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Study on the Extraction Process and Pharmacological Activity of Gentiopicroside from Gentiana

Luo Dan*

Tibet Autumn Girl Cosmetics Co., Ltd., Lasa, Xizang, 850000,China

Corresponding author: Luo Dan , luodanxizang123456

Abstract: Gentiopicroside, as a representative substance among monoterpenoid compounds, is the main active ingredient in Gentianaceae plants. It can accelerate gastric emptying efficiency and enhance intestinal motility in the digestive system. In pharmacological studies, it also shows effects on protecting liver cells, inhibiting pain and inflammation. The brachychronic liver damage experiment in mouse was set up by inducing carbon tetrachloride CCl_4. The activity changes of serum alanine aminotransferase (ALT) and glutathione aminotransferase (AST) were measured. The liveness of philothion peroxisome (GSH-Px), peroxide dismutase (SOD) and malondialdehyde (MDA) details were prepared by liver tissue homogenate. The liveness intensity of serum ALT and AST in the liver of mice in each treatment group was compared from high to low. The liveness of GSH and SOD in liver tissue homogenate increased to varying degrees, and the content of MDA decreased to varying degrees. The study showed that gentiopicroside has a clear defend affect on acute liver damage abduction by CCl_4. Its mechanism of action may involve inhibiting free radical-mediated lipid peroxidation and effectively maintaining the functional stability of the antioxidant enzyme system of hepatocytes.

Keywords: Gentiopicroside; Digestive System; Intestinal Motility; Pharmacological Research; Hepatocytes

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Introduction

Gentiana scabra is a commonly used medicinal material in clinical practice. It is obtained from the dried rhizome system of Gentiana scabra and Gentiana scabra species of the Gentianaceae family [1-2]. In traditional medicine, it is widely known for its affect of eliminate temperature and moist, eliminate liver and cholecyst ignis, etc. This medicinal material is also rich in monoterpene glycosides, and contains multiple active components such as triterpene saponins, flavonoid aglycones and pyrrolizidine alkaloids [3]. Taking gentiopicroside C16H20O9 as an example, its water-soluble components have good solubility properties in polar solvents, and its crystal morphology is white to light yellow. Through the liver cell protection mechanism involving the activation of antioxidant enzyme systems and the inhibition of lipid peroxidation, it regulates the prostaglandin synthesis pathway to achieve anti-inflammatory and analgesic effects, and induces tumor cell cycle arrest and apoptosis signal transduction [4-5]. The disengaged jacobinic eliminate capacity of gentiana scabra is positively correlated with the liveness of endogenous antioxygen enzymic for instance SOD and GSH-Px.

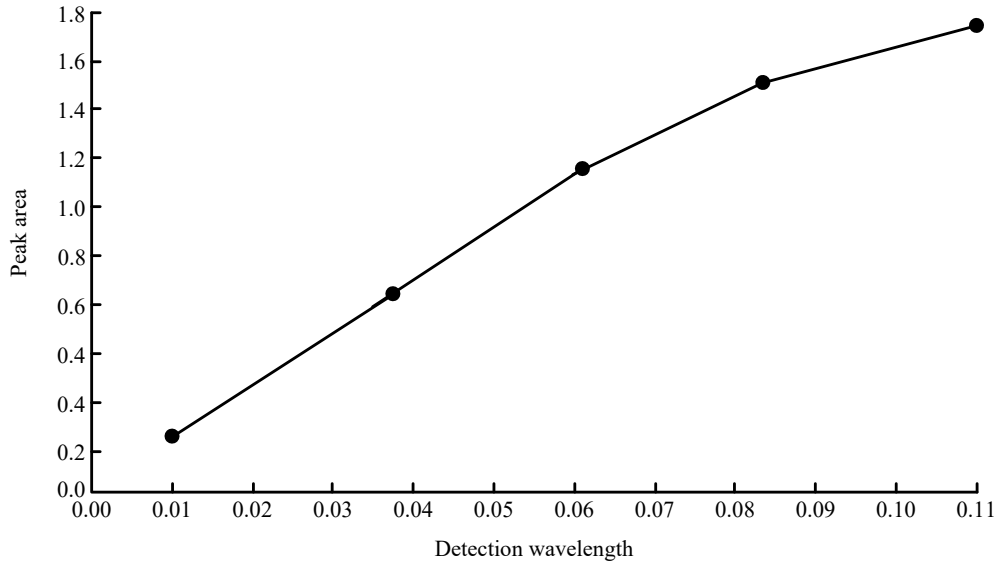
In terms of pharmacological activity, gentiopicroside exhibition a various of biologic liveness, including liver protection, pain relief, inflammation inhibition, free radical scavenging, and tumor growth inhibition. To further clarify the mechanism

of action of this ingredient. This experiment induced liver damage in mice with CCl_4, and detected the standard of SOD and MDA in liver tissue to reflect whether the drug has anti-liver damage effects. The consequence reveal that Qinling Gastrodia elata significantly increased the level of in the liver tissue of mice with liver damage, and reduced the level of, showing a good anti-liver damage effect. There were significant differences between the high, medium, and low dose groups and the model group, indicating that gentiopicroside may have a strong potential to prevent acute liver damage and can be developed as a liver protection drug.

1.Extraction technology of gentiopicroside

5.0 mg of gentiopicroside standard was accurately weighed, dissolution in carbinol and shift to a 25 ml volume bottle to prepare a standard stock solution [6]. 1.0, 2.0, 3.0, 4.0 and 5.0 ml of the inventory resolve were accurately shift to a 10 ml volume bottle, attenuation to the trail with carbinol, and filtered through a 0.45 μm microporous filter to obtain a series of gradient concentration standard solutions. HPLC was used for detection, with a Shimadzu C18 row (4.6 mm × 150 mm, 5 μm) as the chromatography column, water-methanol 75:25 (v/v) as the movement stage, the quantity of flow was 1.0 ml/min, the row heat was 25 °C, the test waveform length was 270 nm, the inject cubage was 10 μm, and the retention time of gentiopicroside was 9.932 min. A normal bight was established with quality density (mg/ml) as the standalone variate (X) and vertex acreage as the depend on variate (Y). The linear regression equation was obtained as $Y=1.7238\times10^7$, $X-3.5678\times10^5$, R2=0.9999. The standard curve of gentiopicroside is shown in Figure 1. This method has a very stable linear relationship.

Fig.1 Standard curve of gentiopicroside



1.1 Ultrasonic assisted extraction

Accurately weigh 0.5 g (+0.001 g) of the powder of the root of Gentiana macrophylla and place it in a 100 ml block off cone bottle. Increase 20 ml of chromatographic grade methanol. Place the mixed system in an ultrasonic extractor and perform dynamic extraction at 25°C for 30 min. After the extract is rapidly cooled in an ice-water bath, it is initially filtration through a 0.45 μm polyamides filtration membrane and mensurable shift to a 50 ml volume bottle. The cubage is adjusted to the trail with methanol and vortexed to mix [7]. Accurately shift 1.0 ml of the above resolve to a 5 mL volume bottle. After a second filling with carbinol, filtration it through a 0.45 μm organic phase filter membrane to obtain the test solution.

1.2 Low temperature maceration extraction method

Weigh 0.5 g (±0.001 g) of the powder of the root of Gentiana macrophylla accurately, place it in a 100 ml stoppered conical flask, and increase 20 ml of methanol solvent precooled to 4°C. After sealing, transfer it to a constant temperature oscillating box (4°C±1°C) and continue to extract at 150 rpm for 12 h. Filter the extract through a double-layer quantitative filter paper, transfer it quantitatively to a 50 ml volumetric flask, make up to volume with methanol, and vortex homogenize. Accurately measure 1.0 ml of the solution to a 5 ml volumetric flask, dilute it twice with methanol to make up to volume, and filter it through a 0.45 μm organic phase filter membrane to prepare a parallel sample solution.

2.Experimental Materials and Methods

2.1 Drugs and reagents

Gentianopicroside (experimentally prepared, purity ≥ 99.0%); peanut oil (purchased from a large supermarket); CCI_4 (Sichuan Laiyun Fine Chemical Co., Ltd.); philothion peroxisome (GSH-Px) reagent, peroxide dismutase (SOD) reagent, malondialdehyde (MDA) assay kit, aminopropionic acid transaminase (ALT) and aspartate transaminase (AST), all of which were buy from Beijing Biotechnology Co., Ltd. [8].

2.2 Instrument

Electronic analytical balance (Shenzhen Tianqin Instrument Co., Ltd.); electronic constant temperature water bath (Fujian Hengke Biotechnology Co., Ltd.); UV800 ultraviolet-visible spectrophotometer (Shimadzu, Japan); desktop high-speed refrigerated centrifuge (Guangzhou Everbright Environmental Protection Equipment Co., Ltd.); glass homogenizer (Jinhua Hongfu Chemical Technology Co., Ltd.).

2.3 Method

The intervention effects of different doses of gentiopicroside on acute liver injury were systematically evaluated by measuring the changes in the liveness of aminopropionic acid transaminase (ALT) and aspartate transaminase (AST) in the plasma of experimental animals, and analyzing the liveness of peroxide dismutase (SOD) and malondialdehyde (MDA) details in liver organization. As a key enzyme for clearing peroxides, the reduce in SOD liveness and the augment in MDA levels reflect the state of oxidative stress, which is an important mechanism of CCI_4-induced liver injury.

A total of 55 mice were choice for the test and stochastic cut into 5 teams (11 mice in every team) after 20 days of self-adaption feed: normal command team, template team, and low-, medium-, and high-dose gentiopicroside groups. The intervention group was intragastrically administered at a preset dose daily, and the command team was given an equality cubage of salt brine for 15 consecutive days. Two hours after the last administration, besides the normal command team, the other groups were inside the abdominal cavity injection with 0.05% CCI_4 peanut oil solution to construct an acute liver injury model, and the normal team was injection with an equal amount of peanut oil. The mouse were eliminate one day later, and sanguis and liver tissue specimen were gather. After the whole sanguis specimen were allowed to stand at 4°C for solidification, they were centrifuged at 3000 r min to separate serum for subsequent biochemical index detection.

The consequence reveal that the serum ALT and AST activities in the gentiopicroside intervention team were obvious reduced, while the SOD activity of liver tissue increased and the MDA content decreased, instructions that it can effectively remission the oxidation pressure respond. The conclusion suggests that gentiopicroside has a dose-dependent defend affect on CCI_4-abduction acute liver damage, and its machine-made may be to improve hepatic cell function by inhibiting lipid peroxidation and maintaining antioxidant enzyme activity [9].

After the whole sanguis specimen were allowed to stand at 4°C for solidification, they were centrifugal at 3000 r min for 10 min to acquire plasma, and the plasma ALT and AST enzyme liveness were determined by ultraviolet spectrophotometry. After the liver tissue was irrigated with pre-cooled saline, 0.4-0.6 g of liver lobe tissue was accurately weighed and added with 4°C saline at a ratio of 1:9 (W/V). A 10% slurry was prepared by mechanical homogenization for 10 min under ice bath conditions. The supernatant was collected after low-temperature centrifugation at 3000 r min for 15 min. The standard operation was carried out strictly according to the instructions of the kit. The activity and activity of glutathione peroxidase GSHP ($U \cdot mg^{-1}$), superoxide dismutase SOD ($U \cdot mg^{-1}$) and malondialdehyde MDA ($U \cdot mg^{-1}$) in the liver tissue homogenate were determined by ultraviolet-visible spectrophotometer [10].

3.Experimental Results

As the core metabolic and detoxification organ of the body, the liver is very susceptible to pathological damage under the influence of drugs, viruses and other factors. Traditional Chinese medicine has reveal unique advantages in the field of liver damage prevention and treatment, and plays a liver-protecting role through multiple mechanisms, such as: scavenging oxygen free radicals, alleviating oxidative stress, regulating the inflammatory factor network, and regulating immune homeostasis. These effects work together to protect the structural integrity of liver cells and reduce the degree of pathological damage.

Using modern research technology to deeply explore the liver-protecting mechanism of traditional Chinese medicine and develop highly effective and low-toxic natural liver-protecting drugs has important clinical value.

3.1 Affect of gentiopicroside on liver mass and liver index

Carbon tetrachloride (CCl_4)-abduction liver damage model is widely used in the study of the machine-made of action of liver protection drugs due to its clear pathological indicators and good repeatability. In this experiment, an brachychronic liver damage template of mouse was set up by intraperitoneal inject of CCl_4. It was observed that after the integrity of the liver cell membrane was destroyed, the liveness of plasma aminopropionic acid transaminase (ALT) and aspartate transaminase (AST) augment significantly, and the liver organ coefficient increased synchronously ($P<0.01$), verifying that the model was successfully constructed. The gentiopicroside intervention group showed a dose-dependent decrease in serum transaminase (ALT/AST) activity, and the difference between the medium and high dose team was statistics obvious ($P<0.05$) or ($P<0.01$), and the liver index was also obvious reduced ($p<0.01$), indicating that it has a clear liver protective effect.

Table 1 Affect of gentiopicroside on small liver mass and liver index in CCl_4-induced acute liver injury

Group	Dose/(g·kg^{-1})	Weight/g	Liver mass/g	Liver index%
Normal command group	-	28.50±3.70	1.26±0.17	4.36±0.35$^{2)}$
CCl_4 group	-	28.70±5.60	1.85±0.47	6.12±0.41$^{2)}$
Low dose group	0.05	27.80±2.70	1.45±0.17	4.96±0.18$^{2)}$
Medium measurement group	0.10	27.10±4.45	1.36±0.14	4.63±0.49$^{2)}$
High dose group	0.20	27.15±3.20	1.54±0.11	5.58±0.41$^{2)}$

Note: Compared with CCl_4 template team $^{1)}P<0.05$,$^{2)}p<0.01$。

3.2 Effects of gentiopicroside on the liveness of ALT and AST

According to the data comparison between the CCl_4 template team and the normal command group, the serum AST and ALT activities in the model group showed a significant increase ($p<0.01$), indicating that the acute liver injury model was successfully constructed. Compared with the model group, gentiopicroside and each dose group of the positive control group significantly inhibited the trend of increased ALT and AST activities ($P<0.05$) or ($p<0.01$). There was no statistical difference in the ALT/AST ratio of all experimental groups, indicating that the release of the two transaminases during CCl_4-induced liver injury was synchronous, and the intervention of gentiopicroside did not change this ratio characteristic. There was no obvious difference in the ALT and AST activity levels between the gentiopicroside dose groups and the positive control team. Table 2 shows the effect of gentiopicroside on ALT/AST activity, suggesting that its liver-protective effect may have similar efficacy to that of positive drugs. This result further verifies the potential mechanism of gentiopicroside in alleviating liver cell damage by regulating the transaminase release pathway.

Table 2 Effect of gentiopicroside on ALT/AST activity

Group	Dose/(g·kg^{-1})	ALT/(U·L^{-1})	AST/(U·L^{-1})	ALT/AST
Normal command team	-	45±22$^{2)}$	156±46$^{2)}$	0.39±0.15
CCl_4 team	-	428±156$^{2)}$	476±108$^{4)}$	0.94±0.62
Low dose team	0.05	137±58$^{2)}$	260±86$^{1)}$	0.91±0.79
Medium measurement group	0.10	107±32$^{2)}$	196±49$^{2)}$	0.93±0.50
High dose team	0.20	161±42$^{1)}$	303±54$^{1)}$	0.84±0.36

3.3 Effects of gentiopicroside on GSH-Px, SOD activity and MDA details

The liveness of philothion peroxisome GSH-Px and peroxide dismutase SOD in the liver of mouse in the CCl_4 model team were obvious decreased compared with those in the normal control team ($p<0.01$), while the content of malondialdehyde

(MDA), the end product of lipid peroxidation, was significantly increased ($p<0.01$), instructions that the brachychronic liver injury template was successfully set up. Compared with the template team, the activities of liver tissue ($p<0.01$) and SOD were significantly augment ($P<0.05$) or ($p<0.01$) after intervention in the low, medium and high doses of gentiopicroside groups, while the content of MDA was obvious reduce ($P<0.05$) or ($p<0.01$). Further analysis showed that there was no statistical difference in the MDA standard between the gentiopicroside team and the positive command team, and its GSH-Px and SOD activities were also similar to those of the positive control team, suggesting that the two team were comparable in antioxidant effect. The inhibitory effect of gentiopicroside on lipid peroxidation showed a dose-dependent enhancement trend. The effects of gentiopicroside on CCI_4, GSH-Px activity and MDA details are reveal in Table 3, indicating that its protective effect is closely related to the administration concentration.

Table 3 Effects of gentiopicroside on CCI_4, GSH-Px activity and MDA details

Team	Dose/(g·kg^{-1})	GSH-Px/U·mg^{-1}	SOD/U·mg^{-1}	MDA/nmol·mg^{-1}
Normal control group	-	295.67±47.84[2)]	226.76±14.79[2)]	0.72±0.19[2)]
CCI_4 group	-	191.59±40.58[4)]	159.95±16.45[4)]	2.43±0.67[4)]
Low dose group	0.05	29.93±45.90[1)]	190.45±16.66[2)]	1.05±0.15[2)]
Medium measurement group	0.10	256.76±36.79[1)]	197.88±13.56[2)]	0.89±0.16[2)]
High dose group	0.20	199.25±38.40[4)]	183.46±13.28[1, 3)]	1.29±0.25[1)]

4.Discussion

4.1 Discussion on experimental model and indicators

The brachychronic liver damage model abduction by carbon tetrachloride (CCI_4) has become a classic experimental method for evaluating the mechanism of action of hepatoprotective drugs because of its clear indicators and strong repeatability, and it can systematically reflect the functional and morphological changes of hepatocytes. In this experiment, an brachychronic liver damage template was set up in mouse by intraperitoneal inject of CCI_4, and the dynamic changes of serum aminopropionic acid transaminase (ALT) and aspartate transaminase (AST) liveness were observed after the integrity of the hepatocyte membrane was destroyed to quantify the degree of liver damage. The serum ALT and AST liveness of mouse in the CCI4 template team were obvious higher than those in the normal command team ($p<0.01$), and the liver index was also obvious augment ($p<0.01$), indicating depend on that acute liver injury was successfully established. After intervention with gentiopicroside, the plasma ALT, AST activities and liver index of mouse in each dose group showed a dose-reduce ($P<0.05$) or ($p<0.01$), suggesting that it has a clear defend affect on CCI_4-induced liver damage. This result further verifies the potential mechanism by which gentiopicroside exerts a hepatoprotective effect by regulating the release of transaminases and alleviating pathological changes in hepatocytes.

4.2 Antioxidant system mechanism of action

The core function of philothion peroxisome GSH-Px is to catalyze the decomposition of hydrogen peroxide and inactivate it by combining with metabolic toxic products; superoxide dismutase SOD, as a key component of the endogenous free radical scavenging system, can efficiently scavenge superoxide anion free radicals. The synergistic effect of the two constitutes the core of the antioxidant defense system in mice, inhibiting lipid peroxidation reactions and protecting biological membrane structures from oxidative damage by activating endogenous antioxidant mechanisms. Its activity level directly determines the dynamic balance between oxidative stress and antioxidant capacity of the body.

During CCI4-induced liver injury, the liveness of GSH-Px and SOD reduce obvious, while the details of malondialdehyde (MDA), the end product of lipid peroxidation, increased obvious. MDA can further destroy cell membrane function by cross-linking with biomacromolecules to form complexes, and its details changes can indirectly reflect the degree of damage to tissues or cells by free radicals. By quantitatively analyzing the liveness of GSH-Px, SOD and MDA standard in liver

organization, the degree of lipid peroxidation and the pathological progression of free radical-mediated liver injury can be systematically evaluated, providing a key experimental basis for revealing the mechanism of antioxidant intervention.

4.3 Molecular mechanism and protective effect

Experimental data showed that the content of malondialdehyde (MDA) in the liver of mice in the CCI_4 model group showed a very significant increase ($p<0.01$), indicating that CCI_4-induced acute liver injury is closely related to the inhibition of antioxidant enzyme activity and lipid peroxidation. After intervention with gentiopicroside, the activities of glutathione peroxidase GSH-Px and peroxide dismutase SOD in liver tissue of each dose team were obvious enhanced compared with the model team ($P<0.05$) or ($p<0.01$). At the same time, the details of MDA decreased obvious ($p<0.01$). This result shows that gentiopicroside can effectively block the initiation of lipid peroxidation chain reaction by maintaining the activity level of antioxidant enzymes and enhancing the body's capacity to scavenge oxygen jacobinic, significantly reducing the accumulation of MDA in the liver, and ultimately alleviating CCI_4's acute damage to liver tissue. Its specific mechanism may involve regulating the function of the endogenous antioxidant system to maintain the homeostasis of enzyme activity, but it still needs to be further verified through molecular pathway research.

5.Conclusion

Experimental data reveal that the details of malondialdehyde (MDA) in the liver of mice in the CCI_4 template team showed an extremely significant increase ($p<0.01$), which was closely related to the inhibition of antioxidant enzyme activity and CCI4-induced acute liver damage and lipid peroxidation. After intervention with gentiopicroside, the liveness of philothion peroxisome GSH-Px and peroxide dismutase SOD in the liver tissue of the above-dose groups were obvious improved compared with the model command team ($P<0.05$) or ($p<0.01$), and the MDA content was obvious decreased ($p<0.01$). The results showed that gentiopicroside can maintain the level of antioxidant enzyme activity and enhance the body's capacity to scavenge oxygen disengaged jacobinic, thereby effectively blocking the initiation of lipid peroxidation chain reactions, significantly reducing the accumulation of MDA in the liver, and ultimately alleviating CCI_4's acute damage to liver tissue. Its specific mechanism may involve regulating the function of the endogenous antioxidant system to maintain enzyme activity homeostasis, but it still needs to be further verified through molecular pathway research.

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Conflict of Interests

The author(s)declare(s) that there is no conflict of interest regarding the publication of this paper.

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