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Research Article
Methanol Extract of *Ficus pumila* L. Inhibits Proliferation, Induces Apoptosis and Arrests the Cell Cycle in HepG2 Live Cancer Cells

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Abstract

Background and Objective: Liver cancer is one of the leading causes of cancer deaths worldwide. Research and development of drugs derived from herbs have always been focused on today. This study aimed to screen and evaluate the effects of the methanol extract of *Ficus pumila* L. leaves on live cancer cells HepG2. **Materials and Methods:** The inhibitory effect of the extract on the proliferation of HepG2 cells was evaluated using an MTT assay. Three dimensions culture model was used to reveal the effect of the extract on the tumorsphere formation ability of HepG2 cells. The apoptosis and cell cycle were analyzed by flow cytometry. Realtime PCR was used for mRNA expression analysis. All data were analyzed using the Mann-Whitney test. **Results:** The *Ficus pumila* L. extract (FPE) has a strong inhibitory activity against liver cancer cells, with an IC_{50} value of 66.42 μg mL^{-1}. The FPE declined the number and size of tumorspheres compared ($p<0.05$) and the apoptosis rate increased by 13.2±2.7 and 35.8±5.0% compared to 4.6±1.6% in control ($p<0.05$). The cell cycle of the FPE-treated cells was arrested at the G2/M phase. The FPE upregulated the expression of several genes involved in cell cycle arrest and apoptosis including p53, p21 and GADD45A and downregulated the expression of cyclin B1 and cyclin D1 genes compared to the control ($p<0.05$). **Conclusion:** The results suggested that *Ficus pumila* L. has potential against liver cancer cells.

Key words: *Ficus pumila* L., HepG2 liver cancer cells, cell cycle, apoptosis, tumorsphere, 3D culture

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Liver cancer has a high mortality rate compared to many other types of cancer in humans. Current therapies for liver cancer have achieved a great deal of progress, including targeted therapy, immunotherapy, cytotoxic therapy or combination therapies[1]. However, besides the advantages of improved efficacy, the cost of these therapies is often expensive for patients[2]. Moreover, the response rate to targeted therapy or immunotherapy is not high, usually less than 20%[3]. Meanwhile, traditional chemotherapy and radiation therapies often cause complications or unwanted effects[4]. Research on the value of herbs in cancer treatment has attracted a lot of attention because it is based on the experience of traditional medicine in many countries around the world[5]. Studying biological mechanisms such as apoptosis, cell cycle and molecular signaling pathways related to anticancer properties is essential for further elucidation of the value of herbs. For liver cancer, some herbs have been used to make drugs in the treatment of liver cancer in China, such as *Trametes robiniophila*, *Brucea javanica*, *Patrinia scabiosaefolia*, *Eupolyphaga steleophaga*, *Citri reticulatae* and *Patrinia scabiosaefolia*[6]. Besides, many other herbs with the potential to inhibit liver cancer have been studied for their molecular mechanisms such as PI3K/AKT signaling pathway[7], cell cycle arrest, apoptosis and reactive oxygen species (ROS)[8].

Ficus pumila L. belongs to the *Ficus* genus with over 800 species reported, widely distributed in tropical and subtropical countries[9]. Over the past several decades, ethnically empiric healing methods along with antibacterial, anti-inflammatory, antioxidant and antidiabetic properties have been shown[10]. Many species in this genus can fight against liver cancer[11], breast cancer[12], colon cancer[13] and lung cancer[14] as mentioned.

Ficus pumila L. is used as an indigenous method for the treatment of hypertension and dyslipidemia[15], liver, kidney and intestinal diseases[16] as well as acts as an anti-inflammatory, antioxidant[17] and antiviral[18].

Several bioactive compounds have been identified in *Ficus pumila* L. including phenolic acids, flavonoids, terpenoids, alcohols and steroids[16]. Their antioxidant, anti-inflammatory, lipolytic, antidiabetic and blood pressure-regulating effects have been reported[15]. For cancer, several studies have shown that the extracts or some certain compounds isolated from *Ficus pumila* L. have the ability to inhibit breast cancer cells, lung cancer cells[19] and leukemia cells[20]. While in some species of the *Ficus* genus, the molecular mechanism of cancer cell inhibition has been studied. Studies on *Ficus pumila* L. have been stopped at determining the ability to inhibit proliferation. Therefore, evaluating the effects of *Ficus pumila* L. on some biological activities in hepatocellular carcinoma needs to be elucidated. Therefore, the present study aimed to investigate the antiproliferative potentials of FPE on HepG2 cells and the underlying mechanism of action through the investigation of apoptosis and cell cycle.

MATERIALS AND METHODS

Collecting leaf samples of medical plants: The study was carried out from April, 2020 to October, 2021. Leaf samples of seven medical plants including *Sambucus javanica* Blume, *Lasia spinosa* Thwaites, *Hovenia dulcis* Thunb, *Asarum petelotii* (Otto Charles Schmidt). The O.C. Schmidt, *Gynura procumbens* (Lour.) Merr., *Ficus pumila* L. and *Sabia parviflora* Wall. ex Roxb. were collected, classified and stored at the National Institutes of Medicinal Materials, Vietnam. These herbs were used according to the indigenous experience of the Dao people in Sapa Mountain, Lao Cai Province of Vietnam to treat several different liver diseases. An amount of 300 g of each leaf sample were washed and dried at 40°C for 72 hrs and then ground with a mortar and pestle into a fine powder. Weigh 15 g of the obtained powder, add 30 mL of 90% methanol and shake the mixture at 200 revolutions per minute (rpm) for 48 hrs. The extract was filtered with Whatman filter paper and then dried in an oven (WFO-520 EYELA, Tokyo, Japan) at 40°C for 48 hrs. The residue obtained after evaporation will be dissolved in Dimethyl Sulfoxide (DMSO) for further analysis.

Cell culture and 2D cell treatment: The HepG2 cell line was grown under single-layer (2D) culture conditions in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% ampicillin/streptomycin (P/S) at 37°C and 5% CO_2. All these chemicals were supplied by Thermo Fisher.

An amount of 10,000 cells were added to each well of a 96-well culture plates containing 100 µL of RPMI 1640 medium. After 24 hrs, the culture medium was completely replaced with 100 µL of the new culture medium containing extracts at different concentrations. Control samples were cells grown in extract-free RPMI 1640 cell culture medium for 24 and 48 hrs.

Cell culture and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay: The HepG2 live cancer cells were cultured in RPMI 1640 medium supplemented with 1% P/S and 10% FBS in 96-well plates at 37°C, 5% CO_2. After 24 hrs, the cells were treated with FPE at different concentrations from 10 to 300 µg mL^{-1}. After 24 or 48 hrs of treatment, the cell phenotype was observed and evaluated using an inverted microscope Ts2 Nikon (Tokyo, Japan).

Cell proliferation was measured by using an MTT assay. Briefly, completely remove the old culture medium, add 100 µL of new culture medium containing 10% MTT at 5 mg mL^{-1} and incubate the culture dish at 37°C for 4 hrs, protected from light, removed culture medium containing MTT and added 100 µL DMSO per well, measuring the optical density (OD) at a wavelength of 570 nm on a spectrophotometer (Multiskan Sky, Thermo Fisher, Waltham, Massachusetts, USA). The cell proliferation rate was calculated by the formula[11]:

$$\text{Cell proliferation (\%)} = \frac{\text{OD value of treated sample}}{\text{OD value of control sample}} \times 100$$

GraphPad Prism 5.0 software (San Diego, California, USA) was used to determine the IC_{50} value for each cell line.

Cell culture and 3D cell treatment: An amount of 1,000 HepG2 cells cultured in each well of a 96-well culture plate were anti-adhesion treated with PolyHEMA (Sigma Aldrich, St. Louis, Missouri, USA) containing 100 µL of DMEMF12 medium (Thermo Fisher, Waltham, Massachusetts, USA), which contains 20 nM Epidermal Growth Factor (EGF) and 20nM Fibroblast Growth Factors (FGF) (Sigma Aldrich, St. Louis, Missouri, USA) as reported by Ramcharan *et al.*[20]. The tumorsphere culture medium was supplemented with FPE at concentrations of 0, 50, 100, 150 and 300 µg mL^{-1}. After 5 days of culture, the number of tumorspheres was counted and photographed under an inverted microscope. Tumorsphere size was measured by using the NIS-Elements imaging software version 3.0 and the Eclipse Ts2 microscope (Nikon, Tokyo Japan). A group of cells was defined as tumorspheres if their diameter was $\geq$100 nm. The number and size of tumorspheres in the treated sample were compared with the control samples.

Analysis of cell cycle and apoptosis using flow cytometry: The HepG2 hepatocellular carcinoma cells were cultured on 12-well culture plates. After 24 hrs, cells were supplemented with a culture medium containing FPE at concentrations of 100, 200 and 300 µg mL^{-1}. The control wells were not added FPE. The treatment time was 48 hrs in the 37°C, 5% CO_2 condition. Subsequently, cells were separated from the plate surface by treatment with trypsin/EDTA and collected by centrifugation at 1,500 rpm for 3 min and fixed in 70% ethanol at -20°C overnight. Then, cells were stained with fluorochrome solution (0.1% sodium citrate (w/v), 0.1% Triton X-100 (v/v), 50 µg mL^{-1} propidium Iodide (PI) in deionized water) for 2 hrs at 4°C before analyses using the BD-Accuri C6 plus Flow cytometry system (BD Biosciences, Bedford, Massachusetts, USA)[21].

Total RNA extraction and real-time PCR: The total RNA of HepG2 hepatocellular carcinoma cells was extracted with Trizol according to the manufacturer's instructions (Invitrogen). Cells cultured on 6-well culture plates were supplemented with 1,000 µL Trizol and mixed with a 1 mL pipette several times to separate cells from the surface of the plate. Incubate cells at room temperature for 5 min, then centrifuge at 12,000 rpm for 10 min at 4°C. Transfer the supernatant to a new Eppendorf tube. Add 2 mL chloroform, vortex the sample for 15 sec and incubate at room temperature for 3 min. Centrifuge at 10,000 rpm for 15 min at 4°C, repeat 2 times. Dry the RNA for 5-10 min and add 100 µL of deionized water. Spectrophotometer at 260/280 nm using a NanoDrop spectrophotometer (Thermo Fisher, Waltham, Massachusetts, USA). Use 1 µg RNA to generate cDNA using SuperScript™ III Reverse Transcriptase (Invitrogen-Thermo Fisher, Waltham, Massachusetts, USA). The Invitrogen™ Platinum™ SYBR™ Green qPCR SuperMix-UDG w/ROX mix was used for Real-time PCR analysis (Invitrogen-Thermo Fisher, Waltham, Massachusetts, USA) in a qTower3 (Analytik Jena, Jena, Germany) following the manufacturer's protocol. Amplification was carried out using target primers (Table 1) for 95°C for 2 min and 40 cycles of 15 sec at 95°C and 30 sec at 60°C and their relative expression was determined by normalizing the expression of each target HPRT1. The change in gene expression level was calculated by method $2^{-\Delta\Delta Ct}$ [22].

Table 1: Primer sequences of genes used for real-time PCR analysis

Gene	Primer sequence (5'-3')
p21	AGGTGGACCTGGAGACTCTCAG
	TCCTCTTGGAGAAGATCAGCCG
p53	CCTCAGCATCTTATCCGAGTGG
	TGGATGGTGGTACAGTCAGAGC
GADD45A	CTGGAGGAAGTGCTCAGCAAAG
	AGAGCCACATCTCTGTCGTCGT
Cyclin B1	GACCTGTGTCAGGCTTTCTCTG
	GGTATTTTGGTCTGACTGCTTGC
Cyclin D1	TCTACACCGACAACTCCATCCG
	TCTGGCATTTTGGAGAGGAAGTG
HPRT1	CATTATGCTGAGGATTTGGAAAGG
	CTTGAGCACACAGAGGGCTACA

Statistical analysis: All samples were evaluated at 3 replicates and presented as Mean±Standard Deviation (SD). Study data were analyzed using Graphpad Prism 5.0 software (Graphpad Inc., San Diego, USA), with Mann-Whitney Test ($p<0.05$).

RESULTS

Inhibitory effect of medical plant extracts on HepG2 liver cancer cells: The inhibitory effect of methanol extracts from seven medical plants on HepG2 liver cancer cells was presented in Table 2. The results showed that the extracts inhibited the proliferation of HepG2 cells in a dose and sample-type-dependent manner. The IC_{50} values recorded were from 66.46-227.7 μg mL^{-1}. *Ficus pumila* L. extract was shown to be a more potent inhibitor of HepG2 cells than the other 6 herbs in the screened group with IC_{50} value of 66.46 μg mL^{-1}.

FPE inhibits the proliferation of HepG2 cells: The first objective of this study was to determine the ability of FPE to inhibit the proliferation of HepG2 cells. The HepG2 cells were cultured in FPE-supplemented media at concentrations ranging from 0 to 300 μg mL^{-1}.

The MTT assay results (Fig. 1a) showed that at the concentration of 50 μg mL^{-1}, HepG2 liver cancer cells grew well on the surface of the culture medium and did not differ from the control group in both 24 and 48 hrs of processing. However, the number of cells significantly declined at concentrations from 100-300 μg mL^{-1} compared to the control group at both 24 hrs ($^{\#}p<0.05$) and 48 hrs treatment time ($^{*}p<0.05$). The cell proliferation rate decreased from 81.9±6.6% (at the concentration of 150 μg mL^{-1}) to 23.8±3.7% (at the concentration of 300 μg mL^{-1}) after 24 hrs of treatment with the FPE and from 40.4±3.9% (at the concentration of 150 μg mL^{-1}) to 15.6±2.1% (at the concentration of 300 μg mL^{-1}) after 48 hrs of treatment with the FPE. The IC_{50} value determined at 24 hrs was 164 μg mL^{-1} and at 48 hrs was 117.8 μg mL^{-1}. Cell images at 48 hrs of treatment (Fig. 1b) also showed a significant decrease in cell density from the concentration of 150-300 μg mL^{-1}. On the other hand, at the concentrations of 200 and 300 μg mL^{-1}, the cells lost their association with the surface of the culture plate and shrank into an oval shape. This image was consistent with the results of the MTT analysis presented above.

FPE inhibits the formation of 3D tumorspheres of HepG2 cells: To evaluate the effect of the FPE on the ability to form tumorspheres in the 3D culture model, cells were incubated in a medium containing different concentrations of the extract. The results (Fig. 2a-b) showed that the FPE at concentrations from 50-150 μg mL^{-1} significantly decreased the number of formed tumor spheres, ranging from 57.7±11.4 to 8.8±1.7, compared to the control which is 82.2±19.1, respectively. Meanwhile, at the higher concentrations (200 and 300 μg mL^{-1}) the tumorspheres were not observed. On the other hand, the size of tumorspheres formed in FPE medium was remarkably reduced in diameter from 65.4±12.9 to 39.6±1.9% compared to the control (100%), respectively.

FPE induces apoptosis of HepG2 cells: Induction of apoptosis is one of the important and common mechanisms of many current anticancer drugs. In this study, the effects of FPE are evaluated at concentrations of 50, 100 and 200 μg mL^{-1} after 48 hrs of treatment on apoptosis of HepG2 cells by Flow cytometry and the change in nuclear phenotype with DAPI (4,6-diamidino-2-phenylindole) dye.

The results (Fig. 3a) showed that the percentage of cells with apoptosis was significantly increased in the cells cultured in the medium supplemented with FPE at a concentration of 100 μg mL^{-1} (13.2±2.7%) and 200 μg mL^{-1} (35.8±5.0%) compared to the control group.

Nuclear staining with DAPI dye (Fig. 3b) indicated that the number of cells where nuclear apoptosis phenotype (arrow) was observed was remarkably increased compared to the control. This result confirmed the above results of Flow cytometry analysis (Fig. 3a). Taken together, these results showed that FPE induced apoptosis in HepG2 cells.

Table 2: Inhibition rate of medical plant extracts on HepG2 cells

Medical plants	100 (μg mL^{-1})	200 (μg mL^{-1})	IC_{50} value (μg mL^{-1})
Sambucus javanica Blume	46.96±2.91%	53.58±2.93%	89.96
Lasia spinosa Thwaites	19.05±9.51%	35.79±8.15%	227.7
Hovenia dulcis Thunb	59.22±11.77%	69.92±7.01%	109.4
Asarum petelotii O.C. Schmidt	45.64±4.24%	54.95±2.03%	184.5
Ficus pumila L.	59.57±3.95%	75.57±4.16%	66.42
Gynura procumbens (Lour) Merr.	17.37±4.21%	53.41±3.13%	142.3
Sabia parviflora Wall. ex Roxb.	1.20±6.14%	35.86±18.89%	185.2

Mean±SD

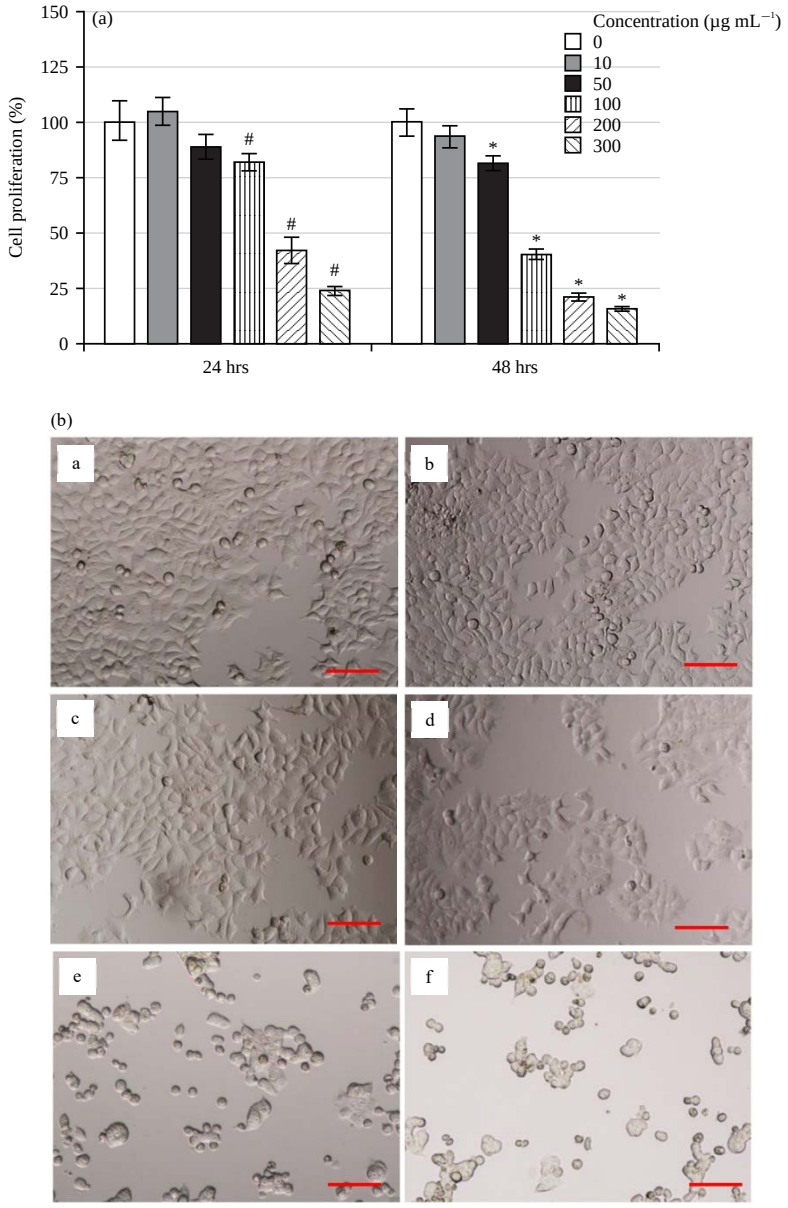

Fig. 1(a-b): Effect of FPE on cell proliferation and morphology. Cells were cultured in the media supplemented with the FPE of 0-300 µg mL^{-1} for 24 and 48 hrs, (a) Cell proliferation rate measured by MTT assay were represented as Mean±SD and (b) Morphologic changes of HepG2 cells at 48 hrs of treatment with FPE

(a) #p<0.05 vs control at 24 hrs, *p<0.05 vs control at 48 hrs, Mann-Whitney Test and (b) a: 0, b: 10, c: 50, d: 100, e: 200, f: 300 µg mL^{-1} at 200X magnification and Scale bar = 50 µm

FPE arrests the cell cycle in the G2/M phase: Evaluation of the effect of FPE on the cell cycle was performed at concentrations of 10, 50 and 100 µg mL^{-1} for 48 hrs of treatment. The results (Fig. 4a) showed that, at the concentration of 10 µg mL^{-1}, there was no significant change in the percentage of cells in different cell cycle phases. However, at the concentrations of 50 and 100 µg mL^{-1}, the percentage of cells in the G2/M phase increased to 24.6±2.5 and 33.6±3.4% compared to 19.7±2.2% in the control. Hence, the FPE arrested the cell division cycle in the G2/M phase. Subsequently, to clarify changes in mRNA expression levels of several cell cycle-related genes, a concentration of 200 µg mL^{-1} FPE was used to treat cells for the analyses of cyclin B1, cyclin D1, p53, p21 and GADD45A genes expression by Real-time PCR. The results

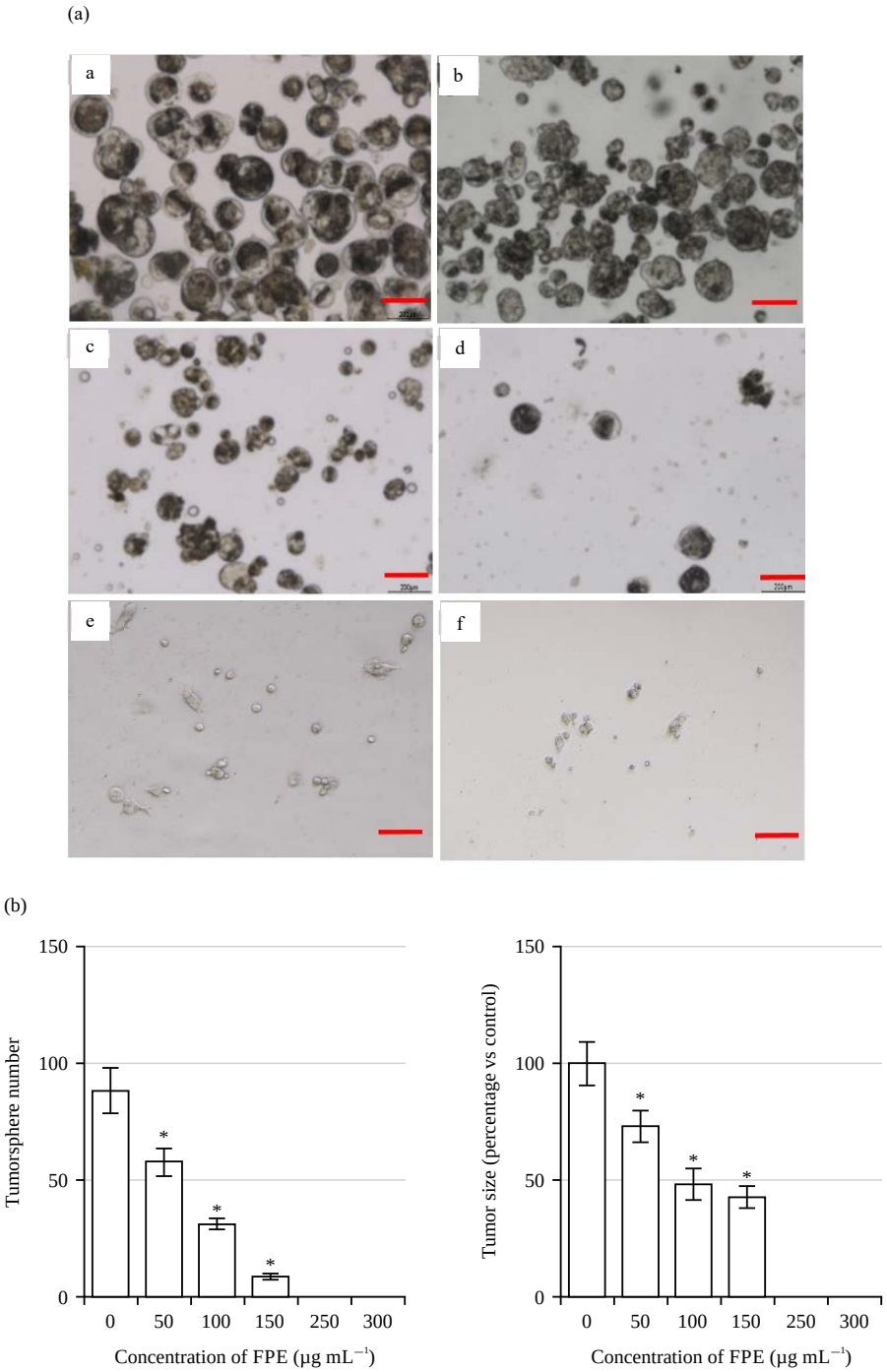

Fig. 2(a-b): Effect of FPE on tumorsphere formation of HepG2 cells, (a) Cells were cultured under non-adhesive conditions and supplemented with extract concentrations of FPE and (b) Change in the number and size of the tumorspheres was expressed as a percentage compared to the control

(a) a: 0, b: 10, c: 50, d: 100, e: 200 and f: 200 µg mL^{-1}, Tumorsphere images at 200X magnification after 5 days of culture, Scale bar = 200 µm and (b) *p<0.05 vs control, Mann-Whitney Test

(Fig. 4b) showed that mRNA levels of GADD45A, p53 and p21 genes were increased approximately 4, 1.5 and 3 times, respectively. While the mRNA levels of cyclin B1 and cyclin D1 were increased approximately 10 and 6 times. Thus, the FPE regulated expression of the genes controlling the cell cycle leading to arrest the of cell division.

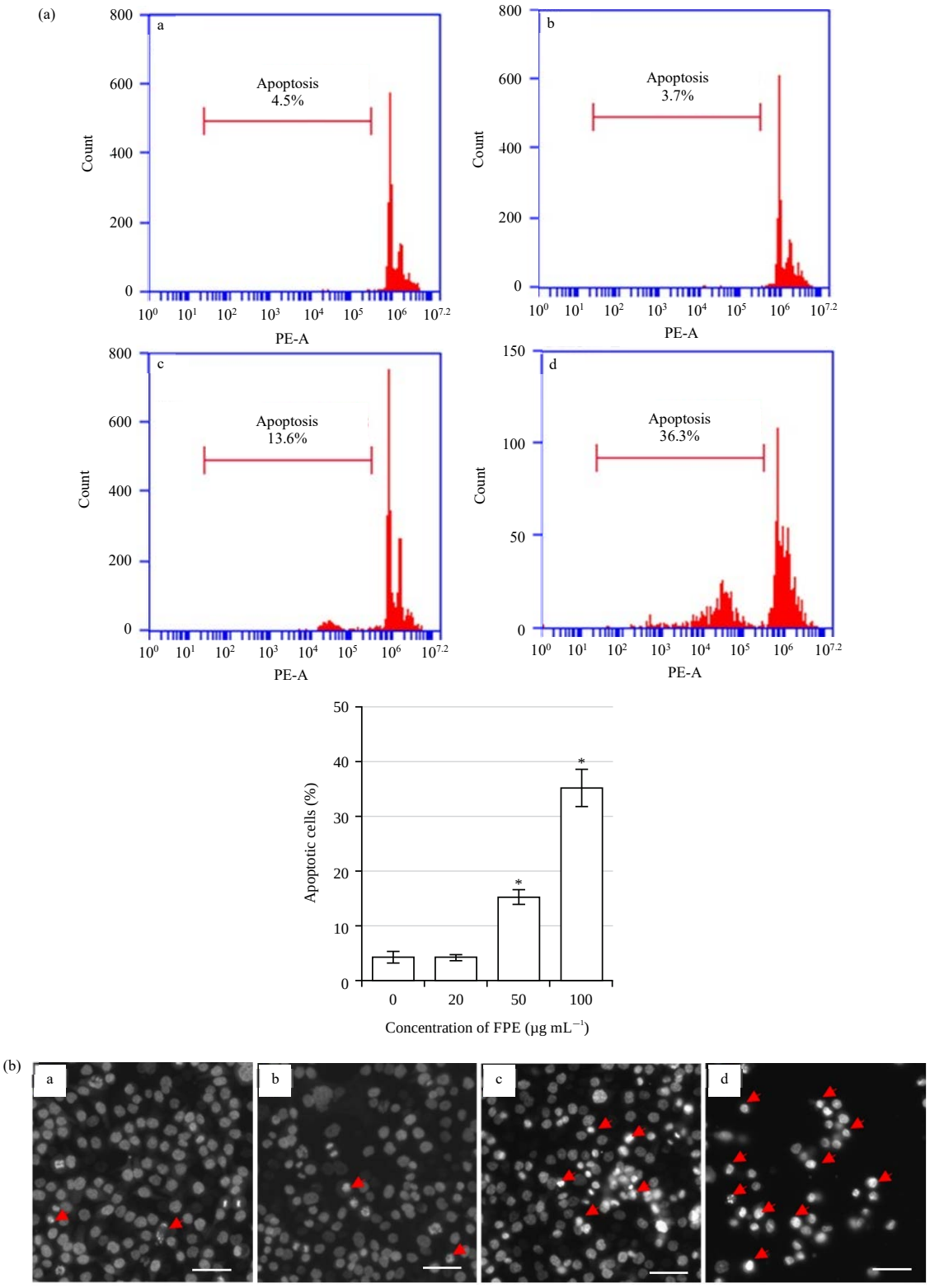

Fig. 3(a-c): FPE induces apoptosis in HepG2 cells, (a) Percentage of apoptotic cells measured by flow cytometry were represented as Mean±SD and (b) Cells were treated with FPE. Cell nuclei were stained with DAPI (4 ,6-diamidino-2-phenylindole) and observed under a T2U fluorescence microscope (Nikon) at 200X magnification

*p<0.05 vs control, Mann-Whitney Test, a: 0, b: 10, c: 50, d: 100 µg mL^{-1} for 48 hrs and Scale bar 50 µm

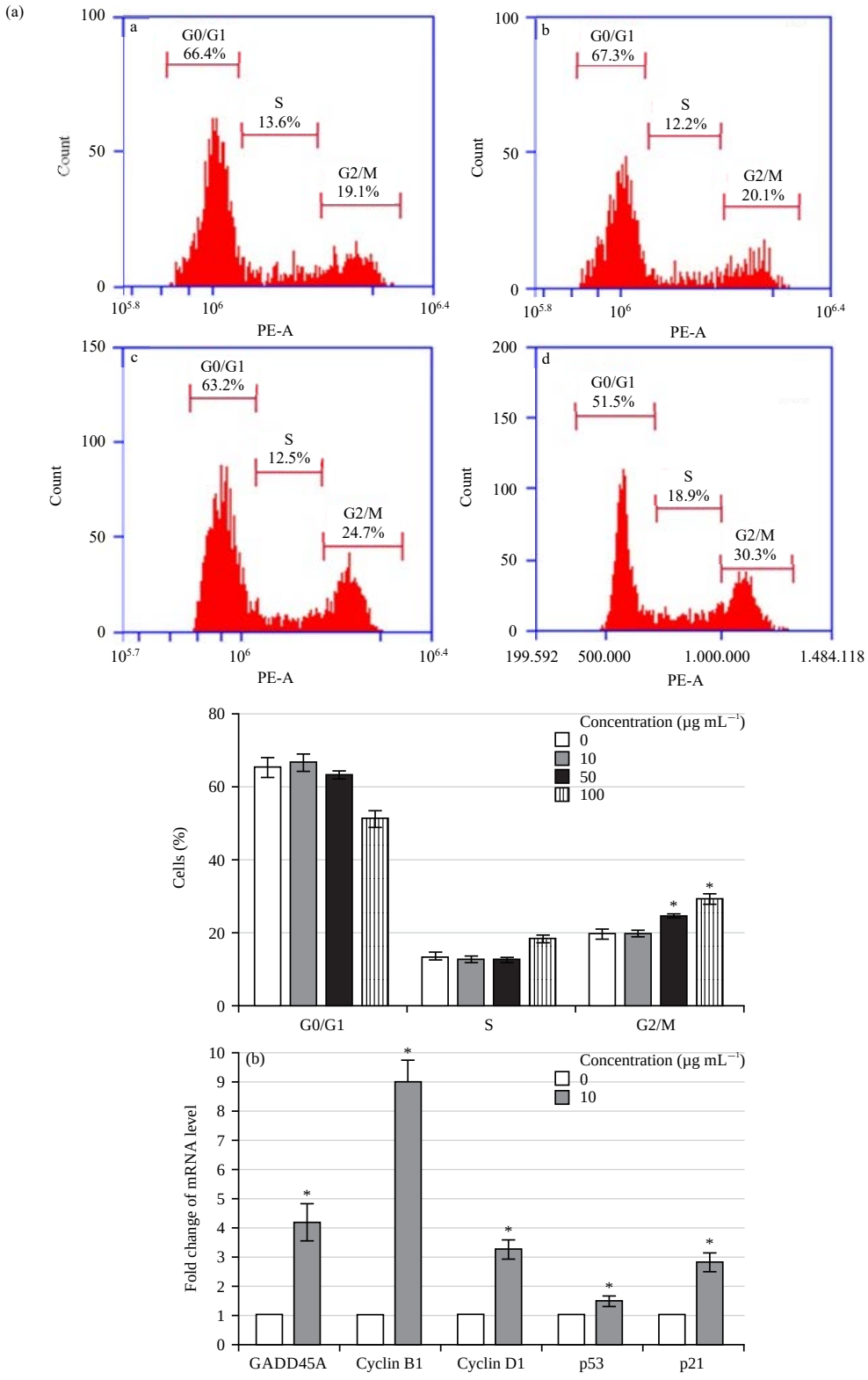

Fig. 4(a-d): FPE arrests the cell cycle in HepG2 cells, (a) Cells were treated with FPE for 48 hrs, were then stained with PI solution and analyzed by flow cytometry and (b) mRNA expression levels of GADD45A, cyclin B1, cyclin D1, p53 and p21 genes were analyzed by real time PCR

(a) a: 0, b: 10, c: 50 and d: 100 μg mL^{-1}, Data represented as cell percentage compared to control, *p<0.05 vs control and (b) *p<0.05 vs control, Mann-Whitney Test

DISCUSSION

Inhibition of proliferation is an important first factor in the evaluation of the anticancer potential of drugs and various compounds. In this study, in a single-layer (2D) culture model, the FPE effectively inhibited HepG2 cell proliferation with an IC_{50} value defined as 164 and 117.8 μg mL^{-1} for 24 and 48 hrs of treatment, respectively. As mentioned above, compared to some other species in the *Ficus* genus, *Ficus pumila* L. is less studied on the activity and mechanism of inhibiting cancer cells. Several previous studies have shown that extracts of *Ficus pumila* L. can inhibit several different types of cancer. However, the effect of *Ficus pumila* L. on apoptosis, the cell cycle of HepG2 cells or the expression of related genes has not been studied. Cancer cells that were cultured under three-dimensional cell cultures are believed to have more similar properties to cancer cells *in vivo* than themselves when cultured in a traditional layer (2D)[23]. The use of 3D culture models is the current trend in drug research[24]. In this model, the effect of drugs on spherical cell masses consisting of many layers of cells is compared to the effect on tumors *in vivo*[25]. Here, it was shown that the FPE not only effectively inhibited the formation of tumorspheres from HepG2 cells, but also significantly declined the size of tumorsphere cells. This may open the prospect of this extract's ability to inhibit tumor formation and tumor proliferation *in vivo*.

Changes in apoptosis can be identified through Flow cytometry analysis or microscopic observation with DAPI dye to detect morphological and structural changes in the cell nucleus[26]. Meanwhile, genes controlling apoptosis can be evaluated through Real-time PCR, Western blot or immunofluorescence analyses[27]. In this study, the FPE-induced cell apoptosis was shown. In addition, nuclear staining with DAPI dye confirmed that the FPE produced cells with an apoptosis phenotype. Although there were little data on the apoptosis-inducing ability of *Ficus pumila* L.in various cancer cell lines, several species of the *Ficus* genus, such as *Ficus virens*, have been shown to induce cell apoptosis of breast cancer MCF7[28], *Ficus septica* induces apoptosis on human oral squamous carcinoma cells[29], *Ficus pumila* L. on human prostate cancer cell[30]. *Ficus dubia* latex on colorectal cancer cell lines[31].

Cancer cell proliferation is closely related to the cell cycle and cell cycle arrest can lead to inhibition of cell replication[32]. This is an important mechanism for the development of current anticancer drugs[33,34]. In this study, FPE induced cell cycle arrest in the G2/M phase. There is earlier evidence that some herbal extracts such as *Costus speciosus*[35], *Vaccinium vitis-idaea*[36], *Trichosanthes kirilowii* tube[37] or potential compounds have the ability to halt HepG2 cancer cell division in the G2/M phase.

The P53, P21 is known to be important protein in cell cycle regulation as well as the induction of apoptosis[38]. Meanwhile, the P53-regulated GADD45A genes have a role in stopping division, promoting nuclear DNA degradation and induction of apoptosis[39]. An earlier study has shown that GADD45, cyclin B1 and cyclin D1 are all strongly associated with a cell cycle arrest in the G2/M phase and enhanced expression of GADD45 has been shown to inhibit the expression of several key proteins that control the cell cycle such as cyclin B1, cyclin A2, cyclin D1 and eventually halt cell division[40]. Real-time PCR analysis showed that FPE upregulated the expression of p53, p21 and GADD45 genes and remarkably increased the expression of cyclin B1 and cyclin D1 genes. Previously, Choudhari *et al.*[41] also showed that an aqueous extract of *Ficus religiosa* induces cell cycle arrest in human cervical cancer cells through the up-regulation of the expression of p53 and p21. *Ficus carica* was shown to upregulate the expression of p53, p21 in MDA-MB-231 breast cancer cells[42]. Meanwhile, *Ficus religiosa* halted the Siha cervical cancer cell cycle through enhanced expression of p53 and p21 and pRb genes[43].

These results suggested that *Ficus pumila* L. has the potential as a treatment option for liver cancer. However, this study also has some limitations such as lacking evaluation changes in expression at the protein level as well as identifying chemical components that play an important role in HepG2 cytotoxicity. Further studies are needed to elucidate the inhibitory mechanism of *Ficus pumila* L. against cancer.

CONCLUSION

This study showed that, under *in vitro* conditions, the FPE has the ability to inhibit proliferation, tumorsphere formation arrests the cell cycle and induce apoptosis of HepG2 liver cancer cells. *Ficus pumila* L. has the potential to inhibit liver cancer cells.

SIGNIFICANCE STATEMENT

This study aims to determine the anti-cancer activity of the *Ficus pumila* L. liver cancer cells. The results showed that the FPE has a strong inhibitory effect on the proliferation of HepG2 liver cancer cells, significantly reducing the number and size of tumorspheres compared to the control. The FPE also increased the apoptosis rate and arrested the cell cycle at the G2/M phase, as well as upregulated the expression of genes involved in cell cycle arrest and apoptosis while downregulating genes associated with tumor growth.

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