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Original Research Article

Effect of *Lonicerae japonicae* caulis extract on the growth and apoptosis of human osteosarcoma U2OS cells

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Abstract

Purpose: *To determine the effect of Lonicerae japonicae Caulis extract (LJCE) on the proliferation, apoptosis, and cycle distribution of human osteosarcoma U2OS cells.*
Methods: *Human osteosarcoma U2OS cells were treated with LJCE at doses of 10, 20, 30 and 40 mg/mL, with untreated U2OS cells as the control group. Cell proliferation was measured using MTT method, while flow cytometric analysis was used for determination of apoptosis and cell cycle distribution.*
Results: *As LJCE concentration increased, the viability of U2OS cells gradually decreased, while the apoptosis rate gradually increased. The LJCE treatment had no marked effect on G1 phase cells ($p > 0.05$). However, the population of G2 cells in each group increased with increasing LJCE concentration, while the S phase cells in each group decreased with increasing concentration. The cells were significantly blocked in the G2 phase ($p < 0.05$).*
Conclusion: *Lonicerae japonicae Caulis extract inhibits the proliferation of U2OS cells and promotes apoptosis of U2OS cells. Therefore, LJCE possesses potentials as a novel therapeutic agent for osteosarcoma.*

Keywords: *Lonicerae japonicae Caulis extract, U2OS, Apoptosis, Proliferation*

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INTRODUCTION

Lonicerae japonicae Caulis is used in Traditional Chinese medicine (TCM) for the treatment of many conditions such as fever, carbuncles, swelling, toxin-induced heat, bloody dysentery, beriberi, myalgia and arthralgia [1,2]. The use of LJCE, which was first reported by celebrated physicians, is contained in Materia Medica. In TCM, LJCE is believed to *clear heat*, *dredge wind*, neutralize toxins, and *unblock collaterals*. Most TCM products are derived from herbs which also serve as food. These products have gained increased public awareness and patronage due to changes in dietary patterns and lifestyle.

Studies have demonstrated that *Lonicerae japonicae* Caulis, which is used, both as a drug and as food, exerts potent pharmacological impacts as anti-inflammatory, antioxidant, anti-tumor, anti-inflammatory, and anti-microbial agent [3,4].

Osteosarcoma, a frequently occurring bone malignancy seen in clinical practice, is more common in adolescents. It has low sensitivity to treatment options such as radiotherapy and chemotherapy, and a poor prognosis [5-7]. Therefore, it is important to evolve more effective prevention and treatment measures for osteosarcoma. This has been a difficult challenge in clinical studies in recent years. Therefore, the present study was aimed at investigating the impact of *Lonicerae japonicae* Caulis extract on growth of human osteosarcoma U-2OS cell line, so as to provide reliable data necessary for clinical management of osteosarcoma.

EXPERIMENTAL

Cell culture

Human osteosarcoma U2OS cells were maintained in McCoy growth medium having 10 % FBS and 1 % antimicrobial agent, at 37 °C in a 5 % CO_2 incubator. The medium was refreshed every 48 h. In addition, HFF-1 cells were maintained at 37 °C in DMEM containing 15 % FBS in a 5 % CO_2 incubator, with fresh medium provided at 48 h intervals. The cells were subjected to sub-culturing at 80 – 90 % confluence.

MTT assay

The MTT assay was used for assessment of cell viability [8]. The cells were plated in 96-well plates, followed by 48-h incubation with graded concentrations of LJCE (10, 20, 30 and 40 mg/mL). The medium was removed after incubation, and then MTT solution (10 µL of 5 mg/mL) was put in, after which the mixture was incubated for 4 h. Thereafter, the MTT was replaced with DMSO (150 µL) to solubilize the resultant formazan crystals. The absorbance of the formazan crystals in every well was determined at 490 nm in a Multiskan Spectrum spectrophotometer (BioTek Instruments, USA). All viability assays were done in triplicate.

Flow cytometry for apoptosis

The U2OS cells were placed in 6-well plates (3×10^5 cells/well), followed by a 24-h incubation with LJCE at doses of 10, 20, 30 and 40 mg/mL. This was followed by a 20-min staining, first with Annexin V-FITC, and then with PI, at laboratory conditions in a dark chamber. Fluorescence intensity was measured flow cytometrically, after which the degrees of apoptosis and necrosis were calculated with DIVA software.

Determination of cell cycle distribution

The U2OS cells were maintained and exposed to the same doses of LJCE (10, 20, 30 and 40 mg/mL) as described in apoptosis measurement. After 48 h, the U2OS cells were subjected to digestion and fixation in 70 % ethanol for 24 h, followed by 30-min staining with PI in a dark chamber. Then, cell cycle distribution was determined flow cytometrically.

Statistics

All analyses were performed with Graph Prism Software 9. Results are presented as mean ± standard deviation (SD). Differences between the 2 groups were subjected to analyses using ANOVA or *t*-test in combination with Tukey's post hoc test. Values of $p < 0.05$ were taken as significant.

RESULTS

As shown in Figure 1, with increasing concentrations, LJCE significantly decreased the viability of U2OS cells. When the LJCE concentration was 40 mg/mL, the cell viability was 33.68 ± 3.71 %.

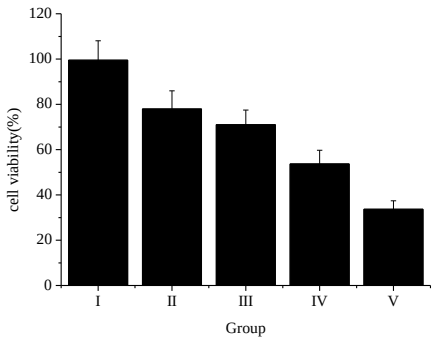

Figure 1: Effect of LJCE on U2OS cell viability

As shown in Figure 2 and Figure 3, increasing concentrations of LJCE significantly increased cell apoptosis rate and significantly decreased cell viability, respectively. When LJCE concentration was 40 mg/ml, the cell apoptosis rate was 68.11 ± 6.58 %. As shown in Figure 4 and Figure 5, the proportion of G1 phase cells in each group was not altered by LJCE. However, the proportion of G2 cells in each group increased with increasing LJCE concentrations, while the number of S phase cells in each group decreased with increasing LJCE concentrations. The cells were blocked at G2 phase, and the extract arrested cell cycle at G2 stage in a dose-reliant fashion.

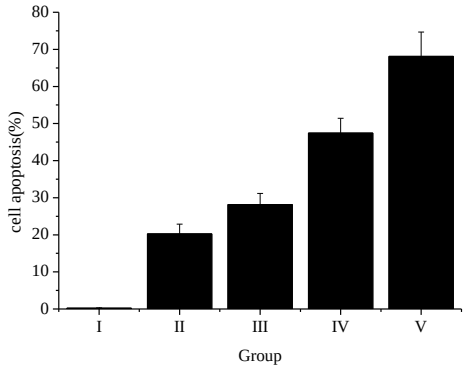

Figure 2: Effect of LJCE on U2OS cell apoptosis

DISCUSSION

Osteosarcoma (OS) is a bone cancer that occurs very frequently in kids and adolescents. It is characterized by high degree of aggressiveness, recurrence and early metastasis to pulmonary tissues, leading to high rates of mortality and morbidity. Osteosarcoma (OS) is characterized by fast metastasis, poor prognosis, and high short-term mortality rate. Due to the rapid spread of OS, the affected patients have very poor 5-year survival rate. The major methods used for treating OS are surgery and chemotherapy [9,10].

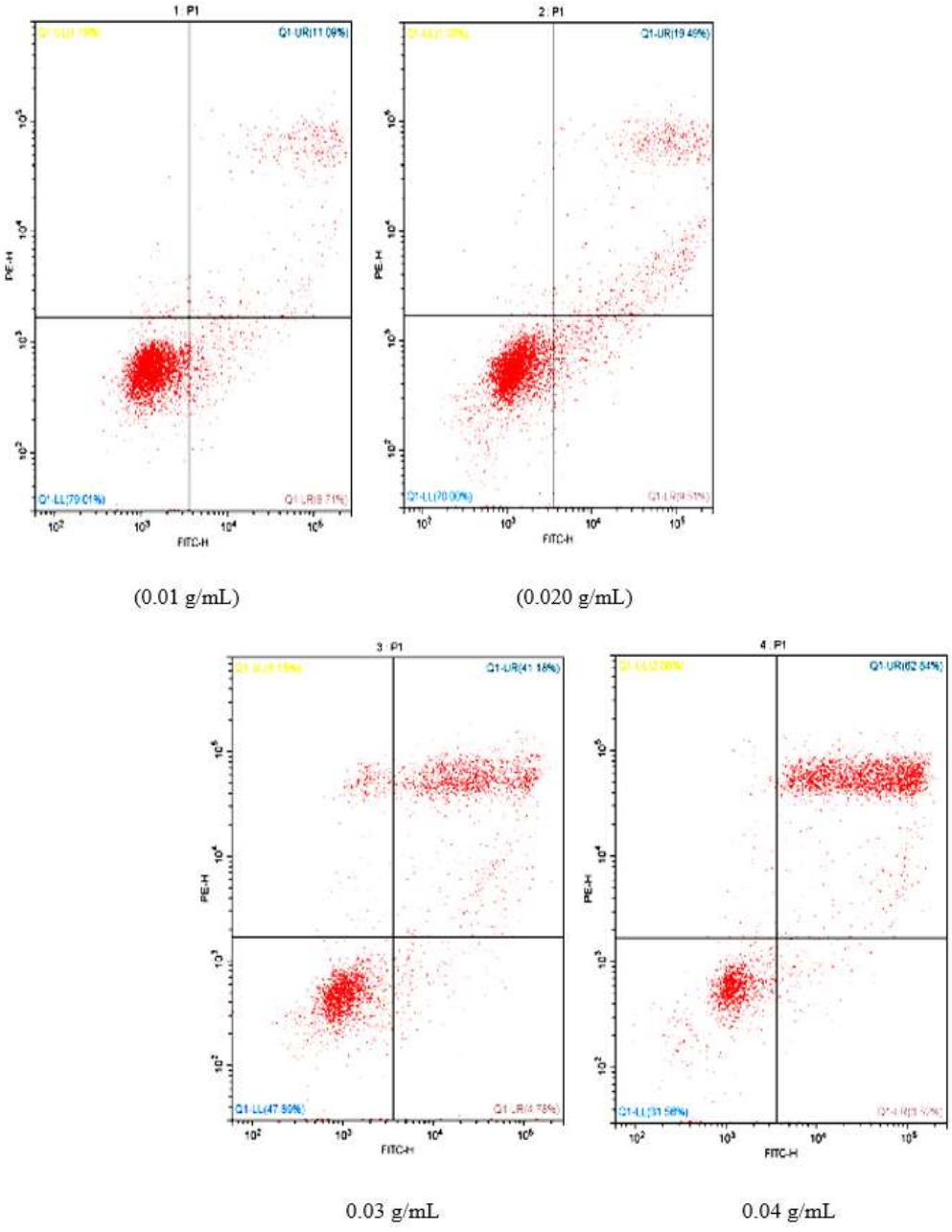

Figure 3: Flow cytometry showing effect of LJCE on U2OS cell apoptosis

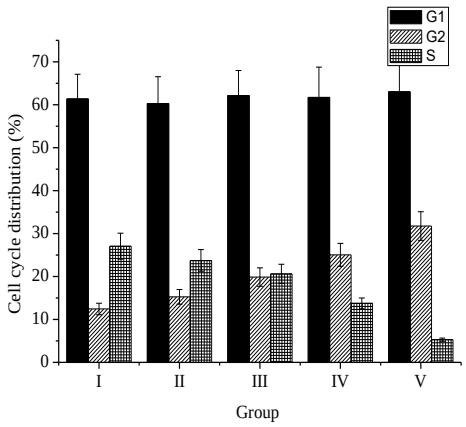

Figure 4: Influence of LJCE on U2OS cell cycle distribution

Unfortunately, chemotherapy-related adverse reactions and postoperative OS relapse lower the quality of life and survival rate of OS patients [11]. Therefore, the current focus is to find safer and more effective therapeutic drugs for OS.

A vast majority of traditional Chinese medicine comes from medicinal plants. The primary and secondary metabolites of these plants are very complex. This complexity is reflected in the different structural types of metabolites. The complex metabolites are an important foundation for the various pharmacological effects and remarkable efficacy exhibited by traditional Chinese medicine [12,13]. *Lonicera japonica* contains organic acids such as chlorogenate and iso-chlorogenic acid, and flavonoids (luteolin, luteolin and quercetin). Moreover, it contains various types of metabolites, e.g., ether terpenes such as deoxygenated loganin and swertiaside. It has been reported that chlorogenic acid may be synthesized through IFN-γ. This signaling pathway inhibited the expression of PD-L1 in both *in vivo* and *in vitro* models of esophageal cancer, thereby exerting anti-esophageal cancer effects [1]. Luteolin regulated the expression of MMP-9 by inhibiting the levels of H3K27Ac and H3K56Ac in the Akt/mTOR signaling pathway, thereby inhibiting the proliferation and metastasis of receptor-positive, triple-negative breast cancer [2].

Swertiside induced mitochondrial apoptosis, interfered with the cell cycle, and targeted the JNK/p38 signaling pathway, ultimately resulting in anti-glioma effects [3]. It is clear from the foregoing that *Lonicera japonica* vine and its metabolites exert anti-tumor effects through various pathways.

The MTT colorimetric method is a simple and accurate method for determination of cell proliferation [14]. It has the advantages of simplicity, speed, economy, and non-use of isotopes. This study used the MTT colorimetric method to determine the effect of LJCE on U2OS cell growth. In the current study, *Lonicerae japonicae* Caulis extract (LJCE) effectively inhibited the growth of human osteosarcoma U2OS cells. This indicates that LJCE has obvious anti-tumor activity.

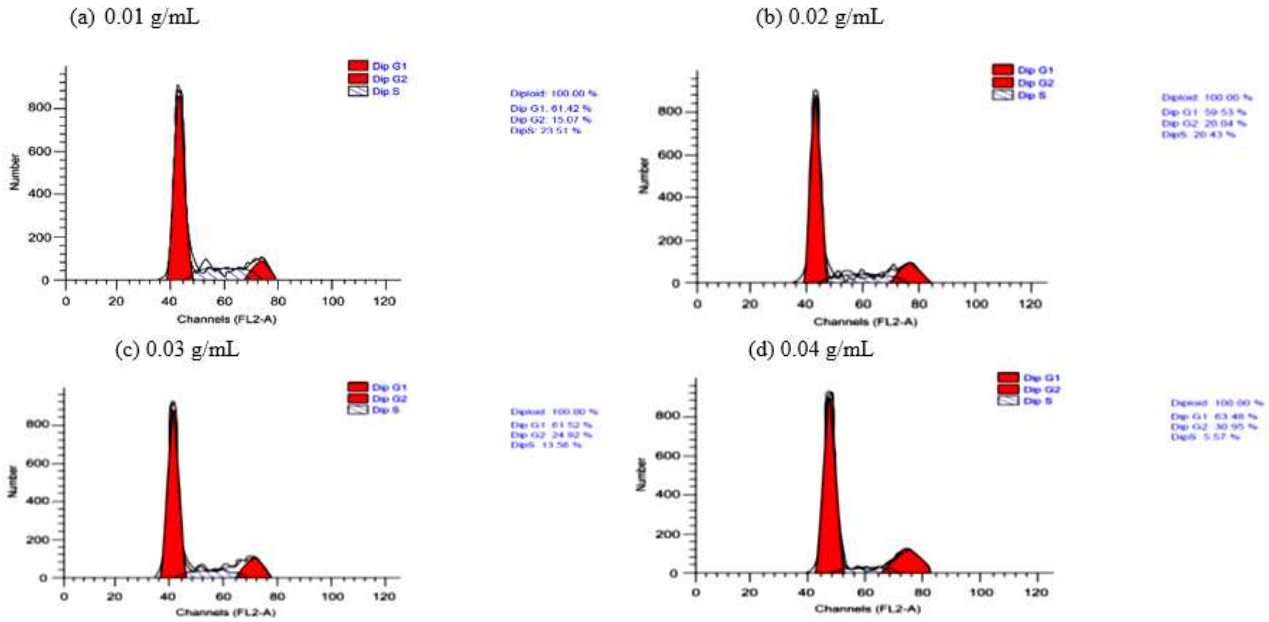

Figure 5: Flow cytometry showing effect of LJCE on U2OS cell cycle, at doses of (a) 0.01 g/mL, (b) 0.02 g/mL, (c) 0.03 g/mL and (d) 0.04 g/mL

Flow cytometry utilizes multiple targets of cascading events related to cell apoptosis to determine cell apoptosis [15]. At the onset of cell apoptosis, phosphatidylserine emerges from the inside layer of the cell to the surface and becomes exposed to the exterior space. Annexin V binds specifically to PS. The combined application of uptake of Annexin V and PI for determination of cell apoptosis makes it easy to distinguish between early-apoptotic and late-apoptotic cells. Therefore, this study used the Annexin-FITC/PI double staining method to measure the rate of apoptosis of gastric cancer cells. Various concentrations of FS-4 induced apoptosis in gastric cancer cells SGC-7901 after 12 h, in an obvious dose-dependent manner [16]. In the current study, *Lonicerae japonicae* Caulis extract (LJCE) effectively enhanced apoptosis of human osteosarcoma U2OS cells. At the same time, LJCE concentration-dependently induced halting of cell cycle in G2 phase. This indicates that LJCE promoted apoptosis of human osteosarcoma U2OS through induction of cell cycle arrest in G2 phase.

CONCLUSION

The present study has demonstrated that LJCE inhibits the growth of U2OS cells through the induction of apoptosis. Therefore, LJCE exhibits potential as a novel therapeutic agent for osteosarcoma. The data reported here provide some experimental basis for the clinical management of osteosarcoma using *Lonicerae japonicae* Caulis extract and its combination.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. XiaoWei Dong and RuShan Yan contributed equally to this work.

HaiDong Liang designed the study and carried it out. XiaoWei Dong, RuShan Yan, QiJun Yao, Yu Lei nad Haidong Liang supervised the data collection, and also analyzed and interpreted the data. XiaoWei Dong, RuShan Yan, QiJun Yao, Yu Lei and Haidong Liang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.

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