A potential oral anticancer drug candidate, *Moringa oleifera* leaf extract, induces the apoptosis of human hepatocellular carcinoma cells

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Abstract. It has previously been reported that cold water-extracts of *Moringa oleifera* leaf have anticancer activity against various human cancer cell lines, including non-small cell lung cancer. In the present study, the anticancer activity of *M. oleifera* leaf extracts was investigated in human hepatocellular carcinoma HepG2 cells. By the analysis of apoptotic signals, including the induction of caspase or poly(ADP-ribose) polymerase cleavage, and the Annexin V and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assays, it was demonstrated that *M. oleifera* leaf extracts induce the apoptosis of HepG2 cells. In the hollow fiber assay, oral administration of the leaf extracts significantly reduced (44-52%) the proliferation of the HepG2 cells and A549 non-small cell lung cancer cells. These results support the potential of soluble extracts of *M. oleifera* leaf as orally administered therapeutics for the treatment of human liver and lung cancers.

Introduction

Plants have been used for millennia in traditional medicine to treat diseases and to supplement various nutrients (1-3). Recently, studies showing that extracts from various types of plants are useful in the medical industry has encouraged the development of novel types of natural products that are effective in various types of diseases, and which may function as antitumor, antioxidant, antiobesity and antimicrobial molecules (3).

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The widely cultivated and fast-growing *Moringa oleifera* (also known as Moringa or drumstick tree) is cultivated in tropical and sub-tropical locales, such as the sub-Himalayan region, Oceania, Latin America, Africa and Asia. *M. oleifera* has been regarded as a 'miracle tree', as it is a significant source of fats, proteins, β-carotene, vitamin C, iron, potassium and other nutrients, and is also effective in the treatment of numerous diseases (4-13). The flowers, roots, leaves and bark of *M. oleifera* have long been used by the public as nutritional supplements and foods, as well as in the manufacture of perfume, skin oil and other products (12,14-19). Certain parts of *M. oleifera* (leaf, stem and root) have been demonstrated to produce various biological activities, including antiatherosclerotic (20), immune-boosting (21), anticardiovascular disease (22), antiviral (1,23-25), antioxidant (2,26-28), antimicrobial (27), anti-inflammatory (29) and tumor-suppressive effects (30). Due to its long history of usage and various biological effects, *M. oleifera* has long been the subject of research interest. A previous study reported on the therapeutic potential of the water-soluble extract from *M. oleifera* leaves (MOL) in the treatment of various types of cancers, including lung, breast and skin cancers (30).

The majority of studies that describe the preparation of bioactive compounds from natural plants have utilized solvent extraction, in which the water insoluble extracts have been typically obtained using methanol and ethanol, in addition to hot water and buffers (1,26,27,31,32). Solvents are widely used, as they allow for effective extraction of a broad range of different phytochemicals, such as bioactive but water insoluble phenolic compounds found in plants. However, these water insoluble compounds may be difficult to orally administer to human patients, and additional efforts to enhance the bioavailability or absorption rates are often required (26). This solvent extraction technique has also been used to extract useful compounds from *M. oleifera* by a number of research groups; however, they have not reported on the effects of the water-soluble extract of *M. oleifera*. In our previous study, the effectiveness of a cold water (4˚C)-soluble extract of MOL in lung cancer prevention was reported (30).

Generally, anticancer drug candidates have bulky hydrophobic groups within their chemical structures that render them water-insoluble and may lead to formulation problems

and serious therapeutic challenges, including serious complications such as embolism and respiratory system failure due to the precipitation of the drug in the case of intravenous administration (33-35). For these reasons, increasing the water solubility of anticancer agents and developing soluble bioactive compounds with strong anticancer activities is vital. Compared with parenteral injections, the continued development of oral anticancer therapies has been fueled by their ease of administration, lack of requirement for hospitalization or clinic visits, acceptable disease outcomes, patient satisfaction, reduced interference in work and social activities, and the paradigm shift that views cancer as a chronic condition. However, orally administered drugs may be degraded by metabolic processes prior to arriving at their target sites (36-38). To address this disadvantage, the development of oral medications with high bioavailability has received much attention in anticancer therapy research.

In the present study, a water-soluble MOL extract was prepared and its anticancer activity was tested against hepatocellular carcinoma cells. Furthermore, its efficacy as an orally administered therapeutic agent in mice with lung and liver cancer was investigated.

Materials and methods

Sample preparation. The leaves of *M. oleifera* (MOL), cultivated in Chiangmai, Thailand, were purchased from Moringa Korea Co. (Milyang, Gyeongsangnam, South Korea). The dried MOL (150 mg) were suspended in 1 ml of cold water (4˚C), vigorously and continuously vortexed for 30 sec, and allowed to stand in the refrigerator (4˚C) for 10 min. After another vigorous vortexing of the MOL extract for 1 min, the water soluble supernatants were collected by centrifugation (13,000 x g for 10 min, twice) and then membrane-filtered (0.2-μm filter). The filtrated MOL extracts were lyophilized at -50˚C for 2 days using a freeze dryer (FD5505; Ilshin Biobase Co., Ltd., Seoul, Korea) then stored at -20˚C. For the experiments, the lyophilized MOL extracts were resuspended in distilled water (DW) at a final concentration of 20 mg/ml of protein.

Cell cultivation. Human non-small cell lung cancer A549 and human hepatocellular carcinoma HepG2 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were seeded at an initial density of $1x10^5$ cells in a 6-well plate containing RPMI-1640 medium (for A549) or DMEM (for HepG2) (GE Healthcare, Pittsburgh, PA, USA) supplemented with 10% fetal bovine serum (GE Healthcare) and 1% penicillin-streptomycin, and incubated at 37˚C.

Flow cytometric analysis. Prior to MOL extract treatment, the HepG2 cells ($1x10^5$) were seeded in a 6-well culture plate and were incubated for 1 day. Following another 2-day incubation, the cells were collected, fixed with 70% ethanol at 4˚C for 2 h and stained with propidium iodide (PI; 50 μg/ml) for 30 min at room temperature. A FACScan system (EPICS XL Flow Cytometry, Beckman Coulter Counter; Beckman Coulter, Inc., Indianapolis, IN, USA) was used to measure the DNA content. The proportion of cells in each cell cycle stage was determined using the Phoenix Multicycler Software (Phoenix Flow System, San Diego, CA, USA) and the numbers in the images indicate the percentages of the total that were sub-G1 cells.

Cell proliferation assay (MTT assay) and colony forming assay. A cell proliferation assay using tetrazolium salt (MTT) was performed to measure the viability of the HepG2 cells (24). The cells were adjusted to a density of $3x10^3$ cells in each well of a 96-well plate and incubated for 1 day. Various concentrations of MOL extracts (0-200 μg/ml) were added, and the cells were incubated for another 2 days. Cell proliferation was measured by Cell Counting Kit-8 (cat. no. CK04, Dojindo Laboratories, Kumamoto, Japan). The percentage ratio is the relative proportion of viable cells compared with the control group (non-MOL extract-treated group).

Colony-forming assay. Clonogenicity was examined by the colony-forming assay, as previously described (30). Briefly, the cells were seeded at an initial density of $1x10^3$ HepG2 cells/well in 6-well culture plates, incubated for 1 day prior to the addition of the MOL extract, and then incubated for a further 7-14 days. Finally, the cells were stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) and images were captured with a Canon digital camera (PowerShot S45; Canon, Inc., Seoul, Korea). Representative images are presented.

Annexin V binding assay. The A549 cells were cultured for 1 day and treated with MOL extract, after which they were incubated for another 2 days. The Annexin V-fluorescein isothiocyanate (FITC)/PI flow cytometric assay kit was purchased from Roche Diagnostics (cat. no. 1185877700; Basel, Switzerland) and used to determine the proportion of apoptotic cells in each MOL-treated group. Briefly, harvested cells were resuspended in incubation buffer at a concentration of 10^4 cells/ml, followed by incubation with Annexin V-FITC for 20 min and subsequent incubation with PI for 5 min in the dark. At least 10,000 stained cells from each sample were analyzed by Cytomics FC 500 (model 175487-FC500; Beckman Coulter Inc., Indianapolis, IN, USA). Data were analyzed with CELL Quest software (BD Biosciences, San Jose, CA, USA).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. Apoptosis was also assessed by utilizing the TUNEL assay to detect DNA strand breaks during apoptosis using an *in situ* cell death detection kit (cat. no. 11684795910; Roche, Basel, Switzerland) according to the manufacturer's instructions. Briefly, the HepG2 cells were treated with different concentrations of MOL extracts (0-300 μg/ml) for 2 days, fixed with freshly prepared 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.4) for 20 min, and incubated in permeabilization solution with 0.3% Triton X-100 for 5 min at room temperature. Subsequent to being washed three times with PBS buffer, the cells were incubated with TUNEL reaction mixture for 60 min at 37˚C and analyzed under a fluorescence microscope (model nos. BX53F and U-RFL-T; Olympus Corporation, Tokyo, Japan), through which images

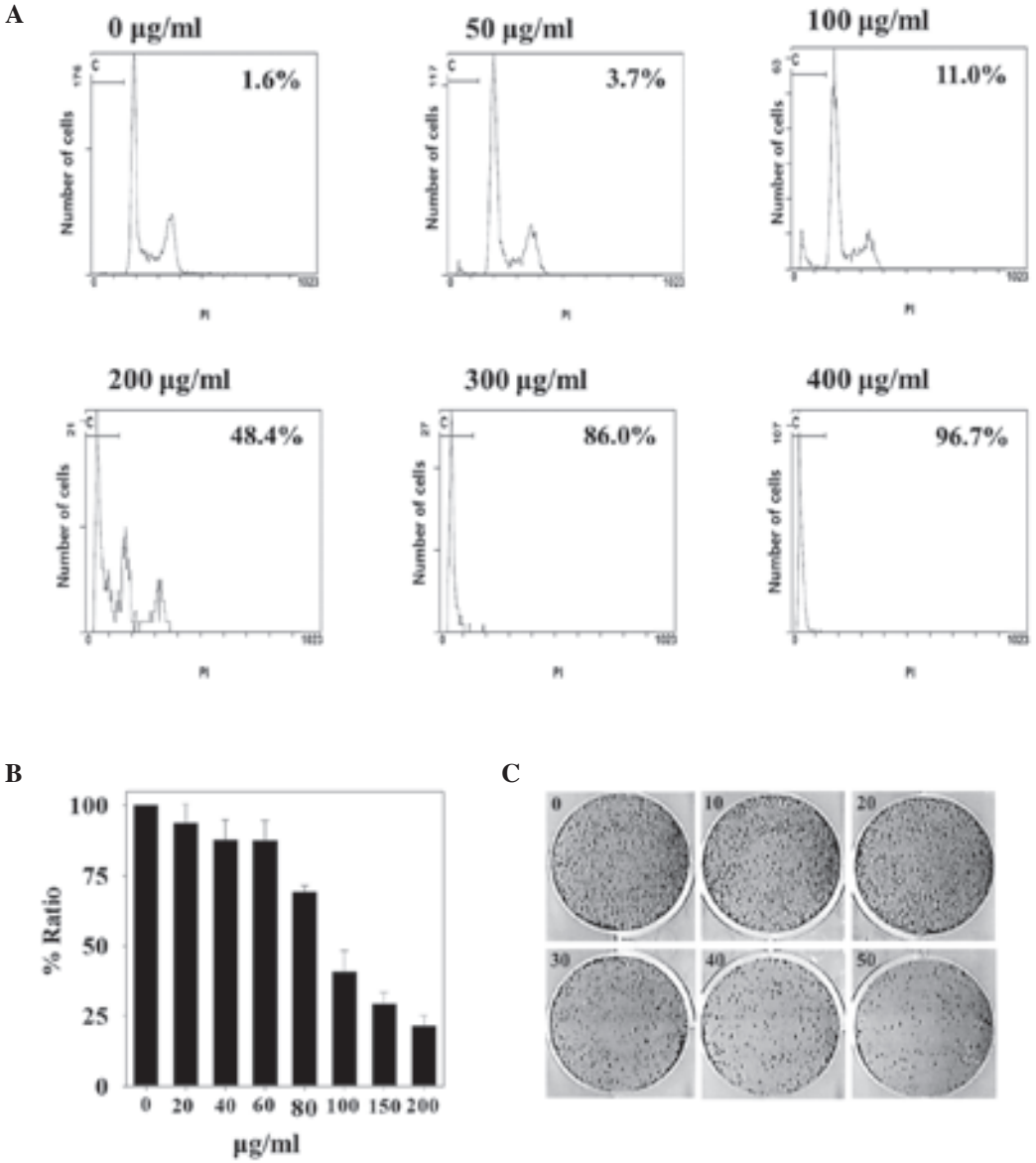

Figure 1. Effects of *Moringa oleifera* leaf extract on cell cycle and cell proliferation. (A) Flow cytometric analysis. Cells ($1x10^5$/well) were seeded and incubated for 1 d prior to treatment with MOL extract (0-400 μg/ml). After incubation for 2 d, the cells were collected and analyzed using the FACScan system. The numbers in the figures indicate the percentages of sub G1 cells. (B) MTT assay. Cells ($3x10^3$/well) were seeded in 96-well plates and incubated for 1 d. Following treatment with various concentrations of MOL extract (0-200 μg/ml), the cells were incubated for 2 d. The percentage ratio was calculated as the relative proportion of viable cells compared with the control group (non-MOL extract treated group). (C) Colony forming assay. Cells ($1x10^3$/well) were seeded in 6-well plates and incubated for 1 d followed by treatment with various concentrations of MOL extract (0-50 μg/ml) and subsequent incubation for 7-14 d. PI, propidium iodide.

of representative fields were captured. The HepG2 cells were also fixed in 4% paraformaldehyde and incubated in 0.5 μg/ml 4,6-diamidino-2-phenylindole solution for 30 min in the dark at room temperature for counterstaining.

Western blot analysis. Prior to adding the MOL extract, the HepG2 cells ($1x10^5$) were seeded in a 6-well culture plate for 1 day. After 2 days of additional incubation, the proteins were collected and their concentrations were determined by the Bradford method (Protein Assay Dye Reagent Concentrate, cat. no. 500-0006; Bio-Rad Laboratories, Hercules, CA, USA). Proteins were then loaded in equal amounts (20 μg) onto an 8-12% SDS polyacrylamide gel and stained with Coomassie Brilliant Blue. The antibodies [polyclonal rabbit anti-β-actin, cat. no. 4967; polyclonal rabbit anti-poly(ADP-ribose) polymerase (PARP), cat. no. 9542; monoclonal rabbit B-cell lymphoma-extra large (Bcl-xL), cat. no. 2764; polyclonal rabbit caspase-3, cat. no. 9662; and polyclonal rabbit anti-cleaved caspase-3, cat. no. 9661] for western blot analysis were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA).

Hollow fiber assay (HFA). An HFA was performed following the standard procedures set by the National Cancer Institute (NCI; Bethesda, MD, USA; https://dtp.cancer.gov/branches/btb/hfa.html) (39-42). Briefly, the two tumor cell lines, A549 and HepG2, were cultured in 75-cm^2 culture flasks. The media were flushed into 1-mm (internal diameter) polyvinylidene fluoride hollow fibers (HFs) with a molecular weight cutoff of 500 kDa (Spectrum

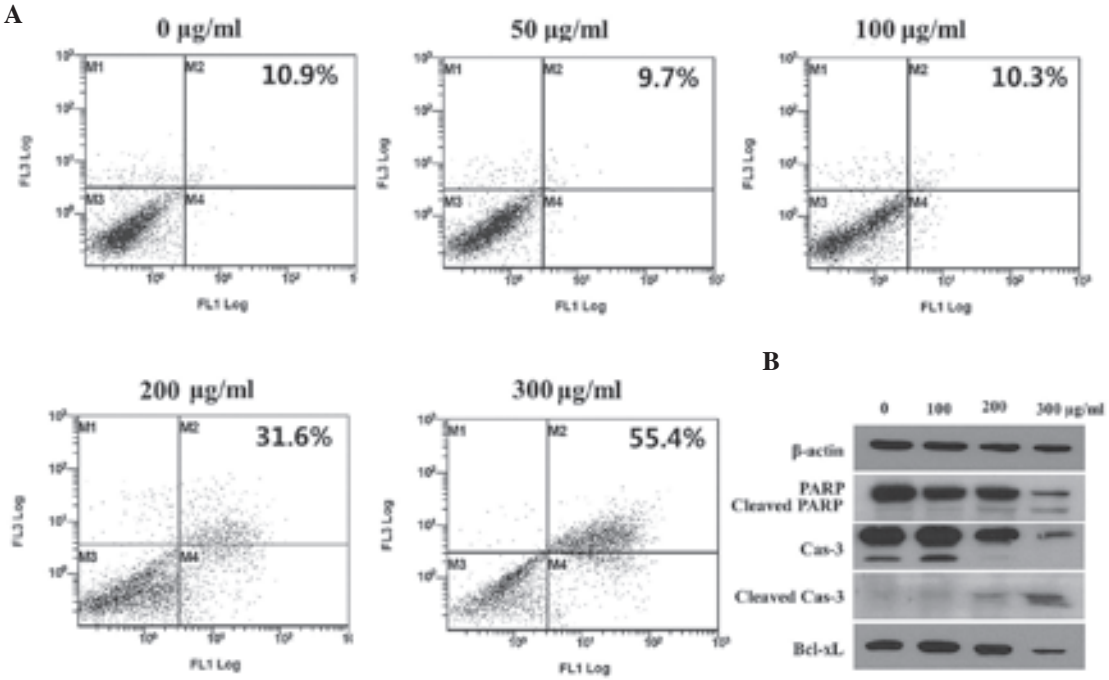

Figure 2. Apoptosis and western blot assays. (A) The number of apoptotic cells was detected and analyzed using flow cytometry. (B) Examination of the levels of proteins associated with the cell cycle and apoptosis. PARP, poly(ADP-ribose) polymerase; Bcl-xL, B-cell lymphoma-extra large; Cas, caspase.

Laboratories, Houston, TX, USA) and then the HFs were loaded with cells ($5x10^5$ cells/ml). Each HF was heat-sealed with a hot smooth-jawed needle holder every 1.5 cm along its length and cut into segments with 2-mm tails for ease of handling; at the highest seeding density, each HF was 1.5 cm long. Each HF segment contained ~$1x10^5$ cells. The fibers were placed into 6-well culture plates containing cell culture medium and were incubated for 1 day prior to surgical implantation in 6-week-old male immunodeficient nude mice (Harlan Laboratories, Horst, Netherlands). All mice were housed under 12-h light-dark cycles in an air-conditioned room with unrestricted access to water and food (Purina 5001 Rodent Chow; Purina, St. Louis, MO, USA). The mice were anesthetized with Zoletil (tiletamine/zolazepam; Virbac Korea Co., Ltd., Seoul, South Korea) and Rompun (xylazine; Bayer, Seoul, South Korea), and then HFs were implanted at subcutaneous sites and the incisions closed using skin staples. The mice were orally administered different concentrations of MOL extract, or were intravenously injected with doxorubicin (cat no. D1515-10MG; Sigma-Adrich) as a control, starting on day 3 following the fiber implantation (each dose of MOL extract and doxorubicin were administered daily for 4 days). After 7 days, the fibers were collected and subjected to the trypan blue exclusion assay. Internationally recognized guidelines on animal welfare were followed and the experiments were approved by the Gachon University Institutional Animal Care and Use Committee (approval no. GIACUC-L2014001).

Statistical analysis. Statistical analysis was performed using SigmaPlot software (version 7.0; Systat Software, Inc., San Jose, CA, USA). A Students's t-test was used to analyze differences in cell viability. $P<0.05$ indicated a statistically significant difference.

Results

Effect of MOL extract on HepG2 hepatocellular carcinoma cells. Dried MOL was extracted with cold DW (4˚C) and the resulting water soluble extract was used in this study. Human hepatocellular carcinoma HepG2 cells were treated with the MOL extract for 2 days, and then analyzed by FACScan. As presented in Fig. 1A, cell cycle analysis demonstrated that MOL at 50, 100, 200, 300 and 400 μg/ml resulted in a concentration-dependent accumulation of HepG2 cells in the sub-G1 phase, which indicated apoptotic cells, as compared to the control (non-MOL extract-treated group). In particular, the HepG2 cells treated with 400 μg/ml of MOL extract showed a sub-G1 fraction of up to 96% compared with the control group.

The cytotoxic inhibitory effect of MOL extract on HepG2 cell proliferation was further verified using the MTT assay (Fig. 1B). Cells treated with different concentrations of MOL extract (0-200 μg/ml) for 2 days were subjected to the MTT assay. The results demonstrated that cell proliferation was significantly inhibited in a concentration-dependent manner by MOL extract ($P<0.05$), and treatment with 200 μg/ml of MOL extract produced inhibition ratios of up to 80%.

The impact of the MOL extract on the clonogenic growth of the HepG2 cells was also examined (Fig. 1C). The cells were seeded onto 6-well plates and treated with MOL extract, and then stained with crystal violet after an incubation period of 7 days. The data showed that the control cells (not treated with MOL extract) formed colonies that were uniformly distributed in the plate (0 μg). By contrast, the cells treated with MOL extract demonstrated an up to 70% reduction in the number of colonies (50 μg/ml of MOL). In conclusion, clonogenic growth was inhibited by MOL extract in a dose-dependent manner.

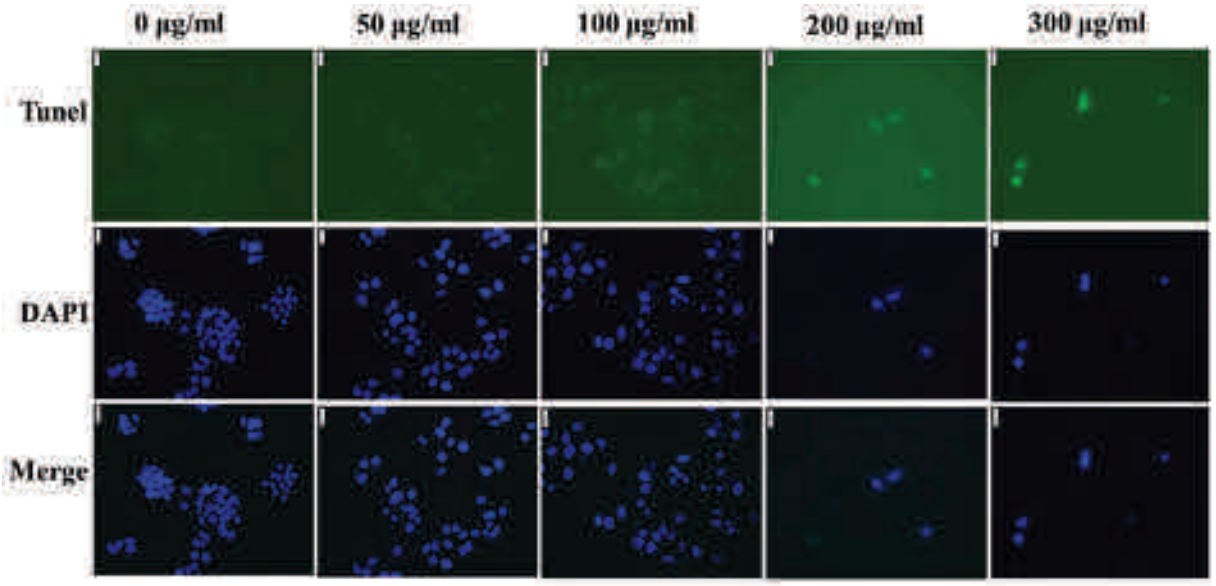

Figure 3. TUNEL assay. HepG2 cells were treated with different concentrations of *Moringa oleifera* leaf extract for 2 days and stained with TUNEL and DAPI. Images of morphological changes in nuclear chromatin were captured with a fluorescence microscope (x100 magnification). TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; DAPI, 4,6-diamidino-2-phenylindole.

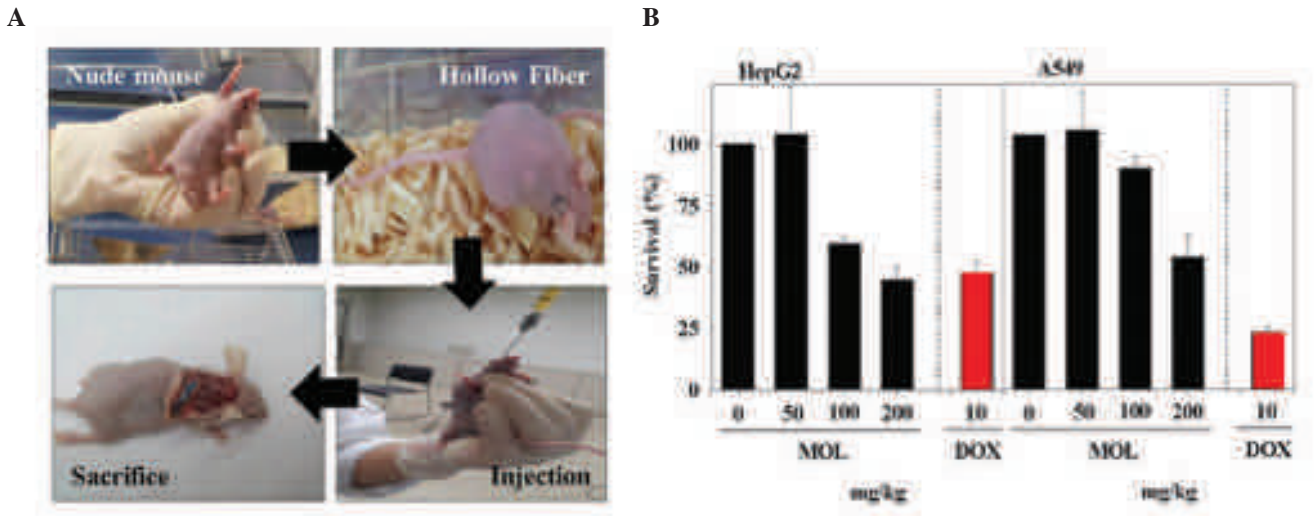

Figure 4. Hollow fiber assay. (A) Experimental procedures. (B) Each fiber for different tumor cell types was implanted, and 3 different concentrations of the MOL extract were orally administered. Doxorubicin was used as a control treatment and was intravenously injected. The average of 3 independent experiments is shown, and the variation within each set of experiment was <20%. The cell preparations were analyzed for viable cell masses by a stable-endpoint MTT assay. DOX, doxorubicin; MOL, *Moringa oleifera* leaf.

Evaluation of apoptosis. As MOL extract treatment produced an accumulation of cells at the G_0/G_1 peak during cell cycle analysis, as well as a reduction in cell viability and the inhibition of clonogenic growth, the apoptotic state of the cells was investigated. In order to detect and quantify apoptosis, the Annexin V-FITC/PI double staining method was used to stain HepG2 cells treated with MOL extract. The cells were collected following 2 days of treatment with MOL extract at different concentrations (0-300 μg/ml), then double-stained and subjected to FACS analysis. The increased dot plots in the late and early apoptotic region demonstrate a dose-dependent effect (Fig. 2A). Subsequent to MOL extract treatment, ~55.4% of cells were in either the early (stained only by Annexin V-FITC) or late (stained by Annexin V-FITC and PI) apoptotic stage. The apoptotic cell ratio of the cells treated with MOL extract at a concentration of 300 μg/ml was 5 times higher than that of the control cells.

Apoptosis in the HepG2 cells was further elucidated by western blotting. As presented in Fig. 2B, the expression of certain apoptotic markers, including cleaved PARP and cleaved caspase-3, were increased by treatment with MOL extract. The antiapoptotic Bcl-xL protein was downregulated by MOL extract treatment, indicating that the MOL extract inhibited cell proliferation (Fig. 2B).

The TUNEL assay is a common method for detecting the DNA fragmentation that results from apoptotic signaling, which allows apoptotic cells with DNA fragmentation to be detected by fluorescence microscopy. In the present

study, TUNEL staining was performed to detect apoptotic morphology alteration in individual HepG2 cells. The presence of TUNEL-positive cells with fragmented DNA in their nuclei was indicated by a green fluorescence signal, indicating that DNA strand breaks had occurred, and that MOL extract induced apoptosis in the HepG2 cells (Fig. 3).

HFA. Considering the *in vitro* results demonstrating that the MOL extract inhibited cancer cell proliferation, an *in vivo* study was crucial to evaluate whether MOL inhibits cancer cell progression. To test this hypothesis, nude mice were implanted with hollow fibers filled with HepG2 or A549 cells. After 2 days, different concentrations of MOL extract were orally administered to the MOL-treated mice, and doxorubicin was intravenously administered to the control mice. On day 5 after administration, the animals were sacrificed and the fibers were collected, and the trypan blue exclusion assay was performed (Fig. 4A). A gradual reduction in cell viability was observed for the HepG2 cells, while a similar kinetic profile was observed for the A549 cells (Fig. 4B). Notably, the effective concentrations of MOL extract in the HFA assay, which inhibited cancer cell proliferation, were similar to those that were effective in the *in vitro* cell-based assay (Fig. 1B) (30), indicating that MOL extract was effectively delivered to the target site via oral administration. As compared to that observed in A549 cells, significantly stronger cytotoxicity was produced by MOL extract in the HepG2 cells, and the rate of cytotoxicity in these cells was higher compared with that produced by doxorubicin (control) treatment ($P<0.05$). Studies involving the administration of higher concentrations of MOL should now be performed.

Discussion

M. oleifera, of the monogeneric family Moringaceae, is distributed worldwide and has been known by a number of different names, including the horseradish tree (English), Soanjna (Hindi), Shobhanjana (Sanskrit), Saragvo (Gujarati), Sajna (Bengali), Munanga (Telugu) and Murangai (Tamil) (43). For millennia, various parts of *M. oleifera* have been used as nutritional supplements (as a rich source of vitamins A and C, iron, calcium, and protein) (44,45), and for their antibiotic (46-49), antihyperlipidemic (50), antidiabetic (51,52), wound healing (53) and antiulcer properties (54). The edible parts of the plant have also been employed traditionally for skin diseases, rheumatism, anemia, cholera and other ailments (53). Previous studies have shown that *M. oleifera* has potential anticancer activities (44,54,55) In particular, it was previously demonstrated that the water-soluble fraction of MOL induced apoptosis in lung cancer cells, and is therefore a novel type of potential anticancer candidate compound (30).

Solvent extraction methods have been widely utilized to obtain extracts from plants, as solvents such as methanol and ethanol effectively extract polar and non-polar bioactive compounds (1,26,27,31,32). However, non-polar compounds are not well absorbed in the body, although they are bioactive. In contrast to the solvent extraction method, compounds obtained via water extraction are often more easily absorbed into the body, although the extraction efficiency and the range of extracted compounds are normally inferior to those that result from the solvent extraction method. Despite these disadvantages, water extraction methods are considered, as the resulting extracts are easily absorbed and offer the possibility of oral administration.

In previous studies, a cold water-soluble extract was prepared from MOL and its anticancer activity was confirmed in human lung cancer A549 cells. In the present study, as a part of an ongoing effort, the anticancer activity of MOL extract against human hepatocellular carcinoma HepG2 cells was investigated to evaluate its potential as an oral medication for lung and liver cancer treatment. The results of the present study showed that MOL extract was effective in the HepG2 cells, in addition to the A549 lung cancer cells. In addition, the oral administration of MOL was effective in the mice with either lung or liver cancer cells, indicating that the cold water MOL extract could be a potential oral anticancer candidate agent.

Recent preclinical studies of anticancer drugs using mice have tended to use parenteral administration methods, including the intravenous or subcutaneous routes. However, for clinical studies, these drugs require further study in order to increase the absorption efficiency, decrease the possible side-effects and reduce the inconvenience to patients. In particular, subcutaneous injection, utilized by a number of studies in the field of anticancer drug development, does not target particular types of cancer, and is thus a poor predictor of the efficacy of its clinical utility, although the candidate agents administered by this method may show efficacy in lab-scale experiments. The present study examined whether a water-soluble MOL extract may be used as an oral medication. As presented in Fig. 4, the oral administration of MOL extract greatly inhibited cancer cell proliferation, indicating that the MOL extract used in the present study is potentially suitable as an oral medication against cancer, although the detailed mechanisms are yet to be elucidated. Future pharmacogenetic and pharmacokinetic studies must be performed in order to further evaluate the clinical potential of MOL extract, in addition to any chronic and acute toxicity.

Although *in vitro* cell-based assays are useful for screening drug candidates and examining their mechanisms of action, further *in vivo* assays are usually necessary prior to clinical testing. For this purpose, researchers developed the traditional xenograft assay, which has been used worldwide. However, the cost of the traditional xenograft assay is high, and it takes a substantial period of time due to the large number of animals required. The *in vivo* HFA in immunodeficient nude mice was designed at the NCI to try to bridge the gap between *in vitro* cell-based assays and human tumor models; the goal of the assay was to predict which compounds would be active in a subsequent xenograft system (56-59). In the HFA, proliferating cancer cells containing hollow fibers with pores, which are small enough to retain the cancer cells, but large enough to permit potential chemotherapeutic drugs to enter, are transplanted into the peritoneum or under the skin of the host mice. Next, test drug candidates are administered to the mice, and the fibers are then retrieved for analysis of any viable cell masses (56). The HFA is similar to the xenograft assay, but is a time- and cost-saving method. For these reasons, the finding that MOL had anticancer activity in the

HFA of the present study supports its potential as a novel anticancer drug. However, future xenograft mouse assays and other non-clinical tests will be required prior to clinical testing.

Drug candidates with anticancer activity often have bulky hydrophobic groups that render them water-insoluble (60). Low water solubility causes a number of problems in the formulation and administration of anticancer agents (61), thus, increasing water solubility and/or developing soluble bioactive compounds with strong anticancer activity have become areas of interest. Oral therapies have the advantage of resulting in the persistent exposure of tumor cells and the tumor environment to the administered cytotoxic drug, and oral anticancer agents allow therapeutic drug treatment in the comfort of the patient's home or in alternative settings, such as retirement homes and assisted-living or extended-care facilities (28). Therefore, efforts to develop soluble drugs that can be orally administered have been considered in the drug discovery processes. However, such a strategy is rarely used by researchers, as the majority of active compounds are water-insoluble. For this reason, a number of researchers are using solvent extraction. Despite this trend, the present study focused on a novel water-soluble MOL extract in order to overcome the problems caused by low solubility, and examined its potential as an oral anticancer drug.

In the present study, the HFA was used to demonstrate that oral administration of MOL, in addition to intravenous injection of doxorubicin, markedly inhibited lung and liver cancer cell proliferation. This significant tumor inhibition produced by oral administration of MOL may be due to the high bioavailability of the extract, as the concentration used in the *in vitro* cell experiment was similar to that used in the *in vivo* mouse experiment. The reasons for the similarity in the effective concentrations in the cell- and mouse-based assays are not clear at this time, however, future preclinical trials, such as pharmacokinetic studies, could uncover them. In the current study, it was demonstrated that the water soluble MOL extract may be a novel and promising natural anticancer drug candidate.

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