinetobacter baumannii	—	24.14±1.23	8.26±0.18	—
Staphylococcus aureus	—	20.25±0.27	8.63±0.12	—
Streptococcus faecalis	—	10.03+/-0.33	10.38+/-0.10	250

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Table 2: (continued).

Methicillin-resistant epidermal staph Grape coccus	—	28.59±1.37	—	—
Bacillus subtilis	—	27.57±1.35	8.27±0.08	—
Methicillin-resistant golden yellow staphylococcus	—	9.26±0.20	8.61±0.13	—
Bacillus thuringiensis	—	22.54+/-0.20	10.01+/-0.11	500
E.coli	—	17.51±0.62	8.14±0.06	—
Micrococcus luteus	—	23.97±0.98	—	—
Klebsiella pneumoniae	—	21.34±0.47	—	—
Polytolerant Pseudomonas aeruginosa	—	19.25+/-0.14	8.98+/-0.11	500

4.1.4. Verification test

According to the response surface analysis, the optimal parameters of solid-liquid ratio (g: mL) 1:16.25, extraction time is 69.04 min, extraction times are 3.18 times, and the yield of total flavonoids was predicted to be 7.502%. During the experiment, the process parameters were adjusted to the solid-liquid ratio (g:mL)1:16, extraction time 69 min, and extraction times 3 times. The experiment verified that the total flavonoids yield obtained by the adjusted process was 7.690%. Compared with the predicted value, RSD<5%, the relative error was small, indicating that the damage prediction ability was good and the reliability of the prediction results was high.[7]

On the basis of single factor experiment, the extraction conditions were optimized and Lg(34) orthogonal experiment was carried out.[8]

Table 3: Extraction rate of ginger root flavonoid components

Flavonoid	**Average recovery rate**	**RSD**	**Extraction ratio**	**In total the proportion of**
	%	**%**	**%**	**%**
3'-hydroxypuerarin	99.64	2.19	0.50+/-0.01	6.55+/-0.13
Puerarin	97.39	3.92	3.31±0.36	43.04±4.68
3'-methoxypuerarin	99.64	3.30	0.77±0.03	10.01±0.39
Puerarin-6''-0-xyloside	101.81	4.16	0.21±0.02	2.73±0.26
Daidzin	99.80	3.06	0.42±0.07	5.46±0.91
Puerarin apigenin	97.92	1.60	1.68±0.20	21.85±2.60
Genistein	100.72	4.12	0.07±0.03	0.91±0.39
On ononin	99.84	2.52	0.03±0.01	0.39±0.13
Daidzein	98.58	2.86	0.08±0.02	1.04+/-0.26

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4.2. Method of model establishment

Forty SPF grade male mice were raised in an animal room with a temperature of (26±2) ℃, humidity of 50%~60%, noise below 60 dB, and lighting for 12h (turn on the light at 8:00 and turn off the light at 20:00) for 7 days. They were randomly divided into 4 groups, which were as follows: Normal control group, CCl4 control group (CCL-induced liver injury in mice can be called model group), biphenyl diester group (biphenyl diester treatment of liver injury can be called positive control group), pueraria flavone extract experimental group, 10 mice in each group. Normal control group and CCl control group were given the same volume of normal saline every day. Bifendate group was given 200mg/kg bifendate and pueraria flavone extract was given 300 mg/kg pueraria flavone extract daily (dosage refer to Pharmacopoeia of the People's Republic of China [9]). Gavage was given once a day for a total of 7 days. Two hours after the last infusion, all groups except the normal control group were intraperitoneally injected with 0.1% CCl4 at the rate of 10mL/kg. After fasting for 16h, the indexes were determined.

4.3. Determination of biochemical indexes of mice

After the last infusion, the eyeball blood of the mice was fasted for 16h, and then the serum was separated by centrifugation under the conditions of 4°, 4 000r/min, and 20 min. The activities of ALT and AST and the content of MDA in the serum were determined. At the same time, liver tissue was taken from mice and placed at -70℃ and frozen for further use. According to the weight of liver tissue: the proportion of normal saline volume =1:9(g/mL), the liver tissue homogenate of 10% was prepared. The activities of SOD and GSH-Px in liver tissue were determined. The contents of ALT, AST and MDA in serum and SOD and GSH-Px in liver tissue were determined according to the operating instructions of the kit.

4.4. Data processing

Each trial was repeated 3 times and the results were averaged. The experimental data were sorted by Excel 2013, and designed and analyzed by Orthogonal Design Assistant Professional edition.

4.5. Assay results of AST and ALT activities in serum of mice

Compared with the CCl4 model group, the AST and ALT activities of pueraria flavonoid extract were significantly different (P<0.01), indicating that pueraria flavonoid extract

significantly reduced the activities of ALT and AST in serum induced by CCIl, and had a hepatoprotective effect. Compared with the normal control group, AST and ALT activities in CCl model group were significantly increased (P<0.01), indicating that CCl4 can cause liver injury in mice. Compared with CCl model group, AST and ALT activities in bifendate group were significantly decreased (P<0.05), but not as much as pueraria flavone extract group, indicating that bifendate can effectively treat liver injury, but the effect is not as good as pueraria flavone extract.

4.6. Determination results of MDA content in serum and GSH-Px and SOD activities in liver tissue of mice

Table 3: Determination results of AST and ALT activities in mice sera(n=10)

Group	AST activity	ALT activity
Normal control group	20.24±1.03	7.68±1.13
CCL model Group	101.58±9.50b	218.56±16.15b
Diphenyl ate group	52.12±5.71b	84.02±6.15b

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Table 3: (continued).

Pueraria flavonoid extract solution experimental group	41.23±4.64b	71.25±5.06b

Compared with the normal control group, the MDA content in serum of CCl4 model group was extremely significantly increased (P<0.01), while the activities of GSH-Px and SOD in liver tissue were extremely significantly decreased (P<0.01). Compared with CCl4 model group, the activities of GSH-Px and SOD in liver tissue of mice in bifendate group were significantly increased (P<0.05); MDA content in serum of experimental group was significantly decreased (P<0.05), and the activities of GSH-Px and SOD were significantly increased (P<0.01). The results indicated that Pueraria flavonoids extract could significantly reduce the MDA content in serum induced by CCI4, and increase the activities of GSH-Px and SOD in liver tissue of mice.

4.7. Discussion

In this study, response surface method was used to optimize the extraction technology of Pueraria root. The yield of total flavonoids was 7.690% under optimized technological conditions, which proved that the extraction technology was stable and feasible. The extracts were qualitatively and quantitatively analyzed. The results showed that The extraction rates of 3-hydroxypuerarin, puerarin, 3' -methoxy puerarin, puerarin -6-0-xyloside, daidzein, puerarin apigenin, genigeniside, ononin and daidzein were 0.50% soil 0.01%, 3.31%±0.36%, 0.77%±0.03%, 0.21%±0.02%, respectively. 0.42% ±0.07%, 1.68%±0.20%, 0.07%±0.03%, 0.03% soil 0.01%, 0.08% soil 0.02%, which accounted for 91.98% of the total pueraria flavonoids. This study is consistent with the research results of Zhao et al. [10]. The yield of puerarin, daidzein and daidzexitin from pueraria root by Hot water extraction method is 2.2%± 0.06%, 0.4%±0.03% and 0.03%±0.02%, respectively. Similar results were also obtained by Yang Qingqing et al. [11], who found that the yield of total flavonoids from pueraria root was 2.11% and 0.44%, respectively, significantly lower than the results of this study. In this study, the qualitative and quantitative analysis of flavonoids in the total flavonoids extract of Pueraria root was carried out, and the content of each flavonoid component and its proportion in the total flavonoids were clarified in detail, which enhanced the representativeness and integrity of the analysis results.

5. Conclusion

The extraction process of Pueraria root was optimized by response surface method, and a matching qualitative and quantitative detection method was established. The optimized extraction process was reasonable, feasible and efficient. The qualitative and quantitative analysis of Pueraria flavone groups showed that pueraria flavone could reduce the activity of ALT, AST and MDA in serum of mice, and increase the activity of GSH-Px and SOD in liver tissue of mice, indicating that Pueraria flavone had an obvious hepatoprotective effect. This experiment can provide reference for the extraction and application of Pueraria flavone. Limitations Complex composition and difficult quality control: The chemical composition of Puerarin is complex, and its content and proportion can be affected by different origins, harvest seasons, and extraction methods, making quality control difficult and difficult to ensure the stability and consistency of therapeutic effects. There is a lack of standardized fingerprint chromatograms and quality standards. Need to optimize extraction process The current extraction process may have problems such as low extraction rate, loss of active ingredients, and residual organic solvents, which need to be further optimized. Explore more efficient and environmentally friendly extraction methods, such as supercritical fluid extraction and enzyme-assisted extraction. Inadequate clinical studies: The current research on the liver-protective effects of

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Puerarin is mainly concentrated on in vitro experiments and animal experiments. There is a lack of high-quality clinical research data, making it difficult to evaluate its therapeutic effects and safety in humans. Inadequate pharmacokinetic studies. There is a lack of pharmacokinetic studies on the absorption, distribution, metabolism, and excretion of Puerarin, making it difficult to determine the optimal dosage and administration regimen. Future Research Directions: Establish an standardized quality control system. Establish a standardized fingerprint chromatogram and quality standard for Puerarin Phosphate, regulate its production and application, and ensure the stability and consistency of its therapeutic effects. Optimize extraction process: Develop more efficient and environmentally friendly extraction methods to improve the extraction rate and purity of active ingredients and reduce organic solvent residues. Deepen research on liver protection mechanism Utilize modern molecular biology techniques to deeply research the mechanism of Puerarin Phosphate's liver protection action, clarify its target sites and signaling pathways. Conduct high-quality clinical studies Conduct randomized, double-blind, placebo-controlled clinical trials to evaluate the efficacy and safety of Puerarin Phosphate in patients with different types of liver diseases. Develop new formulations: Research and develop new formulations of Puerarin Phosphate, such as nanoparticles and liposomes, to improve its bioavailability and therapeutic effects.

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