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ORIGINAL ARTICLE

Lappaconitine Hydrochloride Induces Apoptosis and S Phase Cell Cycle Arrest through MAPK Signaling Pathway in Human Liver Cancer HepG2 Cells

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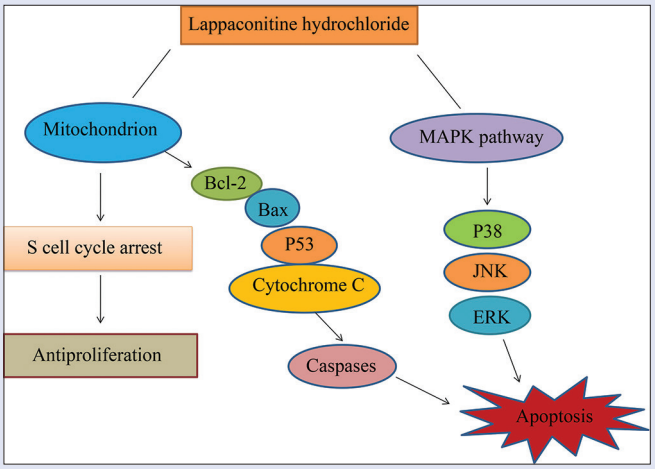
ABSTRACT

Background: Lappaconitine (LA), isolated from the root of *Aconitum sinomontanum* Nakai, had definite pharmacological effects, such as anticancer, analgesia, and anti-inflammation. LA and its derivatives had garnered prevalent consideration due to its analgesic and antitumor effects, but its clinical application was constrained by poor solubility. In this study, a novel LA hydrochloride (LH) was synthesized to upsurge the solubility and improve the efficacy. **Objectives:** The objective of this study was to examine the antitumor effect and primary mechanisms of LH on cell proliferation, cell cycle, and apoptosis in HepG2 cells. **Materials and Methods:** The cell viability and proliferation were assessed using Cell Counting Kit-8 and 5′-ethynyl-2′-deoxyuridine assay. The apoptosis morphological feature of cell was detected with the 4′,6-diamidino-2-phenylindole (DAPI) staining method. The effect of protein expression levels was recognized by Western blot assay. Cell cycle and apoptosis were estimated using flow cytometer. **Results:** LH repressed cell viability and proliferation of HepG2 cells and persuaded apoptosis in a dose-dependent way. Flow cytometry analysis results display that LH could arrest cell cycle of HepG2 cells in S phase, thereby preventing cells entering G2/M phase. LH upregulated the expression of cytochrome C, Bax, P53, cleaved caspase-3, cleaved caspase-9, and cleaved poly ADP-ribose polymerase (PARP) and suppressed the expression of Bcl-2. Furthermore, caspase inhibitor z-VAD-fmk inhibited the activation of cleaved caspase-3 and cleaved caspase-9. Moreover, LH abridged the phosphorylation levels of extracellular signal-regulated kinase and augmented the phosphorylation levels of c-Jun N-terminal kinase and P38. **Conclusion:** LH designated antitumor effect against HepG2 cells through suppressing cell proliferation, inducing apoptosis and cell cycle arrest by aiming mitogen-activated protein kinase signaling pathway.

Key words: Antitumor effect, Chinese medicine, HepG2 cells, lappaconitine hydrochloride, mitogen-activated protein kinase signaling pathway

SUMMARY

- Lappaconitine hydrochloride (LH) delivers the antitumor activity. LH can persuade apoptosis and cell cycle arrest in HepG2 cells. LH may show an antitumor role by activating mitogen-activated protein kinase signaling pathway.



Abbreviations used: LA: Lappaconitine; LH: Lappaconitine hydrochloride; CCK-8: Cell Counting Kit-8; EdU: 5-ethynyl-2′-deoxyuridine; RT: Room temperature; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal bovine serum; PI: Propidium iodide; PBS: Phosphate-buffered saline; FITC: Fluorescein isothiocyanate; OD: Optical density; SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PVDF: Polyvinylidene difluoride.

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INTRODUCTION

Cancer has extremely vulnerable human health due to high occurrence and death. According to the data from the Global Cancer Observatory, there were 36 detailed cancer types by sex and age group. Lung cancer is by far the leading cause of cancer death among both men and women, making up almost 25% of all cancer deaths, followed by female breast cancer (11.6%) and liver cancer (8.2%) for mortality.[1] Liver cancer is a

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common malignancy, which is not delicate to cytotoxic chemotherapy, and the diagnosis of patients is very poor.[2] At present, the treatment of hepatocellular carcinoma contains surgery, liver transplantation, and traditional chemotherapy. However, the side effects of these treatments have many adverse effects on the living standard of patients.[3] It is essential to find more effective substitutes without side effects to prevent and treat liver cancer.

Natural medicines (paclitaxel, camptothecin, etoposide, etc.) possess decent antitumor effects and low side effects, which have become an energetic means of emergent various drugs.[4] The development of natural medicines was originally employed straight without structural modification. In clinical use, these pure natural medicines often have the disadvantages of low solubility, poor pharmacokinetic properties, large toxic and side effects.[5] With the quick development of science and technology, organic synthesis technology and biotechnology have placed the foundation for the conception and promotion of new drugs. Taking natural substances as the precursors and meeting the necessities of drugs after structural modification and performance optimization is the key method for the creation of new natural medicines.[6] Current studies have shown that structural modification of natural medicines could recover physicochemical properties (solubility, distribution, ionizability, etc.), improve pharmacokinetic properties (absorption, distribution, metabolism and excretion), and diminish drug toxicity.[7]

Aconitine is an indispensable bioactive alkaloid resultant from *Aconitum* plants. It is usually used to treat rheumatoid arthritis, heart disease, and tumors.[8] Aconitine is also extensively used in analgesics worldwide, mainly in China and Japan,[9,10] which possesses reliable local esthesia, central analgesic, anti-inflammatory effect, antitumor effect, some refractory pain, and malignant tumor pain.[11,12] Lappaconitine (LA) is consequent from *Aconitum* root, which is mostly employed clinically for LA hydrobromide. It is the first-dependent central analgesic in China which is frequently used to cure gastrointestinal ulcers, gastritis, gallbladder inflammation,[13] rheumatism, sciatic pain, postoperative pain and cancer pain, etc.,[14,15] and possess no addiction, no accumulation of side effects, and mild side effects. Studies have exposed that LA and LA hydrobromide have antitumor effects. LA hydrobromide repressed liver cancer in mice and S180.[16] LA could activate cell apoptosis, leading to the G0/G1 phase arrest in A549 cells.[17] However, LA and LA hydrobromide distress their analgesic effects in clinical applications due to deprived water solubility. The study described that LA could proficiently synthesize with dissimilar inorganic acids, such as SO_4^{2-} and Cl^-. LA sulfate and LH [Figure 1] not only have good solubility but also improve its analgesic consequence and bioavailability.[18] LA sulfate had a perceptible proliferation inhibitory effect on HepG2 cells and HeLa cells.[19,20] Recently, LA sulfate employed the PI3K/Akt/GSK3β signaling pathway as a therapeutic aim, which caused apoptosis in HT-29 cells and A549 cells.[21,22] At present, whether LH has an antitumor effect has not been stated yet.

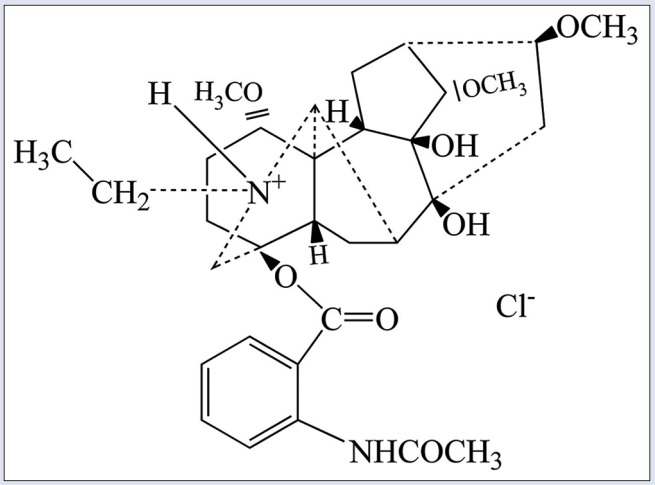

Figure 1: Structure of lappaconitine hydrochloride

Apoptosis is a particular course that leads to programmed cell death and a vigorous target for cancer therapy.[23] Two pathways can trigger apoptosis: mitochondrial pathway and death receptor pathway, both of which are related to mitochondria and Bcl-2 family proteins.[24] Cell apoptosis has a variety of biochemical and morphological structures, touching upon complex signal transduction, for example, PI3K/Akt and mitogen-activated protein kinase (MAPK) pathways.[25] The MAPK pathway contains three significant subfamilies: extracellular signal-regulated kinase (ERK), P38, and c-Jun N-terminal kinase (JNK). It plays a vital part in tumor genesis and regulates many kinds of biological functions, for example, cell apoptosis, proliferation, growth, differentiation, and metastasis.[26-28]

The introductory results of this study showed that LH has a broad-spectrum antitumor effect, which could persuade cell apoptosis of HeLa, A549, U251, and HepG2 cells, and has the superlative antitumor effect on HepG2 cells. This study exposed that LH inhibited cell growth *in vitro* by inducing cell cycle arrest and apoptosis and reconnoitered its potential mechanism. Therefore, LH may be further considered in the clinical trial for treating hepatocellular carcinoma.

MATERIALS AND METHODS

Lappaconitine hydrochloride preparation

LA (purity >97.7%) was delivered by Gansu Xinlan Pharmaceutical Co., Ltd. (Gansu, China). LH was synthesized on the basis of earlier studies.[18] 0.584 g of LA and excess 0.1 mol/L HCl into 10 mL of ethanol. The reaction was stirred at room temperature for 6 h. Consequently, the unreacted HCl solution and solvent were overlooked by decompression distillation. Finally, the repeated recrystallization of acetone resulted in the preparation of LH (Purity >98%), which was stored at −4°C in a dark place for forthcoming use.

Cell culture

HepG2 cells and HeLa cells were procured from the Cell Bank of Shanghai Institute of Cell Biology (Shanghai, China). U251 cells, A549 cells, and HUVEC cells were acquired from the medical experiment center of the 940th Hospital of Joint Logistics Support Force of Chinese People's Liberation Army. The mycoplasma contamination of cell cultures was distinguished by DAPI staining,[29] and the outcomes were negative. HepG2 cells, HeLa cells, A549 cells, HUVEC cells, and U251 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (DMEM, Invitrogen, USA) comprising 10% fetal bovine serum (FBS) (FBS, Invitrogen, USA) at 37°C in 5% CO_2 incubator.

Cell viability assay

Cell Counting Kit-8 (CCK-8) (Biosharp, Hefei, China) assay was showed to perceive cell viability. HepG2 cells, HeLa cells, U251 cells, A549 cells, and HUVEC cells were plated in 96-well plates at a density of approximately 3×10^3 cells/well and incubated with diverse dose of LH (100, 200, 300, 400, 500, and 600 μg/mL) for treating 48 h. Moreover, HepG2 cells (3×10^3 cells/well) were cultured with LA and LH (100, 200, 300, 400, 500, and 600 μg/mL) for 24 h and 48 h in

96-well plates. The old medium was thrown away and added 100 µL of medium containing 10 µL of CCK-8 solution to each well and further cultured for 1 h. The optical density of the samples was measured at 450 nm using a Microplate Reader (Thermo Scientific, USA). The antiproliferative activity is articulated by half-maximal inhibitory concentration (IC_{50}) value. IC_{50} value was calculated using GraphPad Prism 5. The lesser the IC_{50} value, the better the antiproliferative effect. The cell viability (%) = (A_{450} of treated group − A_{450} of blank group)/(A_{450} of untreated group − A_{450} of blank group) × 100%.

Cell proliferation assay

For assessment of cell proliferation, HepG2 cells were sowed in a 24-well plate (5×10^3 cells/well) under ordinary conditions overnight. Following that, HepG2 cells were treated with LH (200, 400, and 600 µg/mL) for 48 h. 5′-ethynyl-2′-deoxyuridine (EdU) cell proliferation exposure kit (RiboBio Co. Ltd., Guangzhou, China) was employed to detect cell proliferation. HepG2 cells were cultured using the EdU reagent for 2 h and fixed with 200 µL phosphate-buffered saline (PBS) comprising 4% paraformaldehyde. Finally, cells were dyed with 1 × Hoechst 33342 for 30 min.[21] Observation stained cells by the fluorescence microscope (Olympus, Tokyo, Japan).

The EdU-positive cells (%) = Number of red EdU/Number of blue Hoechst 33342 × 100%.

Cell morphology assay

HepG2 cells (1×10^4 cells/well) were incubated in a 6-well plate overnight and treated with LH (200, 400, and 600 µg/mL) for 48 h. Thereafter being fixed with 4% tissue cell for 30 min, PBS eroded

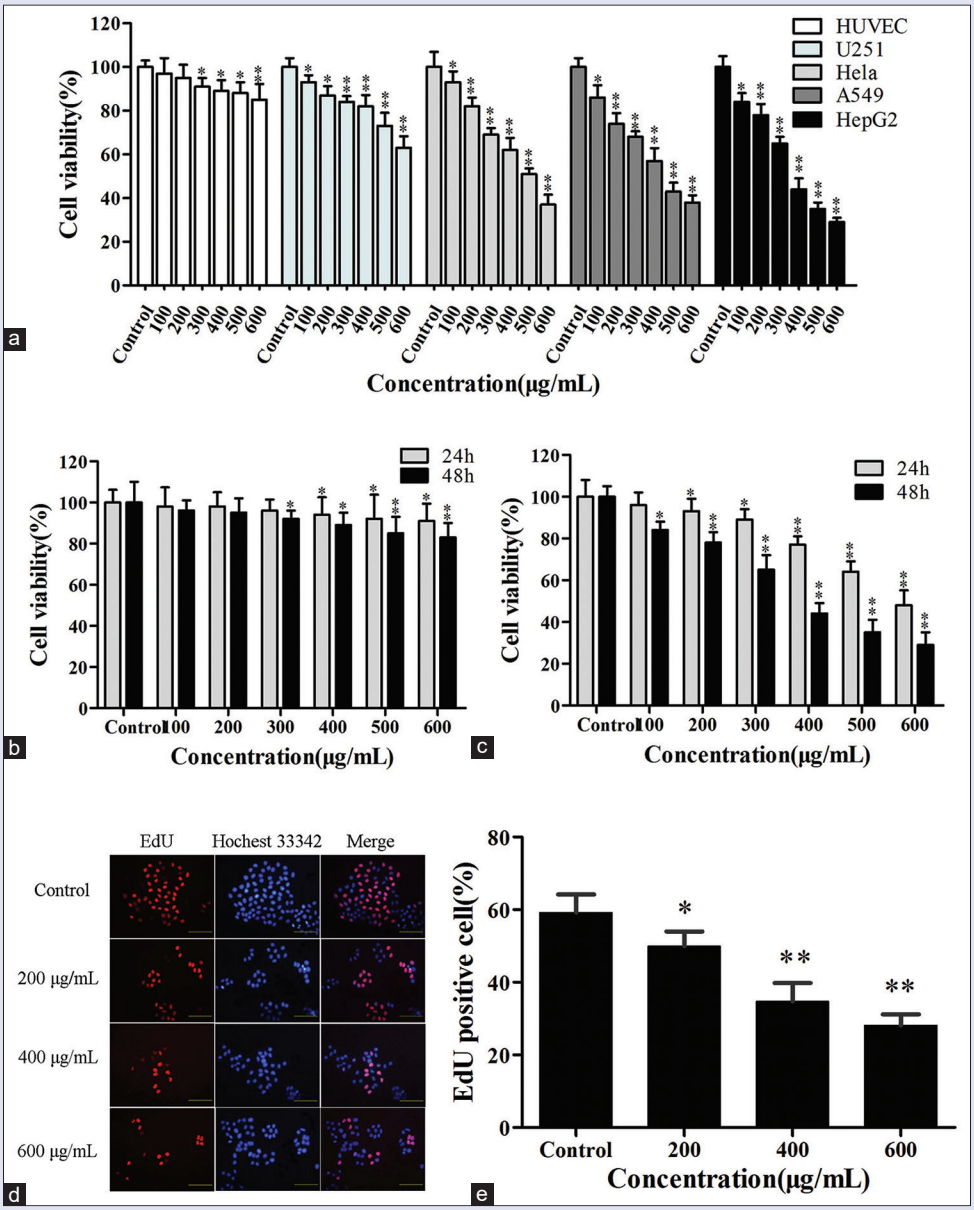

Figure 2: Lappaconitine hydrochloride suppressed HepG2 cell viability and proliferation. (a) HUVEC, U251, HeLa, A549, and HepG2 cells were treated with lappaconitine hydrochloride for 48 h, and cell viability was determined by CCK-8. (b) Cell viability was determined by CCK-8 after incubation with lappaconitine for 24 h and 48 h. (c) Cell viability was determined by CCK-8 after incubation with lappaconitine hydrochloride for 24 h and 48 h. (d) 5-ethynyl-2′-deoxyuridine proliferation assay was used to test cell proliferation. (e) Graph illustrating the ratios of 5-ethynyl-2′-deoxyuridine-positive cells/total cells. Date are reported as mean ± standard deviation ($n = 3$). *$P < 0.05$, **$P < 0.01$ compared with the control. Scale bar = 20 µm

twice. Consequently, the cells were stained with 200 µL of DAPI solution (Solarbio Science and Technology Co. Ltd., Beijing, China) in the dark for 30 min. The stained nuclei were imaged under fluorescence microscopy.

Cell cycle assay

HepG2 cells (1×10^5 cells/well) were cultured in a 6-well plate and treated with different concentrations of LH (200, 400, and 600 µg/mL) for 48 h. After LH treatment, cells were composed and washed and later fixed with 70% ice-cold ethanol at −20°C for 24 h. In the next stage, cells were eroded and stained with propidium iodide (PI) containing 1% RNase A at least for 30 min in darkness.[30] Finally, cell cycle distribution was determined by flow cytometer (BD FACsCanto, NJ, USA).

Apoptosis assay

LH-induced apoptosis of HepG2 cells was evaluated by annexin V/fluorescein isothiocyanate (FITC) and PI (BD Biosciences, USA) co-staining method. Concisely, HepG2 cells (1×10^5 cells/well) were cultured in a 6-well plate with diverse doses of LH (200, 400, and 600 µg/mL) for 48 h. Then, cells were composed and digested with 0.25% trypsin without EDTA. The cells were added 300 µL 1x binding buffer, dyed with annexin V/FITC in the dark for 30 min, and added 5 µL PI for 5 min before flow cytometer.

Western blot analysis

HepG2 cells (1×10^6 cells/well) were plated and exposed with LH (200, 400, and 600 µg/mL) for 48 h in a 6-well plate. Radioimmunoprecipitation assay buffer was used to extract protein. The bicinchoninic acid protein assay kit (Solarbio Science and Technology Co. Ltd., Beijing, China) was examined protein concentration. The protein was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels, shifted to a polyvinylidene difluoride membrane, and blocked with 5% nonfat milk for 2 h. The membranes incubate overnight with appropriate primary antibodies (Cell Signaling Technology, USA) at 4°C and then incubated 2 h using matched secondary antibodies (ZSGB-Bio, Beijing, China). Subsequently, the protein bands were pragmatic by an ECL detection kit (Solarbio Science and Technology Co. Ltd., Beijing, China).

Statistical analysis

All data are articulated as mean ± standard deviation (SD) and reduplicate at least three parallel experiments. One-way ANOVA was

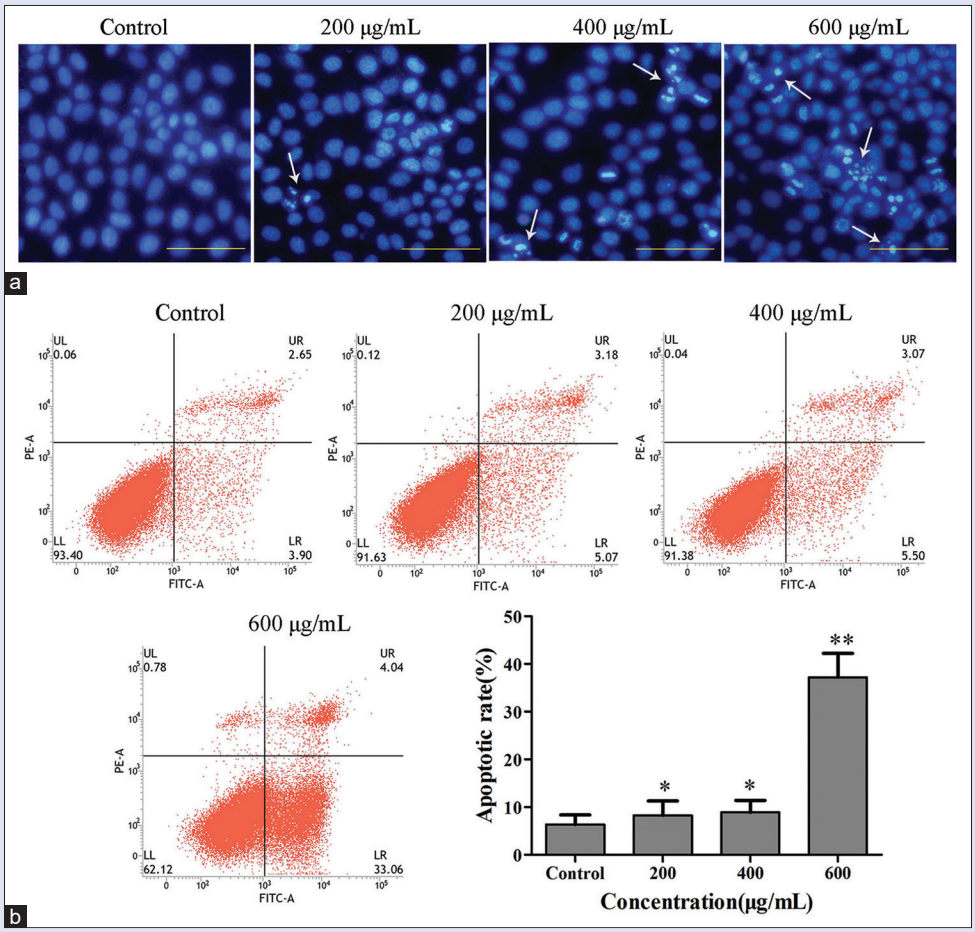

Figure 3: Lappaconitine hydrochloride-induced cell apoptosis in HepG2 cells. (a) Morphological changes in apoptosis of lappaconitine hydrochloride-treated HepG2 cells were observed. The cells were treated with lappaconitine hydrochloride for 48 h and stained with DAPI. Nuclear morphology was observed with a fluorescence microscope. (b) The apoptosis of HepG2 cells treated with lappaconitine hydrochloride for 48 h was analyzed by flow cytometer. The apoptosis rate was shown as mean ± standard deviation ($n = 3$). *$P < 0.05$, **$P < 0.01$ compared with the control. Scale bar = 20 µm

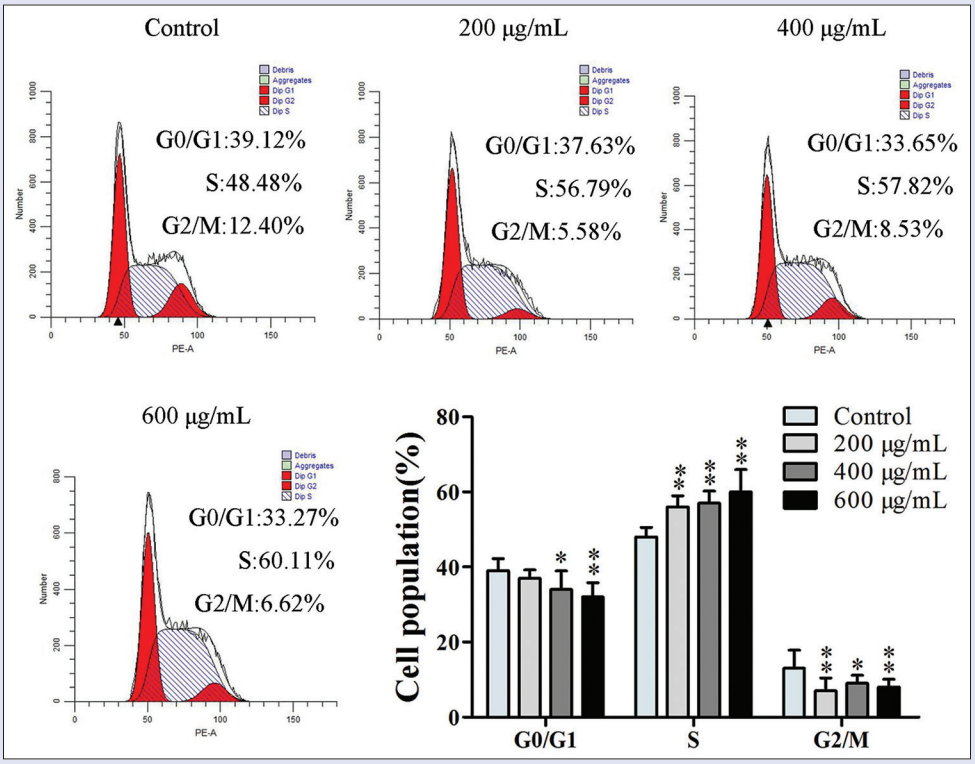

Figure 4: Lappaconitine hydrochloride-induced cell cycle arrest at S phase in HepG2 cells. Flow cytometer was used to analyze cell cycle after treatment with lappaconitine hydrochloride for 48 h. The results were expressed as the mean ± standard deviation ($n = 3$). *$P < 0.05$, **$P < 0.01$ compared with the control

used to analyze the statistical significance tests by SPSS 22.0 SPSS 22.0 (Chicago, IL, USA). $P < 0.05$ was measured statistically significant.

RESULTS

Lappaconitine hydrochloride inhibited cell viability and proliferation in HepG2 cells

CCK-8 assay was accepted to research the growth inhibitory effect. Effects of LH on the cell viability of HUVEC, U251, A549, HeLa, and HepG2 cells are revealed in Figure 2a. The IC_{50} value was employed to examine the inhibitory effect of LH on the proliferation of these cells (low IC_{50} reveals high antitumor effect). The relationship between the antitumor effects was: HepG2 > A549 > HeLa > U251 > HUVEC. Therefore, LH had the most noteworthy antitumor activity on HepG2 cells, so HepG2 cells were selected as the experimental purpose. In addition, at similar doses and experimental time points, LH had no seeming toxicity effect to HUVEC normal cells. It was designated that LH has high selectivity for cancer cells. On the one hand, LA and LH repressed HepG2 cell proliferation in a dose and time-dependent manner ($P < 0.05$), and inhibitory effect of LH is better than LA [Figure 2b and c]. The IC_{50} value of LH on HepG2 cells was 596.2 ± 0.296 μg/mL and 372.7 ± 0.342 μg/mL for 24 h and 48 h. On the other hand, EdU proliferation assay exhibited that the number of EDU-positive cells was noticeably on the diminution in a dose-dependent way ($P < 0.05$) [Figure 2d and e]. In summary, LH could outstandingly conquer cell proliferation in HepG2 cells.

Apoptosis induction by lappaconitine hydrochloride in HepG2 cells

DAPI staining was employed to detect whether HepG2 cells cause cell death through apoptosis or necrosis. The results exposed that the control group had a rounded nucleus and steadily stained. However,

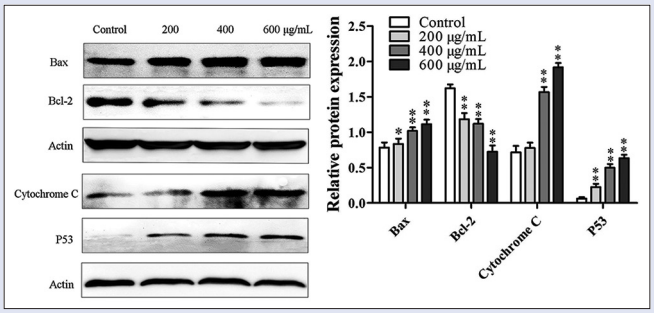

Figure 5: Apoptosis-related proteins were analyzed by Western blot in HepG2 cells. The expression of Bax, Bcl-2, Cytochrome C, and P53 was analyzed by Western blot. The intensity of the bands was presented as the mean ± standard deviation ($n = 3$). *$P < 0.05$, **$P < 0.01$ compared with the control

the LH-treated cells showed nuclear fragmentation, chromatin condensation, and apoptotic bodies initiated to take shape. These alterations were typical of apoptotic death, and they become more pronounced as the LH treatment concentration increases [Figure 3a]. To further examine LH-induced apoptosis, flow cytometer was adopted. Flow cytometer analysis established that the apoptosis rate suggestively augmented after exposure to LH for 48 h in a dose-dependent manner, correspondingly [Figure 3b].

Lappaconitine hydrochloride-induced S phase cell cycle arrest

HepG2 cells were showing to LH for 48 h. Cell cycle analysis was achieved by flow cytometer. LH-treated groups clearly augmented the

S population in a dose-dependent manner by comparing the control group [Figure 4]. Therefore, HepG2 cells were arrested in the S phase in a concentration-dependent manner to avoid cells from entering the G2/M phase, which might impede the proliferation of HepG2 cells.

Lappaconitine hydrochloride-induced apoptosis in HepG2 cells by mitochondrial signaling pathway

To further pursue the molecular mechanism of LH-induced apoptosis, Western blot was conducted to examine the influence of LH on apoptosis-related proteins. As shown in Figure 5, the expression of Bax, cytochrome C and P53 upregulated, while Bcl-2 downregulated with the enhanced concentration of LH. Moreover, the expression of cleaved caspase-3, cleaved caspase-9, and cleaved PARP augmented by LH treatment [Figure 6a and b]. The use of caspase inhibitor z-VAD-fmk further permitted this conclusion. As trails, cells had treated with various drugs: LH, z-VAD-fmk, and LH + z-VAD-fmk. Pretreatment with z-VAD-fmk blatantly lessened the protein expression of cleaved caspase-3 and cleaved caspase-9. When LH was combined with z-VAD-fmk, the two showed a better synergistic effect compared with that of LH alone [Figure 6c and d]. These data established that LH treatment causes the intrinsic apoptosis pathway in HepG2 cells.

Lappaconitine hydrochloride activated the mitogen-activated protein kinase signaling pathway

MAPK is a constituent of the intracellular signal transduction pathway. It has been confirmed to play a vital role in correcting diverse physiological processes, for instance, cell survival and death.[31] This study exemplified that the expression levels of p-P38 and p-JNK were seemingly upregulated ($P < 0.01$); p-ERK was evidently suppressed as the increasing concentration of LH ($P < 0.01$). However, the total expression of JNK, P38, and ERK endured not suggestively different [Figure 7]. The above outcomes discernibly showed that LH-induced cell apoptosis and cycle arrest in HepG2 cells were needy on p-ERK inactivation and activation p-P38 and p-JNK.

DISCUSSION

It is well known that cancer has twisted into the most common health problem in the world. Most anticancer drugs work mainly by activating cell death pathways, comprising apoptosis. Therefore, the regulation of the apoptosis pathway may lead to tumor genesis, drug resistance, and tumor progression, which are the key limiting elements for the attainment of cancer therapy.[32,33]

Traditional Chinese medicine is a brilliant resource for determining new anticancer drugs with low toxic side effects.[34] Preceding research has shown that lariciresinol repressed cell proliferation and persuade apoptosis in HepG2 cells by activating the mitochondrial-mediated apoptosis pathway.[4] LA is a traditional Chinese medicine isolated from the root of *Aconitum sinomontanum* Nakai. CCK-8 assay was employed to examine the effect of LA and LH on cell viability and proliferation of HepG2 cells. This finding displayed that LA and LH could impede the proliferation of HepG2 cells in a dose- and time-dependent manner [Figure 2b and c]. The IC_{50} of LA on HepG2 cells after 24 h and 48 h was 4029 ± 0.148 μg/mL and 2744 ± 0.411 μg/mL. The IC_{50} of LH on HepG2 cells after 24 h and 48 h was 596.2 ± 0.296 μg/mL and 372.7 ± 0.342 μg/mL. The above results prove that LH is more operative than LA. EdU assay revealed that LH could expressively inhibit the proliferation of HepG2 cells in a dose-dependent manner [Figure 2d and e], with the maximum effect at a dose of 600 μg/mL, and dependable with the results stated by Qu *et al.*[22]

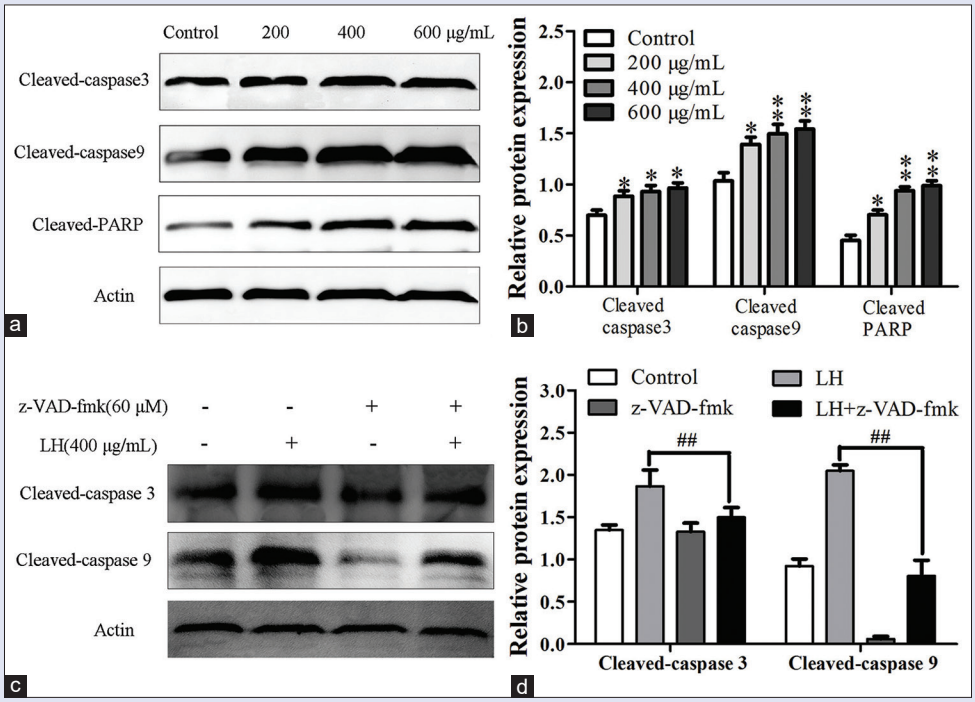

Figure 6: Caspase family proteins were assayed by Western blot. (a) The expression of cleaved caspase-3, cleaved caspase-9, and cleaved PARP was examined by Western blot. (b) Graph illustrating the gray value of the protein. Values were mean ± standard deviation (n = 3). *$P < 0.05$, **$P < 0.01$ compared with the control.(c) HepG2 cells were pretreated with z-VAD-fmk and the expression of cleaved caspase-3 and cleaved caspase-9 was examined by Western blot. (d) Graph illustrating the gray value of the protein. Values were mean ± standard deviation ($n = 3$). ##$P < 0.01$ regard as the significant difference between lappaconitine hydrochloride group and lappaconitine hydrochloride + z-VAD-fmk group.

Earlier in a few decades, killing tumors by inducing apoptosis has been measured as a new drift for the recognition of anticancer drugs. The apoptosis itself plays an energetic part in the development of all kinds of ailments containing cancer.[35] In this study, the double staining of annexin V-FITC/PI displayed that LH could visibly persuade cell apoptosis in early and late apoptosis. Constant with the above results, DAPI staining designated that LH-treated cells had noteworthy apoptotic morphological variations, comprising nuclear shrinkage and chromatin condensation [Figure 3]. Mitochondria-dependent pathways are thought to be one of the essential signaling pathways for various apoptosis.[8] Bcl-2 family proteins are linked to the apoptosis procedure and play an indispensable role in correcting the mitochondrial-dependent apoptosis pathway.[36] Western blot analysis exemplified that the expression of Bax, P_{53}, and cytochrome C augmented and the expression of Bcl-2 diminished [Figure 5]. Caspase family as an intracellular cysteine protease is a critical constituent of apoptosis. It is categorized as two classes of functions, containing promoter caspase, for example, caspase-8, caspase-9, and caspase-12, which can trigger the downstream effects caspase, such as caspase-3.[37] In particular, caspase-3 is widely stared as a critical part of the caspase family, and its activation is a feature of apoptosis.[38] In this study, LH could persuade upregulation of cleaved caspase-3, cleaved caspase-9, and cleaved PARP [Figure 6a]. Moreover, pretreatment with z-VAD-fmk further condensed the activity of cleaved caspase-3 and cleaved caspase-9 [Figure 6c]. In summary, LH tempts apoptosis in HepG2 cells via activating the mitochondrial pathway.

The cell cycle denotes the entire time course that a cell goes through from the accomplishment of one division to the end of the next division. The critical control point for the initiation of proliferation in the cell cycle is between G1 and S phase cycle.[39,40] Chen *et al.*[41] have stated that cannabisin B induces S phase cell cycle arrest in HepG2 cells. Flow cytometry analysis presented that LH arrested HepG2 cells in the S phase [Figure 4], interfering with cells entering G2 and M phase, thereby constraining cell proliferation and persuading apoptosis.

Because tumor metastasis frequently narrates to the coordination of multiple pathways of signal transduction, these pathways regulate cell invasion, migration, and tumor remodeling.[28] MAPK signaling pathway controls the key events in metastasis and incursion of cells.[42] In general, the activation of JNK and P38 kinase helps apoptosis during cell strain and injury, while the activation of ERK is frequently related to cell proliferation and cycle process.[43] Activation of P38 MAPK and JNK also persuades cell apoptosis and cycle arrest.[26,44,45] Earlier founded that pentoxifylline inhibited the proliferation of HepG2 cells and induced apoptosis by activating the MAPK signaling pathway.[46] In this research, to prove whether MAPKs are connected to the cellular mechanism of LH-induced apoptosis, the expression of p-P38, P38, p-JNK, JNK, p-ERK, and ERK was examined by Western blot method. Our results exposed that p-P38 and p-JNK were clearly unregulated while p-ERK was suppressed compared to the control group [Figure 7]. The study designated that MAPK signaling pathway was denoting LH-induced apoptosis.

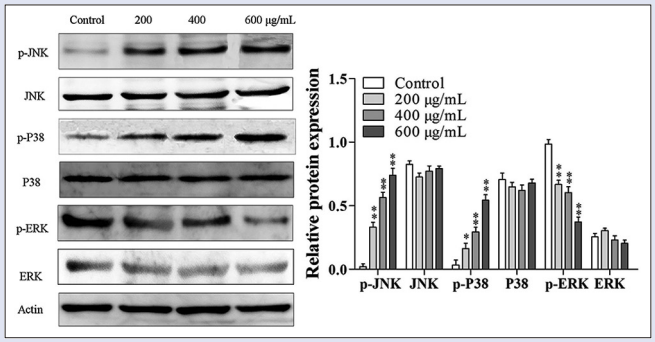

Figure 7: Lappaconitine hydrochloride activated the mitogen-activated protein kinase signaling pathway in HepG2 cells. HepG2 cells were treated with lappaconitine hydrochloride for 48 h. The expression of p-extracellular signal-regulated kinase, extracellular signal-regulated kinase, p-JNK, c-Jun N-terminal kinase, p-P38, and P38 was performed by Western blot analysis. Densitometry analysis values were mean ± standard deviation ($n = 3$). *$P < 0.05$, **$P < 0.01$ compared with the control

CONCLUSION

LH could hinder cell growth, induce S phase cell cycle arrest and apoptosis in human liver cancer HepG2 cells via mitochondrial and MAPK pathway. Although the mechanism of HepG2 cell apoptosis has been considered through a model *in vitro*, there are still some boundaries. Therefore, we will further authenticate our conclusions using animal models in the following study. Besides, LH exerts an outstanding anticancer activity, and it is conceivable to further explore its effect in the treatment of liver cancer in clinical trials.

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Conflicts of interest

There are no conflicts of interest.

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