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ORIGINAL ARTICLE

MECHANISM OF CELASTRUS ORBICULATUS EXTRACT INHIBITING THE INVASION AND METASTASIS OF GASTRIC CANCER CELLS THROUGH RAC1/LIMK1/COFILIN 1 PATHWAY

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Abstract

Celastrus orbiculatus extract contains various biologically active components which have extensive medicinal value. The present work investigated the inhibitory mechanism of *Celastrus orbiculatus* extract on the invasion and metastasis of gastric cancer (GC) cells (GCCs), with particular emphasis on the function of the ras-related C3 botulinum toxin substrate 1 (Rac1)/LIM domain kinase 1 (LIMK1)/cofilin1 signalling pathway (SPW) in this process. First, celastrin was extracted from *Celastrus orbiculatus* using an ultrasonic extraction (ULE) method and detected by high-performance liquid chromatography (HPLC). The human GCC line MGC-803 was then used in the study. The prepared *Celastrus orbiculatus* samples showed the same chromatographic peak as the celastrol standard. In contrast to the Ctrl group, the cell proliferation rates (CPRs) and cell invasion rates (CIRs) in groups A, B and C were greatly reduced, while the cell apoptosis rates (CARs) and cell migration rates (CMRs) were greatly increased ($p < 0.05$). In addition, the Rac1/LIMK1/cofilin1 SPW-associated proteins in the *Celastrus orbiculatus* treatment groups were strongly downshifted compared to the Ctrl group ($p < 0.05$). Meanwhile, there was a continuous downward trend with increased DC and time. *Celastrus orbiculatus* extract, by regulating the Rac1/LIMK1/cofilin1 SPW, suppressed proliferation, invasion and migration of GCCs while facilitating their apoptosis. This discovery provided important evidence for the potential use of *Celastrus orbiculatus* extract in treating GC.

Rezumat

Extractul de *Celastrus orbiculatus* conține multiple componente biologic active cu efecte terapeutice. Studiul a investigat mecanismul inhibitor al extractului de *Celastrus orbiculatus* asupra invaziei și metastazelor celulelor canceroase gastrice (GCC), cu accent deosebit pe funcția substratului 1 (Rac1)/LIM al toxinei botulinice C3 legat de ras 1 (LIMK1)/calea de semnalizare cofilin1 (SPW) în acest proces. În primul rând, celastrin a fost extras din *Celastrus Orbiculatus* folosind o metodă de extracție cu ultrasunete (ULE) și identificat prin cromatografie lichidă de înaltă performanță (HPLC). Linia GCC umană MGC-803 a fost utilizată în studiu. Probele au prezentat același vârf cromatografic ca standardul de celastrol. Spre deosebire de grupul Ctrl, ratele de proliferare celulară (CPR) și ratele de invazie celulară (CIR) din grupurile A, B și C au fost mult reduse, în timp ce ratele de apoptoză celulară (CAR) și ratele de migrare celulară (CMR) au fost mult crescute ($p < 0,05$). În plus, proteinele asociate cu Rac1/LIMK1/cofilin1 SPW din grupurile de tratament cu *Celastrus orbiculatus* au fost puternic reduse în comparație cu grupul Ctrl ($p < 0,05$). Extractul de *Celastrus orbiculatus*, prin reglarea SPW Rac1/LIMK1/cofilin1, a suprimat proliferarea, invazia și migrarea GCC facilitând, în același timp, apoptoza acestora.

Keywords: *Celastrus orbiculatus* extract, Rac1/LIMK1/cofilin1 signalling pathway, gastric cancer, cell invasion, cell metastasis

Introduction

Gastric cancer (GC) is a malignancy arising from gastric mucosal cells and is a frequent cancer worldwide. It is typically categorised into subtypes, including adenocarcinoma, invasive carcinoma and lymphoma [1]. Patients in the early stages may not display noticeable symptoms, but as the disease progresses, they may experience symptoms such as indigestion, abdominal pain, weight loss, vomiting and black stools [2]. GC risk factors include *Helicobacter pylori* infection, dietary habits, smoking and family history [3]. Treatment methods typically involve surgical resection, radiation therapy, chemotherapy and targeted therapy [4].

Celastrus orbiculatus (*Celastraceae* family) is a low-growing herbaceous plant that thrives in damp environments such as stream banks, fields and forest undergrowth. It is known for its heart-shaped leaves and small white flowers [5]. The roots, stems, leaves and flowers of *Celastrus orbiculatus* are widely used in traditional herbal medicines to treat various conditions, including colds, eczema, urinary tract infections and

more [6-8]. *Celastrus orbiculatus* extract contains various bioactive compounds, with key components including flavonoids, alkaloids and various antioxidants [9]. This gives it extensive medicinal value, including the following four aspects: anti-inflammatory and anti-microbial activity [10, 11], antioxidant properties [12, 13], anti-tumour potential [14, 15] and immunomodulatory effects [16, 17]. *Celastrus orbiculatus* has potential value in treating various inflammatory and infectious diseases. Its anti-inflammatory properties are attributed to its ability to regulate the secretion of inflammatory mediators, such as inhibiting the expression of leukocyte chemotactic factors [10, 11]. *Celastrus orbiculatus* is rich in flavonoid compounds and is widely recognised for its antioxidant activity. They help to eliminate free radicals and protect cells from oxidative damage [12, 13].

Anti-tumour potential: Some studies suggest that *Celastrus orbiculatus* extract may have anti-tumour activity against certain types of cancer. It is thought that this effect can be achieved by regulating the growth of tumour cells and inhibiting angiogenesis [14, 15]. *Celastrus orbiculatus* has been shown to regulate the immune system, helping to balance immune responses [16, 17]. As modern medical research advances, we have gained a deeper understanding of the pharmacological actions and potential applications of *Celastrus orbiculatus*. While further research is needed to uncover its molecular mechanisms and broader applications, *Celastrus orbiculatus* extract has demonstrated immense potential as a natural treasure trove of medicinal properties.

The Rac1/LIMK1/cofilin1 signalling pathway (SPW) is an essential component of cell signalling [18]. In cancer, the Rac1/LIMK1/cofilin1 signalling pathway is often over-activated, leading to cytoskeletal re-arrangements that promote cancer cell invasion and metastasis. Highly active Rac1/LIMK1/cofilin1 SPWs contribute to changes in cell morphology, making cancer cells more capable of penetrating tissue barriers and spreading to other sites. Previous research has shown that the Rac1/LIMK1/cofilin1 SPW is involved in inhibiting cancer cell migration, invasion, growth and mechanisms related to drug resistance [19, 20]. Therefore, this SPW has become a focal point of cancer metastasis mechanisms and potential therapeutic targets. Therefore, this work investigated the effects of *Celastrus orbiculatus* extract on the growth and invasive capabilities of GC cells (GCCs) and whether *Celastrus orbiculatus* extract regulated the invasion and metastasis of GCCs through the Rac1/LIMK1/cofilin1 SPW. By uncovering the molecular mechanisms by which *Celastrus orbiculatus* extract inhibits the invasion and metastasis of GCCs, this work provides an important benchmark for future GC treatment.

Materials and Methods

Reagents and instruments

Main reagents employed in this work encompassed *Celastrus orbiculatus* medicinal herb (Beijing Tongrentang Co., Ltd., China); petroleum ether and ethyl acetate (China National Pharmaceutical Group Chemical Reagent Co., Ltd., China); methanol (Merck, China); celastrine (Meilun Biotechnology Co., Ltd., China); RPMI1640, 96-well and 6-well cell culture plates (Beijing Solabao Technology Co., Ltd., China); pancreatin (Beijing Solabao Technology Co., Ltd., China); MTT reagent and Transwell cell culture plates (Corning, China); matrigel and dimethyl sulfoxide (Sigma Aldrich, Germany); Annexin V-FITC kit (Thermo Fisher Sci., USA); crystal violet staining solution (Macgene Biotechnology Co., Ltd., China); RIPA buffer (Solarbio, USA); rabbit polyclonal Phospho-cofilin (Ser3) antibody, mouse monoclonal antibody LMK1, rabbit polyclonal PAK1 antibody, rabbit polyclonal cofilin antibody, GAPDH internal control antibody and BCA reagent kit (Thermo Scientific, USA).

The primary instruments utilised included an ultrasonic cleaner (Kaizheng Ultrasonic Technology Co., Ltd., China), an electronic balance (Mettler Toledo, USA), an HPLC system, an enzyme marker (Thermo Sci., USA), a CO_2 incubator (Eppendorf, Germany), a flow cytometer (FCT, Beckman Coulter, USA), an inverted microscope (Tianjin Wei Yi Optoelectronics Co., Ltd., China) and gel electrophoresis and imaging systems (Beijing 61 Biotechnology Co., Ltd., China).

Preparation of Celastrus orbiculatus extract solution

The test sample solution was prepared according to the following procedures. *Celastrus orbiculatus* dried powder was prepared using the ULE method [21]. Specifically, 10 g of *Celastrus orbiculatus* dried powder was taken, and the ratio of liquid to solid was adjusted to 1:10. It was then extracted with petroleum ether mixed with ethyl acetate (1:1) by ultrasonication for 30 minutes, which should be performed twice, and the solutions, after filtering, were melted and evaporated. The residue obtained was dissolved in methanol and made up to 10 mL. The solution was filtered through a 0.22 μm filter head to obtain the test sample solution. The standard solution was synthesised using the following steps: 0.003 g of celastrine was dissolved in methanol and made up to 10 mL, giving a standard solution containing 0.3 mg/mL of celastrine.

The celastrine content was determined by HPLC. An Extend-C18 chromatographic column (5 μm, 4.6 × 250 mm) was used. The mobile phase consisted of acetonitrile (90) and a 0.1% aqueous solution of phosphoric acid (10), with a flow rate of 0.8 mL/min and a column temperature of room temperature (RT). A 10 μL sample was injected for analysis.

The ULE method was used to evaluate the extraction process. In this procedure, 10 g of *Celastrus orbiculatus* dried powder was used, and the ratio of liquid to solid

was set at 1:10. It was extracted using petroleum ether mixed with ethyl acetate (1:1) by ultrasonication for 30 minutes, which should be done twice. After filtering, the solutions were mixed and evaporated. The residue obtained was dissolved in methanol and made up to 10 mL. The solution obtained was treated with a 0.22 µm filter head. This extraction process was repeated five times, and the extracted celastrine content was analysed each time to validate the stability and repeatability of the extraction method.

Cell culture and passage. GCC line MGC-803 was purchased from Shanghai Huaying Biotechnology Co., Ltd., China. They were cultured in cell culture flasks containing RPMI1640 medium supplemented with 10% foetal bovine serum and maintained in an incubator at 37°C with 5% CO_2. Once the cells adhered to the flask and formed a monolayer on the bottom, they were passaged using 0.25% trypsin digestion.

Cell grouping. Celastrus orbiculatus extract was prepared in various concentrations (10, 50, 100 µg/mL, named Groups A, B and C) by dilution with methanol. According to the intervention method, the selected GCCs were assigned to the Control (Ctrl) group-blank culture medium and to another three treatment groups (A, B and C).

MTT to measure cell proliferation rates (CPR). Log phase MGC-803 cells were harvested, and the cell suspension (CS) was adjusted to 3 × 105 cells/mL. They were then seeded into a 96-well plate. According to each group, 100 µL of the corresponding solution was added. Each group had 6 replicate wells. After 24 and 72 hours of incubation, the supernatant was discarded. Subsequently, 10 µL of MTT reagent was added, and the GCCs were incubated for 4 hours. After this incubation period, 100 µL of dimethyl sulfoxide (DMSO) reagent was added to terminate the incubation. The plate was shaken for 10 minutes, and absorbance was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) reader. CPR was calculated using the equation:

$$CPR (\%) = [(1 - OD \text{ in treatment group})/(OD \text{ in control group})] \times 100.$$

Detection of cell apoptosis rates (CAR). The Annexin V-FITC Assay Kit was used to detect apoptotic and dead cells. Annexin V-FITC is bound to phosphatidyl-serine and stains apoptotic cells positively, while propidium iodide (PI) stains negatively. GCCs were seeded at 3 × 105 cells/mL into a 96-well plate. Depending on the group, 100 µL of the corresponding solution was added. The Ctrl group received an equivalent amount of blank culture medium; each group had 6 replicate wells. After 24 and 72 hours of incubation, the supernatant was discarded. The GCCs were rinsed twice with PBS and then collected by centrifugation at 1,000 rpm/min for 10 minutes. The cells were then resuspended by mixing with 500 µL binding buffer. Next, 5 µL Annexin V-FITC reagent and 10 µL PI reagent were added and gently mixed. The GCCs were then incubated for 5 minutes without light. FCT was applied during this process.

Determination of cell invasion rates (CIR). A mixture of 100 µL Matrigel (1:8 dilution) was applied to the upper chamber of a 24-well Transwell plate. In the treatment groups, 100 µL of the corresponding solution was added. Each group had 6 replicate wells. After 24 and 72 hours of incubation, the supernatant was discarded. The treated cells (4 × 104 cells/well) were then seeded on Matrigel and cultured in a serum-free medium. In the lower chamber, 500 µL of complete culture medium was used. After 48 hours of incubation, the GCCs in the upper chamber were removed, and the remaining GCCs were fixed with 4% paraformaldehyde. The GCCs were stained with 1% crystal violet, washed with PBS to remove excess dye, and observed under a microscope. Cell counts were performed in three fields of view (FOVs) at 100x magnification.

Examination of cell migration rates (CMR). Log phase MGC-803 cells were harvested, and the CS was seeded at 3 × 105 cells/mL. These GCCs were seeded into the upper chamber of the Transwell. Once attached to the chamber, 100 µL of the corresponding solutions were applied in three treatment groups. There were 6 replicate wells in all groups. The GCCs were incubated for 24 and 72 hours to discard the supernatant. The chambers were removed, and the GCCs on the top of the chamber were carefully wiped off. The chambers were rinsed twice with PBS. They were then fixed in 10% paraformaldehyde for 15 minutes and washed twice with PBS. The chambers were stained with crystal violet solution. The number of cells that migrated using the membrane was observed under an inverted microscope with multiple FOVs, and photographs were taken. The CMR (%) = (1 - number of migrated GCCs in the treatment group/number of migrated GCCs in the control group) × 100.

Cell Scratch Assay (CSA). Log phase human gastric cancer MGC-803 cells were harvested, and CS was again determined at 3 × 105 cells/mL. These GCCs were seeded in a 6-well plate. After reaching 90% cell confluence, the medium was changed to 100 µL of the appropriate solution, depending on the group. All groups were assigned 6 replicate wells. The GCCs were incubated for 24 and 72 hours, and a 10 µL sterile pipette tip was used to create a scratch line at the bottom of the culture dish. The GCCs were then incubated for a further 24 hours, cell scratch healing was observed, and the results were photographed.

Determination of Rac1/LIMK1/cofilin1 SPW-related proteins. Western blot analysis was used to measure the levels of Rac1/LIMK1/cofilin1 SPW-related proteins. Log phase MGC-803 cells were harvested, and the CS was set at 3 × 105 cells/mL. These MGC-803 cells were seeded in a 6-well plate. Once attached to the plate, 100 µL of the appropriate solution was added.

6 replicate wells were arranged for all four groups. Similarly, after 24 and 72 hours, the supernatant was removed. HCCs were harvested, and total proteins were extracted using RIPA buffer. Proteins were quantified using a BCA protein assay kit. Proteins were separated by 10% SDS-PAGE gel electrophoresis and then transferred to a nitrocellulose (NC) filter membrane. The membrane was blocked with 5% skim milk at RT for 2 hours. The primary antibodies were incubated with the membrane overnight at 4°C. The membrane was then rinsed three times with TBST. Secondary antibodies were incubated with the membrane for 2 hours, followed by three more washes with TBST. The membrane was then exposed to enhanced chemiluminescence (ECL) reagent without light for 5 minutes, and chemiluminescence signals were detected. The grey scale values of the protein bands were evaluated using Image J software.

Methods for statistical analysis

All experimental data were statistically analysed using SPSS 19.0 (IBM, USA) and presented as means ± standard deviations. Differences between two independent samples were assessed using Student's t-test, and those between multiple groups were determined using one-way analysis of variance (ANOVA). A $p < 0.05$ indicated statistically significant differences.

Results and Discussion

Detection of Celastrus orbiculatus extract

Figure 1 shows the detection results of the *Celastrus orbiculatus* extract. Under chromatographic conditions, the *Celastrus orbiculatus* sample solution exhibited a chromatographic peak identical to the standard celestine, indicating a complete separation. The results of the 5 extractions are presented in Figure 2, which shows no remarkable difference in the extraction yield of celestine ($p > 0.05$).

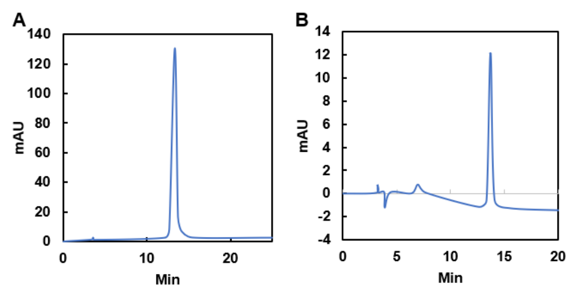

Figure 1.

Chromatogram; A: standard; B: test product

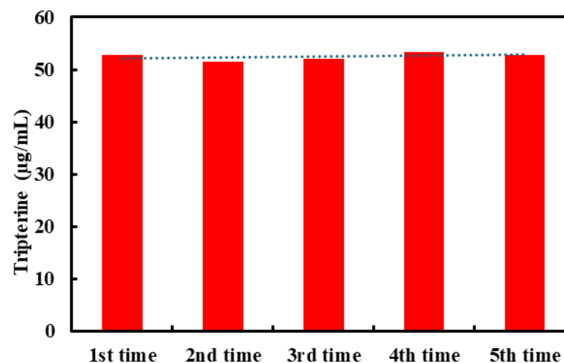

Figure 2.

Comparison of celastrine extraction quantity

Cell Invasion Rates (CIR)

Results of Cell Proliferation Rates (CPR). Figure 3 shows the CPRs in the different groups. In contrast to the Ctrl group, all treatment groups significantly decreased CPR ($p < 0.05$). In addition, CPR decreased with increasing concentration of *Celastrus orbiculatus* extract ($p < 0.05$). Furthermore, the reduction in CPR became more pronounced with increasing incubation time ($p < 0.05$).

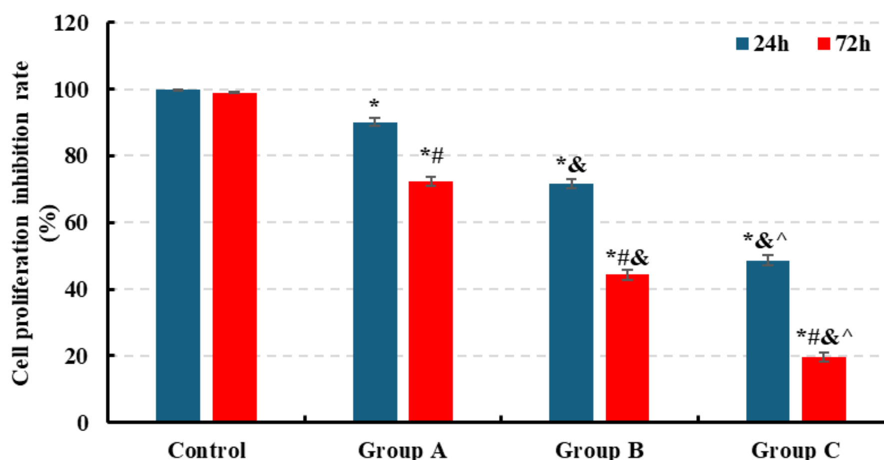

Figure 3.

CPRs in various groups

* = Compared with Ctrl group, $p < 0.05$; & = Compared with group A, $p < 0.05$; ^ = Ccompared with group B, $p < 0.05$;
# = Compared with the rate at 24 hours, $p < 0.05$

Results of Cell Apoptosis Rates (CAR)

The results of the FCT assay are shown in Figure 4. Compared to the Ctrl group, a significant increase in the proportion of apoptotic cells was observed in three treatment groups ($p < 0.05$). In addition, with increasing incubation time and DC of *Celastrus orbiculatus* extract, CARs also increased ($p < 0.05$).

Results of Cell Invasion Rates (CIR)

The results of the Transwell cell invasion experiment are shown in Figure 5. As observed, there was a gradual reduction in the number of GCCs passing through the membrane in groups A, B and C compared to the Ctrl group. The cell count results also indicated that the number of GCCs passing through the membrane was greatly reduced in the *Celastrus orbiculatus* extract treatment groups compared to the Ctrl group ($p < 0.05$). Furthermore, the CIR decreased significantly, and *Celastrus orbiculatus* extract concentration increased ($p < 0.05$).

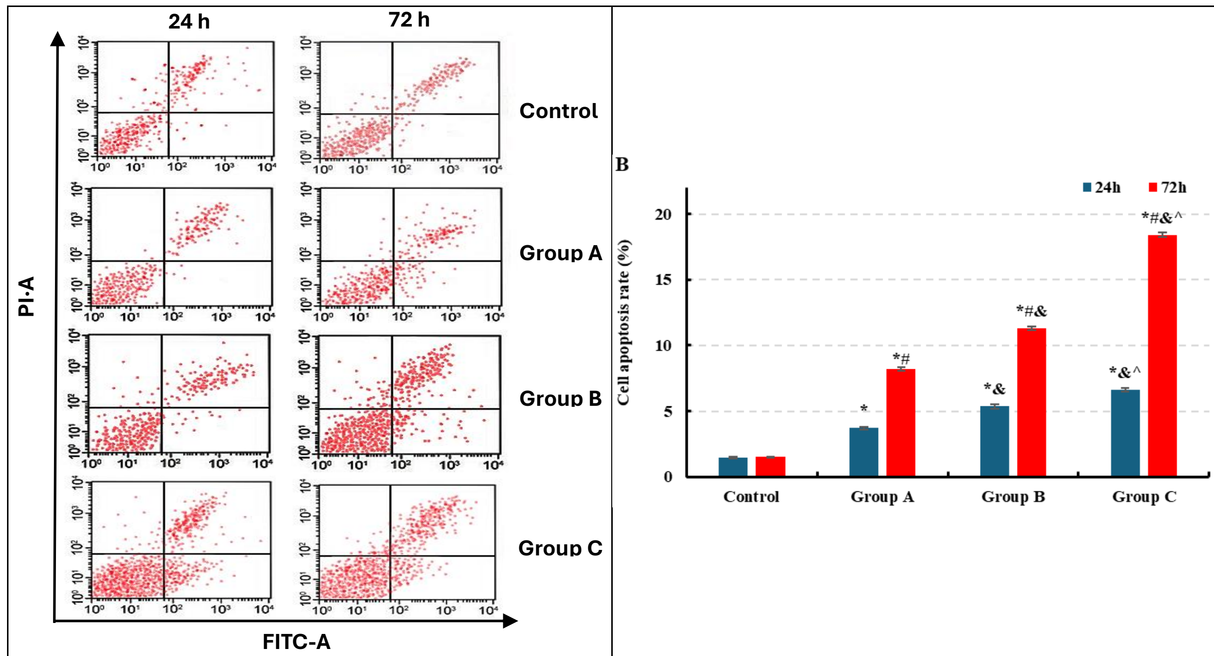

Figure 4.

Comparison of CARs in various groups; A: FCT results; B: CARs

* = Compared with Ctrl group, $p < 0.05$; & = Compared with group A, $p < 0.05$; ^ = Compared with group B, $p < 0.05$; # = Compared with the rate at 24 hours, $p < 0.05$

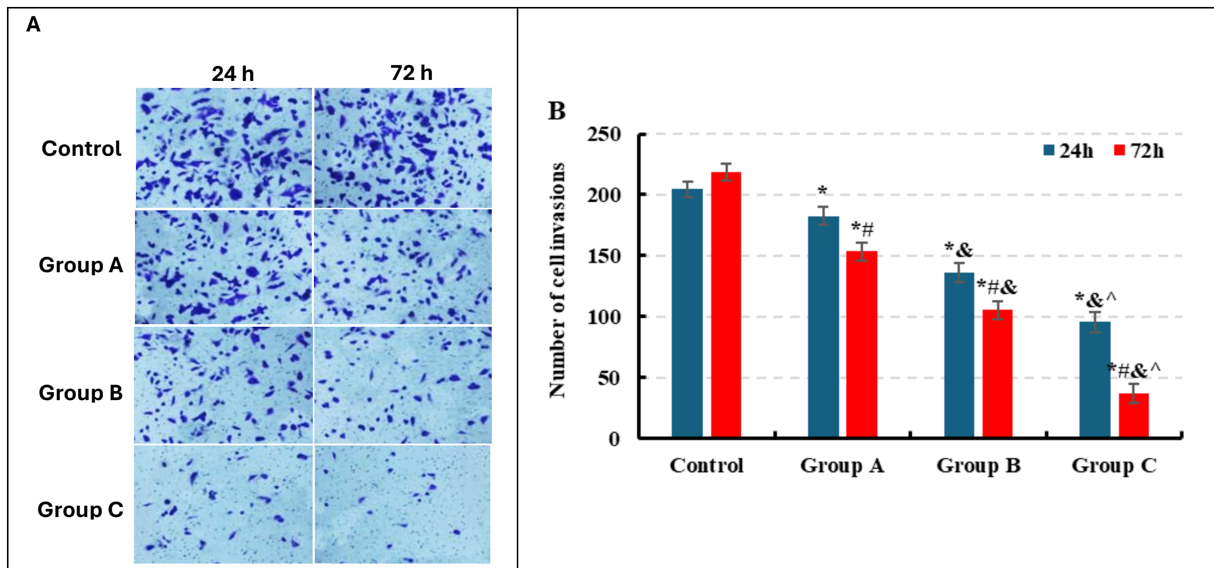

Figure 5.

CIRs in varying groups; A: crystal violet dyeing (×200); B: count of invading GCCs)

* = Compared with Ctrl group, $p < 0.05$; & = Compared with group A, $p < 0.05$; ^ = Compared with group B, $p < 0.05$; # = Compared with the rate at 24 hours, $p < 0.05$

Results of Cell Migration Rates (CMR)

Figure 6 illustrates the observations from the Transwell experiment. Compared to the condition in the Ctrl group, a gradual reduction in the number of migrating cells was observed in the treatment groups. The cell count results also indicated fewer migrating GCCs were observed in groups A, B and C (p < 0.05). Furthermore, cell migration decreased with increasing time and *Celastrus orbiculatus* extract concentration (p < 0.05).

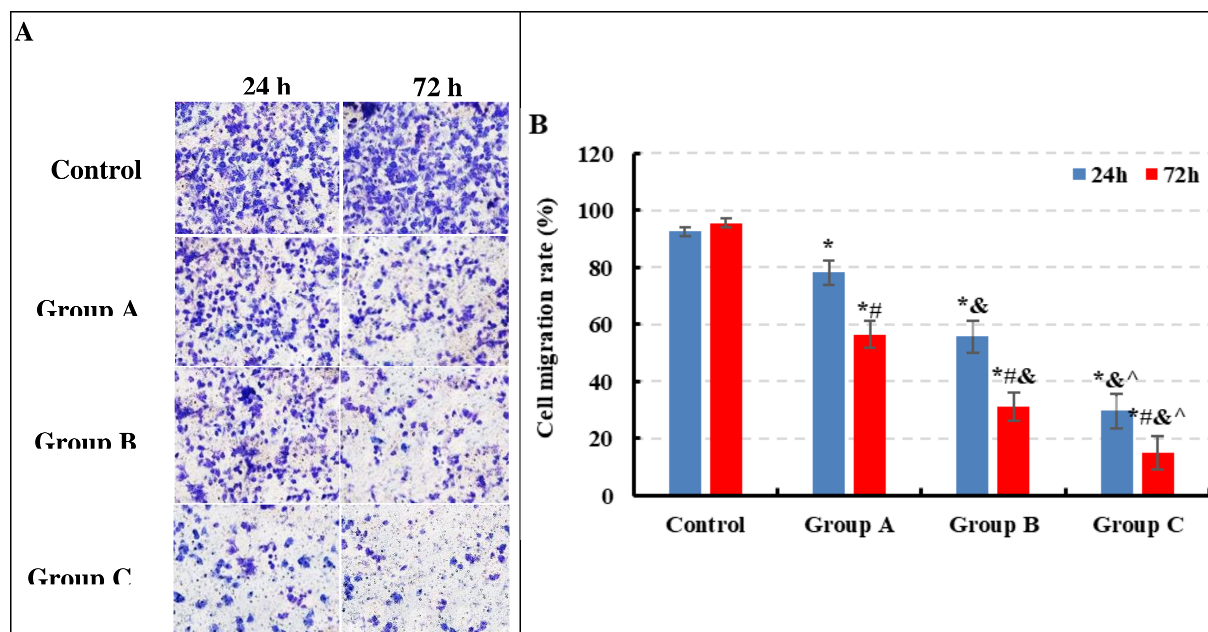

Figure 6.

CMRs in various groups; A: crystal violet dyeing (×200); B: CMRs

* = Compared with Ctrl group, $p < 0.05$; & = Compared with group A, $p < 0.05$; ^ = Compared with group B, $p < 0.05$; # = compared with the rate at 24 hours, $p < 0.05$

The CSA test in Figure 7 suggests that the CSA in the other groups showed a decreasing trend starting from the Ctrl group. Furthermore, this decrease continued with increasing *Celastrus orbiculatus* extract concentration and incubation time. The cell count results showed that the CMRs in the other groups decreased significantly compared to the Ctrl group (p < 0.05).

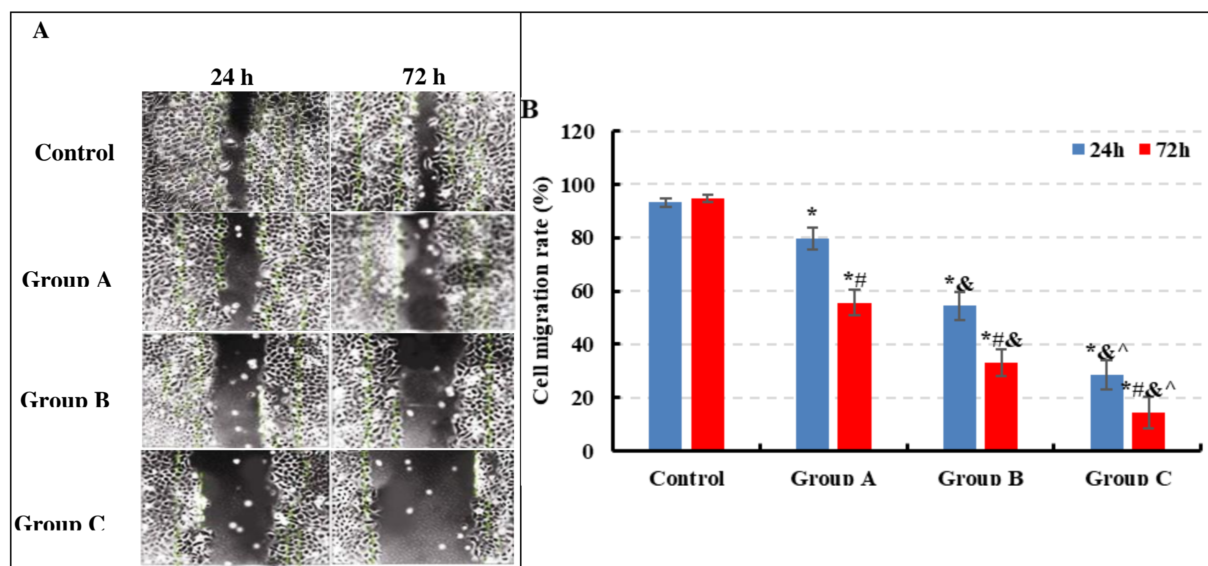

Figure 7.

CSA in different groups; A: CSA; B: CMRs

* = Compared with Ctrl group, $p < 0.05$; & = Compared with group A, $p < 0.05$; ^ = Compared with group B, $p < 0.05$; # = Compared with the rate at 24 hours, $p < 0.05$

Levels of Rac1/LIMK1/cofilin1 SPW-related proteins

Western blot analysis (Figure 8) showed that a decrease in Rac1/LIMK1/cofilin1 SPW-related protein levels was observed in the treatment groups compared to the Ctrl group (p < 0.05). In addition, the protein levels showed a decreasing trend with increasing incubation time and *Celastrus orbiculatus* extract concentration (p < 0.05).

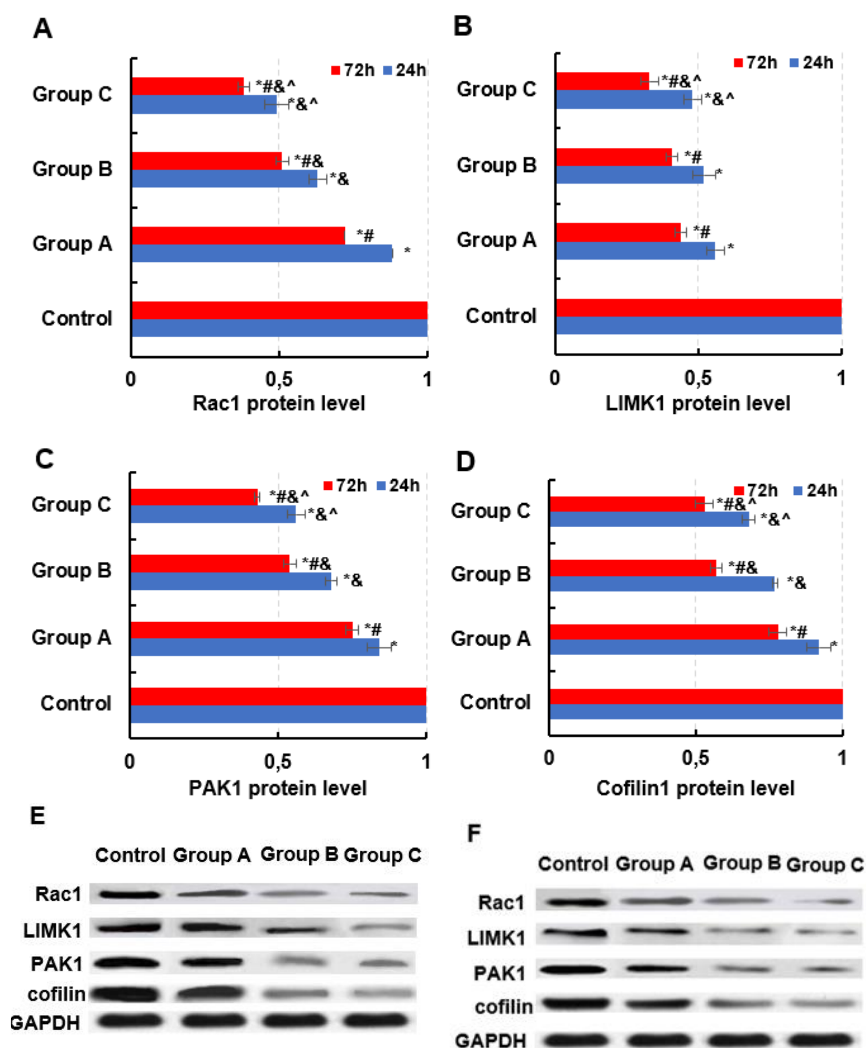

Figure 8.

Levels of Rac1/LIMK1/cofilin1 SPW-related proteins; A: Rac1; B: LIMK1; C: PAK1; D: cofilin; E: Western blot strips at 24 hours and F: Western blot strips at 72 hours

* = Compared with Ctrl group, $p < 0.05$; & = Compared with group A, $p < 0.05$; ^ = Compared with group B, $p < 0.05$; # = Compared with the rate at 24 hours, $p < 0.05$

GC is typically a malignant tumour that arises from the stomach lining and often has no obvious symptoms in its early stages. As a result, it is often discovered at an advanced stage, making it difficult to treat. However, early diagnosis and intervention can significantly improve patient survival. The pathogenesis of this cancer is complex and influenced by a number of factors, including genetics, environment and lifestyle. Epidemiological data show that GC represents a significant global health burden, claiming the lives of thousands of people each year [22]. *Celastrus orbiculatus* extract has been found to contain several bioactive components with potential medicinal value, including analgesic, sedative and anti-inflammatory properties [23, 24]. Recent research suggests that *Celastrus orbiculatus* extract may have potential applications in treating neurological disorders and cancer [25, 26]. Celastrine, the primary active compound in *Celastrus orbiculatus*, belongs to the triterpene class of compounds and has antioxidant, anti-inflammatory, anti-tumour and neuroprotective effects [27]. Studies suggest that celastrine may counteract free radical damage, thereby protecting cells from oxidative stress, with potential implications for preventing chronic diseases such as cardiovascular disease and cancer [28, 29]. This work used the ULE method to extract celastrine from *Celastrus orbiculatus*. The results show that the chromatographic peak in the *Celastrus orbiculatus* extract matches the celastrine

standard, confirming the successful extraction of celastrine. The method demonstrated stability and reproducibility.

Subsequently, the *Celastrus orbiculatus* extract solutions were prepared at different concentrations (10, 50 and 100 μg/mL) and compared with the blank control group to investigate the effect of *Celastrus orbiculatus* extract on invasion and metastasis of GCCs. The research results showed a visible decrease in CPRs of GCCs with increasing time and concentration ($p < 0.05$). Cancer cells exhibit uncontrolled cell proliferation and are not subject to the regulatory mechanisms that govern normal cell growth [30]. The results of this work suggest that *Celastrus orbiculatus* extract may contain anti-cancer components that have a critical effect in inhibiting the proliferation of GCCs. As the concentration increased, CARs increased significantly ($p < 0.05$). Cell apoptosis is a self-destructive cell death process typically considered a mechanism to counteract tumour development. This suggests *Celastrus orbiculatus* extract may inhibit GC growth by promoting cell apoptosis. Compared to the Ctrl group, the other three treatment groups showed a remarkable decrease in transmembrane and migrating cells ($p < 0.05$). Invasion and migration of cancer cells are major contributors to the development and spread of cancer [31]. This work found that as the concentration increased, the migration and invasion capabilities of the cells obviously decreased, suggesting that *Celastrus orbiculatus* extract interferes with these critical processes, thus impeding the spread of cancer cells. In conclusion, this work has provided compelling evidence for the potential role of *Celastrus orbiculatus* extract in inhibiting the proliferation of GCCs, promoting their apoptosis and reducing their migration and invasion. These findings provide a solid scientific basis for using *Celastrus orbiculatus* extract in cancer therapy. SPW research has always focused on biology and medicine because it can reveal the complexity of molecular interactions and cell regulation, providing important clues for understanding disease mechanisms and identifying potential therapeutic targets. In this study, we also investigated the mechanisms by which *Celastrus orbiculatus* extract affects GCCs. In this context, the Rac1/LIMK1/cofilin1 SPW critically affects several cellular processes [32]. Rac1 is a member of the Rho family of small GTPases and is important in cell proliferation, migration, adhesion and morphological changes. LIMK1 is a kinase that can be activated by Rac1, leading to the phosphorylation of cofilin1. Cofilin1 regulates actin filament dynamics, and its phosphorylation status is closely linked to the dynamic stability of the cell's cytoskeleton [33]. In addition, PAK1 is another key component of the Rac1 SPW and is a kinase involved in the initiation and regulation of the SPW. The activity of PAK1 can be activated by Rac1, triggering several downstream events, including the activation of LIMK1 and the phosphorylation of cofilin1 [34]. This SPW is critically involved in physiological and pathological processes. In addition, this SPW is also involved in cell adhesion and cell-cell interactions, which strongly influence tumour invasion and metastasis [35, 36]. In addition, this work showed that the levels of Rac1, LIMK1, PAK1 and cofilin in GCCs in the treatment groups were greatly reduced compared to the conditions in the Ctrl group ($p < 0.05$). This suggests that *Celastrus orbiculatus* extract may achieve the inhibition of GCC proliferation, promotion of GCC apoptosis and suppression of GCC migration and invasion through the inhibition of Rac1/LIMK1/cofilin1 SPW.

Conclusions

In this work, celastrine was successfully extracted from *Celastrus orbiculatus,* and the inhibitory mechanisms of *Celastrus orbiculatus* extract for invasion and metastasis of GCCs were investigated. The observations indicated that *Celastrus orbiculatus* extract remarkably suppressed the proliferative capacity of GCCs, increased CAR and reduced CRI and CMR of GCCs. This process may be achieved by inhibiting the Rac1/LIMK1/cofilin1 SPW. However, this work was primarily based on cell experiments, and future research should include *in vivo* experiments and clinical trials to validate the efficacy and safety of *Celastrus orbiculatus* extract in the treatment of GC, thus providing greater support for its clinical application. Furthermore, in addition to Rac1/LIMK1/cofilin1 SPW, other potential signalling pathways can be further investigated in future research to fully understand the effects of *Celastrus orbiculatus* extract on GCC. In conclusion, this work provides strong evidence for the potential therapeutic value of *Celastrus orbiculatus* extract in treating GC, but further research and validation are required to ensure its successful application in clinical practice.

Conflict of interest

The authors declare no conflict of interest.

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