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Hepatoprotective Activity of the *Odontites vulgaris* Moench herb against Carbon Tetrachloride Toxicity and Evaluating its Standardization Parameters

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O.vulgaris Moench is known for its medicinal properties, mainly to remove blood fever and inflammation. The extent of investigation aims to study the activity of O.vulgaris (OV), a medicinal plant, on hepatic acute injury-induced CCl4 in rats and establish the requirements of the standard for this medicinal plant. Wistar rats were induced to the CCl4 model with or without OV (112, 224, 560 mg/kg b.w) co-administration for 7 days. Identifications for standardization study were that the diagnostic cellular structures in O vulgaris were defined by light microscopy, and apigenin, luteolin, and aucubin were revealed using the TLC method. The flavonoids were measured 4.3±0.62% and iridoid content was 4.86±0.93%. Some parameters for quality and safety of the medicinal plant were estimated as moisture 5.3±0.5%, ash 4.5±0.3%, insoluble ash in hydrochloric acid 1.2±0.1%, extractable in water 23±1.5%, total aerobic microbial 3*102 and total yeast and mold 2*10. OV administration (112 and 224 mg/kg b.w.) decreased hepatocellular damage, AST, ALT in CCl4-treated rats. OV clearly lowered the direct bilirubin and, MIP-1a and MCP-1 in the serum of CCl4-treated rats. OV treated group lowered the level of CTGF increased the concentration of SOD and reduced structural changes in liver tissue all over the acute liver injury. We concluded that the study results in the hepatoprotective activity of CCl4-caused acute injury by O.vulgaris in rats, the quality control criteria for O.vulgaris were defined, and the monograph's draft Mongolian National Pharmacopeia for the medicinal plant was updated.

Keywords: Apigenin; Aucubin; Carbon tetra chloride; Hepatoprotective activity; Luteolin.

Liver injury induced by carbon tetrachloride (CCl_4) is a widely used experimental model to study hepatotoxicity.[1] CCl_4 causes acute liver injury primarily through the generation of reactive metabolites that induce oxidative stress, lipid peroxidation, inflammation, mitochondrial

dysfunction, and hepatic fibrosis. The model is valuable for studying mechanisms of liver injury and testing potential therapeutic agents. [2-3]

O.vulgaris Moench or Red bartsia a common medicinal plant that belongs to the family *Orobanchaceae* and is distributed in several parts of Europe and Asia including Mongolia. [4] In Mongolia, *O. vulgaris* is recognized for its medicinal effects mostly, to remove blood fever and inflammation. Four Tantras mention this herb as a traditional Tibetan and Mongolian medicine for such as cooling fever of blood, lung, and liver acute diseases. *O.vulgaris* is included in 25 traditional drugs, and it is the 17th most frequently used in Mongolian and Tibetan medical prescriptions, among Mongolian medicinal plants. [5-9]

The stem of *O. vulgaris* grows to a height of 15-40 cm and is branched from the base the from below, hairy in some places, leaves are 1.5-2.5 cm long, 1.5-7 mm wide, the central vein is thick, and with small teeth along the margin. The flower is pink-red, the petals are 10 mm long, the pistil-silique seeds are oblong 7-8 mm long, and the pollen is densely located inside the flower.

Distributions in phytogeographical regions of Mongolia Khentei; Khangai; Mongolian Dauria; Mongolian Altai; Middle Khalkha; East Mongolia; Depression of Great Lakes; Valley of Lakes; Dzungarian Gobi.[5]

The main biologically active ingredients are *O.vulgaris* flavonoids, iridoids, and phenol carboxylic acids. Additionally, O. vulgaris contains a diverse range of bioactive compounds, including terpenoids, sterols, flavonoids, phenols, lipids, lignans, alkaloid, and benzene sulfonic acid, iridoid glycosides.[10-14] These chemical components have many areas of biological effects such as reducing inflammation, antibacterial, inhibiting oxidation, and anticancer. Also, some types of terpenoids have antimicrobial, antioxidant, inhibiting the formation tumors, protect the liver damage, anti-inflammatory, and flavonoids have a lot of biological effects such as anti-inflammatory, antioxidant, anticancer, antimicrobial and antiviral activities. [15-20] Recent studies have showed that *O.vulgaris* has anti-rheumatoid arthritis, antioxidant, cytoprotective, and aflatoxin B1 inhibitory properties. [11.13.14]

This study aims to evaluate the hepatoprotective activity of flavonoid-rich extracts of *O. vulgaris* in CCl_4, -induced acute liver injury in rats and standardize its active phytochemical components.

MATERIALS AND METHODS

Chemicals and reagents

Standards, chemicals, and pharmacological reagents

Luteolin, apigenin, and aucubin standards were used from Sigma Aldrich (USA). All other reagents and solvents were analytical grade. The ELISA kits for macrophage inflammatory protein-1a (MIP-1á), monocyte chemotactic protein-1 (MCP-1), and superoxide dismutase, connective tissue growth factor (CTGF) were purchased from MLBIO Biotechnology Co.Ltd (Shanghai, China) and used in this study.

Plant material and extraction

Plant samples of *O.vulgaris* were collected from Bulgan province, Mongolia in 2023. Its species was identified by T.Munkh-erdene, a botanical curator and taxonomist at Botanic Garden and Research Institute, MAS. We extracted 1:10 infusum from the O.vulgaris (OV) herb and used it in the hepatoprotective effect experiment. 10 g crushed dried plant material was suspended in 250ml water and boiled till **water evaporated** to **100 ml.**

Microscopic examination

Tissue micro-sections were prepared using a freezing microtome (VCM-202III). The micro-preparations are prepared using clarifying fluid ($C_2H_3CI_3O_2$) and 5-15% NaOH. The cell wall is dyed by alcian blue, methylene blue, saffron, and glycerin ($C_2H_5(OH)_3$). The anatomical structure is determined using a light microscope "NOVEL". Images are taken with a digital camera for the microscope. [21]

TLC identification of apigenin and luteolin

To identify the flavonoids in a sample of *O. vulgaris*, 1 g of the sample was extracted using 20 mL of 40% ethanol through a reflux for 20 minutes. After cooling, 10 mL of 10% hydrochloric acid was added, and the mixture was refluxed again for another 30 minutes. Once cooled, the extract was shaken with 20 mL of chloroform in two separate rounds. The chloroform fractions were collected, combined, and evaporated to obtain dry residues, which were then dissolved in methanol to create

sample solutions for Thin-Layer Chromatography (TLC). Reference solutions of apigenin and luteolin were prepared at 1 µL/mL in methanol. For the TLC, 10 µL of the sample and reference solutions were applied onto TLC plates (Merck Silica Gel 60 GF 254). The chromatography was conducted using an eluent consisting of hexane, ethyl acetate, and acetic acid in a ratio of 30:15:5 (v/v). After allowing the plate to dry at 20^0C-25^0C temperature, it was sprayed with a 3% aluminum chloride solution in ethanol and analyzed under UV light at a wavelength of 365 nm. The retardation factor (Rf) value which is the ratio of the standard's distance developed to the solvent's distance developed was calculated.[3]

TLC identification of the iridoids

A 1 g sample of *O.vulgaris* was extracted with 25 mL of methanol via reflux for 20 minutes. After cooling, the extract was used as the sample solution for TLC. A reference solution of aucubin was prepared at a concentration of 5 µL/mL in methanol. Both 10 µL of the sample solution and 10 µL of the reference solution were applied to TLC plates (Merck Silicagel 60 GF 254). The chromatographic separation was carried out using an eluent consisting of formic acid, water, ethyl acetate, and acetone in a 1:1:5:5 (v/v) ratio. Once the plate had dried at 20^0C-25^0C temperature, it was sprayed with a 5% anisaldehyde-sulfuric acid solution and heated at 105°C for 5-10 minutes for visualization.[4]

Fig. 1. *O.vulgaris* Moench

Total Flavonoid Content

In this procedure, 1 g of *O.vulgaris* was extracted with 50 mL of 70% ethanol by refluxing for 40 minutes. After the extraction, the solution was allowed to cool and was then filtered. To assess the total flavonoid content, 3 mL of the test solution was transferred into a 25 mL flask. To this, 1 mL of 5% sodium nitrite, 1 mL of 10% aluminum nitrate, and 10 mL of 4% sodium hydroxide were added. The absorbance of the solution was measured at 500 nm using a spectrophotometer. The total flavonoid content of the extract was quantified and expressed as the equivalent of luteolin (mg of LU/g of extract) [24].

Total Iridoid Content

The total iridoid content was determined using a colorimetric method. A 0.4 mL test solution was transferred to a 10 mL volumetric flask, to which 4 mL mixture of acetic acid, copper II sulfate, and concentrated hydrochloric acid 10:1:0.5 was added. The mixture was then heated at 70°C for 10 minutes. After cooling, the absorbance of the solution was measured at 609 nm. The iridoid content was quantified utilizing a standard curve prepared with aucubin as the reference standard [25,26].

Induction of CCL4 acute injury and animal treatment

The acute CCL4 model developed by Handa and Sharma and Idris Türel et al. was utilized to plan the dosing schedule. To induce acute liver toxicity, an injection of 0.8 ml/kg of CCL4 (diluted 1:1 with olive oil) was administered intraperitoneally.[27,28] 5 groups and each contains (n = 7) 1. The control group was administered only physiologic saline, 2. CCL4 group olive oil 1:1 (0.8 ml/kg) received physiologic saline, 3. CCL4+*O.vulgaris* consistently 112 mg/kg (CCL4+OV 112 mg/kg) olive oil (1:1) (0.8 ml/kg) intraperitoneal injection, 4. CCL4+*O.vulgaris* 224 mg/kg (CCL4+OV 224 mg/kg) olive oil (1:1) (0.8 ml/kg) intraperitoneal injection, 5. CCL4+*O. vulgaris* 560 mg/kg (CCL4+OV 560 mg/kg) olive oil (1:1) (0.8 ml/kg) intraperitoneal injection respectively. The regimen was once daily and all injections were given once daily with CCl4 for seven days. At the end of the study (day 8), blood samples were collected by cardiocentesis,

and the rats were sacrificed with an overdose of pentobarbital sodium (100 mg/kg, IP), and liver samples were taken for histopathological examination.

Measurement of serum aminotransferase activity, bilirubin amount, and assay of MIP-1á, and MCP-1, CTGF levels in serum

Blood samples were collected , centrifuged at 2500 rpm for 15 minutes, and the serum was stored at -20^0 C for further analysis . Assay of enzymes aspartate aminotransferase and alanine aminotransferase, certain inflammatory cytokines macrophage inflammatory protein-1á, and monocyte chemoattractant protein-1 and anti-oxidation, growth factors as superoxide dismutase, and connective tissue growth factor were determined by enzyme-linked immune sorbent assay. (Elisa Shanghai MLBIO Biotechnology Co. Ltd.) kits specific for the detection of these factors, and the absorbance was measured at 450 nm by a plate reader (Chromate 4300 microplate, Shanghai MLBIO Biotechnology Co. Ltd., China).[29,30]

Histopathological examination

After the hepatic specimens were fixed in 10% formalin for 24 hours. Histopathological specimens were fixed in a 10% neutral buffered formalin solution for more than 24 hours, washed with running water for 24 hours, dehydrated with graded ethanol, embedded in melted paraffin wax, sectioned (3-5 ìm thick) using a sliding microtome (Yamato Kohki, Japan), and stained with haematoxylin and eosin (HE) (Sigma Aldrich). Sections were examined and photographed by light microscopy (Nikon Eclipse Ci, Japan).[21] A total 30 slides were subjected to histological assessment.[27,28]

Statistical analysis

Data are presented as the mean ± standard deviation (SD). Statistical analyses were conducted using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). The statistical significance of differences was analyzed exerting the Kruskall-Wallis test, where $P<0.05$ was considered to indicate a statistically significant difference.

RESULTS

Microscopic structure of the *O.vulgaris*

The transverse section of the stem exhibits a diamond-shape, with a a single layers epidermis on the outermost surface.. Underneath the epidermis is a superficial parenchyma layer consisting of densely 3-4 layers with a thick cell wall. Simple trichomes are located evenly on the surface of the stem. The xylem and phloem of the vascular bundle are formed by a closed ring surrounding the stem. The outer vascular bundle has sclerenchyma cells with more developed. The central region of stem contains sparsely distributed pithy parenchyma cells. The xylem of the vascular bundle is more developed. Outside, the vascular bundle is endodermis composed of a single layer (Figure 1, A, A1, A2).

The leaf is dorsaventral. The chlorenchyma is sparsely distributed palisade tissue, composed of a single layer, and sparsely spongy tissue composed of 4-5 layers. The epidermal cell wall is obtuse. Both simple unicellular trichomes and multicellular trichomes are present on the adaxial and abaxial epidermis. Sunken anomocytic stomata are evenly distributed across all surfaces of the leaf. The collateral vascular bundle is located between the spongy tissues. The main vascular bundle is more developed (Figure 1, B1, B2, B3).

Thin-layer chromatography (TLC) analysis

Luteolin, apigenin, and aucubin were identified in *Odontites vulgaris* from Mongolia by TLC for standardization (Figure 2).

Rf of apigenin and luteolin were 0.43 and 0.31, respectively. When flavonoids were hydrolyzed, they were revealed and a suitable eluent was acetic acid-ethyl acetate-hexane (5:15:30, v/v) for flavonoids. Aucubin was available for identification in methanol extract directly and the Rf value was 0.51.

The total content of biologically active compounds and general requirement

The flavonoid content of the *O.vulgaris*, expressed as luteolin equivalent, ranged from 4.0 to 40.0, based on the standard curve (equation: $y = 0.0108x - 0.0012$, $r^2 = 0.9963$). The flavonoid concentration was estimated to be between 3.9% and 4.79%. For iridoids, quantified as aucubin equivalent utilizing the calibration curve (equation: $y = 9.5981x + 0.0132$, $r^2 = 0.966$), the content ranged from 3 to 18 µg/mL.

Quality control parameters of the phytochemicals were conducted in accordance with WHO guidelines for assessing the quality of herbal medicines, including references to contaminants and residues, *Quality control*

methods for medicinal plant materials, and Mongolian national pharmacopeia. [31-33]

Hepatoprotective effects of *O.Vulgaris*

Effect on ALT,ASTand Bilirubin levels

In the rat model of CCL4 induced acute liver injury , the evidence of severe liver cell damage and lysis is that the serum AST level in the CCl4-treated group was three times higher than in the control group, indicating severe hepatocellular damage ($p<0.001$), which is an acute injury of acute liver cell injury and necrosis indicates that it has arisen. The group treated with *O. vulgaris* was reduced by 53.2%, 58.2% at 112 and 224 mg/kg doses, and 41.6% at 560 mg/kg dose($p<0.001$).

ALT enzyme activity in serum is an important parameter in determining severe damage and destruction of liver cells in the group of intoxication by CCL4 in rats. This result shows that the level of ALT enzyme increased 3.4 times ($p<0.001$) in the CCL4-treated group compared to the non-treated or control group, indicating the worsening of hepatocellular injury. However, the group treated with *O. vulgaris* was reduced by 84.5%-89.3% ($p<0.001$) at all doses compared to the CCL4 group. The level of direct bilirubin in the serum, of the CCL4 group (0.568±0.23 mg/dL) compared to the control group (0.146±0.04 mg/dL) was statistically significantly increased by 3.8 times ($p<0.05$). In comparison to the CCL4 group (0.568±0.23 mg/dL), the group treated with *O. vulgaris* showed a reduction of 61.7% at the dose of 224 mg/kg (0.217±0.05 mg/dL) and 56.3% at

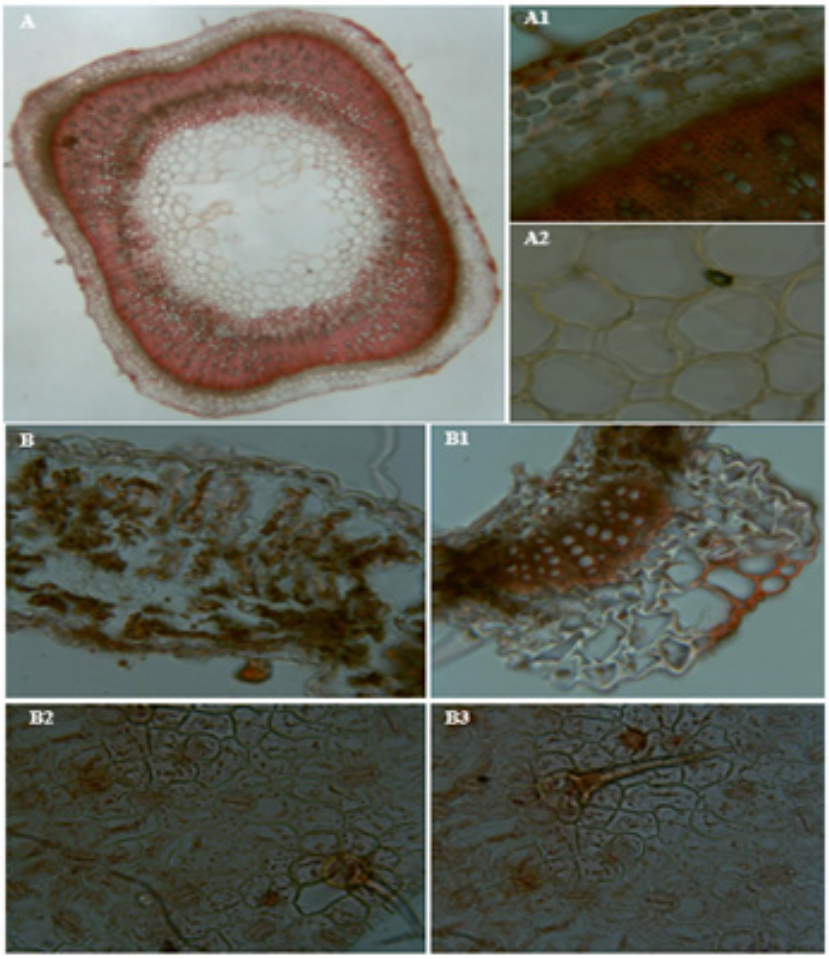

Fig. 2. Microstructure of the *O.vulgaris*. A. Stem cross-section (10x4), A1. Stem external part (10x40), A2. Pithy parenchyma of the stem (10x40), B. Leaf microscopic structure (10x40), B1. Main vascular bundle (10x40), B2. Adaxial epidermis (10x40), B3. Abaxial epidermis (10x40)

the dose of 560 mg/kg (0.239±0.09 mg/dL), which was statistically significant ($p<0.05$). According to the outcomes of the study, *O. vulgaris* has activity in reducing the necrosis of liver cells, inhibiting the activity of AST and ALT in serum, and lessening the concentration of direct bilirubin.

Anti inflammatory effects of *O. Vulgaris* (MIP-1a and MCP-1)

MCP-1 levels increased by 26% ($p<0.05$) in the CCl_4 -intoxicated group (147.0±9.3 pg/ml) compared to the control (108.9±2.9 pg/ml), indicating an inflammatory response to liver injury. However, the OV-treated group decreased by 18%, 28%, and 26% In the *O. vulgaris* -treated group, there was a decrease of 18%, 28%, and 26% in measurements, recorded as 124.7±8.2 pg/ml at the 112 mg/kg dose, 106.2±9.5 pg/ml at the 224 mg/kg dose, and 108.8±6.3 pg/ml at the 560 mg/kg dose ($p<0.05$).

Table 1. Parameters for standardization and safety

Parameter	Result
Total flavonoids	4.3±0.62%
Total iridoids	4.86±0.93%
Foreign matter	Absent
Loss on drying	5.3±0.5%
Ash	4.5±0.3%
Insoluble ash in hydrochloric acid	1.2±0.1%
Extractable in water	23±1.5%
Total aerobic microbial	$3*10^2$
Total yeast and mold	2*10

In CCL4 intoxication, the MIP-1á cytokine showed a significant increase of 62% ($p<0.05$), reaching levels of 71.81±9.51 pg/ml in the CCL4 group, compared to 44.33±3.39 pg/ml in the control group. Treatment with a 112 mg/kg dose of *O. vulgaris* resulted in a decrease to 50.54±9.28 pg/ml, reflecting a 30% reduction. Additionally, at doses of 224 mg/kg and 592 mg/kg of OV, MIP-1á levels were further reduced to 49.65±7.62 pg/ml (31%) and 53.92±5.48 pg/ml (25%), respectively, both with statistical significance ($p<0.05$)

Additionally, the CTGF levels were found to be 354.4±8.2 pg/ml in control rats, and this increased by 21.2% to 429.8±10.3 pg/ml in the CCL4 model ($p=0.05$). Significantly, all doses of *O. vulgaris* showed a substantial decrease in CTGF levels compared to the CCL4 group ($p<0.05$). Similarly, the superoxide dismutase levels were determined to be 5.18±0.69 ng/l in control rats, and these decreased by 25% to 3.84±0.43 ng/ml in the CCL4 intoxicated group ($p<0.05$). However, the SOD levels of medium and high doses of *O. vulgaris* increased significantly compared to the CCL4 group ($p<0.05$). These results, presented well, have important implications for our understanding of the subject and the potential for future research and applications.

Histopathological analysis

The livers in the control group (Figure 5A) displayed a well-defined structure of the surface capsule (Glisson). The borders of the lobules were indistinct, and the hepatocytes, which serve as the functional units of the liver, were organized

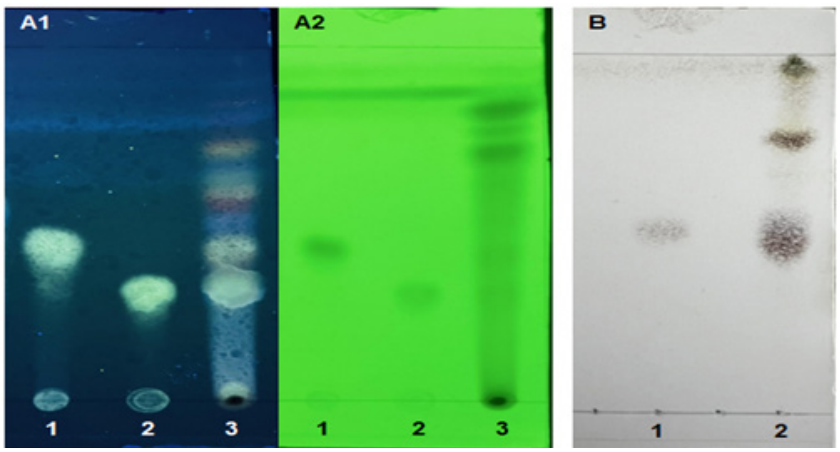

Fig. 3. Result of TLC of *O.vulgaris*. A1-2. TLC of flavonoids, 1-apigenin, 2-luteolin, 3-*O.vulgaris.* B. TLC of iridoid, 1-aucubin, 2- *Odontites vulgaris*

in a columnar arrangement surrounding the central vein, along with the artery and bile duct. In the CCL4 group (Figure 5B), extensive fatty degeneration was observed. This was characterized by numerous cytoplasmic lipid droplets, hepatocyte enlargement, and the displacement of nuclei toward the periphery. Additionally, some nuclei exhibited signs of dissolution, leading to granular necrosis. The sinusoidal spaces of cells displaying these pathological changes were reduced. These microstructural alterations suggest the establishment of a pathological model in the liver. In the experimental group receiving *O.vulgaris* at a dose of 224 mg/kg, a relatively small number of cells in the peripheral region of the liver showed signs of necrosis. Their cytoplasm contained fat-like droplets of varying sizes, with some cells appearing enlarged and their nuclei displaced to the edges. There was also a minor amount of hyperemia present in the liver sinusoids. However, there was a significant reduction in both hepatocyte necrosis and fatty degeneration in the groups treated with *O. vulgaris* doses of 112 and 224 mg/kg.

DISCUSSION

The liver plays a crucial role in digestion, detoxification, excretion of harmful substances, and

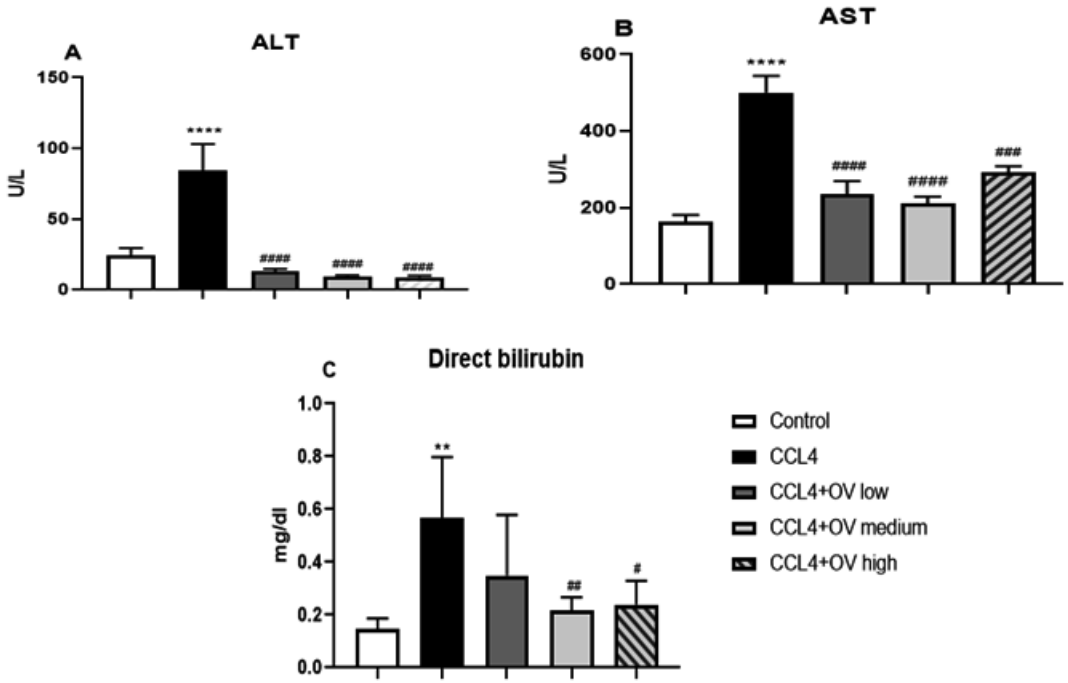

Fig. 4. Effect of *O.Vulgaris* on CCL-induced acute liver injury in rats (n = 7)
A. ALT level, B. AST, C. Direct bilirubin concentration

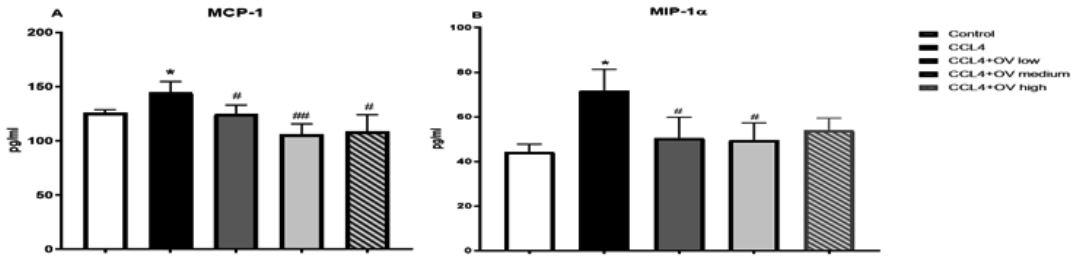

Fig. 5. Anti-inflammatory Effect of O.vulgaris on CCL-induced intoxicated rats (n = 7) A.MCP-1, B.MIP-1α

protein synthesis. However, it is highly susceptible to damage from drugs, toxins, and viral infections [34.35]

According to traditional Mongolian medicine, liver diseases are classified into 18 types, primarily attributed to imbalance in blood, bile and heat.. In Mongolian traditional medicine, herbal raw materials are commonly used in liver disorders.[7.8] Our team is currently studying the quality and standards of the *O. vulgaris* plant and its liver protective effects. We selected a model by carbon tetrachloride-induced acute liver injury in rats. The CCl4 induced liver injury model is widely used to evaluate hepatoprotective agents due to its well-characterized mechanisms of oxidative stress, inflammation, and fibrosis formation . [2,27.28]

Table 2. Effect of *O.Vulgaris* on CTGF and SOD levels in CCI4-induced acute liver injury (n=7)

	Groups				
CTGF (pg/ml)	Control	CCL4	CCL4+OV low	CCL4+OV medium	CCL4+OV High
	353.5±16.65	410.0±20.0*	362.1±26.7#	363.8±33.6#	359.1±8.3#
SOD (ng/ml)	5.18±0.69	3.84±0.43	3.87±0.26	5.21±0.87	4.54±0.33

*p<0.05 differentiated with the control group; #p<0.05 compared with the CCL4 intoxicated group

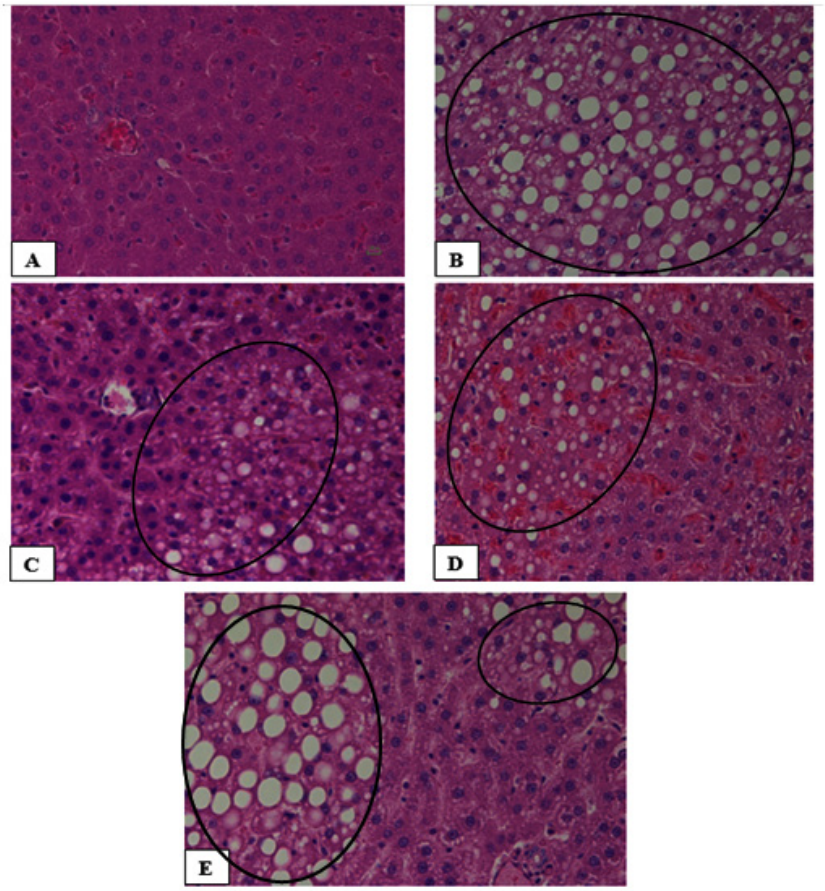

Fig. 6. Liver pathological changes in rats. A. Liver microstructure of the control group. B. Liver microstructure of the CCL4 group. C. Liver microstructure of the OV low dose group. D. Liver microstructure of the OV medium dose group Liver microstructure of the OV high dose group. n=5 magnification 400 H&E Round -fatty degeneration of hepatocytes

In our study, the administration of *O. vulgaris* reduced the elevated levels of ALT and AST caused by CCl4 in rats, indicating that the size of the injured hepatocytes decreased due to the effects of *O.vulgaris*. Our results showed that serum levels of AST and ALT, which are biomarkers of liver injury, along with direct bilirubin, significantly increased after repeated doses of CCl4 in rats. However, these levels decreased in all groups treated with OV. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) are enzymes primarily found in the liver, although AST is also present in other tissues, such as the heart and muscles. Elevated levels of ALT and AST indicate hepatocellular disease, while an increase in bilirubin suggests cholestatic changes. [27,28]

MIP-1á is a chemokine produced primarily by activated macrophages and other immune cells. It plays a crucial role in the immune response, particularly in the recruitment and activation of immune cells. MIP-1á is elevated in response to liver injury and aids in attracting inflammatory cells, such as macrophages and neutrophils, to the damaged tissue. These immune cells then release more pro-inflammatory cytokines, which contribute to additional liver damage and fibrosis. MIP-1á, produced by activated macrophages, plays a crucial role in immune cell recruitment and inflammation. Elevated levels in the CCl4 model suggest a strong inflammatory response, which was significantly reduced by *O. vulgaris* treatment.

MCP-1 is a cytokine that plays a key role in immune responses, particularly by recruiting monocytes, memory T cells to sites of inflammation. It is also implicated in fibrosis, where it promotes the accrue of leucocytes in tissues like the liver, lungs, and kidneys, potentially contributing to scar formation and chronic tissue damage. [29,30]

Additionally, critical liver injury and inflammatory response were also stimulated by CCl4.[29,30] Determination of inflammatory chemokines level also revealed the decreased concentration of MIP 1á, and MCP-1, respectively, in *O. vulgaris* -treated rats treated with CCl4, when compared to the model group.

The connective tissue growth factor plays a crucial role in various cellular processes. Its primary function is the proliferation and differentiation of connective tissue. It is often implicated in tissue repair and fibrosis, but when overexpressed, it can lead to scarring fibrosis in tissues liver.[30] In the present study, serum CTGF levels were increased in the model group compared to the control group and significantly decreased in all *O. vulgaris* -treated groups.

Superoxide dismutase is an essential enzyme that plays an important role in protecting cells from oxidative stress. Its main function is to accelerate the conversion of superoxide radicals (O, {), highly reactive molecules that are byproducts of cellular respiration and other metabolic processes, into oxygen (O,) and hydrogen peroxide (H, O,). [36] Also, superoxide dismutase was decreased by CCL4 intoxicated group when but the SOD of medium and high doses of OV-treated groups significantly increased by than CCL4 group.

In summary, *O. vulgaris* reduced the cytolysis of hepatic cells, ameliorated direct bilirubin marker of bile stasis, affected some, especially of inflammation chemokines, augmented indicators of some oxidation markers, but decreased assessment of fibrosis, and protected liver tissue damage caused by carbon tetrachloride acute liver injury. We attribute the *O. vulgaris*'s hepatoprotective activity to its biologically active compounds, including total flavonoids and iridoids. In this regard, many previous studies can confirm the above hypothesis. Aucubin is an iridoid glycoside commonly used in traditional medicinal remedies. Research has investigated the protective effects and mechanisms of aucubin against LPS-induced acute hepatitis. Findings suggest that this biologically active substance possesses anti-inflammatory and antioxidant properties, indicating its potential effectiveness in treating acute hepatitis caused by LPS.[15]

Additionally, the effects of secoiridoid compounds extracted from Gentianella turkestanerum on carbon tetrachloride (CCl4)-induced liver injury in mice have been studied. The extract of G. turkestanerum exhibited protective effects against CCl4-induced acute liver damage, significantly reducing serum levels of ALT, AST, and ALP in mice with acute liver injury. Although serum total protein (TP) and malondialdehyde (MDA) levels increased in the experimental group, this effect was reversed with the application of G. turkestanerum extract in a dose-dependent manner.

Thus, the extract proved to be protective against CCl4-induced acute liver injury in mice.[37] Based on these results, it is suggested that the total flavonoid compounds found in O. vulgaris may exert a hepatoprotective effect. During the standardization study of O. vulgaris, researchers conducted a microscopic examination of its anatomical structure and performed both qualitative and quantitative analyses of the dominant biologically active substances in the plant. The main ingredients selected for standardization were flavonoids and iridoids, with the total flavonoid content determined to be 4.3 ± 0.62%, and iridoids at 4.86 ± 0.93%, as measured by spectrophotometry. Researchers in Inner Mongolia identified 35 compounds in O. vulgaris using UPLC-MS. These included flavonoids such as luteolin, luteolin-7-O-glucuronide, apigenin, apigenin-7-O-glucoside, diosmetin, and hydroxygenkwanin, as well as other compounds like adenosine, syringaresinol, D-mannitol, and esculetin.[10] Nayan G. Patel conducted a study focusing on the quantitative determination of flavonoids (apigenin and luteolin) in Premna mucronata Roxb. using the HPTLC method. In this study, the mobile phase consisted of ethyl acetate, toluene, and formic acid in a ratio of 4:6:0.3, with NP-PEG as the spray reagent.[38] Past research has indicated that important ingredients in Odontites species include flavonoids, iridoids, phenolic acids, and phenylethanolic glycosides.[11-14,39] Furthermore, quality control and safety parameters are closely associated with the findings regarding Odontites ruber Gilib, as documented in medicinal plants of Mongolia.[40]

CONCLUSION

The quality control criteria for *O. vulgaris* were established, including flavonoid and iridoid content, and the findings contributed to updating its monograph in the Mongolian National Pharmacopeia. The study demonstrated that *O. vulgaris* effectively protects against CCl_4-induced acute liver injury by reducing serum ALT, AST, and direct bilirubin levels, increasing SOD activity, and inhibiting inflammatory markers (MIP-1á, and MCP-1) and fibrotic marker (CTGF). Histopathological analysis confirmed a reduction liver tissue damage .

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Conflict of Interest

The author(s) do not have any conflict of interest

Data Availability Statement

This statement does not apply to this article.

Ethics statement

This study was approved by the Research Ethics Committee of the National University of Medical Sciences of Mongolia. (Approval No 2022/3-09).

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable.

Author Contributions

Zolzaya Bilegsaikhan: Data collection, Analysis, Writing- Original Draft; Dejidmaa Buyantogtokh: Data collection, Analysis, review and & Editing, and Project Administration; Erdenechimeg Chuluunbaatar: Data collection, Analysis, Review& Editing; Anu Altangerel: Data collection, Analysis; Tserenkhand Gundsambuu: Data collection, Analysis, Review, & Editing; Tserentsoo Byambaa: Visualization, Supervision; Chimedragchaa Chimedtseren: Funding Acquisition, Resources, Supervision

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